ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 January 24; 14(1): 1-39

Published by Baishideng Publishing Group Inc

WJCO

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 1 January 24, 2023

MINIREVIEWS

1 Oncologic impact of colonic stents for obstructive left-sided colon cancer

Suzuki H, Tsujinaka S, Sato Y, Miura T, Shibata C

ORIGINAL ARTICLE

Basic Study

13 Identification of a three-gene prognostic signature for radioresistant esophageal squamous cell carcinoma

Wang XY, Beeraka NM, Xue NN, Yu HM, Yang Y, Liu MX, Nikolenko VN, Liu JQ, Zhao D

27 5-mRNA-based prognostic signature of survival in lung adenocarcinoma

Xia QL, He XM, Ma Y, Li QY, Du YZ, Wang J

World Journal of Clinical Oncology

Monthly Volume 14 Number 1 January 24, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Oncology*, Mohamed Elbadawy, BSc, MD, Ph.D., (Med), Associate Professor, Department of Pharmacology, Faculty of Veterinary Medicine, Benha University, 13736 Moshtohor, Toukh, Elqaliobiya, Egypt. mohamed.elbadawy@fvtm.bu.edu.eg

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 edition of Journal Citation Reports® cites the 2021 Journal Citation Indicator (JCI) for *WJCO* as 0.35.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Yu-Jie Ma*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
January 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 January 24; 14(1): 1-12

DOI: 10.5306/wjco.v14.i1.1

ISSN 2218-4333 (online)

MINIREVIEWS

Oncologic impact of colonic stents for obstructive left-sided colon cancer

Hideyuki Suzuki, Shingo Tsujinaka, Yoshihiro Sato, Tomoya Miura, Chikashi Shibata

Specialty type: Surgery

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Endo S; Rajer M, Slovenia

Received: September 19, 2022
Peer-review started: September 19, 2022
First decision: November 25, 2022
Revised: December 7, 2022
Accepted: December 31, 2022
Article in press: December 31, 2022
Published online: January 24, 2023

Hideyuki Suzuki, Shingo Tsujinaka, Yoshihiro Sato, Tomoya Miura, Chikashi Shibata, Department of Gastroenterological Surgery, Tohoku Medical and Pharmaceutical University, Sendai 983-8536, Miyagi, Japan

Corresponding author: Shingo Tsujinaka, MD, Associate Professor, Department of Gastroenterological Surgery, Tohoku Medical and Pharmaceutical University, 1-15-1 Fukumuro, Miyagino-ku, Sendai 983-8536, Miyagi, Japan. tsujinakas@tohoku-mpu.ac.jp

Abstract

Colonic stenting has had a significant positive impact on the management of obstructive left-sided colon cancer (OLCC) in terms of both palliative treatment and bridge-to-surgery (BTS). Notably, many studies have convincingly demonstrated the effectiveness of stenting as a BTS, resulting in improvements in short-term outcomes and quality of life, safety, and efficacy in subsequent curative surgery, and increased cost-effectiveness, whereas the safety of chemotherapy after stenting and the long-term outcomes of stenting as a BTS are controversial. Several studies have suggested an increased risk of perforation in patients receiving bevacizumab chemotherapy after colonic stenting. In addition, several pathological analyses have suggested a negative oncological impact of colonic stenting. In contrast, many recent studies have demonstrated that colonic stenting for OLCC does not negatively impact the safety of chemotherapy or long-term oncological outcomes. The updated version of the European Society of Gastrointestinal Endoscopy guidelines released in 2020 included colonic stenting as a BTS for OLCC as a recommended treatment. It should be noted that the experience of endoscopists is involved in determining technical and clinical success rates and possibly oncological outcomes. This review discusses the positive and negative impacts of colonic stenting on OLCC treatment, particularly in terms of oncology.

Key Words: Colonic stents; Obstructive left-sided colon cancer; Bridge to surgery; Chemotherapy; Long-term outcomes; European Society of Gastrointestinal Endoscopy guidelines

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Colonic stenting has been widely used in the management of obstructive left-sided colon cancer, and its effectiveness has been convincingly demonstrated. However, some controversies remain, including the safety of chemotherapy after stenting and the long-term outcomes of stenting as a bridge to surgery (BTS). Nevertheless, many recent studies have demonstrated that colonic stenting exerts no negative impact on long-term oncological outcomes, and this technique is recommended as a BTS in the European Society of Gastrointestinal Endoscopy guidelines. Herein, we review and discuss the positive and negative effects of colonic stenting in colon cancer treatment.

Citation: Suzuki H, Tsujinaka S, Sato Y, Miura T, Shibata C. Oncologic impact of colonic stents for obstructive left-sided colon cancer. *World J Clin Oncol* 2023; 14(1): 1-12
URL: https://www.wjgnet.com/2218-4333/full/v14/i1/1.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i1.1

INTRODUCTION

Colorectal cancer remains one of the most common malignant diseases worldwide. Among all patients with colorectal cancer, approximately 10% present with large bowel obstruction[1]. The most common location for obstructive colon cancer (OCC) is the sigmoid colon, and more than 75% of OCC are located on the left side, i.e., distal to the splenic flexure[2].

Emergency surgery (ES) has traditionally been the mainstay of OCC management. There are several options for ES procedures to treat obstructive left-sided colon cancer (OLCC); however, a stoma is often needed in any case. Patients with clinically severe instability or in whom resection is not possible should be treated with diverting loop colostomy[3]. Hartmann's procedure, that is, resection of the diseased colon or rectum with end colostomy, has been widely performed for resectable OLCC[4]. Resection with primary anastomosis could be considered an option during ES for resectable cases; due to the risk of anastomotic leakage, a temporary diverting stoma can be created simultaneously in many cases. However, the reversal rate of stomas is relatively low when created under these conditions. Öistämö *et al*[5] retrospectively analyzed acute cases of OLCC and demonstrated that 35% of stomas created with the intention of being temporary were never reversed. Stomas can have a negative impact on the patient's body image and quality of life (QOL). Additionally, diverting stoma formation in colorectal resection for OCC is related to increased postoperative complications, failure to wean off the ventilator, and longer hospital stays[6].

Colonic stenting is a powerful modality for intestinal decompression to resolve problems associated with ES. In addition, recent advances in stent technology have profoundly impacted OLCC management. Herein, we review the current state of colonic stenting and discuss its impact on colorectal cancer treatment, particularly focusing on its relationship with oncology.

HISTORY AND INDICATION

Palliative purpose and bridge to surgery

There are two main purposes of colonic stenting for OCC: palliative treatment and bridge to surgery (BTS). In this context, palliative treatment involves stenting applied to patients with an unresectable lesion, while BTS comprises preoperative stenting for intestinal decompression until the condition suitable for curative surgery is improved[7]. In comparison, colonic stenting for palliative purposes has a long history of use. Colonic stents were first reported by Dohmoto *et al*[8] in 1991. This study reported using stents for palliative treatment of OCC. Since then, many studies have elucidated the usefulness of colonic stents for palliative treatment in patients requiring intestinal decompression. In addition, the effectiveness of short-term outcomes of stent placement for unresectable colorectal cancer has been widely recognized, at least in the late 20th century[9,10].

Recently, self-expandable metallic stents (SEMS) as BTS have been widely used. Relief from obstruction with BTS enables restoration of dilated intestinal conditions prior to surgery, decreases mortality and morbidity, avoids stoma, and improves the quality of life[11]. Importantly, colonic stenting as a BTS should be performed under strict indications compared with stenting for palliative treatment, as BTS ultimately aims at a radical cure and requires long-term safety.

Left-sided vs right-sided colon

Stents can be placed not only in the left-sided colon but also in the right-sided colon. Although some reports have suggested that obstructive right-sided colonic cancer is also a good indication of SEMS, the effectiveness of SEMS for right-sided colonic obstruction has been less reported than that for left-sided

colonic obstruction[9]. Morita *et al*[12] analyzed the advantages of SEMS as a BTS over primary surgery in a retrospective, multicenter cohort study. When patients with left-sided colon cancer were compared, the rates of primary resection with anastomosis and stoma-free surgery were significantly higher in the SEMS group, whereas when patients with right-sided colon cancer were compared, no significant difference in the rates was observed between the SEMS and primary surgery groups. In addition, several disadvantages of SEMS placement in the right-sided colon have been pointed out, including a lower technical success rate and longer procedure time[13-15]. The authors of the European Society of Gastrointestinal Endoscopy (ESGE) guidelines also suggested the difficulty of stenting in the colon proximal to the splenic flexure and emphasized that SEMS recommendations should be applied to left-sided colon cancer[16].

TECHNICAL AND CLINICAL SUCCESS RATES

Recent studies have reported high technical and clinical success rates of SEMS placement for OCC. In a meta-analysis published in 2021, Neo *et al*[17] examined the technical and clinical success rates of SEMS for colorectal obstruction. In this study, technical success was defined as successful placement and deployment of the stent, and clinical success was defined as colonic decompression within 96 h after the stent was successfully placed. The technical and clinical success rates of SEMS were 92% in 1550 patients [95% confidence interval (CI): 0.88-0.95] and 82% in 1105 patients (95%CI: 0.77-0.87), respectively. In another meta-analysis published in 2021, the success rates were compared between SEMS and transanal decompression tubes (TDT). The overall success rates of SEMS and TDT were 92.1% and 71.9%, respectively, and both the technical and clinical success rates of SEMS were significantly better than those of TDT[18].

Some reports have suggested that technical and clinical success rates depend on the operators' experience, with experience of at least 20-30 cases required to ensure safety and effectiveness[15,19]. In addition, Boyle *et al*[20] identified short strictures and wide angulations distal to the stricture as factors indicating successful stenting in colonic obstruction. A post hoc analysis of a multicenter clinical trial in Japan identified several factors related to the difficulty of SEMS placement, including peritoneal carcinomatosis or expansive strictures[13].

The Japan Colonic Stent Safe Procedure Research Group proposed a scoring system for the clinical features of colorectal obstruction according to the patient's oral intake status, termed the ColoRectal Obstruction Scoring System (CROSS). This system scores patients on a scale of 0-4 as follows: 0, requiring continuous decompression; 1, no oral intake; 2, liquid or enteral nutrition; 3, oral intake of soft solids, low-residue diet, or full diet with symptoms of stricture; and 4, oral intake of soft solids, low-residue diet, or full diet without symptoms of stricture[21]. The above-mentioned post-hoc analysis suggested that CROSS 0 before stenting was one of the factors related to the difficulty of SEMS placement[13]. In contrast, another post-hoc analysis of multicenter clinical trials showed that SEMS as BTS in CROSS 0 patients showed comparable technical and clinical success rates, safety, and short-term outcomes to those in CROSS 1 and 2 patients[22]. Thus, it is inconclusive whether CROSS 0 before SEMS placement affects the technical and clinical success rates of SEMS.

COMPLICATIONS

Perforation

Perforation is one of the most common and critical complications of SEMS placement. A recent meta-analysis demonstrated that the overall perforation rate of colonic stenting for OLCC is 5%[17]. In addition, several studies have reported the outcomes of patients with stent-related perforations or factors related to stent-related perforations.

According to the meta-analysis mentioned above, when the studies were compared between perforation rates > 8% and ≤ 8%, the perforation rate > 8% group showed poorer technical success rates, although the 5-year overall and disease-free survival rates were not significantly different[17]. In a Dutch randomized clinical trial, the SEMS in the BTS group tended to have a lower 4-year disease-free survival rate than that in the ES group. In addition, the subgroup with stent-related perforation had a significantly poorer disease-free survival than the ES group, which suggested that stent-related perforation exacerbated oncological outcomes. However, it should be noted that in this trial, the number of patients was small, and the stent-related perforation rate was high (approximately 23%)[23]. Furthermore, it should also be noted that ES had better postoperative outcomes than BTS by stent because of the lower success rate of stent placement reported prior to 2014.

Datye *et al*[24] aggregated articles on perforation after SEMS placement for OCC until 2008 and analyzed data such as causes and mortality. The overall perforation rate was 4.9%, and concomitant chemotherapy, steroids, and radiotherapy were identified as risk factors for perforation; however, no significant difference was observed in the perforation rate between palliative treatment and BTS. The

authors also emphasized a high mortality rate of perforation cases (16%); however, the data did not necessarily show a negative impact of SEMS itself, considering the low overall perforation-related mortality rate (0.8%) and high mortality rate of ES (15%-20%).

van Halsema *et al*[25] pointed to the stent type as a risk factor for perforation. The authors defined stent types with high (< 10%) (WallFlex, Comvi, and Niti-S D-type) and low (< 5%) (Hanarostent and Niti-S covered) perforation rates. In fact, the perforation rates of certain stent types, especially the WallFlex stent, vary across reports. For example, Meisner *et al*[26] demonstrated that the overall perforation rate of WallFlex stent placement for OCC was 5.1% in 255 cases in prospective and multicenter studies. In a prospective multicenter study using WallFlex stent in Japan, the perforation rate was reported to be 1.6%[27]. van Halsema *et al*[25] reported a relatively high occurrence of delayed perforation after WallFlex stent placement and considered that the short follow-up period may have reduced the overall perforation rate of the stent.

Migration and re-obstruction

According to a systematic review, the rate of stent migration is approximately 10% (interquartile range 3%-22%). In this report, laser pretreatment and chemotherapy were identified as factors that promote stent migration[28]. Because the high risk of perforation and migration has been mentioned, laser or balloon dilation prior to stent placement is not recommended[28-30]. The overall re-obstruction rate was reported to be 10% (interquartile range 0%-15%), and when the cases were limited to palliative treatment, the re-obstruction rate was 16% (interquartile range 0%-23%)[28].

Safety of chemotherapy

The negative impact of SEMS on colorectal cancer management has been demonstrated in several respects, including chemotherapy after SEMS placement, which raised the concern that chemotherapy after SEMS placement may increase the risk of perforation. In theory, chemotherapy destroys proliferating cancer cells in the colonic wall; therefore, it can provoke stent-related perforation[25]. Although the safety of chemotherapy after SEMS placement remains to be fully elucidated[31], several recent studies have suggested an answer.

In a retrospective study that reviewed patients who underwent SEMS placement, the perforation rates were 13% in patients receiving no chemotherapy, 6% in patients receiving chemotherapy without bevacizumab, and 20% in patients receiving chemotherapy with bevacizumab[32]. Another retrospective study also suggested that subsequent bevacizumab therapy increased the risk of complications after SEMS insertion, and the perforation risk increased nearly threefold[33]. A meta-analysis of studies between 2005 and 2011 further revealed that the perforation rate in patients receiving bevacizumab-based chemotherapy was significantly higher than that in patients receiving no chemotherapy, whereas the perforation rate in patients receiving non-bevacizumab-based chemotherapy was significantly lower than that in patients who received no chemotherapy[25].

Some reports have demonstrated that chemotherapy does not affect the SEMS complications. However, a recent retrospective analysis indicated that chemotherapy before SEMS increased the risk of stent-related complications, whereas chemotherapy after SEMS had no impact on complications[34]. In a single-center retrospective study, Lee *et al*[35] compared the adverse events of SEMS as a palliative treatment for OCC between patients receiving bevacizumab therapy and those not receiving bevacizumab therapy. In this study, the perforation rate in the bevacizumab group was only 1%, which was equivalent to that of the non-bevacizumab group (3%). The authors considered that the low perforation rate might be related to the many years of experience of endoscopists. Additionally, one retrospective study showed the effectiveness and safety of SEMS before neoadjuvant chemotherapy and curative surgery, although the sample size was small. This study suggested the relatively low toxicity and high tolerability of neoadjuvant chemotherapy with two cycles of CAPOX or three cycles of mFOLFOX6 after SEMS. The resected specimens were also analyzed, suggesting a low risk of perineural invasion[36].

POSITIONING IN GUIDELINES

The degree of recommendation for SEMS as palliative management or BTS for OLCC has been described in many international guidelines, and the description seems to change with time. Herein, recent changes in the positioning of SEMS in the guidelines and the impact of changes in the description of SEMS are discussed below.

Webster *et al*[37] reviewed 19 international guidelines for the management of OLCC between 2010 and 2018. Stenting for palliative management was recommended in most guidelines, whereas opinions regarding the recommendation of emergency stenting as a BTS were divided. Eight guidelines recommended ES, two from the United States recommended emergency stenting as BTS, and nine suggested either ES or stenting as BTS could be selected. Guidelines from countries other than the United States did not actively recommend SEMS as a BTS until recently.

However, the description of the recommendations in the ESGE guidelines has recently changed. In the ESGE guidelines published in 2014, SEMS as BTS for OLCC was not recommended because of the risk of stent-related complications, particularly perforation[38]. In recent years, many studies have revealed the long-term safety of SEMS as a BTS; therefore, the description of the ESGE guidelines regarding the use of SEMS for OCC was updated in 2020, and SEMS as a BTS for OLCC has become a recommended treatment[16].

The impact of these updated recommendations in the guidelines has also been reported. The national colorectal cancer guidelines were updated in the Netherlands in 2014, and SEMS as a BTS for OLCC is clearly recommended. Consequently, the application rates of ES and SEMS for OLCC were reversed, and some changes occurred after 2014 in the Netherlands: the proportion of laparoscopic surgery increased, and the permanent stoma rate and total hospital stay decreased[39].

Despite the major impact of the guidelines on treatment, it should be noted that concerns regarding the quality of the guidelines have also been reported. Gavriilidis *et al*[40] used the Appraisal of Guidelines for Research and Evaluation II instrument to evaluate the quality of the 14 current guidelines describing the management of OLCC. The authors pointed out a poor applicability score in many guidelines and concerns regarding variations in guideline quality. Further research may trigger more changes to the description of guidelines and improve their quality.

SHORT-TERM AND LONG-TERM OUTCOMES

Short-term outcomes

Traditionally, in many cases of OCC, emergency decompression surgery was performed without adequate evaluation of preoperative staging and comorbidity. As a result, the risk of morbidity and mortality was unavoidably involved. SEMS as a BTS is considered a valid option for these cases as it can offer plenty of time to evaluate preoperative problems and improve the medical condition of patients [9]. Based on this perspective, it is not surprising that SEMS as a BTS has been reported to be advantageous in terms of short-term outcomes compared to ES. In a meta-analysis of randomized controlled trials comparing SEMS as BTS and ES for OLCC, the need for stoma creation, the incidence of postoperative complications, and the occurrence of wound infection were significantly reduced in the SEMS group[41].

TDT is another option for BTS of OCC; however, TDT has more disadvantages than SEMS: Slow decompression, bad odor, complicated management, difficult oral intake, and poor QOL[42]. Furthermore, several meta-analyses have compared the short-term outcomes between SEMS and TDT, and TDT was found to have poorer short-term outcomes. TDT showed lower clinical and technical success rates, solid food intake, and temporal discharge in a subsequent operation; TDT increased blood loss, prolonged operative time, and enhanced stoma rates[18,43]. In the context of these circumstances, the ESGE guidelines updated in 2020 do not recommend TDT placement over SEMS placement[16].

Negative reports on long-term outcomes

The advantages of SEMS as a BTS in short-term outcomes have been convincingly demonstrated, whereas the long-term outcomes of SEMS as a BTS have been controversial. In other words, the oncological safety of SEMS as a BTS remains unclear. However, high-quality research on the long-term outcomes of SEMS as a BTS has been increasing in recent years. Thus far, several studies have suggested the negative oncological impact of SEMS placement (Table 1). In a meta-analysis of randomized control trials, although no significant differences were observed in 3-year disease-free survival or overall survival between the SEMS as BTS group and ES group, the risk of systemic recurrence was significantly higher in the SEMS group than in the ES group[44]. Katsuki *et al*[45] analyzed a nationwide inpatient database in Japan and conducted a retrospective cohort study using propensity score-matching. The authors compared the long-term outcomes of patients with OLCC between SEMS as BTS and ES and demonstrated that the SEMS group showed significantly worse overall survival than the ES group. Gorissen *et al*[46] analyzed OLCC patients aged 75 years and younger from a prospective cohort study. In this study, the local recurrence rate in the SEMS group was significantly higher than that in the ES group, and the authors concluded that SEMS was associated with an increase in local recurrence, particularly in younger patients. Uehara *et al*[47] retrospectively evaluated the oncological outcomes of SEMS in patients with stage II or III OCC. The authors reported a higher distant metastatic recurrence rate in the SEMS group than in the ES group. Mege *et al*[48] examined the overall and disease-free survival of patients who underwent SEMS placement or creation of decompression stoma as a BTS for OLCC in a multicenter retrospective study. The authors demonstrated a significantly lower overall survival rate in the SEMS group, which may be related to an increase in worse pathological findings, such as tumor perforation. Sabbagh *et al*[49] reported significantly lower overall survival and significantly higher cancer-specific mortality in the SEMS group than in the ES group.

Table 1 Recent reports on long-term outcomes of colonic stenting as a bridge to surgery for obstructive left-sided colon cancer

Ref.	Publication year	Study type	Number of stent placements	Disease-free survival	Overall survival	Overall recurrence	Systemic recurrence	Local recurrence
Foo *et al* [44]	2019	Meta-analysis	222	NS	NS	SEMS > ES	SEMS > ES	NS
Katsuki *et al*[45]	2021	Multi-center retrospective study	498	NA	SEMS < ES	NA	NA	NA
Gorissen *et al*[46]	2013	Single-center prospective study	62	NS	NS	NS	NS	SEMS > ES in patients aged ≤ 75
Uehara *et al* [47]	2022	Single-center retrospective study	43	NS	NS	NA	SEMS > ES	NS
Mege *et al* [48]	2019	Multi-center retrospective study	191	NS	SEMS < DS	NA	NA	NA
Sabbagh *et al*[49]	2013	Multi-center retrospective study	48	NS	SEMS < ES	NS	NA	NA
Cirocchi *et al*[56]	2021	Meta-analysis	102-148	NS	NS	NS	NS	NS
Arezzo *et al* [57]	2017	Multi-center RCT	56	NS	NS	NS	NS	NS
Amelung *et al*[58]	2019	Multi-center retrospective study	222	NS	NS	NS	NS	NS
Veld *et al* [59]	2020	Multi-center retrospective study	121	NS	NS	NS	NS	NS
Endo *et al* [60]	2021	Multi-center retrospective study	113	TDT > ES (SEMS *vs* ES: NS)	NA	TDT > ES (SEMS *vs* ES: NS)	NS	NS
Kim *et al* [61]	2022	Single-center retrospective	98	NS	NS	NA	NA	NA

RCT: Randomized clinical trial; SEMS: Self-expandable metallic stent; ES: Emergency surgery; DS: Decompression stoma; TDT: Transanal decompression tube; NS: Not significant; NA: Not available.

Negative reports in pathological studies

As mentioned above, the potential negative impact of SEMS on oncological outcomes has also been suggested through histopathological examinations. Sabbagh *et al*[50] conducted a pathological analysis and revealed that tumor and peritumor ulceration, perineural invasion, and lymph node invasion were seen more frequently in resected specimens after SEMS placement than in cases of surgery only. These pathological features are associated with poor prognosis. Other authors have also reported negative factors for SEMS placement from a pathological viewpoint. Zhang *et al*[51] analyzed the histopathological findings of specimens resected after SEMS or TDT for OLCC. The authors reported that vascular invasions, wound abscesses, and ulcer formation was more frequently observed in the SEMS group than in the TDT group.

Some reports have also indirectly suggested the negative impact of SEMS on colorectal cancer treatment through analysis of the peripheral blood of patients. Maruthachalam *et al*[52] reported that circulating cytokeratin 20 mRNA levels after stent placement for left-sided colon cancer was significantly higher than before stenting, suggesting the possibility of tumor manipulation by inserting a guidewire or dilating and deploying the stent. Yamashita *et al*[53] showed an increase in viable circulating tumor cells after SEMS placement for OCC, which suggested that SEMS placement and expansion could allow the release of colorectal cancer cells into circulation. Recent technological developments in genome sequencing and molecular diagnosis have allowed the measurement of circulating tumor DNA (ctDNA), which is released from tumor cells undergoing apoptosis or necrosis into the systemic circulation[54]. The use of ctDNA has been extensively evaluated as a promising biomarker for the treatment of colorectal cancer. Takahashi *et al*[55] demonstrated that the plasma levels of ctDNA in patients with OCC increased after SEMS placement, although this increase was not

observed after TDT insertion. These findings indicate that SEMS placement may induce tumor cell dissemination. However, it remains unclear whether these changes in peripheral blood are related to the long-term oncological prognosis of patients.

Positive reports on long-term outcomes

As mentioned below, the oncological prognosis of SEMS as BTS is equivalent to that of ES and has been increasing in recent years (Table 1). In a meta-analysis of randomized controlled trials comparing SEMS as BTS and ES for OCC, SEMS showed the same mortality and significantly lower morbidity than ES. In addition, recurrence and survival outcomes were not significantly different between SEMS and ES[56]. Arezzo *et al*[57] demonstrated no significant differences in 3-year overall survival rates or progression-free survival rates observed between SEMS as a BTS and ES in a large multicenter randomized controlled trial. In addition, considering the significantly lower stoma rate in the SEMS group, the authors concluded that SEMS as a BTS was a viable approach for OCC. Amelung *et al*[58] retrospectively compared the long-term outcomes of patients with OLCC between SEMS as BTS and ES using propensity score matching, showing no significant differences in the 3-year disease-free survival rates, overall survival rates, or locoregional recurrence rates, whereas the SEMS group showed a lower permanent stoma risk. In a cohort study in the Netherlands, decompressing stoma and SEMS were compared to determine which has advantages as a BTS for OLCC. The study showed no significant differences in the 3-year locoregional recurrence rates, disease-free survival rates, or overall survival rates[59]. Endo *et al*[60] reported that the long-term oncologic outcome of SEMS as BTS for patients with OLCC was comparable to that of ES, whereas the long-term outcome of TDT was poorer than that of ES.

A recent Korean retrospective study examining the long-term outcomes of SEMS as BTS for OCC further found no significant difference in the 5-year overall survival and 5-year disease-free survival between the SEMS and ES groups. The authors emphasized the high technical and clinical success rates (99% and 92.9%, respectively) and a low perforation rate (1%) in the study, which could be due to the highly experienced endoscopist. Similarly, SEMS placement performed by experienced endoscopists may improve oncological outcomes[61]. Thus, endoscopist experience also seems to influence the long-term prognosis of patients. Amelung *et al*[62] performed a systematic review and meta-analysis of patients with OLCC to compare the long-term oncological outcomes after SEMS as a BTS with those after ES. The authors demonstrated that SEMS placement showed a significant survival benefit in more than 40 patients. The ESGE also recommends that an experienced endoscopist should perform or directly supervise stent placement[16].

CURATIVE SURGERY AFTER COLONIC STENTING

In cases of resectable OLCC, SEMS can facilitate the performance of minimally invasive one-stage surgery safely and effectively, which is one of the major benefits of SEMS as a BTS. Enomoto *et al*[63] compared laparoscopic and open surgery after SEMS insertion for OCC. Blood loss in the laparoscopic surgery group was less than that in the open surgery group, whereas the operative time was significantly shorter in the open surgery group.

The safety and efficacy of robot-assisted laparoscopic surgery after SEMS placement have also been reported recently[64]. Li *et al*[65] analyzed 79 cases where SEMS placement was performed for OCC in the largest single center in Singapore from 2013 to 2020. The authors showed that 14% of the patients underwent robot-assisted surgery for curative surgery. The progression and spread of minimally invasive surgery for colorectal cancer can strengthen the benefits of SEMS as BTS.

No consensus has yet been reached regarding the proper waiting period between SEMS insertion and curative surgery. Sato *et al*[66] retrospectively analyzed the long-term oncological outcomes of patients with OCC who underwent SEMS placement and curative surgery. The authors found that relapse-free survival was significantly shortened when the interval between stenting and curative surgery was longer than 16 d. Another retrospective study examining long-term outcomes after SEMS as a BTS for OCC further demonstrated that the risk of recurrence is associated with a long interval (longer than 18 d) between stenting and curative surgery[67]. In a nationwide cohort study in the Netherlands, patients with OLCC receiving SEMS as a BTS were divided into three groups according to the interval between stenting and surgery, as follows: 5-10 d group, 11-17 d group, and > 17 d. No significant differences were observed in 3-year disease-free survival or overall survival between the groups, although short-term outcomes were generally better in the 11-17 d group than in the 5-10 d and > 17 d groups[68]. In the ESGE guidelines published in 2014, the suggested time interval from colonic stenting as BTS to elective surgery was 5-10 d in patients with left-sided colon cancer; however, recent ESGE guidelines suggested a time interval of approximately 2 wk until resection[16,38]. In addition, the authors of the recent ESGE guidelines further described that the time interval should be determined considering the balance between stent-related adverse events and surgical outcomes because a short interval can reduce stent-related adverse events, whereas a long interval can improve surgical outcomes[16]. It should also be noted that ctDNA concentration was reported to increase over time following SEMS placement, which implies that a long interval may worsen the oncological outcome[55]. At any rate, as there is no

prospective comparative study on this matter[16], the optimal time interval between SEMS and curative surgery remains uncertain, and further research is required.

COST-EFFECTIVENESS

Many reports have shown that SEMS is cost-effective for both palliative intervention and BTS. Quinn *et al*[69] analyzed the costs and effectiveness in patients with unresectable or metastatic colorectal cancer who received SEMS or ES for acute colonic obstruction using decision tree analysis. The authors demonstrated that SEMS is a more cost-effective treatment for palliative intervention than ES. In a Japanese single-center retrospective study, short-term outcomes and total healthcare costs were compared between the SEMS, curative surgery, and ES groups. The study showed earlier oral intake, shorter total hospital stay, and lower total costs in the SEMS group than in the ES group, which suggested that SEMS as BTS was a more cost-effective treatment[70]. A Canadian decision analysis performed in 2006 elucidated the cost-effectiveness of SEMS as a BTS compared with the conventional surgical approach for acute OLCC[71].

Despite these studies, many clinicians may still regard SEMS for BTS as a treatment with lower cost-effectiveness. Suen *et al*[72] administered a questionnaire to Oceanian surgeons, surveying their intention to participate in randomized controlled trials on stent placement for OCC. Most surgeons gave a positive response to using stents for palliative treatment, whereas the majority of surgeons gave a negative response to using stents as BTS because they considered stenting as a BTS less cost-effective than ES.

CONCLUSION

Colonic stenting has had a positive impact on the management of OLCC, including facilitating the avoidance of stoma and reducing postoperative complications in the subsequent curative surgery, whereas a negative impact of colonic stenting on long-term oncologic outcomes seemed to have been emphasized until a decade ago. Many recent studies have demonstrated the long-term safety of colonic stenting for OLCC, which led to a change in the ESGE guidelines updated in 2020 as follows: SEMS as a BTS for OLCC is a recommended treatment. It should be noted that the experience of endoscopists is involved in determining the technical and clinical success rates and possibly the oncological outcomes. Uncertainty remains regarding SEMS placement for OLCC, including the long-term oncologic prognosis and safety of chemotherapy after SEMS; further investigation will be needed to clarify these points in the future.

FOOTNOTES

Author contributions: Suzuki H wrote the paper; Tsujinaka S supervised and critically revised the manuscript; Sato Y, Miura T, and Shibata C critically revised the manuscript; all authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors declare no conflicts of interest in regards to this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Japan

ORCID number: Hideyuki Suzuki 0000-0003-0696-2799; Shingo Tsujinaka 0000-0002-8554-3869; Yoshihiro Sato 0000-0003-3722-6815; Tomoya Miura 0000-0001-9092-460X; Chikashi Shibata 0000-0001-8191-4784.

S-Editor: Chang KL
L-Editor: A
P-Editor: Chang KL

REFERENCES

1 **Yeo HL**, Lee SW. Colorectal emergencies: review and controversies in the management of large bowel obstruction. *J*

Gastrointest Surg 2013; **17**: 2007-2012 [PMID: 24048614 DOI: 10.1007/s11605-013-2343-x]

2 **Frago R**, Ramirez E, Millan M, Kreisler E, del Valle E, Biondo S. Current management of acute malignant large bowel obstruction: a systematic review. *Am J Surg* 2014; **207**: 127-138 [PMID: 24124659 DOI: 10.1016/j.amjsurg.2013.07.027]

3 **Pisano M**, Zorcolo L, Merli C, Cimbanassi S, Poiasina E, Ceresoli M, Agresta F, Allievi N, Bellanova G, Coccolini F, Coy C, Fugazzola P, Martinez CA, Montori G, Paolillo C, Penachim TJ, Pereira B, Reis T, Restivo A, Rezende-Neto J, Sartelli M, Valentino M, Abu-Zidan FM, Ashkenazi I, Bala M, Chiara O, De' Angelis N, Deidda S, De Simone B, Di Saverio S, Finotti E, Kenji I, Moore E, Wexner S, Biffl W, Coimbra R, Guttadauro A, Leppäniemi A, Maier R, Magnone S, Mefire AC, Peitzmann A, Sakakushev B, Sugrue M, Viale P, Weber D, Kashuk J, Fraga GP, Kluger I, Catena F, Ansaloni L. 2017 WSES guidelines on colon and rectal cancer emergencies: obstruction and perforation. *World J Emerg Surg* 2018; **13**: 36 [PMID: 30123315 DOI: 10.1186/s13017-018-0192-3]

4 **Meyer F**, Marusch F, Koch A, Meyer L, Führer S, Köckerling F, Lippert H, Gastinger I; German Study Group "Colorectal Carcinoma (Primary Tumor)". Emergency operation in carcinomas of the left colon: value of Hartmann's procedure. *Tech Coloproctol* 2004; **8** Suppl 1: s226-s229 [PMID: 15655630 DOI: 10.1007/s10151-004-0164-3]

5 **Öistämö E**, Hjern F, Blomqvist L, Falkén Y, Pekkari K, Abraham-Nordling M. Emergency management with resection *vs* proximal stoma or stent treatment and planned resection in malignant left-sided colon obstruction. *World J Surg Oncol* 2016; **14**: 232 [PMID: 27577887 DOI: 10.1186/s12957-016-0994-2]

6 **Shwaartz C**, Fields AC, Prigoff JG, Aalberg JJ, Divino CM. Should patients With obstructing colorectal cancer have proximal diversion? *Am J Surg* 2017; **213**: 742-747 [PMID: 27742029 DOI: 10.1016/j.amjsurg.2016.08.005]

7 **Seo SY**, Kim SW. Endoscopic Management of Malignant Colonic Obstruction. *Clin Endosc* 2020; **53**: 9-17 [PMID: 31906606 DOI: 10.5946/ce.2019.051]

8 **Dohmoto M**. New method-endoscopic implantation of rectal stent in palliative treatment of malignant stenosis. *Endoscopia Digestiva* 1991; **3**: 1507-1512

9 **Kim EJ**, Kim YJ. Stents for colorectal obstruction: Past, present, and future. *World J Gastroenterol* 2016; **22**: 842-852 [PMID: 26811630 DOI: 10.3748/wjg.v22.i2.842]

10 **Liberman H**, Adams DR, Blatchford GJ, Ternent CA, Christensen MA, Thorson AG. Clinical use of the self-expanding metallic stent in the management of colorectal cancer. *Am J Surg* 2000; **180**: 407-11; discussion 412 [PMID: 11182388 DOI: 10.1016/s0002-9610(00)00492-x]

11 **Lauro A**, Binetti M, Vaccari S, Cervellera M, Tonini V. Obstructing Left-Sided Colonic Cancer: Is Endoscopic Stenting a Bridge to Surgery or a Bridge to Nowhere? *Dig Dis Sci* 2020; **65**: 2789-2799 [PMID: 32583222 DOI: 10.1007/s10620-020-06403-2]

12 **Morita S**, Yamamoto K, Ogawa A, Naito A, Mizuno H, Yoshioka S, Matsumura T, Ohta K, Suzuki R, Matsuda C, Hata T, Nishimura J, Mizushima T, Doki Y, Mori M; Clinical Study Group of Osaka University (CSGO), Colorectal Group. Benefits of using a self-expandable metallic stent as a bridge to surgery for right- and left-sided obstructive colorectal cancers. *Surg Today* 2019; **49**: 32-37 [PMID: 30105529 DOI: 10.1007/s00595-018-1701-4]

13 **Kuwai T**, Yamaguchi T, Imagawa H, Yoshida S, Isayama H, Matsuzawa T, Yamada T, Saito S, Shimada M, Hirata N, Sasaki T, Koizumi K, Maetani I, Saida Y. Factors related to difficult self-expandable metallic stent placement for malignant colonic obstruction: A post-hoc analysis of a multicenter study across Japan. *Dig Endosc* 2019; **31**: 51-58 [PMID: 30113095 DOI: 10.1111/den.13260]

14 **Yoon JY**, Jung YS, Hong SP, Kim TI, Kim WH, Cheon JH. Clinical outcomes and risk factors for technical and clinical failures of self-expandable metal stent insertion for malignant colorectal obstruction. *Gastrointest Endosc* 2011; **74**: 858-868 [PMID: 21862005 DOI: 10.1016/j.gie.2011.05.044]

15 **Lee HJ**, Park SJ, Cheon JH, Kim TI, Kim WH, Hong SP. What is the necessity of endoscopist for successful endoscopic stenting in patients with malignant colorectal obstruction? *Int J Colorectal Dis* 2015; **30**: 119-125 [PMID: 25376335 DOI: 10.1007/s00384-014-2060-2]

16 **van Hooft JE**, Veld JV, Arnold D, Beets-Tan RGH, Everett S, Götz M, van Halsema EE, Hill J, Manes G, Meisner S, Rodrigues-Pinto E, Sabbagh C, Vandervoort J, Tanis PJ, Vanbiervliet G, Arezzo A. Self-expandable metal stents for obstructing colonic and extracolonic cancer: European Society of Gastrointestinal Endoscopy (ESGE) Guideline - Update 2020. *Endoscopy* 2020; **52**: 389-407 [PMID: 32259849 DOI: 10.1055/a-1140-3017]

17 **Neo VSQ**, Jain SR, Yeo JW, Ng CH, Gan TRX, Tan E, Chong CS. Controversies of colonic stenting in obstructive left colorectal cancer: a critical analysis with meta-analysis and meta-regression. *Int J Colorectal Dis* 2021; **36**: 689-700 [PMID: 33495871 DOI: 10.1007/s00384-021-03834-9]

18 **Chen F**, Dong Q, Zhang F. Is self-expandable metallic stents superior to transanal decompression tubes for the treatment of malignant large-bowel obstruction: a meta-analysis. *Ann Palliat Med* 2021; **10**: 7378-7387 [PMID: 34263636 DOI: 10.21037/apm-20-2600]

19 **Williams D**, Law R, Pullyblank AM. Colorectal stenting in malignant large bowel obstruction: the learning curve. *Int J Surg Oncol* 2011; **2011**: 917848 [PMID: 22312531 DOI: 10.1155/2011/917848]

20 **Boyle DJ**, Thorn C, Saini A, Elton C, Atkin GK, Mitchell IC, Lotzof K, Marcus A, Mathur P. Predictive factors for successful colonic stenting in acute large-bowel obstruction: a 15-year cohort analysis. *Dis Colon Rectum* 2015; **58**: 358-362 [PMID: 25664716 DOI: 10.1097/DCR.0000000000000243]

21 **Japan Colonic Stent Safe Procedure Research (JCSSPR) Group**. CROSS: ColoRectal Obstruction Scoring System 2012. Available from: https://colon-stent.com/en

22 **Ohki T**, Yoshida S, Yamamoto M, Isayama H, Yamada T, Matsuzawa T, Saito S, Kuwai T, Tomita M, Shiratori T, Shimada M, Hirakawa T, Koizumi K, Saida Y. Determining the difference in the efficacy and safety of self-expandable metallic stents as a bridge to surgery for obstructive colon cancer among patients in the CROSS 0 group and those in the CROSS 1 or 2 group: a pooled analysis of data from two Japanese prospective multicenter trials. *Surg Today* 2020; **50**: 984-994 [PMID: 32025817 DOI: 10.1007/s00595-020-01970-3]

23 **Sloothaak DA**, van den Berg MW, Dijkgraaf MG, Fockens P, Tanis PJ, van Hooft JE, Bemelman WA; collaborative Dutch Stent-In study group. Oncological outcome of malignant colonic obstruction in the Dutch Stent-In 2 trial. *Br J Surg* 2014; **101**: 1751-1757 [PMID: 25298250 DOI: 10.1002/bjs.9645]

24 **Datye A**, Hersh J. Colonic perforation after stent placement for malignant colorectal obstruction--causes and contributing factors. *Minim Invasive Ther Allied Technol* 2011; **20**: 133-140 [PMID: 20929424 DOI: 10.3109/13645706.2010.518787]

25 **van Halsema EE**, van Hooft JE, Small AJ, Baron TH, García-Cano J, Cheon JH, Lee MS, Kwon SH, Mucci-Hennekinne S, Fockens P, Dijkgraaf MG, Repici A. Perforation in colorectal stenting: a meta-analysis and a search for risk factors. *Gastrointest Endosc* 2014; **79**: 970-982 [PMID: 24650852 DOI: 10.1016/j.gie.2013.11.038]

26 **Meisner S**, González-Huix F, Vandervoort JG, Repici A, Xinopoulos D, Grund KE, Goldberg P; Registry Group TW. Self-Expanding Metal Stenting for Palliation of Patients with Malignant Colonic Obstruction: Effectiveness and Efficacy on 255 Patients with 12-Month's Follow-up. *Gastroenterol Res Pract* 2012; **2012**: 296347 [PMID: 22761609 DOI: 10.1155/2012/296347]

27 **Saito S**, Yoshida S, Isayama H, Matsuzawa T, Kuwai T, Maetani I, Shimada M, Yamada T, Tomita M, Koizumi K, Hirata N, Kanazawa H, Enomoto T, Sekido H, Saida Y. A prospective multicenter study on self-expandable metallic stents as a bridge to surgery for malignant colorectal obstruction in Japan: efficacy and safety in 312 patients. *Surg Endosc* 2016; **30**: 3976-3986 [PMID: 26684205 DOI: 10.1007/s00464-015-4709-5]

28 **Khot UP**, Lang AW, Murali K, Parker MC. Systematic review of the efficacy and safety of colorectal stents. *Br J Surg* 2002; **89**: 1096-1102 [PMID: 12190673 DOI: 10.1046/j.1365-2168.2002.02148.x]

29 **Lee JM**, Byeon JS. Colorectal Stents: Current Status. *Clin Endosc* 2015; **48**: 194-200 [PMID: 26064818 DOI: 10.5946/ce.2015.48.3.194]

30 **Sebastian S**, Johnston S, Geoghegan T, Torreggiani W, Buckley M. Pooled analysis of the efficacy and safety of self-expanding metal stenting in malignant colorectal obstruction. *Am J Gastroenterol* 2004; **99**: 2051-2057 [PMID: 15447772 DOI: 10.1111/j.1572-0241.2004.40017.x]

31 **Matsuda A**, Yamada T, Matsumoto S, Shinji S, Ohta R, Sonoda H, Takahashi G, Iwai T, Takeda K, Sekiguchi K, Yoshida H. Systemic Chemotherapy is a Promising Treatment Option for Patients with Colonic Stents: A Review. *J Anus Rectum Colon* 2021; **5**: 1-10 [PMID: 33537495 DOI: 10.23922/jarc.2020-061]

32 **Imbulgoda A**, MacLean A, Heine J, Drolet S, Vickers MM. Colonic perforation with intraluminal stents and bevacizumab in advanced colorectal cancer: retrospective case series and literature review. *Can J Surg* 2015; **58**: 167-171 [PMID: 25799132 DOI: 10.1503/cjs.013014]

33 **Small AJ**, Coelho-Prabhu N, Baron TH. Endoscopic placement of self-expandable metal stents for malignant colonic obstruction: long-term outcomes and complication factors. *Gastrointest Endosc* 2010; **71**: 560-572 [PMID: 20189515 DOI: 10.1016/j.gie.2009.10.012]

34 **Zeng Z**, Liu Y, Wu K, Li D, Lai H, Zhang B. Efficacy and Safety of Fluoroscopy-Guided Self-Expandable Metal Stent Placement for Treatment of Malignant Colorectal Obstruction. *Dig Dis Sci* 2022 [PMID: 35653010 DOI: 10.1007/s10620-022-07557-x]

35 **Lee JH**, Emelogu I, Kukreja K, Ali FS, Nogueras-Gonzalez G, Lum P, Coronel E, Ross W, Raju GS, Lynch P, Thirumurthi S, Stroehlein J, Wang Y, You YN, Weston B. Safety and efficacy of metal stents for malignant colonic obstruction in patients treated with bevacizumab. *Gastrointest Endosc* 2019; **90**: 116-124 [PMID: 30797835 DOI: 10.1016/j.gie.2019.02.016]

36 **Han JG**, Wang ZJ, Zeng WG, Wang YB, Wei GH, Zhai ZW, Zhao BC, Yi BQ. Efficacy and safety of self-expanding metallic stent placement followed by neoadjuvant chemotherapy and scheduled surgery for treatment of obstructing left-sided colonic cancer. *BMC Cancer* 2020; **20**: 57 [PMID: 31992260 DOI: 10.1186/s12885-020-6560-x]

37 **Webster PJ**, Aldoori J, Burke DA. Optimal management of malignant left-sided large bowel obstruction: do international guidelines agree? *World J Emerg Surg* 2019; **14**: 23 [PMID: 31139245 DOI: 10.1186/s13017-019-0242-5]

38 **van Hooft JE**, van Halsema EE, Vanbiervliet G, Beets-Tan RG, DeWitt JM, Donnellan F, Dumonceau JM, Glynne-Jones RG, Hassan C, Jiménez-Perez J, Meisner S, Muthusamy VR, Parker MC, Regimbeau JM, Sabbagh C, Sagar J, Tanis PJ, Vandervoort J, Webster GJ, Manes G, Barthet MA, Repici A; European Society of Gastrointestinal Endoscopy. Self-expandable metal stents for obstructing colonic and extracolonic cancer: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2014; **46**: 990-1053 [PMID: 25325682 DOI: 10.1055/s-0034-1390700]

39 **Veld JV**, Amelung FJ, Borstlap WAA, Eise van Halsema E, Consten ECJ, Siersema PD, Ter Borg F, Silvester van der Zaag E, Fockens P, Bemelman WA, Elise van Hooft J, Tanis PJ; Dutch Snapshot Research Group. Changes in Management of Left-Sided Obstructive Colon Cancer: National Practice and Guideline Implementation. *J Natl Compr Canc Netw* 2019; **17**: 1512-1520 [PMID: 31805533 DOI: 10.6004/jnccn.2019.7326]

40 **Gavriilidis P**, Askari A, de'Angelis N, Gavriilidis EP, Wheeler J, Davies J. Appraisal of the Current Guidelines for Management of Malignant Left-Sided Colonic Obstruction Using the Appraisal of Guidelines Research and Evaluation II Instrument. *Dig Surg* 2021; **38**: 177-185 [PMID: 33756480 DOI: 10.1159/000514446]

41 **Allievi N**, Ceresoli M, Fugazzola P, Montori G, Coccolini F, Ansaloni L. Endoscopic Stenting as Bridge to Surgery *vs* Emergency Resection for Left-Sided Malignant Colorectal Obstruction: An Updated Meta-Analysis. *Int J Surg Oncol* 2017; **2017**: 2863272 [PMID: 28761765 DOI: 10.1155/2017/2863272]

42 **Saida Y**. Current status of colonic stent for obstructive colorectal cancer in Japan; a review of the literature. *J Anus Rectum Colon* 2019; **3**: 99-105 [PMID: 31583324 DOI: 10.23922/jarc.2019-009]

43 **Matsuda A**, Yamada T, Matsumoto S, Sakurazawa N, Kawano Y, Sekiguchi K, Matsutani T, Miyashita M, Yoshida H. Short-term outcomes of a self-expandable metallic stent as a bridge to surgery vs. a transanal decompression tube for malignant large-bowel obstruction: a meta-analysis. *Surg Today* 2019; **49**: 728-737 [PMID: 30798434 DOI: 10.1007/s00595-019-01784-y]

44 **Foo CC**, Poon SHT, Chiu RHY, Lam WY, Cheung LC, Law WL. Is bridge to surgery stenting a safe alternative to emergency surgery in malignant colonic obstruction: a meta-analysis of randomized control trials. *Surg Endosc* 2019; **33**: 293-302 [PMID: 30341649 DOI: 10.1007/s00464-018-6487-3]

45 **Katsuki R**, Jo T, Yasunaga H, Ishimaru M, Sakamoto T. Outcomes of self-expandable metal stent as bridge to surgery *vs* emergency surgery for left-sided obstructing colon cancer: A retrospective cohort study. *Am J Surg* 2021; **221**: 168-173 [PMID: 32600844 DOI: 10.1016/j.amjsurg.2020.06.012]

46 **Gorissen KJ**, Tuynman JB, Fryer E, Wang L, Uberoi R, Jones OM, Cunningham C, Lindsey I. Local recurrence after

stenting for obstructing left-sided colonic cancer. *Br J Surg* 2013; **100**: 1805-1809 [PMID: 24227368 DOI: 10.1002/bjs.9297]

47 **Uehara H**, Yamazaki T, Iwaya A, Kameyama H, Komatsu M, Hirai M. Comparison of the oncological outcomes of stenting as a bridge to surgery and surgery alone in stages II to III obstructive colorectal cancer: a retrospective study. *Ann Coloproctol* 2022; **38**: 235-243 [PMID: 34256426 DOI: 10.3393/ac.2020.01067.0152]

48 **Mege D**, Sabbagh C, Manceau G, Bridoux V, Lakkis Z, Momar D, Sielezneff I, Karoui M; AFC (French Surgical Association) Working Group. What is the Best Option Between Primary Diverting Stoma or Endoscopic Stent as a Bridge to Surgery with a Curative Intent for Obstructed Left Colon Cancer? *Ann Surg Oncol* 2019; **26**: 756-764 [PMID: 30623342 DOI: 10.1245/s10434-018-07139-0]

49 **Sabbagh C**, Browet F, Diouf M, Cosse C, Brehant O, Bartoli E, Mauvais F, Chauffert B, Dupas JL, Nguyen-Khac E, Regimbeau JM. Is stenting as "a bridge to surgery" an oncologically safe strategy for the management of acute, left-sided, malignant, colonic obstruction? *Ann Surg* 2013; **258**: 107-115 [PMID: 23324856 DOI: 10.1097/SLA.0b013e31827e30ce]

50 **Sabbagh C**, Chatelain D, Trouillet N, Mauvais F, Bendjaballah S, Browet F, Regimbeau JM. Does use of a metallic colon stent as a bridge to surgery modify the pathology data in patients with colonic obstruction? *Surg Endosc* 2013; **27**: 3622-3631 [PMID: 23572218 DOI: 10.1007/s00464-013-2934-3]

51 **Zhang S**, Liu G, Wu GH, Zhang SW, Zhao YJ, Xu J. Transanal decompression tube is superior to self-expandable metallic colonic stent for malignant colorectal obstruction: a retrospective study. *ANZ J Surg* 2022; **92**: 140-145 [PMID: 34636468 DOI: 10.1111/ans.17274]

52 **Maruthachalam K**, Lash GE, Shenton BK, Horgan AF. Tumour cell dissemination following endoscopic stent insertion. *Br J Surg* 2007; **94**: 1151-1154 [PMID: 17541987 DOI: 10.1002/bjs.5790]

53 **Yamashita S**, Tanemura M, Sawada G, Moon J, Shimizu Y, Yamaguchi T, Kuwai T, Urata Y, Kuraoka K, Hatanaka N, Yamashita Y, Taniyama K. Impact of endoscopic stent insertion on detection of viable circulating tumor cells from obstructive colorectal cancer. *Oncol Lett* 2018; **15**: 400-406 [PMID: 29391884 DOI: 10.3892/ol.2017.7339]

54 **Yoo RN**, Cho HM, Kye BH. Management of obstructive colon cancer: Current status, obstacles, and future directions. *World J Gastrointest Oncol* 2021; **13**: 1850-1862 [PMID: 35070029 DOI: 10.4251/wjgo.v13.i12.1850]

55 **Takahashi G**, Yamada T, Iwai T, Takeda K, Koizumi M, Shinji S, Uchida E. Oncological Assessment of Stent Placement for Obstructive Colorectal Cancer from Circulating Cell-Free DNA and Circulating Tumor DNA Dynamics. *Ann Surg Oncol* 2018; **25**: 737-744 [PMID: 29235008 DOI: 10.1245/s10434-017-6300-x]

56 **Cirocchi R**, Arezzo A, Sapienza P, Crocetti D, Cavaliere D, Solaini L, Ercolani G, Sterpetti AV, Mingoli A, Fiori E. Current Status of the Self-Expandable Metal Stent as a Bridge to Surgery Versus Emergency Surgery in Colorectal Cancer: Results from an Updated Systematic Review and Meta-Analysis of the Literature. *Medicina (Kaunas)* 2021; **57** [PMID: 33804232 DOI: 10.3390/medicina57030268]

57 **Arezzo A**, Balague C, Targarona E, Borghi F, Giraudo G, Ghezzo L, Arroyo A, Sola-Vera J, De Paolis P, Bossotti M, Bannone E, Forcignanò E, Bonino MA, Passera R, Morino M. Colonic stenting as a bridge to surgery *vs* emergency surgery for malignant colonic obstruction: results of a multicentre randomised controlled trial (ESCO trial). *Surg Endosc* 2017; **31**: 3297-3305 [PMID: 27924392 DOI: 10.1007/s00464-016-5362-3]

58 **Amelung FJ**, Borstlap WAA, Consten ECJ, Veld JV, van Halsema EE, Bemelman WA, Siersema PD, Ter Borg F, van Hooft JE, Tanis PJ; Dutch Snapshot Research Group. Propensity score-matched analysis of oncological outcome between stent as bridge to surgery and emergency resection in patients with malignant left-sided colonic obstruction. *Br J Surg* 2019; **106**: 1075-1086 [PMID: 31074507 DOI: 10.1002/bjs.11172]

59 **Veld JV**, Amelung FJ, Borstlap WAA, van Halsema EE, Consten ECJ, Siersema PD, Ter Borg F, van der Zaag ES, de Wilt JHW, Fockens P, Bemelman WA, van Hooft JE, Tanis PJ; Dutch Snapshot Research Group. Comparison of Decompressing Stoma *vs* Stent as a Bridge to Surgery for Left-Sided Obstructive Colon Cancer. *JAMA Surg* 2020; **155**: 206-215 [PMID: 31913422 DOI: 10.1001/jamasurg.2019.5466]

60 **Endo S**, Kumamoto K, Enomoto T, Koizumi K, Kato H, Saida Y. Comparison of survival and perioperative outcome of the colonic stent and the transanal decompression tube placement and emergency surgery for left-sided obstructive colorectal cancer: a retrospective multi-center observational study "The CODOMO study". *Int J Colorectal Dis* 2021; **36**: 987-998 [PMID: 33247313 DOI: 10.1007/s00384-020-03806-5]

61 **Kim SH**, Jang SH, Jeon HJ, Choi HS, Kim ES, Keum B, Jeen YT, Chun HJ, Kim J. Colonic stenting as a bridge to surgery for obstructive colon cancer: is it safe in the long term? *Surg Endosc* 2022; **36**: 4392-4400 [PMID: 35075522 DOI: 10.1007/s00464-021-08789-0]

62 **Amelung FJ**, Burghgraef TA, Tanis PJ, van Hooft JE, Ter Borg F, Siersema PD, Bemelman WA, Consten ECJ. Critical appraisal of oncological safety of stent as bridge to surgery in left-sided obstructing colon cancer; a systematic review and meta-analysis. *Crit Rev Oncol Hematol* 2018; **131**: 66-75 [PMID: 30293707 DOI: 10.1016/j.critrevonc.2018.08.003]

63 **Enomoto T**, Saida Y, Takabayashi K, Nagao S, Takeshita E, Watanabe R, Takahashi A, Nakamura Y, Asai K, Watanebe M, Nagao J, Kusachi S. Open surgery *vs* laparoscopic surgery after stent insertion for obstructive colorectal cancer. *Surg Today* 2016; **46**: 1383-1386 [PMID: 27017599 DOI: 10.1007/s00595-016-1331-7]

64 **Takeyama H**, Danno K, Nishigaki T, Yamashita M, Yamazaki M, Yamakita T, Nishihara A, Taniguchi H, Mizutani M, Nakamichi I, Yura M, Ikeda K, Oka Y. Robot-assisted laparoscopic surgery after placing a self-expanding metallic stent for malignant rectal obstruction: a case report. *Surg Case Rep* 2019; **5**: 156 [PMID: 31654242 DOI: 10.1186/s40792-019-0719-1]

65 **Li JW**, Ngu JC, Lim KR, Tay SW, Jiang B, Wijaya R, Yusof S, Ong CJ, Kwek ABE, Ang TL. Colonic stenting in acute malignant large bowel obstruction - an audit of efficacy and safety in a tertiary referral centre in Singapore. *Singapore Med J* 2021 [PMID: 34600451 DOI: 10.11622/smedj.2021127]

66 **Sato R**, Oikawa M, Kakita T, Okada T, Abe T, Yazawa T, Tsuchiya H, Akazawa N, Yoshimachi S, Ohira T, Harada Y, Okano H, Ito K, Tsuchiya T. A longer interval after stenting compromises the short- and long-term outcomes after curative surgery for obstructive colorectal cancer. *Surg Today* 2022; **52**: 681-689 [PMID: 34648067 DOI: 10.1007/s00595-021-02385-4]

67 **Broholm M**, Kobborg M, Frostberg E, Jeppesen M, Gögenür I. Delay of surgery after stent placement for resectable

malignant colorectal obstruction is associated with higher risk of recurrence. *Int J Colorectal Dis* 2017; **32**: 513-516 [PMID: 27853888 DOI: 10.1007/s00384-016-2705-4]

68 **Veld JV**, Kumcu A, Amelung FJ, Borstlap WAA, Consten ECJ, Dekker JWT, van Westreenen HL, Siersema PD, Ter Borg F, Kusters M, Bemelman WA, de Wilt JHW, van Hooft JE, Tanis PJ; Dutch Snapshot Research Group. Time interval between self-expandable metal stent placement or creation of a decompressing stoma and elective resection of left-sided obstructive colon cancer. *Endoscopy* 2021; **53**: 905-913 [PMID: 33339059 DOI: 10.1055/a-1308-1487]

69 **Quinn PL**, Arjani S, Ahlawat SK, Chokshi RJ. Cost-effectiveness of palliative emergent surgery *vs* endoscopic stenting for acute malignant colonic obstruction. *Surg Endosc* 2021; **35**: 2240-2247 [PMID: 32430522 DOI: 10.1007/s00464-020-07637-x]

70 **Kaida T**, Doi K, Yumoto S, Kinoshita S, Takeyama H, Ishiodori H, Baba H. Cost-effectiveness of self-expandable metallic stents as bridge to surgery for obstructive colorectal cancer. *Int J Clin Oncol* 2021; **26**: 1485-1491 [PMID: 33937958 DOI: 10.1007/s10147-021-01928-6]

71 **Singh H**, Latosinsky S, Spiegel BM, Targownik LE. The cost-effectiveness of colonic stenting as a bridge to curative surgery in patients with acute left-sided malignant colonic obstruction: a Canadian perspective. *Can J Gastroenterol* 2006; **20**: 779-785 [PMID: 17171197 DOI: 10.1155/2006/307324]

72 **Suen MK**, Zahid A, Young JM, Rodwell L, Solomon MJ, Young CJ. How to decide to undertake a randomized, controlled trial of stent or surgery in colorectal obstruction. *Surgery* 2015; **157**: 1137-1141 [PMID: 25796417 DOI: 10.1016/j.surg.2015.01.022]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 January 24; 14(1): 13-26

DOI: 10.5306/wjco.v14.i1.13

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

Identification of a three-gene prognostic signature for radioresistant esophageal squamous cell carcinoma

Xiao-Yan Wang, Narasimha M Beeraka, Nan-Nan Xue, Hui-Ming Yu, Ya Yang, Mao-Xing Liu, Vladimir N Nikolenko, Jun-Qi Liu, Di Zhao

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Bagheri-Mohammadi S, Iran; Kao JT, Taiwan

Received: July 25, 2022
Peer-review started: July 25, 2022
First decision: October 24, 2022
Revised: October 25, 2022
Accepted: December 6, 2022
Article in press: December 6, 2022
Published online: January 24, 2023

Xiao-Yan Wang, Di Zhao, Department of Endocrinology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China

Narasimha M Beeraka, Nan-Nan Xue, Ya Yang, Jun-Qi Liu, Department of Radiation Oncology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China

Narasimha M Beeraka, Vladimir N Nikolenko, Department of Human Anatomy, I. M. Sechenov First Moscow State Medical University, Moscow 119991, Russia

Narasimha M Beeraka, Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Mysuru 570015, India

Hui-Ming Yu, Department of Radiation Oncology, Peking University Cancer Hospital & Institute, Beijing 065005, China

Mao-Xing Liu, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Gastrointestinal Surgery IV, Peking University Cancer Hospital & Institute, Beijing, China

Vladimir N Nikolenko, M.V. Lomonosov Moscow State University, Moscow 119991, Russia

Corresponding author: Di Zhao, MD, Department of Endocrinology, The First Affiliated Hospital of Zhengzhou University, No. 1 Jianshedong Street, Zhengzhou, 450052, Henan Province, China. zhaodi0110@126.com

Abstract

BACKGROUND

Esophageal squamous cell carcinoma (ESCC) is causing a high mortality rate due to the lack of efficient early prognosis markers and suitable therapeutic regimens. The prognostic role of genes responsible for the acquisition of radioresistance in ESCC has not been fully elucidated.

AIM

To establish a prognostic model by studying gene expression patterns pertinent to radioresistance in ESCC patients.

METHODS

Datasets were obtained from the Gene Expression Omnibus and The Cancer Genome Atlas databases. The edgeR, a Bioconductor package, was used to analyze mRNA expression between different groups. We screened genes specifically responsible for radioresistance to estimate overall survival. Pearson correlation analysis was performed to confirm whether the expression of those genes correlated with each other. Genes contributing to radioresistance and overall survival were assessed by the multivariate Cox regression model through the calculation of βi and risk score using the following formula: $\sum_{i=1}^{n} \beta i \times \mathrm{PSI}$.

RESULTS

We identified three prognostic mRNAs (cathepsin S [*CTSS*], cluster of differentiation 180 [*CD180*], and SLP adapter and CSK-interacting membrane protein [*SCIMP*]) indicative of radioresistance. The expression of the three identified mRNAs was related to each other ($r > 0.70$ and $P < 0.05$). As to 1-year and 3-year overall survival prediction, the area under the time-dependent receiver operating characteristic curve of the signature consisting of the three mRNAs was 0.716 and 0.841, respectively. When stratifying patients based on the risk score derived from the signature, the high-risk group exhibited a higher death risk and shorter survival time than the low-risk group ($P < 0.0001$). Overall survival of the low-risk patients was significantly better than that of the high-risk patients ($P = 0.018$).

CONCLUSION

We have developed a novel three-gene prognostic signature consisting of *CTSS, CD180*, and *SCIMO* for ESCC, which may facilitate the prediction of early prognosis of this malignancy.

Key Words: Esophageal squamous cell carcinoma; CTSS; CD180; SCIMP; Radioresistance; TNM stage; Prognosis

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The current study identified a novel three-gene prognostic signature consisting of *CTSS, CD180*, and *SCIMO* for esophageal squamous cell carcinoma, which may facilitate the prediction of early prognosis of this malignancy.

Citation: Wang XY, Beeraka NM, Xue NN, Yu HM, Yang Y, Liu MX, Nikolenko VN, Liu JQ, Zhao D. Identification of a three-gene prognostic signature for radioresistant esophageal squamous cell carcinoma. *World J Clin Oncol* 2023; 14(1): 13-26
URL: https://www.wjgnet.com/2218-4333/full/v14/i1/13.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i1.13

INTRODUCTION

Esophageal cancer is one of the most commonly occurring gastrointestinal tumors and ranks 7th in incidence and 6th in death among all malignancies worldwide. The highest incidence rate was reported in China[1]. Esophageal cancer includes two main pathological types, namely, esophageal adenocarcinoma and esophageal squamous cell carcinoma (ESCC), and 88% of ESCC cases originate in central and southern Asia[2]. Surgery is the conventional method of treatment for early-stage esophageal cancer patients. Neoadjuvant radiotherapy is also reported to be a crucial therapeutic modality for treating advanced stage ESCC patients[3]. However, the differences in sensitivity of each patient to radiation therapy result in variable prognoses among ESCC patients. ESCC is an aggressive malignancy with a poor overall survival[4]. The available staging system is not very satisfactory in predicting the treatment outcome in ESCC patients, and the application of cancer genomics to predict clinical outcomes may improve the treatment of ESCC[5,6].

Tumor radiotherapy can induce either direct damage to DNA by inducing DNA double-strand breaks, or indirectly modulate cell signaling cascades to foster tumor cell death[7]. However, the clinical outcomes of radiotherapy in most esophageal tumor patients predominantly depend on the inherent sensitivity of tumor cells to radioactive rays. Furthermore, tumor cell insensitivity can lead to the occurrence of radioresistance, which involves several cellular mechanisms including cell cycle checkpoint regulation[8], stemness acquisition[9,10], epithelial mesenchymal transformation (EMT)[11], and activation of multiple pro-survival and pro-proliferation signaling pathways[12,13]. Furthermore, radioresistance is also mediated by tumor-associated microenvironment factors, such as hypoxia-

induced HIF-1 signaling factors[14,15], tumor-associated fibroblasts (CAFs)[16], and tumor-associated macrophages[17,18]. Hence, radioresistance is one of the significant reasons for the failure of radiotherapy in ESCC patients. High-throughput sequencing technology is a promising novel approach to identify genes that are related to tumor radioresistance in ESCC. Maher *et al*[19] identified a set of five genes including *EPB41L3, RTKN, STAT5B, NMES1*, and *RNPC1* as biomarkers for response to neoadjuvant radiotherapy in esophageal cancer. Overexpression of PTK7 can activate NF-kB to enhance raidoresistance in radiosensitive ESCC cells[20]. Transcriptome analysis delineated that the *MALAT1-ATG9B* and *DDIT4-MB-PLAT* genes could regulate radioresistance in *in vitro* models of ESCC cells by modulation of autophagy and hypoxia pathways[21]. The prognostic role and underlying genomic pathways pertinent to the acquisition of radioresistance in ESCC patients have not yet been fully unraveled. Therefore, it is crucial to identify biomarkers and genes pertaining to radioresistance in ESCC for selecting novel therapeutic modalities to mitigate radioresistance in this malignancy.

The current study identified mRNAs as potential radioresistance markers in ESCC cells with the aid of merged mRNA data collected from the Gene Expression Omnibus (GEO) and Cancer Genome Atlas (TCGA) databases. The study identified a three-gene signature, including *CTSS, CD180*, and *SCIMP*, that may predict the development of radioresistance in ESCC cells. Furthermore, we constructed a prognostic model for radioresistant ESCC based on the risk scores derived from clinical features and the three-gene signature.

MATERIALS AND METHODS

GEO database search: Identifying 'radioresistance-promoting mRNAs'

Primarily, the microarray profiles in GSE81812 dataset pertaining to 'non-radiated KYSE-180 cells' and "12 and 30 Gy radiated KYSE-180 cells" were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/) to identify mRNAs contributing to radioresistance in ESCC cells. The edgeR package (www.bioconductor.org/packages/release/bioc/html/edgeR.html) was used to analyze the differential expression of mRNAs between different groups ('0 Gy group *vs* 12 Gy group' and '0 Gy group *vs* 30 Gy group') to identify genes related to radioresistance. The cutoff parameters were false discovery rate < 0.05 and $|\text{Log}_2 \text{ fold change}| > 2$.

TCGA database search: Identification of 'radioresistance-promoting mRNAs' associated with overall survival

Gene expression profile and clinical information of ESCC patients in the TCGA database were downloaded (https://gdc-portal.nci.nih.gov/). Overall survival rates were determined to ascertain the prognostic significance of the identified radioresistance promoting mRNAs in the TCGA database; the overall survival rates were analyzed by using survival package in R through Kaplan-Meier analysis and finally compared using the Log-rank test and Cox proportional hazards regression analysis. Then, radioresistance-promoting mRNAs associated with overall survival were screened.

Multivariate Cox regression analysis: Construction of prognostic model based on 'radioresistance-promoting mRNAs' associated with overall survival

The association of radioresistance-promoting mRNAs with overall survival was estimated using the multivariate Cox regression model, adjusted for age, gender, grade, and stage, to calculate βi. The forest plot was plotted to exhibit the hazards regression (HR) of the multivariate Cox regression model results. Later, risk score was estimated by using the following formula: $\sum_{i=1}^{n} \beta i \times \text{PSI}$. By using the maximally selected rank statistics from the 'survminer' package in R, all samples were divided into a low-risk group and a high-risk group subsequently, and survival analysis was conducted to assess prognosis differences between the two groups.

Confirmation of relationship of 'radioresistance-promoting mRNAs' with overall survival, tumor stage, and tumor grade

Pearson correlation coefficients ($P < 0.05$) were calculated using r.test () in R to confirm whether the identified radioresistance-associated mRNAs were typically related to the stage and grade of ESCC. The results are shown in violin plots.

Kyoto Encyclopedia of Genes and Genomes pathway analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of mRNAs associated with radioresistance in ESCC was performed using the ClusterProfile package (http://www.bioconductor.org/packages/release/bioc/html/clusterProfiler.html) for a more comprehensive understanding of biological features. A P value < 0.05 was set as the cut-off criterion in the KEGG pathway. Data pertinent to KEGG pathway analysis is attached as a supporting file.

Statistical analysis

Statistical analyses were executed with the aid of SPSS 22.0 software (IBM, Chicago, IL, United States) and R version 3.6.0. Overall survival rate was estimated using the Kaplan-Meier method. Multivariate Cox proportional HR analysis was executed to identify prognostic factors (the three-gene signature, age, gender, tumor stage, and tumor grade). Differences between groups were compared using the Student's *t*-test or paired samples *t*-test. $P < 0.05$ was considered to have statistical significance.

RESULTS

GEO database search of mRNA expression profiles to identify radioresistance-promoting mRNAs

The gene count data of expression profiles of 22456 mRNAs in 41 samples of 0 Gy, 92 samples of 12 Gy, and 89 samples of 30 Gy were obtained from the GSE81812 dataset downloaded from GEO. We identified upregulation of 1168 mRNAs in the '0 Gy group *vs* 12 Gy group' comparison and 497 mRNAs in the '0 Gy group *vs* 30 Gy group' comparison by using the edgeR package. To distinguish the differentially expressed mRNAs at different X-ray levels, the top 50 mRNAs are shown in a heatmap and principal component analysis (PCA) was performed (Figure 1A-D). A total of 379 intersection mRNAs were identified from the 0-12 Gy and 0-30 Gy comparisons as radioresistance associated genes.

Prognostic significance of radioresistance-promoting mRNAs from TCGA database

Log-rank test and Cox proportional hazards regression were adjusted for other confounding factors such as gender, age, stage, and grade. These statistical analyses were used to screen for prognostic genes, and a total of 5293 mRNAs were selected. Among them, 44 mRNAs were significantly associated with radioresistance. We selected 23 mRNAs that were negatively correlated with prognosis for further analysis. The intersection of radioresistant prognostic mRNAs is visualized in a Venn diagram (Figure 1E).

Determination of correlations among radioresistance-promoting mRNAs

For the 23 mRNAs mentioned above, we primarily investigated whether their expression correlated with each other based on the data in the TCGA database. Although they were expressed at different levels in ESCC patients, the results showed strong correlations among three mRNAs, namely, *CTSS*, *CD180*, and *SCIMP* ($r > 0.70$ and $P < 0.05$). The correlations of 23 mRNAs are shown in a heatmap (Figure 2A). Hence, we selected these three mRNAs as radioresistance-promoting mRNAs of interest. Correlations of these three mRNAs are shown in a scattergram (Figure 2B-D).

Establishment of a gene signature as prognostic model for radioresistance

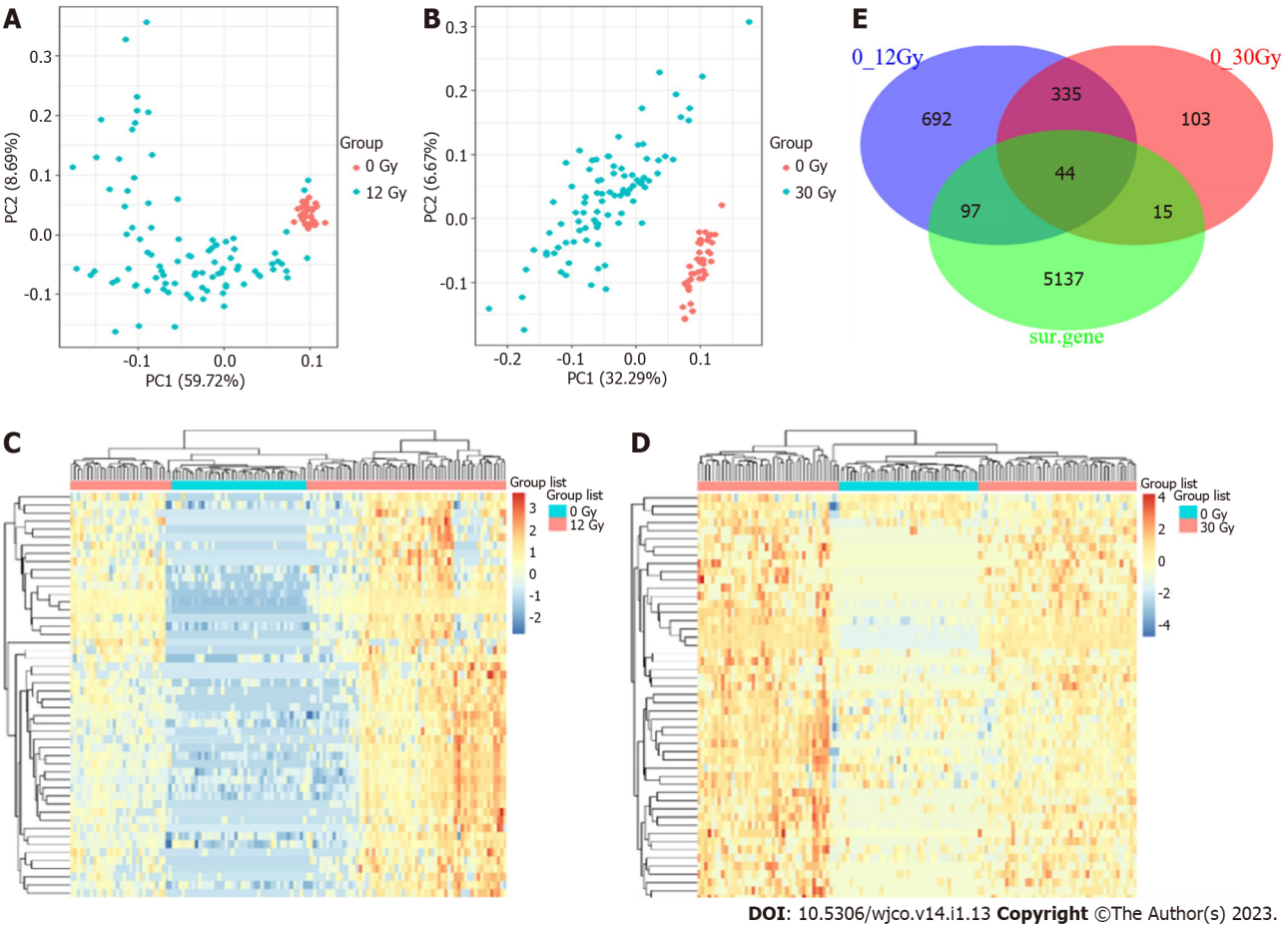
To explore the potential prognostic value of the above three mRNAs pertinent to radioresistance, we evaluated the overall survival rates of ESCC patients based on the expression patterns of these three mRNAs based on the data in the TCGA database by using Kaplan-Meier curves. As shown in Figure 3A, their low expression was associated with a good overall survival (TCGA database), and the median survival time was statistically significant ($P < 0.05$) for all the three mRNAs.

Subsequently, the connection between the three-gene signature and overall survival was explored through multivariate Cox regression model adjusted for patient age, gender, tumor grade, and tumor stage, for which, the HR with 95% confidence interval was depicted through the forest plot (Figure 3B). ROC analysis for the model is shown in Figure 3C (area under the curve: 0.716 and 0.841 for 1- and 3-year survival, respectively). Accordingly, the risk score of each patient was calculated, and all the patients were divided into either a high risk group or a low risk group based on the risk score.

The patients of the high-risk group exhibited a 'higher death risk and shorter survival time' than the patients in the low-risk group; the heatmap of the three genes (*CTSS, CD180*, and *SCIMP*) showed that the high-risk patients typically had higher expression of these genes than the low-risk patients (Figure 3D-F). The Kaplan-Meier curves revealed that the low-risk patients typically with low expression of these three genes exhibited a good overall survival (Figure 3G).

External validation based on GEO dataset

To further validate the prognostic value of the three mRNAs, GSE53625 dataset was downloaded from the GEO database. As shown in Figure 4A, downregulation of *SCIMP* expression was associated with a good survival outcome. When patients were divided into two groups based on *CTSS* expression, there was no statistically significant difference in their survival. CD180 expression also showed no significant correlation with survival. In the same manner, the risk score of GEO samples was calculated, and the overall survival of patient samples in the low-risk group was also higher than that of patient samples in the high-risk group (Figure 4B). The risk curve, scatter plot, and heatmap results were also similar to those obtained based on TCGA dataset (Figure 4C-E).



DOI: 10.5306/wjco.v14.i1.13 **Copyright** ©The Author(s) 2023.

Figure 1 Comparative principal component analysis and heatmap analysis of up-regulated mRNAs between non-radiated and radiated KYSE-180 cell samples. A and B: Principal component analysis. Samples were clustered into two groups: 0 Gy group *vs* 12 Gy group (A) and 0 Gy group *vs* 30 Gy group (B); C and D: Heatmap analysis. Upregulated genes are indicated in red whereas downregulated ones are indicated in green. The expression of mRNAs in radiated samples was comparatively higher than that in non-radiated samples: 0 Gy group *vs* 12 Gy group (C) and 0 Gy group *vs* 30 Gy group (D); E: Venn diagram of radioresistance-promoting mRNAs associated with prognosis in esophageal squamous cell carcinoma.

Association of the three mRNAs with pathological grade and tumor-node-metastasis stage

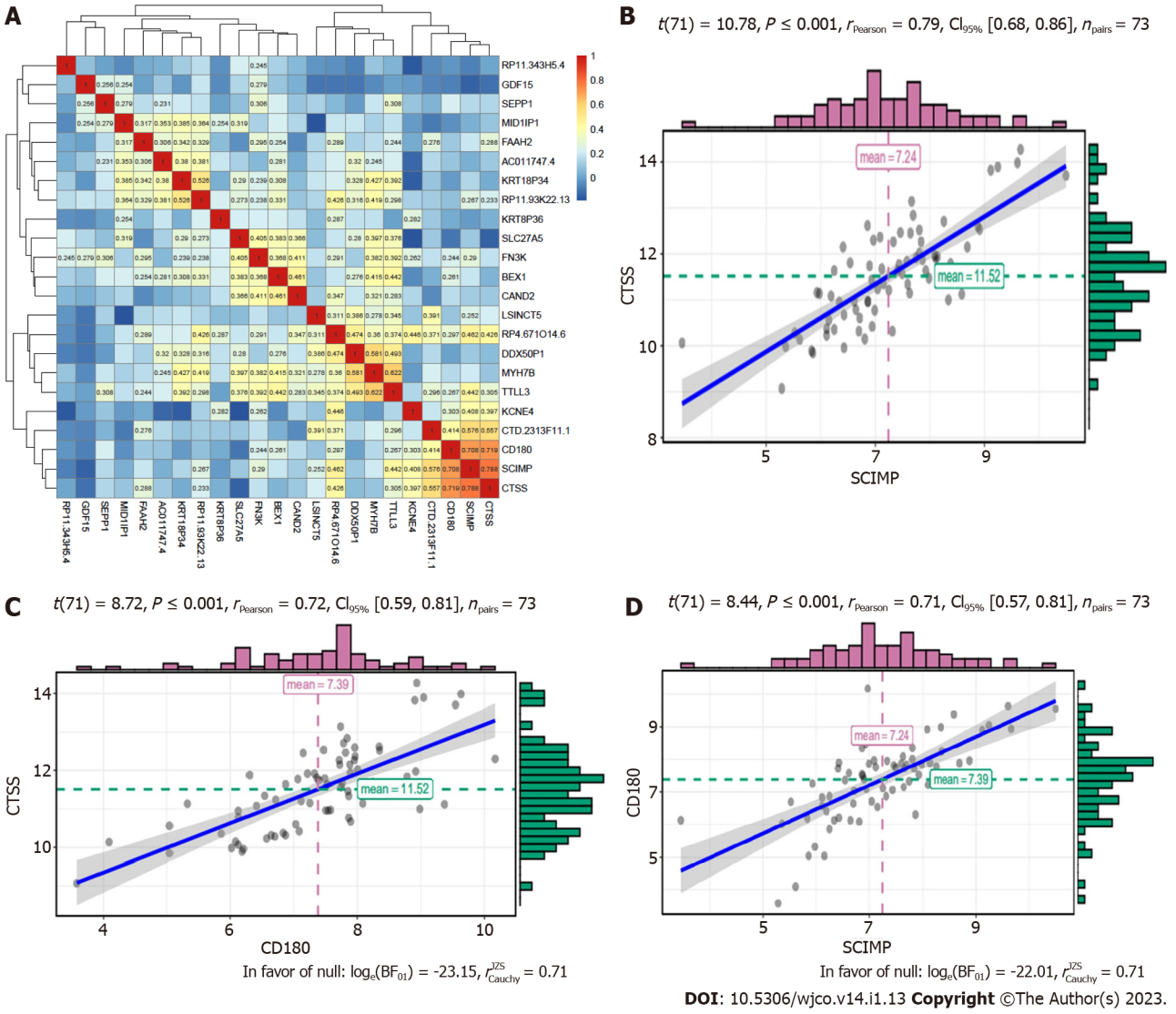
We next explored the association between the three radioresistance-promoting mRNAs and pathological grade (Figure 5A-C) and tumor-node-metastasis (TNM) stage (Figure 5D-F). *CTSS, CD180*, and *SCIMP* exhibited significantly higher expression in advanced pathological grades (2-3 *vs* 1) and tumor stages (II-IV *vs* I).

Functional characteristics of CTSS, CD180, and SCIMP mRNAs

To further explore the underlying biological features of the three mRNAs in ESCC, we performed Pearson correlation between the three mRNAs, namely, *CTSS, CD180*, and *SCIMP*, and the other mRNAs to identify co-expressed mRNAs. A total of 539 mRNAs were selected for KEGG pathway enrichment analysis ($P < 0.01$, $r > 0.4$). Our results showed that the co-expressed mRNAs were mainly enriched in 50 pathways, including NF-kB, JAK-STAT, cell adhesion molecules signaling, and PD-L1 expression & PD-1 checkpoint pathways (Figure 6).

DISCUSSION

Prolonged and fractionated irradiation during radiotherapy in ESCC patients could confer radioresistance and result in distant metastasis, which may lead to treatment failure[22,23]. CAFs can foster radioresistance in ESCC tumor cells through the long noncoding RNA DNM3OS by modulating the PDGFβ/PDGFRβ/FOXO1 signaling pathway, suggesting that CAFs-promoted DNM3OS could be a crucial target to reverse radioresistance in ESCC tumor cells. A study by Zhao *et al*[24] in 2020, showed that three genes (*FOXL2, TCF4*, and *NR2F2*) exhibited a significant correlation with the prognosis of endometrial carcinoma; biological pathways associated with the low expression of these three genes were significantly enriched in cell cycle and fatty acid metabolism of cancer cells. However, there is

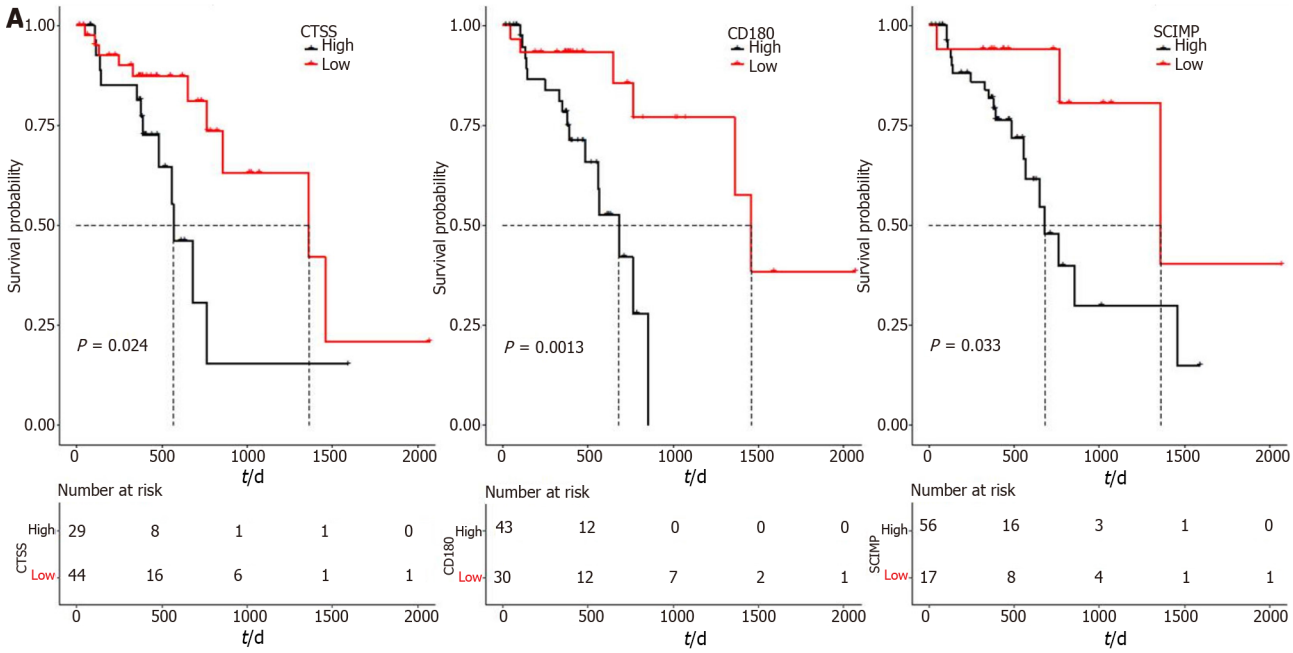


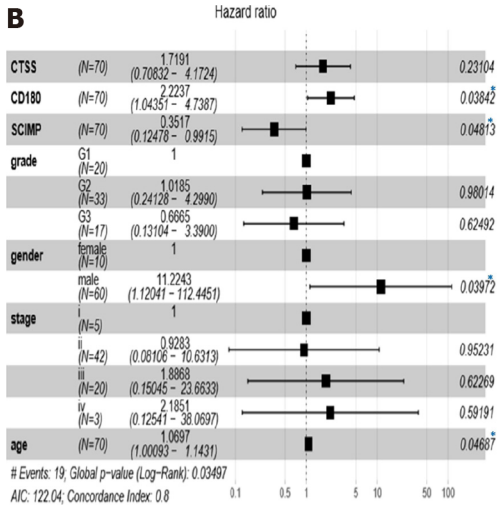
DOI: 10.5306/wjco.v14.i1.13 **Copyright** ©The Author(s) 2023.

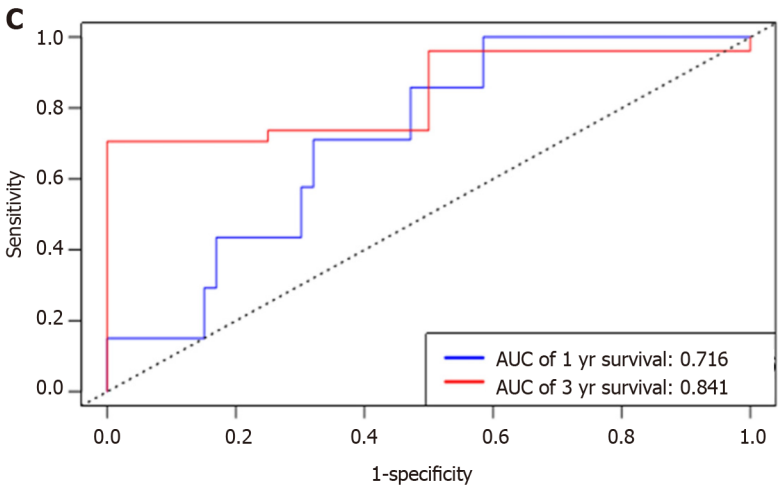
Figure 2 Heatmap and scattergram depicting the correlations of mRNAs. A: Heatmap showing correlations of 23 mRNAs contributing to radioresistance; B-D: Scattergram showing correlations between two of the three mRNAs: *CTSS vs SCIMP* (B), *CTSS vs CD180* (C), and *CD180 vs SCIMP* (D).

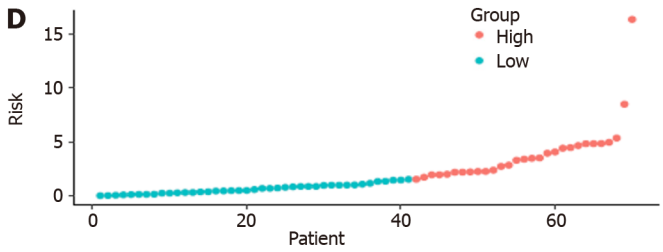
limited evidence to validate the gene signatures involved in conferring radioresistance in ESCC patients to delineate accurate and efficient disease prognosis[25]. Ma *et al*[26] demonstrated that HMGB1 promotes radioresistance through the activation of autophagy. Furthermore, differentially expressed genes (DEGs) including '*CFLAR, LAMA5, ITGA6, ITGB4*, and *SDC4*' in five signaling cascades (PI3K-AKT pathway, CYCS gene-based apoptosis pathway, S100AX–AKT3-related pathway, SDC4 and HSPG2 pathway, and mTOR signaling pathway) were reported to be associated with radioresistance in *in vitro* ESCC models, and tissue biopsies of ESCC patients[27]. In the present study, we, for the first time, constructed a risk score model based on three radioresistance-associated mRNAs (*CTSS, CD180*, and *SCIMP*) and clinical features of ESCC patients; this model could facilitate oncologists to predict overall survival of ESCC patients with acquired radioresistance in radiotherapy.

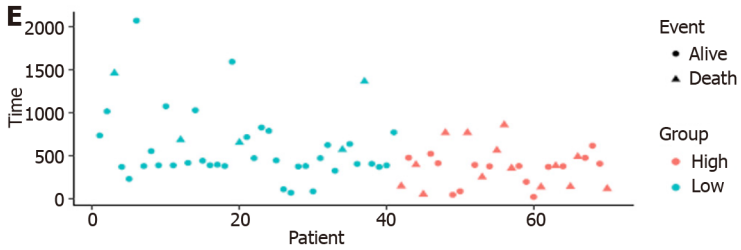
A research study showed that the insulin-like growth factor 2 mRNA-binding protein 3 can contribute to the development of radioresistance in ESCC[28]. miR-205 promotes radioresistance in ESCC typically through enhancing DNA repair, impairing apoptosis, and stimulating EMT[29]. Another factor *i.e.*, eEF2K, could foster the progression of radioresistance in ESCC[30]. In our study, the involvement of three mRNAs (*CTSS, CD180*, and *SCIMP*) in radioresistance was analyzed through the transcriptome profiling of ESCC samples between non-radiated KYSE-180 cells and 12 or 30 Gy far infrared radiation-treated KYSE-180 cells and by constructing a risk score model. However, the overall survival information in GSE81812 dataset is unavailable, so we conducted univariate and multivariate Cox regression analysis based on the TCGA database, and identified 49 radioresistance-associated mRNAs associated with survival, of which 23 were inversely correlated with survival. After comprehensive correlation analysis, we selected three radioresistance-associated mRNAs (*CTSS, CD180*, and *SCIMP*) that were strongly correlated with each other based on the data in the TCGA database. Subramanian *et al*[31] deciphered that the well-developed genomic signatures are significantly

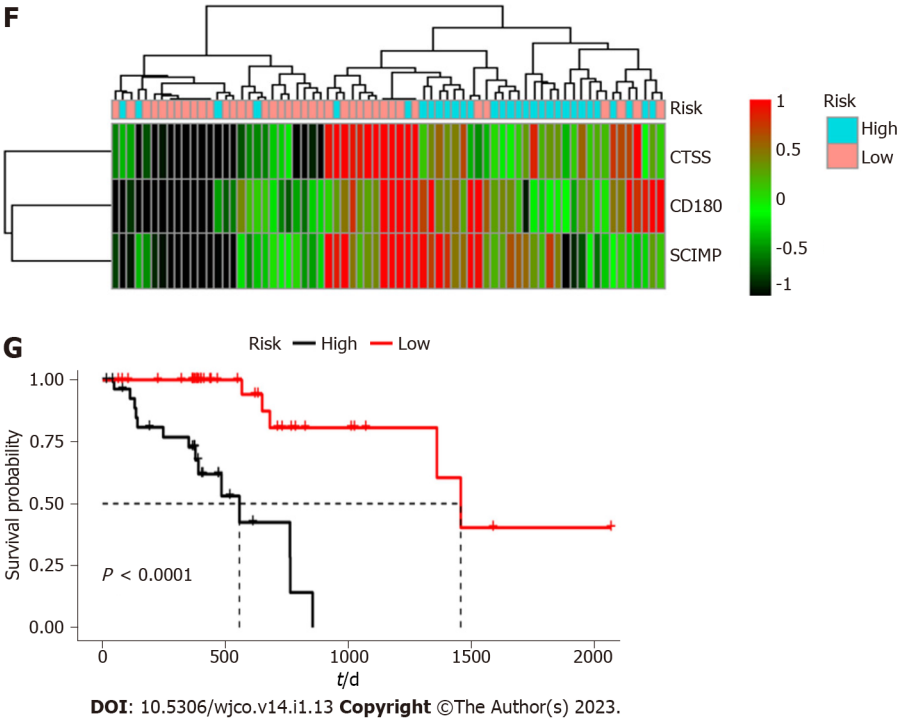

A
CTSS
High
Low
Survival probability
P = 0.024
t/d
Number at risk
High 29 8 1 1 0
Low 44 16 6 1 1
CD180
P = 0.0013
High 43 12 0 0 0
Low 30 12 7 2 1
SCIMP
P = 0.033
High 56 16 3 1 0
Low 17 8 4 1 1


B
Hazard ratio
CTSS (N=70) 1.7191 (0.70832 - 4.1724) 0.23104
CD180 (N=70) 2.2237 (1.04351 - 4.7387) 0.03842
SCIMP (N=70) 0.3517 (0.12478 - 0.9915) 0.04813
grade G1 (N=20) 1
G2 (N=33) 1.0185 (0.24128 - 4.2990) 0.98014
G3 (N=17) 0.6665 (0.13104 - 3.3900) 0.62492
gender female (N=10) 1
male (N=60) 11.2243 (1.12041 - 112.4451) 0.03972
stage i (N=5) 1
ii (N=42) 0.9283 (0.08106 - 10.6313) 0.95231
iii (N=20) 1.8868 (0.15045 - 23.6633) 0.62269
iv (N=3) 2.1851 (0.12541 - 38.0697) 0.59191
age (N=70) 1.0697 (1.00093 - 1.1431) 0.04687
Events: 19; Global p-value (Log-Rank): 0.03497
AIC: 122.04; Concordance Index: 0.8


C
Sensitivity
1-specificity
AUC of 1 yr survival: 0.716
AUC of 3 yr survival: 0.841


D
Group
High
Low
Risk
Patient


E
Time
Patient
Event
Alive
Death
Group
High
Low



DOI: 10.5306/wjco.v14.i1.13 **Copyright** ©The Author(s) 2023.

Figure 3 Kaplan-Meier curves. A: Kaplan-Meier survival curves by expression of *CTSS*, *SCIMP*, and *CD180* based on data from TCGA database; B: Forest plot established with a hazard ratio calculated through multivariate Cox regression model adjusting for age, gender, grade, and stage ($P < 0.05$); C: Receiver operating characteristic analysis for 1- and 3-year overall survival prediction; D-F: Risk score distribution for patients in high risk group and low risk group. *SCIMP*, *CD180*, and *CTSS* had higher expression in high risk group than in low risk group; G: Kaplan–Meier survival curves for the high risk group and low risk group.

beneficial for improving clinical outcomes in ESCC patients. Results of the overall survival of patients in this study suggested that patients with a higher risk score exhibited a poorer prognosis. Moreover, we downloaded the GSE53625 dataset as independent validation data to validate the prognostic role of the three-mRNA signature. Our result confirmed that the risk score model could also predict the survival outcome based on the external validation datasets.

Among the three mRNAs investigated, *CTSS* encodes a cysteine protease. Seo *et al*[32] showed radiation-induced *CTSS* overexpression, which can consequently promote radioresistance, and knockdown of *CTSS* could induce impairment of radioresistance by modulating the ROS-IFN-γ pathway[32]. Additionally, a plethora of research studies have found that CTSS is particularly involved in modulating autophagy pathways[33], PI3K/Akt and Ras/Raf/MAPK signaling pathways[34], and EGFR-ERK signaling pathway[35] as these signaling cascades are more or less involved in conferring radioresistance. However, there are no reports available in the literature to delineate that CD180 and SCIMP are involved in causing radioresistance in ESCC patients. CD180 belongs to the family of Toll-like receptors. Its expression has been reported to be associated with acute or chronic leukemia[36]. *SCIMP* encodes a transmembrane adaptor protein that shapes host defense and inflammation *via* direct modulation of TLR4[37].

A report by Yang *et al*[27] described the activation of the PI3K-Akt signaling pathway (KEGG ID: hsa05200) with upregulation of DEGs such as *LAMA5, LAMB2, LAMB3, ITGA6,* and *ITGB4* at 12-Gy and 30-Gy fractionated irradiation. Thus, PI3K-Akt is reported to be involved in protecting KYSE-180 cells from undergoing apoptosis after irradiation. *CYCS* gene-based apoptosis pathway (KEGG ID: hsa04210) is impaired after 12-Gy irradiation due to the induction of *CYCS* downregulation. KEGG pathway analysis of S100AX–AKT3 signaling depicted that the activation of this pathway could enhance the migration and metastasis of HSCC KYSE-180-12 Gy and KYE-180-30 Gy cells[27,38]. SDC4 and HSPG2 [KEGG ID: hsa05205] are two proteoglycans that were reported to be upregulated during the irradiation of KYSE-180 cells at doses of 12 Gy and 30 Gy. These genes are responsible for tumor cell invasion and metastasis[27]. In the present study, KEGG pathway analysis was performed to clarify the underlying mechanisms of the three mRNAs contributing to the radioresistance of ESCC cells. Our results showed that these mRNAs were mainly enriched in pathways that are related to radioresistance, such as the JAK-STAT signaling pathway[39] and NF-kB signaling pathway[40]. Our results also demonstrated the radioresistance-promoting ability of these three mRNAs. Besides, these mRNAs were enriched in immune-related pathways, such as antigen processing and presentation, cytokine-cytokine receptor interaction, and Th17 cell differentiation. Hence, these three radioresistance-associated mRNAs might be involved in the regulation of immune pathways contributing to ESCC cell radioresistance.

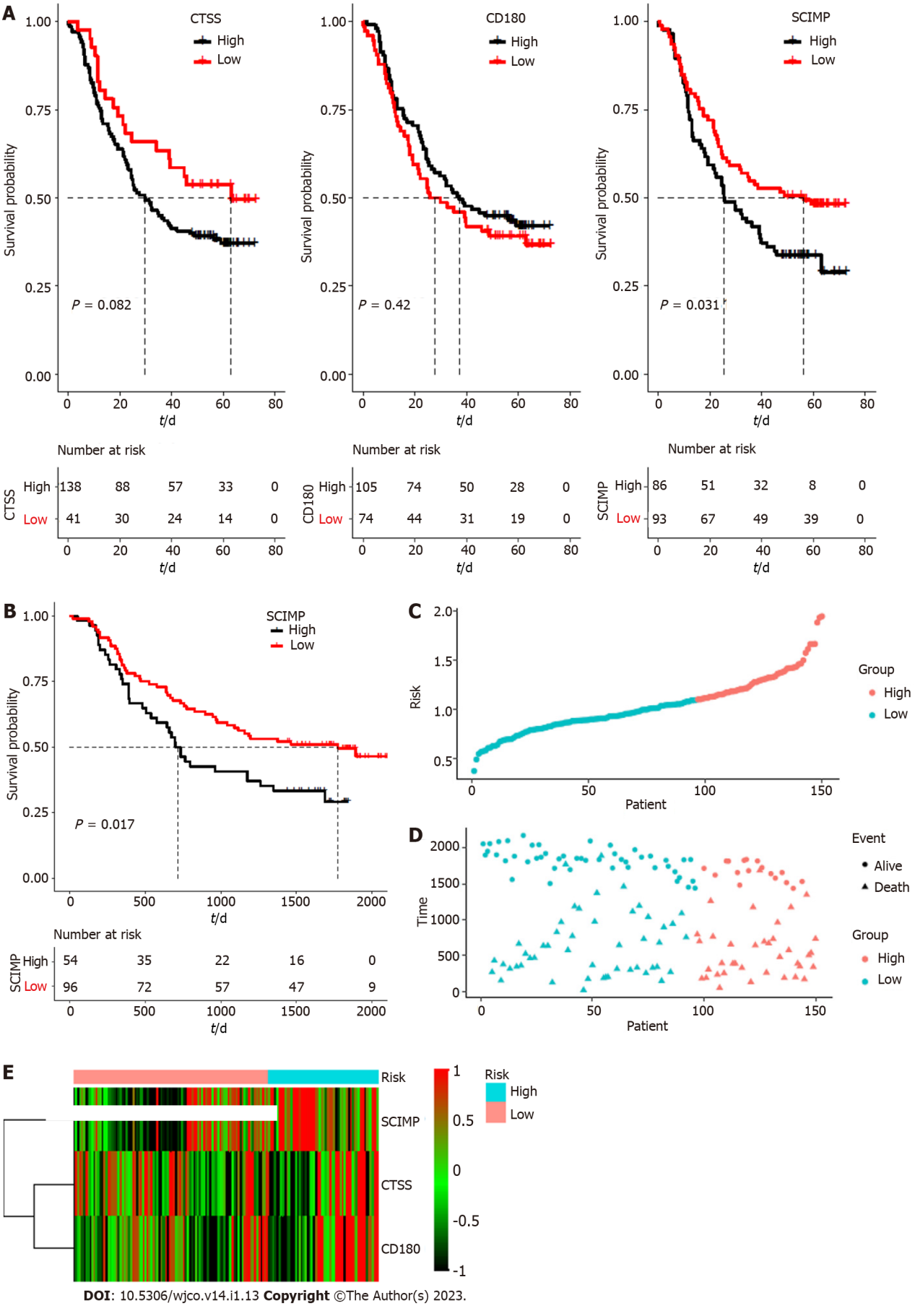
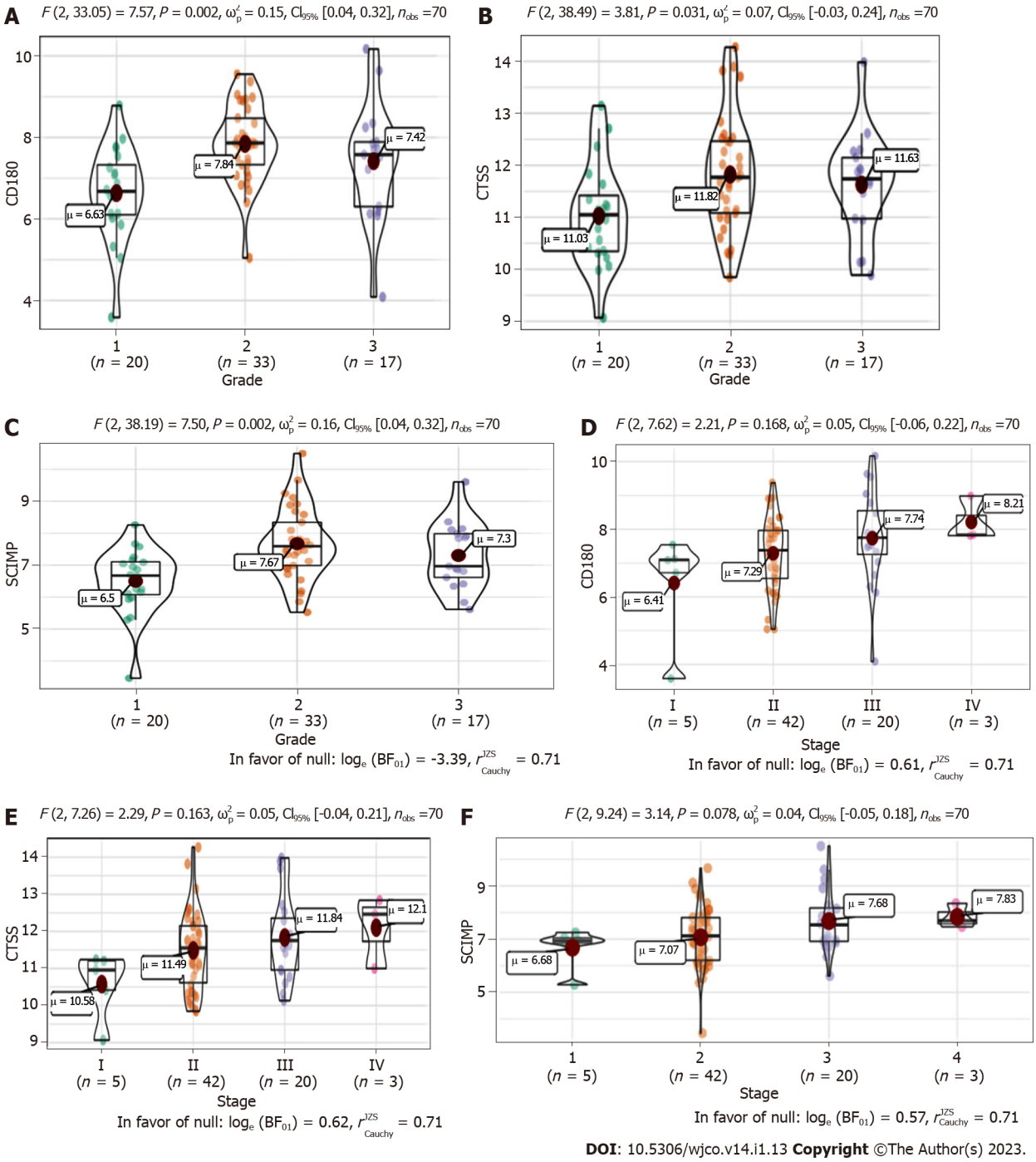


Figure 4 Validation of prognostic value of the three mRNAs based on dataset downloaded from the Gene Expression Omnibus database.

A: Kaplan–Meier survival curves for *SCIMP*, *CD180*, and *CTSS* based on GSE53625 dataset; B: Kaplan–Meier survival curves for the high risk group and low risk

group; C-E: Risk score distribution for patients in high risk group and low risk group. *SCIMP*, *CD180*, and *CTSS* had higher expression in high risk group than in low risk group.

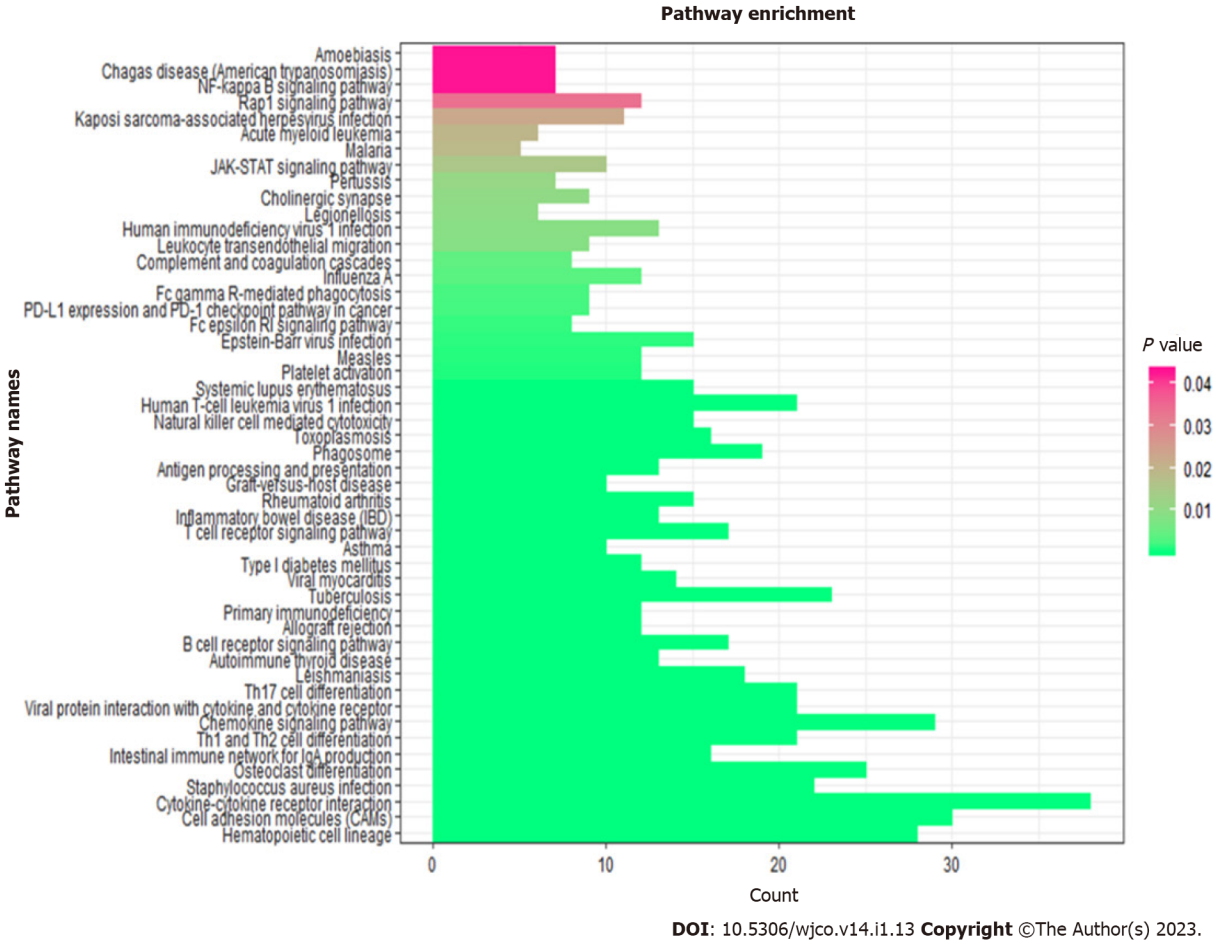


DOI: 10.5306/wjco.v14.i1.13 **Copyright** ©The Author(s) 2023.

Figure 5 Association of the three radioresistance-promoting mRNAs (*CTSS*, *CD180*, and *SCIMP*) with tumor characteristics. A-C: Box plots showing a positive correlation of *SCIMP*, *CD180*, and *CTSS* with pathological grade; D-F: Box plots showing a positive correlation of *SCIMP*, *CD180*, and *CTSS* with tumor-node-metastasis stage.

CONCLUSION

In summary, our study proved that CTSS, CD180 and SCIMP can promote the development of radioresistance in ESCC patients. The novel three-gene signature developed based on the three genes can be used as a prognostic model to predict the prognosis of patients with radioresistant ESCC.



DOI: 10.5306/wjco.v14.i1.13 **Copyright** ©The Author(s) 2023.

Figure 6 Kyoto Encyclopedia of Genes and Genomes enrichment analysis of co-expressed mRNAs. *CTSS*, *CD180*, and *SCIMP* genes can interact with other co-expressed mRNAs, which were mainly enriched in 50 pathways, such as NF-kB, JAK-STAT, PD-L1 expression & PD-1 checkpoint pathway, and cell adhesion molecules signaling pathways.

ARTICLE HIGHLIGHTS

Research background

Esophageal squamous cell carcinoma (ESCC) is causing a high mortality rate due to the lack of efficient early prognosis markers and suitable therapeutic regimens.

Research motivation

The prognostic role of genes responsible for the acquisition of radioresistance in ESCC has not been fully elucidated.

Research objectives

To establish a prognostic model by studying gene expression patterns pertinent to radioresistance in ESCC patients.

Research methods

Datasets were obtained from the Gene Expression Omnibus and The Cancer Genome Atlas databases. The edgeR, a Bioconductor package, was used to analyze mRNA expression between different groups. We screened genes specifically responsible for radioresistance to estimate overall survival. Pearson correlation analysis was performed to confirm whether the expression of those genes correlated with each other. Genes contributing to radioresistance and overall survival were assessed by the multivariate Cox regression model through the calculation of βi and risk score using the following formula: $\sum_{i=1}^{n} \beta i \times \mathrm{PSI}$.

Research results

We identified three prognostic mRNAs (cathepsin S [*CTSS*], cluster of differentiation 180 [*CD180*], and SLP adapter and CSK-interacting membrane protein [*SCIMP*]) indicative of radioresistance. The

expression of the three identified mRNAs was related to each other ($r > 0.70$ and $P < 0.05$). As to 1-year and 3-year overall survival prediction, the area under the time-dependent receiver operating characteristic curve of the signature consisting of the three mRNAs was 0.716 and 0.841, respectively. When stratifying patients based on the risk score derived from the signature, the high-risk group exhibited a higher death risk and shorter survival time than the low-risk group ($P < 0.0001$). Overall survival of the low-risk patients was significantly better than that of the high-risk patients ($P = 0.018$).

Research conclusions

We have developed a novel three-gene prognostic signature consisting of *CTSS*, *CD180*, and *SCIMO* for ESCC.

Research perspectives

The three-gene signature developed in this study may facilitate the prediction of early prognosis of this malignancy.

FOOTNOTES

Author contributions: Wang XY, Beeraka NM, Xue NN, Yu HM, Yang Y, Liu MX, Nikolenko VN, Liu JQ, and Zhao D conceptualized and designed the study; Beeraka NM, Wang XY, Liu JQ, Zhao D, Xue NN, Yu HM, Nikolenko VN, and Yang Y performed the literature analysis and drafted the manuscript; Beeraka NM, Liu JQ, and Zhao D revised, edited, and extended the final draft; all authors have reviewed and approved the manuscript before submission; Wang XY and Beeraka NM contributed equally to this work.

Institutional review board statement: Since the data of this study were obtained from the TCGA and GEO public databases, in which no personal identification information was included, informed consent was waived by the First Affiliated Hospital of Zhengzhou University.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest to disclose.

Data sharing statement: All the supplementary files can be provided upon request by the editorial office as the data was obtained from the TCGA and GEO databases.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Jun-Qi Liu 0000-0002-6015-1033.

S-Editor: Liu JH
L-Editor: Wang TQ
P-Editor: Liu JH

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Wang Y**, Lyu Z, Qin Y, Wang X, Sun L, Zhang Y, Gong L, Wu S, Han S, Tang Y, Jia Y, Kwong DL, Kam N, Guan XY. FOXO1 promotes tumor progression by increased M2 macrophage infiltration in esophageal squamous cell carcinoma. *Theranostics* 2020; **10**: 11535-11548 [PMID: 33052231 DOI: 10.7150/thno.45261]

3 **Ajani JA**, D'Amico TA, Bentrem DJ, Chao J, Corvera C, Das P, Denlinger CS, Enzinger PC, Fanta P, Farjah F, Gerdes H, Gibson M, Glasgow RE, Hayman JA, Hochwald S, Hofstetter WL, Ilson DH, Jaroszewski D, Johung KL, Keswani RN, Kleinberg LR, Leong S, Ly QP, Matkowskyj KA, McNamara M, Mulcahy MF, Paluri RK, Park H, Perry KA, Pimiento J, Poultsides GA, Roses R, Strong VE, Wiesner G, Willett CG, Wright CD, McMillian NR, Pluchino LA. Esophageal and Esophagogastric Junction Cancers, Version 2.2019, NCCN Clinical Practice Guidelines in Oncology. *J Natl ComprCancNetw* 2019; **17**: 855-883 [PMID: 31319389 DOI: 10.6004/jnccn.2019.0033]

4 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]

5 **Ohashi S**, Miyamoto S, Kikuchi O, Goto T, Amanuma Y, Muto M. Recent Advances From Basic and Clinical Studies of Esophageal Squamous Cell Carcinoma. *Gastroenterology* 2015; **149**: 1700-1715 [PMID: 26376349 DOI:

10.1053/j.gastro.2015.08.054]

6 **Zhan XH**, Jiao JW, Zhang HF, Li CQ, Zhao JM, Liao LD, Wu JY, Wu BL, Wu ZY, Wang SH, Du ZP, Shen JH, Zou HY, Neufeld G, Xu LY, Li EM. A three-gene signature from protein-protein interaction network of LOXL2- and actin-related proteins for esophageal squamous cell carcinoma prognosis. *Cancer Med* 2017; **6**: 1707-1719 [PMID: 28556501 DOI: 10.1002/cam4.1096]

7 **Bayo J**, Tran TA, Wang L, Peña-Llopis S, Das AK, Martinez ED. Jumonji Inhibitors Overcome Radioresistance in Cancer through Changes in H3K4 Methylation at Double-Strand Breaks. *Cell Rep* 2018; **25**: 1040-1050.e5 [PMID: 30355483 DOI: 10.1016/j.celrep.2018.09.081]

8 **Zhou Y**, Chu L, Wang Q, Dai W, Zhang X, Chen J, Li L, Ding P, Zhang L, Gu H, Lv X, Zhang W, Zhou D, Zhang P, Cai G, Zhao K, Hu W. CD59 is a potential biomarker of esophageal squamous cell carcinoma radioresistance by affecting DNA repair. *Cell Death Dis* 2018; **9**: 887 [PMID: 30166523 DOI: 10.1038/s41419-018-0895-0]

9 **Qian D**, Zhang B, Zeng XL, Le Blanc JM, Guo YH, Xue C, Jiang C, Wang HH, Zhao TS, Meng MB, Zhao LJ, Hao JH, Wang P, Xie D, Lu B, Yuan ZY. Inhibition of human positive cofactor 4 radiosensitizes human esophageal squmaous cell carcinoma cells by suppressing XLF-mediated nonhomologous end joining. *Cell Death Dis* 2014; **5**: e1461 [PMID: 25321468 DOI: 10.1038/cddis.2014.416]

10 **Croagh D**, Frede J, Jones PH, Kaur P, Partensky C, Phillips WA. Esophageal stem cells and genetics/epigenetics in esophageal cancer. *Ann N Y AcadSci* 2014; **1325**: 8-14 [PMID: 25266010 DOI: 10.1111/nyas.12521]

11 **He E**, Pan F, Li G, Li J. Fractionated Ionizing Radiation Promotes Epithelial-Mesenchymal Transition in Human Esophageal Cancer Cells through PTEN Deficiency-Mediated Akt Activation. *PLoS One* 2015; **10**: e0126149 [PMID: 26000878 DOI: 10.1371/journal.pone.0126149]

12 **Hein AL**, Ouellette MM, Yan Y. Radiation-induced signaling pathways that promote cancer cell survival (review). *Int J Oncol* 2014; **45**: 1813-1819 [PMID: 25174607 DOI: 10.3892/ijo.2014.2614]

13 **Su H**, Jin X, Zhang X, Zhao L, Lin B, Li L, Fei Z, Shen L, Fang Y, Pan H, Xie C. FH535 increases the radiosensitivity and reverses epithelial-to-mesenchymal transition of radioresistant esophageal cancer cell line KYSE-150R. *J Transl Med* 2015; **13**: 104 [PMID: 25888911 DOI: 10.1186/s12967-015-0464-6]

14 **Zhu H**, Yang X, Ding Y, Liu J, Lu J, Zhan L, Qin Q, Zhang H, Chen X, Yang Y, Liu Z, Yang M, Zhou X, Cheng H, Sun X. Recombinant human endostatin enhances the radioresponse in esophageal squamous cell carcinoma by normalizing tumor vasculature and reducing hypoxia. *Sci Rep* 2015; **5**: 14503 [PMID: 26412785 DOI: 10.1038/srep14503]

15 **Matsuo M**, Matsumoto S, Mitchell JB, Krishna MC, Camphausen K. Magnetic resonance imaging of the tumor microenvironment in radiotherapy: perfusion, hypoxia, and metabolism. *SeminRadiatOncol* 2014; **24**: 210-217 [PMID: 24931096 DOI: 10.1016/j.semradonc.2014.02.002]

16 **Underwood TJ**, Hayden AL, Derouet M, Garcia E, Noble F, White MJ, Thirdborough S, Mead A, Clemons N, Mellone M, Uzoho C, Primrose JN, Blaydes JP, Thomas GJ. Cancer-associated fibroblasts predict poor outcome and promote periostin-dependent invasion in oesophageal adenocarcinoma. *J Pathol* 2015; **235**: 466-477 [PMID: 25345775 DOI: 10.1002/path.4467]

17 **Shigeoka M**, Urakawa N, Nakamura T, Nishio M, Watajima T, Kuroda D, Komori T, Kakeji Y, Semba S, Yokozaki H. Tumor associated macrophage expressing CD204 is associated with tumor aggressiveness of esophageal squamous cell carcinoma. *Cancer Sci* 2013; **104**: 1112-1119 [PMID: 23648122 DOI: 10.1111/cas.12188]

18 **Izawa S**, Mimura K, Watanabe M, Maruyama T, Kawaguchi Y, Fujii H, Kono K. Increased prevalence of tumor-infiltrating regulatory T cells is closely related to their lower sensitivity to H2O2-induced apoptosis in gastric and esophageal cancer. *Cancer ImmunolImmunother* 2013; **62**: 161-170 [PMID: 22865268 DOI: 10.1007/s00262-012-1327-0]

19 **Maher SG**, Gillham CM, Duggan SP, Smyth PC, Miller N, Muldoon C, O'Byrne KJ, Sheils OM, Hollywood D, Reynolds JV. Gene expression analysis of diagnostic biopsies predicts pathological response to neoadjuvant chemoradiotherapy of esophageal cancer. *Ann Surg* 2009; **250**: 729-737 [PMID: 19801928 DOI: 10.1097/SLA.0b013e3181bce7e1]

20 **Park M**, Yoon HJ, Kang MC, Kwon J, Lee HW. PTK7 regulates radioresistance through nuclear factor-kappa B in esophageal squamous cell carcinoma. *TumourBiol* 2016; **37**: 14217-14224 [PMID: 27557627 DOI: 10.1007/s13277-016-5288-3]

21 **Wu H**, Yu J, Kong D, Xu Y, Zhang Z, Shui J, Li Z, Luo H, Wang K. Population and singlecell transcriptome analyses reveal diverse transcriptional changes associated with radioresistance in esophageal squamous cell carcinoma. *Int J Oncol* 2019; **55**: 1237-1248 [PMID: 31638164 DOI: 10.3892/ijo.2019.4897]

22 **Sjoquist KM**, Burmeister BH, Smithers BM, Zalcberg JR, Simes RJ, Barbour A, Gebski V; Australasian Gastro-Intestinal Trials Group. Survival after neoadjuvant chemotherapy or chemoradiotherapy for resectableoesophageal carcinoma: an updated meta-analysis. *Lancet Oncol* 2011; **12**: 681-692 [PMID: 21684205 DOI: 10.1016/S1470-2045(11)70142-5]

23 **Ding M**, Zhang E, He R, Wang X. Newly developed strategies for improving sensitivity to radiation by targeting signal pathways in cancer therapy. *Cancer Sci* 2013; **104**: 1401-1410 [PMID: 23930697 DOI: 10.1111/cas.12252]

24 **Zhao H**, Jiang A, Yu M, Bao H. Identification of biomarkers correlated with diagnosis and prognosis of endometrial cancer using bioinformatics analysis. *J Cell Biochem* 2020 [PMID: 32692884 DOI: 10.1002/jcb.29819]

25 **Zhang H**, Hua Y, Jiang Z, Yue J, Shi M, Zhen X, Zhang X, Yang L, Zhou R, Wu S. Cancer-associated Fibroblast-promoted LncRNA*DNM3OS* Confers Radioresistance by Regulating DNA Damage Response in Esophageal Squamous Cell Carcinoma. *Clin Cancer Res* 2019; **25**: 1989-2000 [PMID: 30463848 DOI: 10.1158/1078-0432.CCR-18-0773]

26 **Ma H**, Zheng S, Zhang X, Gong T, Lv X, Fu S, Zhang S, Yin X, Hao J, Shan C, Huang S. High mobility group box 1 promotes radioresistance in esophageal squamous cell carcinoma cell lines by modulating autophagy. *Cell Death Dis* 2019; **10**: 136 [PMID: 30755598 DOI: 10.1038/s41419-019-1355-1]

27 **Yang L**, Zhang X, Hou Q, Huang M, Zhang H, Jiang Z, Yue J, Wu S. Single-cell RNA-seq of esophageal squamous cell carcinoma cell line with fractionated irradiation reveals radioresistant gene expression patterns. *BMC Genomics* 2019; **20**: 611 [PMID: 31345182 DOI: 10.1186/s12864-019-5970-0]

28 **Yoshino K**, Motoyama S, Koyota S, Shibuya K, Sato Y, Sasaki T, Wakita A, Saito H, Minamiya Y, Sugiyama T, Ogawa J. Identification of insulin-like growth factor 2 mRNA-binding protein 3 as a radioresistance factor in squamous esophageal cancer cells. *Dis Esophagus* 2014; **27**: 479-484 [PMID: 22989274 DOI: 10.1111/j.1442-2050.2012.01415.x]

29 **Pan F**, Mao H, Bu F, Tong X, Li J, Zhang S, Liu X, Wang L, Wu L, Chen R, Wei H, Li B, Li C, Yang Y, Steer CJ, Zhao J, Guo Y. Sp1-mediated transcriptional activation of miR-205 promotes radioresistance in esophageal squamous cell carcinoma. *Oncotarget* 2017; **8**: 5735-5752 [PMID: 27974696 DOI: 10.18632/oncotarget.13902]

30 **Zhu H**, Song H, Chen G, Yang X, Liu J, Ge Y, Lu J, Qin Q, Zhang C, Xu L, Di X, Cai J, Ma J, Zhang S, Sun X. eEF2K promotes progression and radioresistance of esophageal squamous cell carcinoma. *RadiotherOncol* 2017; **124**: 439-447 [PMID: 28431753 DOI: 10.1016/j.radonc.2017.04.001]

31 **Subramanian J**, Simon R. What should physicians look for in evaluating prognostic gene-expression signatures? *Nat Rev ClinOncol* 2010; **7**: 327-334 [PMID: 20421890 DOI: 10.1038/nrclinonc.2010.60]

32 **Seo HR**, Bae S, Lee YS. Radiation-induced cathepsin S is involved in radioresistance. *Int J Cancer* 2009; **124**: 1794-1801 [PMID: 19101991 DOI: 10.1002/ijc.24095]

33 **Hsin MC**, Hsieh YH, Wang PH, Ko JL, Hsin IL, Yang SF. Hispolon suppresses metastasis *via* autophagic degradation of cathepsin S in cervical cancer cells. *Cell Death Dis* 2017; **8**: e3089 [PMID: 28981104 DOI: 10.1038/cddis.2017.459]

34 **Gautam J**, Bae YK, Kim JA. Up-regulation of cathepsin S expression by HSP90 and 5-HT_7 receptor-dependent serotonin signaling correlates with triple negativity of human breast cancer. *Breast Cancer Res Treat* 2017; **161**: 29-40 [PMID: 27796714 DOI: 10.1007/s10549-016-4027-1]

35 **Chen KL**, Chang WS, Cheung CH, Lin CC, Huang CC, Yang YN, Kuo CP, Kuo CC, Chang YH, Liu KJ, Wu CM, Chang JY. Targeting cathepsin S induces tumor cell autophagy *via* the EGFR-ERK signaling pathway. *Cancer Lett* 2012; **317**: 89-98 [PMID: 22101325 DOI: 10.1016/j.canlet.2011.11.015]

36 **Chaplin JW**, Kasahara S, Clark EA, Ledbetter JA. Anti-CD180 (RP105) activates B cells to rapidly produce polyclonal Ig *via* a T cell and MyD88-independent pathway. *J Immunol* 2011; **187**: 4199-4209 [PMID: 21918197 DOI: 10.4049/jimmunol.1100198]

37 **Luo L**, Bokil NJ, Wall AA, Kapetanovic R, Lansdaal NM, Marceline F, Burgess BJ, Tong SJ, Guo Z, Alexandrov K, Ross IL, Hibbs ML, Stow JL, Sweet MJ. SCIMP is a transmembrane non-TIR TLR adaptor that promotes proinflammatory cytokine production from macrophages. *Nat Commun* 2017; **8**: 14133 [PMID: 28098138 DOI: 10.1038/ncomms14133]

38 **Grottke A**, Ewald F, Lange T, Nörz D, Herzberger C, Bach J, Grabinski N, Gräser L, Höppner F, Nashan B, Schumacher U, Jücker M. Downregulation of AKT3 Increases Migration and Metastasis in Triple Negative Breast Cancer Cells by Upregulating S100A4. *PLoS One* 2016; **11**: e0146370 [PMID: 26741489 DOI: 10.1371/journal.pone.0146370]

39 **Park SY**, Lee CJ, Choi JH, Kim JH, Kim JW, Kim JY, Nam JS. The JAK2/STAT3/CCND2 Axis promotes colorectal Cancer stem cell persistence and radioresistance. *J ExpClin Cancer Res* 2019; **38**: 399 [PMID: 31511084 DOI: 10.1186/s13046-019-1405-7]

40 **Hou Y**, Liang H, Rao E, Zheng W, Huang X, Deng L, Zhang Y, Yu X, Xu M, Mauceri H, Arina A, Weichselbaum RR, Fu YX. Non-canonical NF-κB Antagonizes STING Sensor-Mediated DNA Sensing in Radiotherapy. *Immunity* 2018; **49**: 490-503.e4 [PMID: 30170810 DOI: 10.1016/j.immuni.2018.07.008]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 January 24; 14(1): 27-39

DOI: 10.5306/wjco.v14.i1.27

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

5-mRNA-based prognostic signature of survival in lung adenocarcinoma

Qian-Lin Xia, Xiao-Meng He, Yan Ma, Qiu-Yue Li, Yu-Zhen Du, Jin Wang

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Moussa BS, Egypt; Yu Y, China

Received: October 5, 2022
Peer-review started: October 5, 2022
First decision: October 24, 2022
Revised: November 2, 2022
Accepted: December 13, 2022
Article in press: December 13, 2022
Published online: January 24, 2023

Qian-Lin Xia, Yu-Zhen Du, Laboratory Medicine, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200233, China

Xiao-Meng He, Yan Ma, Qiu-Yue Li, Jin Wang, Scientific Research Center, Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China

Corresponding author: Jin Wang, PhD, Professor, Scientific Research Center, Shanghai Public Health Clinical Center, Fudan University, No 2901 Caolang Road, Jinshan District, Shanghai 201508, China. wjincityu@yahoo.com

Abstract

BACKGROUND

Lung adenocarcinoma (LUAD) is the most common non-small-cell lung cancer, with a high incidence and a poor prognosis.

AIM

To construct effective predictive models to evaluate the prognosis of LUAD patients.

METHODS

In this study, we thoroughly mined LUAD genomic data from the Gene Expression Omnibus (GEO) (GSE43458, GSE32863, and GSE27262) and the Cancer Genome Atlas (TCGA) datasets, including 698 LUAD and 172 healthy (or adjacent normal) lung tissue samples. Univariate regression and LASSO regression analyses were used to screen differentially expressed genes (DEGs) related to patient prognosis, and multivariate Cox regression analysis was applied to establish the risk score equation and construct the survival prognosis model. Receiver operating characteristic curve and Kaplan-Meier survival analyses with clinically independent prognostic parameters were performed to verify the predictive power of the model and further establish a prognostic nomogram.

RESULTS

A total of 380 DEGs were identified in LUAD tissues through GEO and TCGA datasets, and 5 DEGs (TCN1, CENPF, MAOB, CRTAC1 and PLEK2) were screened out by multivariate Cox regression analysis, indicating that the prognostic risk model could be used as an independent prognostic factor (Hazard ratio = 1.520, $P < 0.001$). Internal and external validation of the model confirmed that the prediction model had good sensitivity and specificity (Area under the

curve = 0.754, 0.737). Combining genetic models and clinical prognostic factors, nomograms can also predict overall survival more effectively.

CONCLUSION

A 5-mRNA-based model was constructed to predict the prognosis of lung adenocarcinoma, which may provide clinicians with reliable prognostic assessment tools and help clinical treatment decisions.

Key Words: Lung adenocarcinoma; Differentially expressed genes; Prognostic signature; Risk score; Nomogram

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Five differentially expressed genes (DEGs) (TCN1, CENPF, MAOB, CRTAC1, and PLEK2) selected by multiple Cox regression analysis in the prognostic risk models could be considered as independent prognostic factors for lung adenocarcinoma.

Citation: Xia QL, He XM, Ma Y, Li QY, Du YZ, Wang J. 5-mRNA-based prognostic signature of survival in lung adenocarcinoma. *World J Clin Oncol* 2023; 14(1): 27-39
URL: https://www.wjgnet.com/2218-4333/full/v14/i1/27.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i1.27

INTRODUCTION

Lung adenocarcinoma (LUAD) is a common histological type of lung cancer that is a malignant tumor that seriously threatens human health, accounting for approximately 40% of lung cancers[1]. In recent years, some progress has been made in diagnostic and treatment strategies of clinical and experimental oncology for lung cancer. However, LUAD patients with localized or locally advanced disease still have a high risk of death, and their 5-year overall survival rate is still less than 15%[2]. Assessing the patient's prognosis can help choose effective treatments to balance side effects with treatment benefits and decide whether to give more aggressive treatment. Although tumor–node–metastasis (TNM) classification plays an important role in the prognosis assessment of LUAD patients, the prognosis of some patients is significantly different even if the stages are similar. Therefore, the identification of reliable prognostic biomarkers to predict clinical outcomes and help make accurate clinical treatment decisions is clearly critical. The rapid development of gene chips and high-throughput sequencing have facilitated the development of new predictive tools based on prognostic genes for lung cancer. These relevant studies involved in prognostic genes of lung cancer have identified several prognostic models that have predicted the overall survival rate of LUAD patients (Table 1)[3-14]. For example, a six-gene model (RRAGB, RSPH9, RPS6KL1, RXFP1, RTL1 and RRM2) based on the weighted gene coexpression network predicted the overall survival rate of non-small-cell lung cancer patients[13]. A 3-gene prognostic model (URKA, CDC20, and TPX2A) also accurately predicted overall survival in smoking-related lung adenocarcinoma[14]. In addition, through analysis of TCGA data, the risk score of the 12-mRNA signature was correlated with poor prognosis in patients with lung adenocarcinoma[3]. Therefore, the in-depth exploration of public databases such as the Gene Expression Omnibus (GEO) and the Cancer Genome Atlas (TCGA) databases, discovery of other genes related to the prognosis of LUAD and development of a comprehensive prognosis assessment system including multiple biomarkers may be effective ways to predict the prognosis of lung adenocarcinoma and individual treatment.

Here, we first integrated three lung adenocarcinoma datasets from the GEO database to screen for differentially expressed genes (DEGs). Then, the TCGA-LUAD data set was used to identify DEGs. Univariate Cox and LASSO regression analyses were further used to determine the DEGs associated with overall survival. The risk score was calculated by multiplying multiple Cox coefficients by gene expression. The prognostic model was also combined with clinical parameters to construct a prognostic nomogram to predict overall survival. Finally, Gene set enrichment analysis (GSEA) was performed to identify the potential biological pathways of the five genes in the model.

Table 1 Data and studies involved several prediction models for the prognostic signature of non-small cell lung cancer/ lung adenocarcinoma

Datasets	Model-related genes	Application	Ref.
GEO (GSE19188, GSE33532)	DLGAP5, KIF11, RAD51AP1, CCNB1, AURKA, CDC6, OIP5, NCAPG	Prognostic signature for predicting overall survival in lung adenocarcinoma	Li *et al*[3], 2018
GEO (GSE31210, GSE37745, GSE50081) + TCGA	PLEKHH2, ISCU, CLUL1, CHRDL1, PAIP2B, CDCP1	Prognostic signature for predicting both disease-free and overall survival in non-small cell lung cancer	Zuo *et al*[4], 2019
GEO (GSE31210, GSE37745, GSE50081) + TCGA	CDCP1, HMMR, TPX2, CIRBP, HLF, KBTBD7, SEC24B-AS1, SH2B1	Prognostic Signature for predicting overall survival of early-stage non-small cell lung cancer	He *et al*[5], 2019
TCGA	RHOV, CD109, LINC00941, FRRS1	Prognostic signature for predicting overall survival in lung adenocarcinoma	Shukla *et al*[6], 2016
GEO (GSE3141, GSE30219, GSE50081) + TCGA	ADAM12, BTK, ERG	Prognostic gene signature associated with the microenvironment of lung adenocarcinoma	Yue *et al*[7], 2019
GEO (GSE50081, GSE30219, GSE31210, GSE19188, GSE37745, GSE3141, GSE31908)	ABCC4, ADRBK2, KLHL23, PDS5A, UHRF1, ZNF551	Prognostic signature for predicting overall survival in non-small cell lung cancer	Huang *et al*[8], 2016
GEO (GSE50081, GSE31210, GSE30219, GSE29013, GSE68465, GSE42127) + E-MTAB-923	STAT1, CLU, GTSE1, NUSAP1, ABCA8, TNNT1, ENTPD3, CPA3	Prognostic signature for predicting overall survival in non-small cell lung cancer	Shahid *et al*[9], 2016
GEO (GSE8894, GSE14814, GSE30219, GSE31210, GSE37745, GSE50081)	KIF15, DLGAP5, ASPM, ADAM10, RAD51AP1, FGFR10P, NCGAP	Prognostic gene expression signature for early stage lung adenocarcinoma	Krzystanek *et al* [10], 2016
TCGA	BCHE, CCNA1, CYP24A1, DEPTOR, MASP2, MGLL, MYO1A, PODXL2, RAPGEF3, SGK2, TNNI2, ZBTB16	Prognostic signature for predicting overall survival in lung adenocarcinoma	Zengin *et al* [11], 2020
TCGA	PTPRH, OGFRP1, LDHA, AL365203.1, LINC02178, AL512488.1, LINC01312, AL353746.1, DRAXINP1, LINC02310	Prognostic signature for predicting overall survival in lung adenocarcinoma	Li *et al*[12], 2018
14 GEO datasets	ABCC4, ADRBK2, KLHL23, PDS5A, UHRF1, ZNF551	Prognostic signature for predicting overall survival in non-small cell lung cancer	Xie *et al*[13], 2019
GEO (GSE31210, GSE32863, GSE40791, GSE43458, GSE75037) + TCGA	AURKA, CDC20, TPX2	Prognostic signature for predicting overall survival in smoking-related lung adenocarcinoma	Zhang *et al*[14], 2019

GEO: the Gene Expression Omnibus; TCGA: the Cancer Genome Atlas.

MATERIALS AND METHODS

Gene expression profile data collection from the GEO and TCGA databases

The GEO database (https://www.ncbi.nlm.nih.gov/geo/) was used for the mRNA expression and clinical data of lung adenocarcinoma that needed to meet the following criteria: (1) Human lung adenocarcinoma tissue samples; (2) tumor and nontumor lung control tissue samples; and (3) ≥ 50 samples. Finally, three gene expression microarray data sets (GSE43458, GSE32863 and GSE27262), which included 163 LUAD tumor tissue samples and 113 adjacent normal tissue samples, were downloaded for DEG analysis. On the other hand, the original count data and corresponding clinical data of LUAD patients in the training set and test set, which includes 535 LUAD patient samples and 59 control samples, were downloaded from TCGA project (https://tcga-data.nci.nih.gov/tcga/). Complete survival information and gene expression profile data of 494 patients were obtained from the TCGA database after excluding samples that could not be assessed for tumor histological grade or had no overall survival (OS) information. The model was validated using transcriptome analysis of 90 LUAD patients from the GSE11969 dataset. The workflow of our LUAD biomarker analysis process is shown in Supplementary Figure 1.

Screening and verification of DEGs in lung adenocarcinoma tissue

To identify DEGs between LUAD and lung tissues, GE02R was used for differential expression analysis of the GSE43458, GSE32863, and GSE27262 data sets. The DEGs of the TCGA-LUAD dataset were analyzed using the "limma" software package of R software, and the threshold of DEG screening was $|\log FC| > 2$ and $P < 0.05$ according to our previous study[15]. Human protein mapping (https://www.proteinatlas.org/) evaluates lung adenocarcinoma and DEG protein expression in normal lung tissue[16]. Mutation data in lung adenocarcinoma patients were obtained from cBioPortal (

https://www.cbioportal.org/)[17].

Identification of prognostic differential genes and establishment of prognostic models

TCGA-LUAD data were randomly divided into a training set (n = 346) and a test set (n = 148). In the test set, we performed univariate Cox regression analysis for DEGs determined by a comprehensive analysis of the GEO data set to determine the relationship between patient survival and gene expression. $P < 0.01$ was considered statistically significant and was included in subsequent analysis. Next, we applied LASSO regression to further reduce the number of DEGs in the selected panel with the best predictive performance by 10-fold cross-validation of the R-based glmnet package. Finally, multivariate Cox regression analysis was performed to obtain the five optimal prognostic gene regression coefficients from the multivariate Cox proportional hazard regression model. A prognostic risk score for the five genes was then established based on the multivariate Cox regression model regression coefficient multiplied by a linear combination of its mRNA expression level.

Identification of prognostic models and related genes

The Lung Adenocarcinoma (TCGA, PanCancer Atlas) database in cBioPortal was used to analyze the genetic mutation model. We used data from the TCGA to analyze model-related gene expression. The THPA (http://www.proteinatlas.org) database was used to analyze the protein expression of model-related genes[16]. Patients in the training set were divided into high-risk and low-risk groups according to the median risk score as the cutoff point. Kaplan-Meier (KM) survival curves and Wilcoxon tests combined with the R package "survival" were used to compare the survival differences between the high-risk and low-risk groups. Time-dependent receiver operating characteristic (ROC) curve analysis was conducted using the R software package "survivalROC" to assess the prediction model's forecasting capacity. $P < 0.05$ was considered statistically significant. The test cohort and the entire cohort were used for internal validation, the GSE11969 dataset was downloaded from the GEO database for external validation, and the risk score of each patient was calculated using the same model based on the prognostic gene signature to further verify the predictive value of the prognostic gene signature.

Establish and verify the forecast nomograms

To provide clinicians with a quantitative method for predicting 1-year, 3-year, and 5-year overall survival in LUAD patients, we used a combined model of all independent prognostic factors selected by multivariate Cox regression analysis to construct a nomogram. KM analysis, area under the curve (AUC), consistency index (C-index), and comparison of predicted and observed overall survival were used to evaluate the prognostic nomographs' performance[18].

Functional enrichment analysis of model genes

GSEA was used to analyze the signaling pathways of relevant genes involved in the development of lung adenocarcinoma to clarify the molecular mechanism of the prognostic gene signature. GSEA software (GSEA 4.0.3) was downloaded from the Broad Institute website (http://software.broadinstitute.org/gsea/index.jsp), and the analyzed access was from the c2.cp.kegg.v7.0.symbols.gmt data set in the Molecular Signature Database (MsigDB). The enrichment analysis was carried out by the weighted enrichment method, and the number of random combinations was set as 1000. All other parameters were set as default values. Gene sets with $P < 0.05$ were regarded as significantly enriched gene sets.

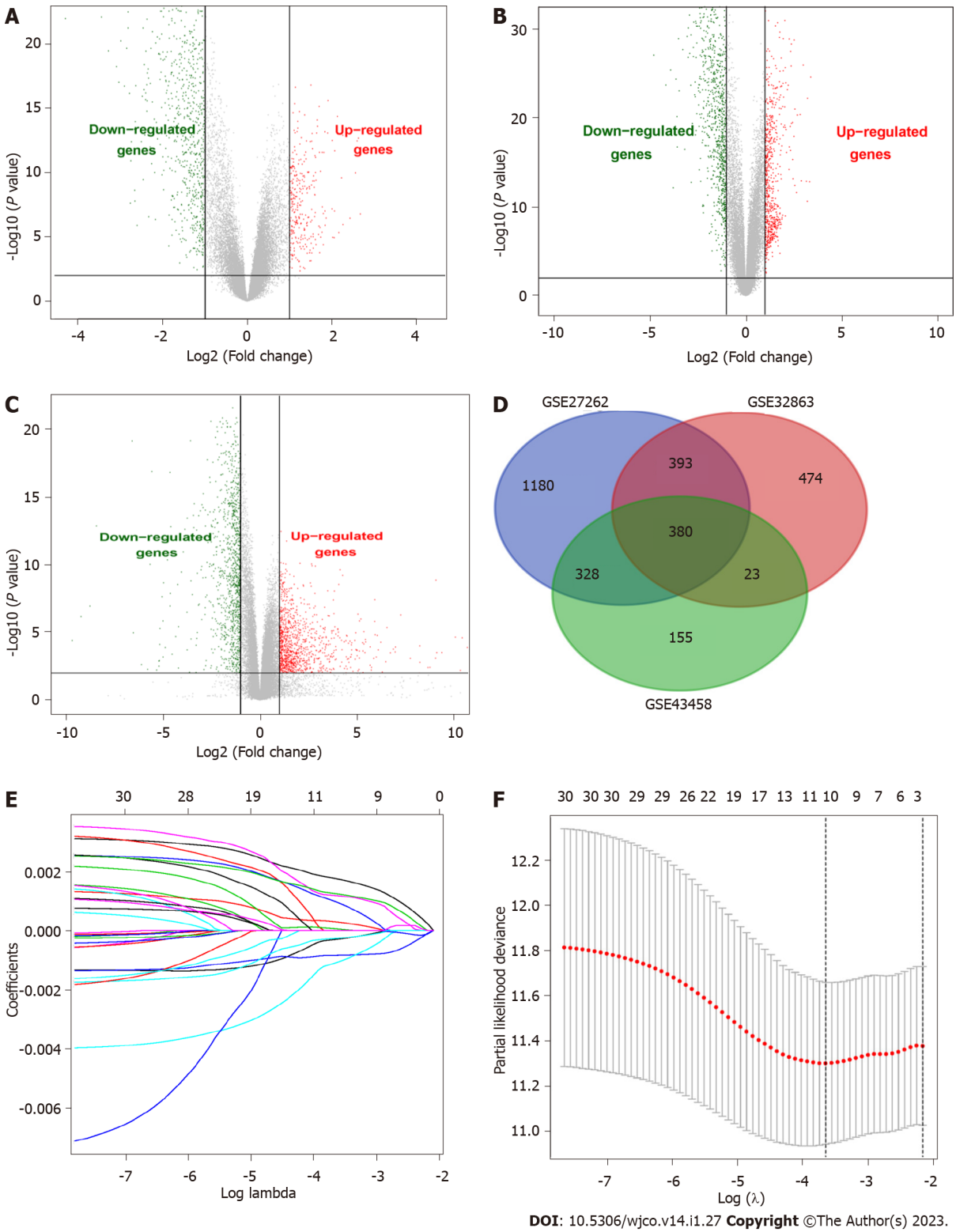
Statistical analysis

Statistical analysis and corresponding graph drawing were performed using R3.6.3 software, and Cox regression analysis of the hazard ratio (HR) and 95% confidence interval (CI) was used to evaluate the association between DEG expression and prognosis. A t-test of paired samples or a nonparametric Wilcoxon rank sum test of unpaired samples was used for analysis of continuous variables, and categorical variables were tested by the chi-square test or Fisher's exact test. $P < 0.05$ indicated a significant difference.

RESULTS

Screening and identification of differentially expressed genes in the TCGA-LUAD database

We researched the results as described in the flowchart (Supplementary Figure 1). This study analyzed three GEO datasets (GSE43458, GSE32863, and GSE27262), and 886, 1270, and 1921 DEGs were found, respectively. Then, we found 380 common DEGs by Venn diagram analysis. DEGs were verified in the TCGA-LUAD database (535 Lung adenocarcinoma tissues and 59 Lung cancer tissues), further confirming the differential expression of these 380 genes in lung adenocarcinoma and normal lung tissues (Figure 1A-D).



DOI: 10.5306/wjco.v14.i1.27 **Copyright** ©The Author(s) 2023.

Figure 1 Screening of differential genes and establishing LASSO regression. A: Volcano map of the differential genes in the GSE43458; B: Volcano map of the differential genes in the GSE32863; C: Volcano map of the differential genes in the GSE27262; D: Venn diagram of the three Gene Expression Omnibus datasets; E: LASSO coefficients profiles of 380 common differential genes; F: LASSO regression with ten- fold cross-validation constructed the models.

Screening of prognostic differential genes and establishment of prognostic models

Univariate Cox regression analysis was performed on 380 DEGs in the training set. A total of 30 DEGs were related to the survival of patients with lung adenocarcinoma ($P < 0.05$) and further screened by LASSO regression (Figure 1E). Cross-validation was used to establish the model, as shown in Figure 1F. A total of 5 mRNAs (TCN1, CENPF, MAOB, CRTAC1, and PLEK2) were included in the model. Multivariate Cox regression analysis was performed for the above 5 mRNAs, and the risk scoring equation was established according to the corresponding regression coefficient. Risk score (RS) =

(0.00288* TCN1 EXP) + (0.0387* CENPF EXP) + (-0.0291* MAOB EXP) + (-0.0198 *CRTAC1 EXP) + (0.0214* PLEK2 EXP).

Verification of mRNA expression and genetic changes associated with 5 prognostic genes

Among the 566 patients included in the cBioPortal for Lung Adenocarcinoma (TCGA, PanCancer Atlas) database, 93 patients (16.4%) showed genetic changes in these 5 genes, among which missense mutations were the most common mutation type (Figure 2A). In the TCGA LUAD cohort, the mRNA expression levels of TCN1, CENPF, and PLEK2 were significantly increased in lung adenocarcinoma tissues compared with those in normal lung tissues, while MAOB and CRTAC1 were significantly decreased in lung adenocarcinoma tissues (Figure 2B). A human protein mapping database (http://www.proteinatlas.org) was used to explore the protein expression level. Immunohistochemical (IHC) results of four genes (TCN1 was not included in the database) in lung cancer and normal lung gland tissues are shown in Figure 2C. Consistent with the mRNA results, IHC results showed that CENPF and PLEK2 had significantly higher mean expression levels in lung adenocarcinoma tissue than in normal lung tissue. In contrast, the CRTAC1 expression level was higher in normal lung tissue than in lung adenocarcinoma tissue. MAOB showed no difference between normal and lung adenocarcinoma tissues (Figure 2C).

Evaluation of five-mRNA prognostic model

Each patient's risk score in the training group was calculated based on the above risk score function. The "SurvMiner" R software package was used to obtain the median critical point, and the patients were divided into a high-risk group and a low-risk group (Figure 3A). As the RS score increased, the patients' survival time was shortened, and the number of deaths increased significantly (Figure 3B). Figure 3C shows the heatmap of 5 prognostic genes in the high- and low-risk groups. The KM survival curve indicated a lower overall survival in the high-risk group than in the low-risk group ($P < 0.001$) (Figure 3D). To further verify the prognostic assessment model's accuracy, we used the R "survival ROC" package to draw the ROC curve (Figure 3E). The results showed that the AUC values of the risk score model for predicting the overall survival at 1, 3 and 5 years in patients with lung adenocarcinoma were 0.711, 0.668 and 0.728, respectively, indicating that the multigene model had a good predictive ability for the OS of patients with lung adenocarcinoma. Multiple Cox regression analysis showed that RS, along with patient age and stage, could be independent prognostic factors for lung adenocarcinoma patients (Figure 3F).

Internal and external validation of the five-mRNA prognostic signature

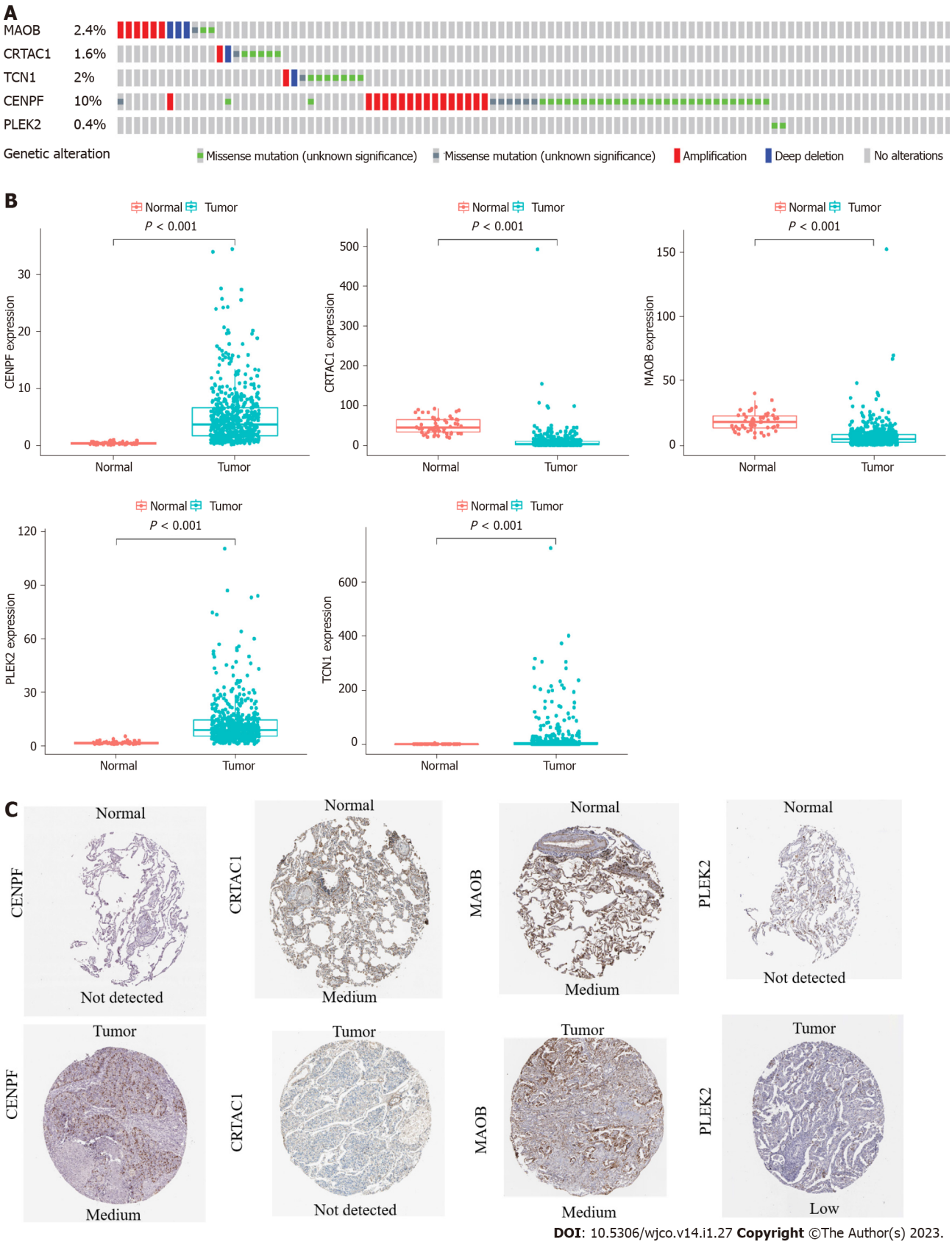
To verify the predictive value of the 5-mRNA prognostic signature, we used the same formula to calculate risk scores for patients with the internal validation set ($n = 160$), entire validation set ($n = 535$), and external validation set (GSE11969, $n = 90$). Consistent with the training group results, the OS of LUAD patients in the high-risk group was significantly lower than that in the low-risk group (Figure 4A-C). The KM survival analysis of the prognostic signature showed that the AUC values of the 1-year, 3-year, and 5-year OS of the internal validation set, the overall validation set and external validation set were 0.754, 0.630, 0.684, and 0.737, 0.701, 0.680, and 0.779, 0.752, 0.715, respectively (Figure 4D-F). Taken together, our results suggest that this 5-gene signature performs well in predicting overall survival in patients with lung adenocarcinoma.

Establish and verify the nomogram

To establish clinically applicable methods for predicting survival in patients with lung adenocarcinoma, we established a nomogram using three independent prognostic factors (including age, stage, and risk score) to predict 1-year, 3-year, and 5-year OS in patients with lung adenocarcinoma (Supplementary Figure 2A). The calibration diagram showed that the nomogram performs well (Supplementary Figure 2B). The AUC values of the 1-year, 2-year, and 3-year overall survival predictions of the nomograph were 0.760, 0.712, and 0.709, respectively (Supplementary Figure 3A). The KM chart effectively distinguishes the various risks of these categories, with people with higher scores having significantly poorer overall survival ($P < 0.001$) (Supplementary Figure 3B). The C-index (95%CI) of the age, stage, and risk score and combination models was 0.501 (0.480-0.522), 0.684 (0.662-0.076), 0.625 (0.604-0.646), and 0.726 (0.702-0.750), respectively (Supplementary Table 1). Thus, the nomogram performs well in predicting overall survival in patients with lung adenocarcinoma, which may be useful for patient counseling and clinical decision-making.

Biological pathways of the five prognostic model genes were identified

GSEA was performed to identify the potential biological processes of the 5 prognostic genes and showed that the samples with highly expressed TCN1, CENPF, and PLEK2 were enriched with focal adhesion, the p53 signaling pathway, and Toll-like sensors, respectively. MAOB and CRTAC1 samples were mediated in the cell cycle and ubiquitin-mediated proteolysis (Supplementary Figure 4), respectively.

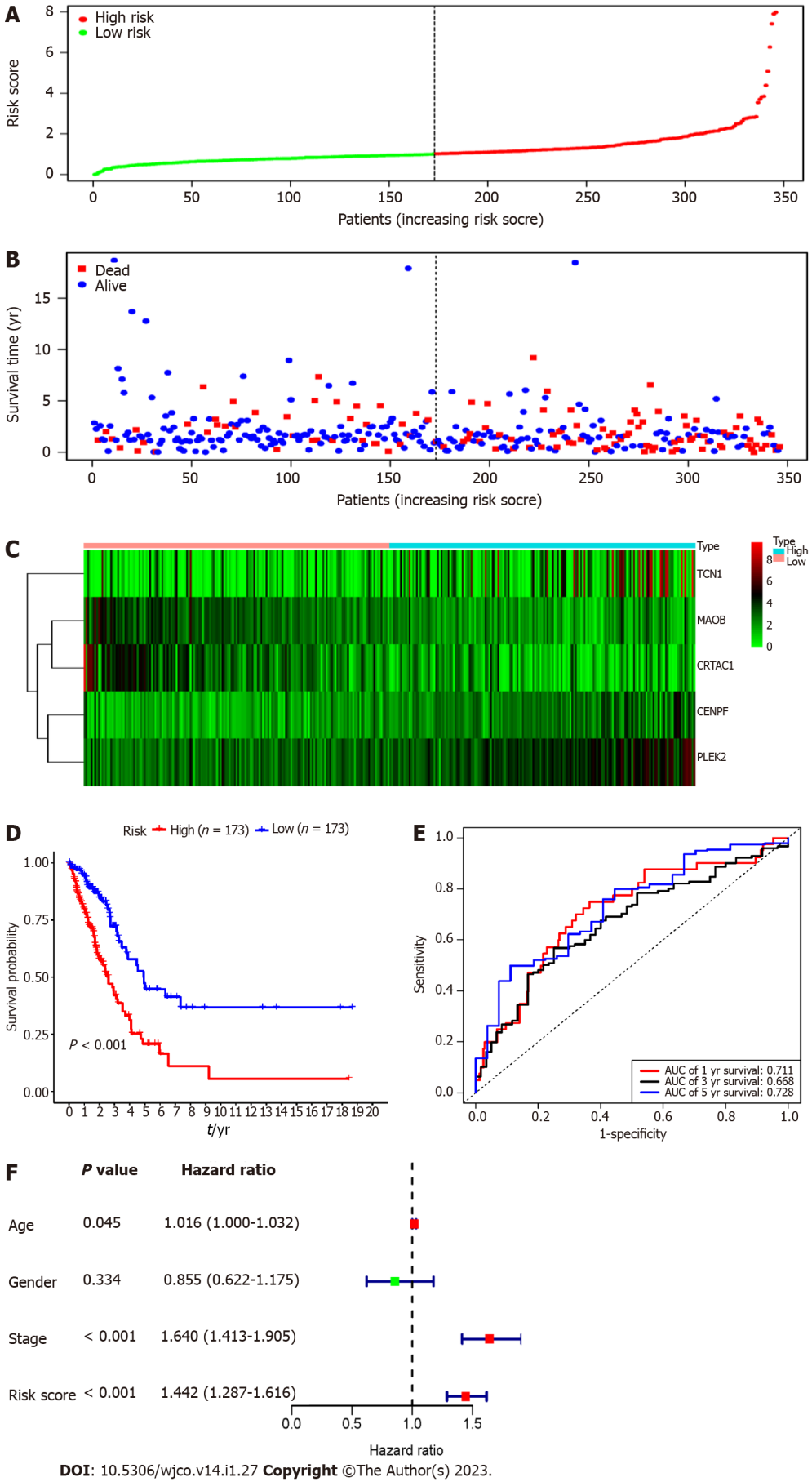


DOI: 10.5306/wjco.v14.i1.27 **Copyright** ©The Author(s) 2023.

Figure 2 The expression and genetic alterations of the 5 prognostic genes in Lung adenocarcinoma. A: Genetic alterations rate of 5 model genes; B: Differential expression of the mRNA levels in lung adenocarcinoma tissues; C: Differential expression at the protein levels of the five model genes.

DISCUSSION

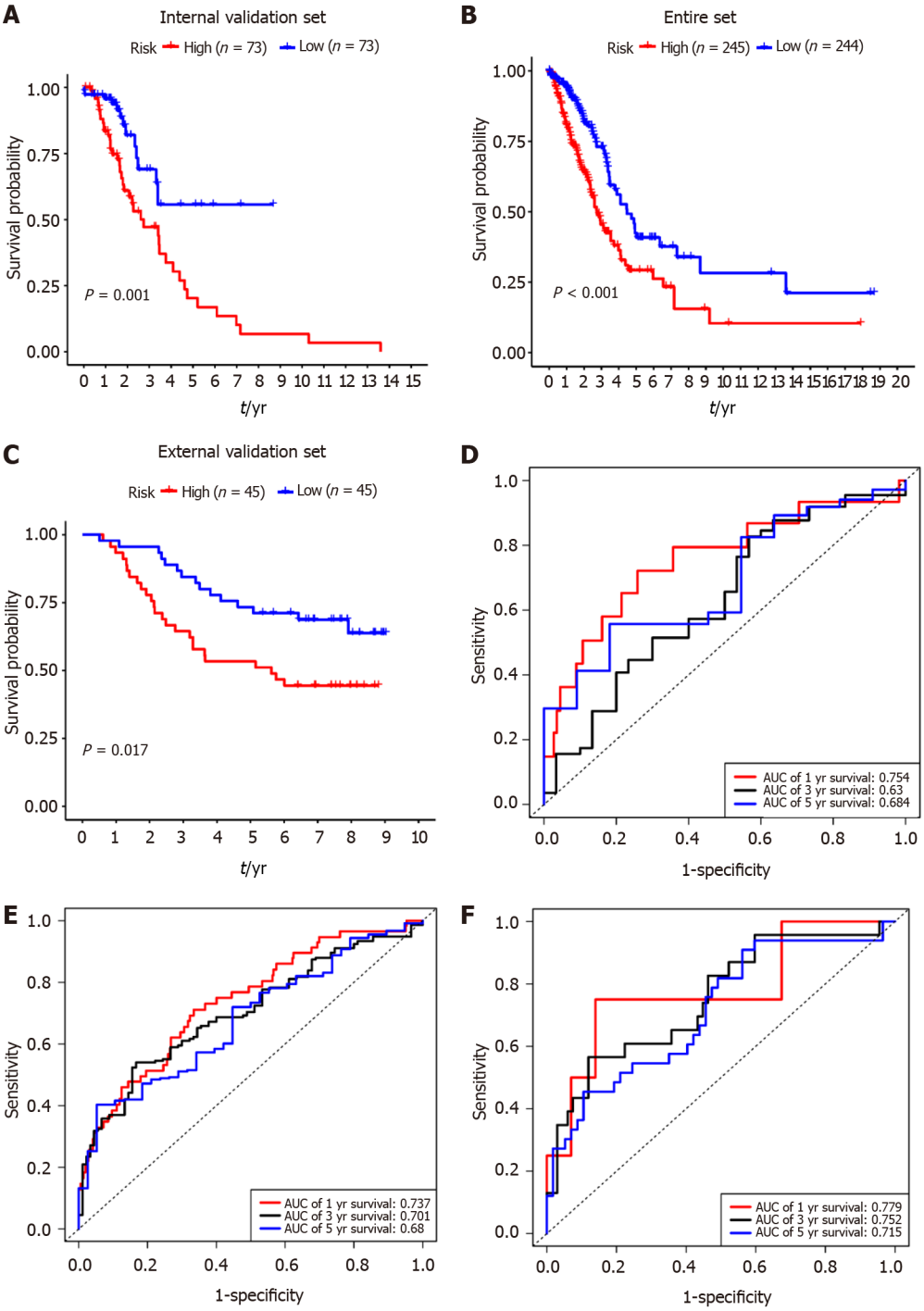
Recently, the tumor prognosis model based on the abnormal gene mRNAs has shown great potential due to its high prediction accuracy. Traditional clinicopathological parameters, such as tumor stage,



DOI: 10.5306/wjco.v14.i1.27 **Copyright** ©The Author(s) 2023.

Figure 3 Risk score model, time-dependent receiver operating characteristic analysis, and survival analysis for the 5-gene signature in

Lung adenocarcinoma. A: The risk score curve divided the patients into the high-risk and low-risk groups; B: Distribution map of the patient's survival status; C: Heatmap of model genes in high and low risk groups; D: Kaplan-Meier survival analysis of the 5-mRNA-based prognostic signature; E: Receiver operating characteristic curves to evaluate the prognostic signature; F: Multiple cox regression analysis of the risk scores and clinical parameters.



DOI: 10.5306/wjco.v14.i1.27 **Copyright** ©The Author(s) 2023.

Figure 4 Internal and external validation of the 5-gene prognostic signature. A: Internal validation by survival analysis; B: The entire dataset validation by survival analysis; C: GSE11969-based external validation by survival analysis; D: Internal validation by receiver operating characteristic (ROC) curves; E: The entire dataset validation by ROC curves; F: GSE11969-based external validation by ROC curves.

have been used to reflect and predict disease progression. However, a single clinical parameter has poor predictive ability for prognosis[3,6,7,10]. In this study, we identified 380 reliable lung adenocarcinoma differential genes by comprehensive analysis of three GEO datasets combined with data from the TCGA-LUAD. Univariate, LASSO and multivariate Cox analyses of DEGs were performed to establish a

prognostic risk model for lung adenocarcinoma based on 5 mRNAs (TCN1, CENPF, MAOB, CRTAC1, and PLEK2). These five new genes were significantly correlated with the prognosis of LUAD patients. MAOB and CRTAC1 were negative prognostic genes, while TCN1, CENPF and PLEK2 were positive prognostic genes. Recently, several studies have revealed the important role of these five genes in cancer progression. Monoamine oxidase B (MAOB) is an enzyme located on the outer mitochondrial membrane. It is responsible for catalyzing monoamine oxidation to produce hydrogen peroxide and is mainly involved in the metabolism of neurotransmitters[19]. The relationship between MAOB and tumors is less reported. It has been reported that MAOB mRNA is significantly lower in the saliva of oral squamous cell carcinoma patients than in that of healthy controls[20]. Xu *et al*[21] found that MAOB is a key DNA methylation driver gene for prostate cancer and plays an important role in the DNA methylation of prostate cancer patients through a comprehensive analysis of the TCGA methylation data. There is no previous report about MAOB in lung adenocarcinoma. CRTAC1 encodes human chondrogenic acid protein 1, which can be used as a marker of chondrocytes to distinguish human chondrocytes from osteoblasts and mesenchymal stem cells in cultures[22]. Currently, this gene is rarely reported in tumors. TCN1 is a member of the vitamin B12-binding protein family and is a 60-70 kDa molecular weight protein. High levels of TCN1 are primarily related to abnormal granulocyte proliferation. TCN1 is overexpressed in a variety of malignancies, such as pancreas, breast, and colon cancer, and is associated with tumor progression and metastasis[23-25]. TCN1 was significantly associated with advanced colorectal cancer[24] and laryngeal cancer[26]. Centromere protein F (CENPF), as an important member of the centromere protein family, is a component of the centromere complex and plays an important regulatory role in mitosis[27]. CENPF expression is abnormally increased in a variety of malignant tumors and is associated with the prognosis of patients[28,29]. Using bioinformatics and immunohistochemical analysis, CENPF overexpression was associated with poor prognosis of breast cancer and tumor bone metastasis[30]. Through comprehensive analysis of three GEO databases, CENPF was identified as a key gene with prognostic value in lung adenocarcinoma, which was consistent with our research results[31]. Pleckstrin-2 (PLEK2) is a 353 amino acid protein encoded by the PLEK2 gene in the human genome and is widely expressed in various tissues. Its overexpression contributes to the formation of large apolipoproteins, thereby promoting cell proliferation[32]. PLEK2 has been found to be related to the invasion and metastasis of multiple tumors. In gallbladder cancer (GBC), PLEK2 overexpression enhances the epithelial-mesenchymal transformation (EMT) process in GBC cells, leading to subsequent higher rates of cell migration, invasion, and liver metastasis[33]. The overexpression of PLEK2 also significantly promoted the EMT and migration of non-small cell lung cancer and destroyed the vascular endothelial barrier[34]. After identifying the five prognostic gene markers, we also conducted internal and external validation to confirm their predictive value and revealed that the prognostic signatures had good prognostic diagnostic value. To improve the prognostic predictive power of the five prognostic gene markers, a predictive nomogram combining risk scores and conventional clinical prognostic parameters (including age and tumor stage) was constructed to enable clinicians to determine the prognosis of each patient. Its graphical scoring system is easy to understand and helps customize treatment and medical decisions. The prognostic models and nomograms associated with five-gene characteristics have not been reported. Hence, our study may be useful prognostic and diagnostic classification tools for lung adenocarcinoma. Our study still has some limitations. First, the study only focuses on transcriptome sequencing data. If other omics techniques, such as DNA methylation and single nucleotide polymorphisms, can be analyzed together, more favorable results may be obtained. Second, our research is limited to the bioinformatics analysis of the TCGA and GEO databases. Although we have verified the accuracy of the models internally and externally, the verification of large samples in the clinical diagnosis and treatment process will further enhance their diagnostic accuracy and clinical value.

CONCLUSION

In summary, our study identified a 5-gene model and prognostic nomogram that combined gene models and clinical prognostic factors to predict the overall survival rate of lung adenocarcinoma patients, and this nomogram may be of great significance for the selection of personalized treatment options and clinical medical decisions in patients with lung adenocarcinoma.

ARTICLE HIGHLIGHTS

Research background

Lung adenocarcinoma patients with localized or locally advanced disease have a high risk of death, and their 5-year overall survival rate is less than 15%.

Research motivation

To evaluate the prognosis of Lung adenocarcinoma (LUAD) patients and optimize treatment, effective clinical research prediction models.

Research objectives

To identify reliable prognostic biomarkers to predict clinical outcomes and to help clinicians to make accurate clinical treatment decisions.

Research methods

The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) were used to screen for differential genes for lung adenocarcinoma. Univariate regression analysis combined with LASSO regression analysis was used to screen for prognostic-related genes. Multivariate Cox regression analysis was applied to establish the risk score equation and construct the survival prognosis model.

Research results

We establish a prognostic risk model for lung adenocarcinoma based on 5 mRNAs (TCN1, CENPF, MAOB, CRTAC1, and PLEK2). These five new genes were significantly correlated with the prognosis of LUAD patients. To improve the prognostic predictive power of the five prognostic gene markers, a predictive nomogram combining risk scores and conventional clinical prognostic parameters (including age and tumor stage) was constructed to enable clinicians to determine the prognosis of each patient.

Research conclusions

A 5-mRNA-based model was constructed to predict the prognosis of lung adenocarcinoma, which may provide clinicians with reliable prognostic assessment tools and help clinical treatment decisions.

Research perspectives

Our study identified a 5-gene model and constructed a nomogram which may have important implications for clinical medical decision and personalized treatment of patients with lung adenocarcinoma.

FOOTNOTES

Author contributions: Wang J, Du YZ, and Xia QL conceived and designed the experiments; Xia QL, He XM and Ma Y analyzed the data; Li QY contributed to analysis tools; Xia QL wrote the manuscript; Wang J and Xia QL revised the manuscript; all authors have read and approved the final manuscript.

Supported by the Science and Technology Development Fund of the Pudong New Area, No. PKJ2021-Y53; and the National Natural Science Foundation of China, No. 81974315.

Institutional review board statement: The study was reviewed and approved by the Shanghai Public Health Clinical Center Laboratory Animal Welfare & Ethics Committee Institutional Review Board [(Approval No. 2020-A006-01]).

Conflict-of-interest statement: All the authors declare no competing financial interests.

Data sharing statement: The mRNA expression and clinical data of lung adenocarcinoma analyzed during the current study are available on the GEO (https://www.ncbi.nlm.nih.gov/geo/) and TCGA databases (https://www.cbioportal.org/). The protein expression of model-related genes of LUAD analyzed in this study is also available on the THPA database (http://www.proteinatlas.org).

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Yu-Zhen Du 0000-0002-3395-4471; Jin Wang 0000-0002-0062-2489.

S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

REFERENCES

1 **Meng W**, Ye Z, Cui R, Perry J, Dedousi-Huebner V, Huebner A, Wang Y, Li B, Volinia S, Nakanishi H, Kim T, Suh SS, Ayers LW, Ross P, Croce CM, Chakravarti A, Jin VX, Lautenschlaeger T. MicroRNA-31 predicts the presence of lymph node metastases and survival in patients with lung adenocarcinoma. *Clin Cancer Res* 2013; **19**: 5423-5433 [PMID: 23946296 DOI: 10.1158/1078-0432.CCR-13-0320]

2 **Drosten M**, Barbacid M. Modeling K-Ras-driven lung adenocarcinoma in mice: preclinical validation of therapeutic targets. *J Mol Med (Berl)* 2016; **94**: 121-135 [PMID: 26526121 DOI: 10.1007/s00109-015-1360-5]

3 **Li S**, Xuan Y, Gao B, Sun X, Miao S, Lu T, Wang Y, Jiao W. Identification of an eight-gene prognostic signature for lung adenocarcinoma. *Cancer Manag Res* 2018; **10**: 3383-3392 [PMID: 30237740 DOI: 10.2147/CMAR.S173941]

4 **Zuo S**, Wei M, Zhang H, Chen A, Wu J, Wei J, Dong J. A robust six-gene prognostic signature for prediction of both disease-free and overall survival in non-small cell lung cancer. *J Transl Med* 2019; **17**: 152 [PMID: 31088477 DOI: 10.1186/s12967-019-1899-y]

5 **He R**, Zuo S. A Robust 8-Gene Prognostic Signature for Early-Stage Non-small Cell Lung Cancer. *Front Oncol* 2019; **9**: 693 [PMID: 31417870 DOI: 10.3389/fonc.2019.00693]

6 **Shukla S**, Evans JR, Malik R, Feng FY, Dhanasekaran SM, Cao X, Chen G, Beer DG, Jiang H, Chinnaiyan AM. Development of a RNA-Seq Based Prognostic Signature in Lung Adenocarcinoma. *J Natl Cancer Inst* 2017; **109** [PMID: 27707839 DOI: 10.1093/jnci/djw200]

7 **Yue C**, Ma H, Zhou Y. Identification of prognostic gene signature associated with microenvironment of lung adenocarcinoma. *PeerJ* 2019; **7**: e8128 [PMID: 31803536 DOI: 10.7717/peerj.8128]

8 **Huang P**, Cheng CL, Chang YH, Liu CH, Hsu YC, Chen JS, Chang GC, Ho BC, Su KY, Chen HY, Yu SL. Molecular gene signature and prognosis of non-small cell lung cancer. *Oncotarget* 2016; **7**: 51898-51907 [PMID: 27437769 DOI: 10.18632/oncotarget.10622]

9 **Shahid M**, Choi TG, Nguyen MN, Matondo A, Jo YH, Yoo JY, Nguyen NN, Yun HR, Kim J, Akter S, Kang I, Ha J, Maeng CH, Kim SY, Lee JS, Kim SS. An 8-gene signature for prediction of prognosis and chemoresponse in non-small cell lung cancer. *Oncotarget* 2016; **7**: 86561-86572 [PMID: 27863408 DOI: 10.18632/oncotarget.13357]

10 **Krzystanek M**, Moldvay J, Szüts D, Szallasi Z, Eklund AC. A robust prognostic gene expression signature for early stage lung adenocarcinoma. *Biomark Res* 2016; **4**: 4 [PMID: 26900477 DOI: 10.1186/s40364-016-0058-3]

11 **Zengin T**, Önal-Süzek T. Analysis of genomic and transcriptomic variations as prognostic signature for lung adenocarcinoma. BMC bioinformatics 2020, 21(Suppl 14):368[DOI: 10.1186/s12859-020-03691-3].

12 **Li YY**, Yang C, Zhou P, Zhang S, Yao Y, Li D. Genome-scale analysis to identify prognostic markers and predict the survival of lung adenocarcinoma. *J Cell Biochem* 2018; **119**: 8909-8921 [PMID: 30105759 DOI: 10.1002/jcb.27144]

13 **Xie H**, Xie C. A Six-Gene Signature Predicts Survival of Adenocarcinoma Type of Non-Small-Cell Lung Cancer Patients: A Comprehensive Study Based on Integrated Analysis and Weighted Gene Coexpression Network. *Biomed Res Int* 2019; **2019**: 4250613 [PMID: 31886214 DOI: 10.1155/2019/4250613]

14 **Zhang MY**, Liu XX, Li H, Li R, Liu X, Qu YQ. Elevated mRNA Levels of AURKA, CDC20 and TPX2 are associated with poor prognosis of smoking related lung adenocarcinoma using bioinformatics analysis. *Int J Med Sci* 2018; **15**: 1676-1685 [PMID: 30588191 DOI: 10.7150/ijms.28728]

15 **Xia Q**, Li Z, Zheng J, Zhang X, Di Y, Ding J, Yu D, Yan L, Shen L, Yan D, Jia N, Chen W, Feng Y, Wang J. Identification of novel biomarkers for hepatocellular carcinoma using transcriptome analysis. *J Cell Physiol* 2019; **234**: 4851-4863 [PMID: 30272824 DOI: 10.1002/jcp.27283]

16 **Thul PJ**, Lindskog C. The human protein atlas: A spatial map of the human proteome. *Protein Sci* 2018; **27**: 233-244 [PMID: 28940711 DOI: 10.1002/pro.3307]

17 **Huang JT**, Wang J, Srivastava V, Sen S, Liu SM. MicroRNA Machinery Genes as Novel Biomarkers for Cancer. *Front Oncol* 2014; **4**: 113 [PMID: 24904827 DOI: 10.3389/fonc.2014.00113]

18 **Li Z**, Chen Z, Hu G, Zhang Y, Feng Y, Jiang Y, Wang J. Profiling and integrated analysis of differentially expressed circRNAs as novel biomarkers for breast cancer. *J Cell Physiol* 2020; **235**: 7945-7959 [PMID: 31943203 DOI: 10.1002/jcp.29449]

19 **Edmondson DE**. Hydrogen peroxide produced by mitochondrial monoamine oxidase catalysis: biological implications. *Curr Pharm Des* 2014; **20**: 155-160 [PMID: 23701542 DOI: 10.2174/13816128113190990406]

20 **Oh SY**, Kang SM, Kang SH, Lee HJ, Kwon TG, Kim JW, Lee ST, Choi SY, Hong SH. Potential Salivary mRNA Biomarkers for Early Detection of Oral Cancer. *J Clin Med* 2020; **9** [PMID: 31963366 DOI: 10.3390/jcm9010243]

21 **Xu N**, Wu YP, Ke ZB, Liang YC, Cai H, Su WT, Tao X, Chen SH, Zheng QS, Wei Y, Xue XY. Identification of key DNA methylation-driven genes in prostate adenocarcinoma: an integrative analysis of TCGA methylation data. *J Transl Med* 2019; **17**: 311 [PMID: 31533842 DOI: 10.1186/s12967-019-2065-2]

22 **Steck E**, Bräun J, Pelttari K, Kadel S, Kalbacher H, Richter W. Chondrocyte secreted CRTAC1: a glycosylated extracellular matrix molecule of human articular cartilage. *Matrix Biol* 2007; **26**: 30-41 [PMID: 17074475 DOI: 10.1016/j.matbio.2006.09.006]

23 **Chong LY**, Cheok PY, Tan WJ, Thike AA, Allen G, Ang MK, Ooi AS, Tan P, Teh BT, Tan PH. Keratin 15, transcobalamin I and homeobox gene Hox-B13 expression in breast phyllodes tumors: novel markers in biological classification. *Breast Cancer Res Treat* 2012; **132**: 143-151 [PMID: 21574054 DOI: 10.1007/s10549-011-1555-6]

24 **Chu CM**, Yao CT, Chang YT, Chou HL, Chou YC, Chen KH, Terng HJ, Huang CS, Lee CC, Su SL, Liu YC, Lin FG, Wetter T, Chang CW. Gene expression profiling of colorectal tumors and normal mucosa by microarrays meta-analysis using prediction analysis of microarray, artificial neural network, classification, and regression trees. *Dis Markers* 2014; **2014**: 634123 [PMID: 24959000 DOI: 10.1155/2014/634123]

25 **Wu Y**, Wei J, Ming Y, Chen Z, Yu J, Mao R, Chen H, Zhou G, Fan Y. Orchestrating a biomarker panel with lncRNAs and mRNAs for predicting survival in pancreatic ductal adenocarcinoma. *J Cell Biochem* 2018; **119**: 7696-7706 [PMID: 29923223 DOI: 10.1002/jcb.27119]

26 **Wang Y**, Yue C, Fang J, Gong L, Lian M, Wang R, Feng L, Ma H, Ma Z, Liu H. Transcobalamin I: a novel prognostic biomarker of neoadjuvant chemotherapy in locally advanced hypopharyngeal squamous cell cancers. *Onco Targets Ther* 2018; **11**: 4253-4261 [PMID: 30100732 DOI: 10.2147/OTT.S166514]

27 **Varis A**, Salmela AL, Kallio MJ. Cenp-F (mitosin) is more than a mitotic marker. *Chromosoma* 2006; **115**: 288-295 [PMID: 16565862 DOI: 10.1007/s00412-005-0046-0]

28 **Brendle A**, Brandt A, Johansson R, Enquist K, Hallmans G, Hemminki K, Lenner P, Försti A. Single nucleotide polymorphisms in chromosomal instability genes and risk and clinical outcome of breast cancer: a Swedish prospective case-control study. *Eur J Cancer* 2009; **45**: 435-442 [PMID: 19008095 DOI: 10.1016/j.ejca.2008.10.001]

29 **Chen WB**, Cheng XB, Ding W, Wang YJ, Chen D, Wang JH, Fei RS. Centromere protein F and survivin are associated with high risk and a poor prognosis in colorectal gastrointestinal stromal tumours. *J Clin Pathol* 2011; **64**: 751-755 [PMID: 21613637 DOI: 10.1136/jcp.2011.089631]

30 **Sun J**, Huang J, Lan J, Zhou K, Gao Y, Song Z, Deng Y, Liu L, Dong Y, Liu X. Overexpression of CENPF correlates with poor prognosis and tumor bone metastasis in breast cancer. *Cancer Cell Int* 2019; **19**: 264 [PMID: 31632198 DOI: 10.1186/s12935-019-0986-8]

31 **Song YJ**, Tan J, Gao XH, Wang LX. Integrated analysis reveals key genes with prognostic value in lung adenocarcinoma. *Cancer Manag Res* 2018; **10**: 6097-6108 [PMID: 30538558 DOI: 10.2147/CMAR.S168636]

32 **Hu MH**, Bauman EM, Roll RL, Yeilding N, Abrams CS. Pleckstrin 2, a widely expressed paralog of pleckstrin involved in actin rearrangement. *J Biol Chem* 1999; **274**: 21515-21518 [PMID: 10419454 DOI: 10.1074/jbc.274.31.21515]

33 **Luo Y**, Robinson S, Fujita J, Siconolfi L, Magidson J, Edwards CK, Wassmann K, Storm K, Norris DA, Bankaitis-Davis D, Robinson WA, Fujita M. Transcriptome profiling of whole blood cells identifies PLEK2 and C1QB in human melanoma. *PLoS One* 2011; **6**: e20971 [PMID: 21698244 DOI: 10.1371/journal.pone.0020971]

34 **Wu DM**, Deng SH, Zhou J, Han R, Liu T, Zhang T, Li J, Chen JP, Xu Y. PLEK2 mediates metastasis and vascular invasion *via* the ubiquitin-dependent degradation of SHIP2 in non-small cell lung cancer. *Int J Cancer* 2020; **146**: 2563-2575 [PMID: 31498891 DOI: 10.1002/ijc.32675]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 February 24; 14(2): 40-98

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 2 February 24, 2023

REVIEW

40 Hereditary cancer syndromes

Imyanitov EN, Kuligina ES, Sokolenko AP, Suspitsin EN, Yanus GA, Iyevleva AG, Ivantsov AO, Aleksakhina SN

69 Significance of music therapy in treating depression and anxiety disorders among people with cancer

Eseadi C, Ngwu MO

MINIREVIEWS

81 Therapeutic challenge for immunotherapy targeting cold colorectal cancer: A narrative review

Ma SX, Li L, Cai H, Guo TK, Zhang LS

89 New trends in the surgical management of soft tissue sarcoma: The role of preoperative biopsy

Pavlidis ET, Pavlidis TE

World Journal of Clinical Oncology

Monthly Volume 14 Number 2 February 24, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Oncology*, Budhi S Yadav, MD, (Radiation Oncology), Professor, Department of Radiotherapy & Oncology, Post Graduate Institute of Medical Education & Research, Chandigarh, 160012, India. drbudhi@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 edition of Journal Citation Reports® cites the 2021 Journal Citation Indicator (JCI) for *WJCO* as 0.35.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Yu-Jie Ma*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
February 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 February 24; 14(2): 40-68

DOI: 10.5306/wjco.v14.i2.40

ISSN 2218-4333 (online)

REVIEW

Hereditary cancer syndromes

Evgeny N Imyanitov, Ekaterina S Kuligina, Anna P Sokolenko, Evgeny N Suspitsin, Grigoriy A Yanus, Aglaya G Iyevleva, Alexandr O Ivantsov, Svetlana N Aleksakhina

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A, A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Stabellini N, United States; Zhang X, China

Received: November 17, 2022
Peer-review started: November 17, 2022
First decision: November 30, 2022
Revised: December 9, 2022
Accepted: February 14, 2023
Article in press: February 14, 2023
Published online: February 24, 2023

Evgeny N Imyanitov, Ekaterina S Kuligina, Anna P Sokolenko, Evgeny N Suspitsin, Grigoriy A Yanus, Aglaya G Iyevleva, Alexandr O Ivantsov, Svetlana N Aleksakhina, Department of Tumor Growth Biology, N.N. Petrov Institute of Oncology, St.-Petersburg 197758, Russia

Evgeny N Imyanitov, Ekaterina S Kuligina, Anna P Sokolenko, Evgeny N Suspitsin, Grigoriy A Yanus, Aglaya G Iyevleva, Alexandr O Ivantsov, Svetlana N Aleksakhina, Department of Clinical Genetics, St.-Petersburg Pediatric Medical University, St.-Petersburg 194100, Russia

Corresponding author: Evgeny N Imyanitov, DSc, Professor, Department of Tumor Growth Biology, N.N. Petrov Institute of Oncology, Pesochny, Leningradskaya Str. 68, St.-Petersburg 197758, Russia. evgeny@imyanitov.spb.ru

Abstract

Hereditary cancer syndromes (HCSs) are arguably the most frequent category of Mendelian genetic diseases, as at least 2% of presumably healthy subjects carry highly-penetrant tumor-predisposing pathogenic variants (PVs). Hereditary breast-ovarian cancer and Lynch syndrome make the highest contribution to cancer morbidity; in addition, there are several dozen less frequent types of familial tumors. The development of the majority albeit not all hereditary malignancies involves two-hit mechanism, *i.e.* the somatic inactivation of the remaining copy of the affected gene. Earlier studies on cancer families suggested nearly fatal penetrance for the majority of HCS genes; however, population-based investigations and especially large-scale next-generation sequencing data sets demonstrate that the presence of some highly-penetrant PVs is often compatible with healthy status. Hereditary cancer research initially focused mainly on cancer detection and prevention. Recent studies identified multiple HCS-specific drug vulnerabilities, which translated into the development of highly efficient therapeutic options.

Key Words: Hereditary cancer syndromes; Germline pathogenic variants; Cancer predisposition; Cancer treatment; Next-generation sequencing

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: There are many reviews describing particular types of hereditary cancer syndromes (HCSs) (*e.g.*, hereditary breast-ovarian cancer, Lynch syndrome, Li-Fraumeni syndrome, *etc.*). However, for the last 15-20 years there were no publications providing a general overview on familial cancers. Our paper describes mechanisms underlying genetic cancer predisposition, lists major types of HCSs, and comments on therapeutic advances in the management of hereditary tumors.

Citation: Imyanitov EN, Kuligina ES, Sokolenko AP, Suspitsin EN, Yanus GA, Iyevleva AG, Ivantsov AO, Aleksakhina SN. Hereditary cancer syndromes. *World J Clin Oncol* 2023; 14(2): 40-68
URL: https://www.wjgnet.com/2218-4333/full/v14/i2/40.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i2.40

INTRODUCTION

Hereditary cancer syndromes (HCSs) are a heterogeneous group of genetic diseases, which are associated with significantly increased risk of tumor development. There is a number of severe inborn disorders characterized by profound multiorgan failures, where cancer susceptibility constitutes only a part of clinical presentation of the disease (*e.g.*, Bloom syndrome, Fanconi anemia, Nijmegen breakage syndrome, ataxia-telangiectasia, *etc.*). Most of these syndromes involve biallelic inactivation of genes involved in DNA repair and are characterized by severe immune deficiency[1,2]. Subjects affected by "genuine" HCSs usually do not have any detectable phenotypic malfunctions, they differ from truly healthy people only by a highly elevated propensity to develop malignant disease in certain organs.

Hereditary cancers apparently represent the most common category of vertically transmitted disorders. Indeed, while the occurrence of the best known genetic diseases, *e.g.*, cystic fibrosis or phenylketonuria, usually falls below 1:10000, the population frequency of *BRCA1/2*-associated hereditary breast-ovarian cancer (HBOC) or *MLH1/MSH2*-linked Lynch syndrome is about 25-30 times higher and approaches approximately 1:300–1:400[3-6]. Collectively, at least 2% of presumably healthy subjects carry germline PVs associated with highly increased and often a nearly-fatal risk of a certain cancer type, and these estimates can be significantly higher in populations with pronounced founder effect[5,7].

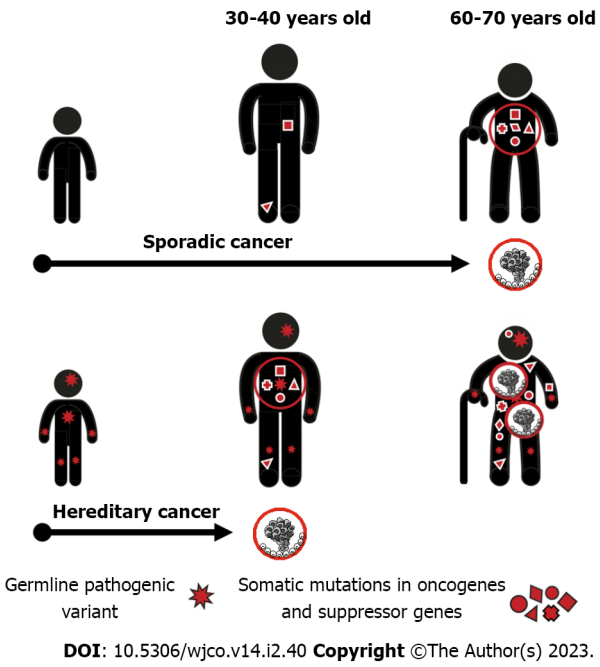
Earlier studies on HCSs usually assumed that almost all carriers of pathogenic alleles are destined to develop cancer, *i.e.* they considered mainly families and genes with almost 100% disease penetrance. The development of genetic technologies and the availability of large collections of cancer patients and healthy subjects resulted in the discovery of genes, whose alteration is associated with less pronounced but still medically relevant (2-3-fold) increase of cancer risk. These moderately penetrant alleles rarely cause familial clustering of malignancies and present a challenge for defining disease-preventive strategies. Furthermore, unbiased case-control studies revealed that earlier family-based HCS investigations overestimated disease risks for the majority of cancer genes; in fact, seemingly none of the well-established HCS genes has a complete penetrance, with the most of estimates falling within 40%–80% probability of tumor development for germline pathogenic variant (PV) carriers[4-6,8,9].

Virtually all HCSs are more or less organ-specific, *i.e.* they mainly manifest by cancers arising in particular anatomic sites or tissues. However, the development of hereditary cancer registries and large data sets led to the understanding that many HCSs are associated with a wider spectrum of cancers than was initially suggested, although most of the newly added tumor types are characterized only by a marginal increase of their lifetime risk. For example, *BRCA1* and *BRCA2* were discovered as breast-ovarian cancer genes. Recent data indicate that carriers of *BRCA1/2* PVs may have a borderline elevation of the probability of development for almost all major cancer types[10-16].

MECHANISMS OF HEREDITARY CANCER PREDISPOSITION

The acquisition of a single mutation in oncogene or suppressor gene is usually fully tolerable for a human cell due to the existence of multiple cancer-protecting biological mechanisms. The process of malignant transformation ultimately requires accumulation of several cancer-driving events in the same cell clone. Consequently, when a single cancer-associated PV is inherited from the parents, its carrier remains phenotypically healthy despite the presence of the pathogenic allele in every cell of the body. However, the number of additional events necessary for cancer manifestation decreases by one, therefore the probability of tumor development in this subject is manifold higher as compared to general population (Figure 1).

The majority of known HCS genes are suppressor genes, which require biallelic inactivation to exert their action. When inactivating PV in a single allele is inherited, the remaining copy of the gene retains its function and the normal health status is preserved. The process of malignant transformation is



DOI: 10.5306/wjco.v14.i2.40 **Copyright** ©The Author(s) 2023.

Figure 1 Mechanisms of hereditary cancer predisposition. Single cancer-driving mutation is usually fully compensated, therefore carriers of germline pathogenic variants may remain healthy during a prolonged period of time. However, since every cell in the target organ already contains one alteration in cancer gene, the probability of accumulation of a critical mass of additional oncogenic mutations in any given cell clone is high, and cancer manifestation often occurs at a relatively young age.

usually triggered by the "second hit", *i.e.* by a somatic inactivation of the remaining allele occurring in any cell located within the target organ. This mechanism is highly characteristic for the best known HCS genes, *e.g.*, *RB1*, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *etc*[4,17-19]. There are examples of mutated suppressor genes, which contribute to the development of hereditary cancers without mandatory inactivation of the remaining gene copy. It is suggested that the reduced gene dosage, so-called haploinsufficiency, is a primary cause of malignant transformation in these situations. Interestingly, some genes, *e.g.*, *PALB2* and *CHEK2*, may utilize both mechanisms: Indeed, instances of both monoallelic and biallelic inactivation of these genes in human tumors have been described in the literature, and there are clear biological differences between carcinomas associated with haploinsufficiency *vs* second-hit loss-of-function of the above genes[20,21].

A few human cancers are caused by the inheritance of activated oncogene. The best known example is the syndrome of multiple endocrine neoplasia (MEN) type 2A and 2B (now sometimes classified as MEN2 and MEN3, respectively), which is associated with gain-of-function PVs in *RET* receptor tyrosine kinase[22].

HCSs have a Mendelian mode of inheritance. Most of currently described hereditary cancers are transmitted by autosomal-dominant mechanisms. Recessive inheritance of cancer predisposition is more difficult to study, especially for common tumor types, therefore only a few examples of biallelic cancer-predisposing gene defects have been identified so far[23,24]. There are also reports describing instances of oligogenic inheritance, *i.e.* the combination of genetic variants resulting in significant increase of cancer risks[25-28].

Hereditary cancers usually have peculiar phenotypic characteristics attributed to their mechanisms of development[29]. Most of HCSs arising in adults manifest after the peak of reproductive activity, so cancer predisposition is transmitted through generations virtually without negative selection and HCS patients often describe multiple instances of the same disease in their relatives. Presence of the first cancer-predisposing mutation in every cell of the human organism ensures highly increased risk of cancer disease as long as target organs or their parts remain in the body. Consequently, HCSs often manifest by multiple primary malignancies[30]. Furthermore, given that the cancer development in PV carriers requires less additional somatic events as compared to genetically healthy subjects, hereditary cancers commonly demonstrate younger age at onset. The development of HCS usually involves gene-specific pathways, therefore these cancers are often distinguished by predetermined molecular portrait and histological appearance. For example, *BRCA1*-associated breast carcinomas are usually triple-negative, chromosomally unstable and carry somatic mutation in the *TP53* suppressor gene[31-34]. All these features, *i.e.*, family cancer history, presence of multiple primary tumors, young age at onset, and especial phenotypic characteristics, represent well-recognized clinical signs of HCSs[29].

MAJOR TYPES OF HCSs

Breast and ovarian carcinomas

It is difficult to discuss hereditary breast cancer (BC) and hereditary ovarian cancer (OC) as two separate disease entities, because the best known and the most frequent genetic causes for these diseases are represented by PVs located within the same genes, *BRCA1* and *BRCA2* (Figure 2). Nevertheless, there are essential differences between BC and OC, which may critically affect genetic investigations of these diseases. The lifetime risk for BC in Western countries is around 1:8, therefore about 1 out of 60-70 mother-daughter or sister-sister pairs would share this disease just by chance[35,36]. OC is significantly less common, with population occurrence approaching close to 1:60–1:70; therefore, the probability of "random" co-occurrence of OC in two female first-degree relatives is very low, falling within 1:3500–1:5000[36,37]. Furthermore, while two-thirds of OC cases belong to its major histological entity, *i.e.* high-grade serous ovarian carcinoma, breast carcinomas are characterized by significant biological diversity manifested by differences in their receptor status and other essential tumor features[38,39]. It appears that hereditary BC research has more confounding factors as compared to the analysis of OC familial clustering.

The causes of HBOC syndrome are considerably better understood than the genetic basis of hereditary BC alone. There are two major contributors to BC and OC predisposition, *BRCA1* and *BRCA2* (Table 1). Both these genes are involved in double-strand DNA repair by homologous recombination. *BRCA2*-associated cancers tend to have older age at onset as compared to *BRCA1*-driven malignancies. PVs in both *BRCA1* and *BRCA2* genes confer approximately 70% lifetime risk for BC; the cumulative risk for OC is estimated to be 44% and 17% for *BRCA1* and *BRCA2* genes, respectively[40]. Importantly, these collective calculations may somehow be misleading, because some PVs located within these genes predispose preferentially to BC, while others are associated with more pronounced OC risk; in fact, there are so-called BC and OC cluster regions located within these genes[41]. There are multiple genetic and non-genetic factors, which modify the risk of cancer disease in *BRCA1/2* PV carriers[42]. *BRCA1/2* make significant contribution to cancer morbidity: These PVs are observed in approximately 2%–5% of BC patients and up to 25%–30% of women diagnosed with high-grade serous OC[5,6,43-46]. In addition to *BRCA1* and *BRCA2*, some *RAD51* paralogs, namely *RAD51C* and *RAD51D*, predispose both to BC and OC[5,47,48]. Recent data also suggest the involvement of *RAD51B* germline PVs in breast- OC susceptibility[49]. The occurrence of PVs in newly described HBOC genes is an order of magnitude lower as compared to *BRCA1/2*[5,47].

PALB2 is the third most important BC-predisposing gene after *BRCA1* and *BRCA2*[50]. Its penetrance towards BC is similar to *BRCA2*, while the data regarding the role of *PALB2* PVs in OC predisposition are conflicting[47,51]. There are two middle-penetrance genes, *ATM* and *CHEK2*, which are associated with 2-3-fold elevation of the risk of BC development but are unlikely to contribute to increased OC susceptibility[47]. Moderate BC predisposing roles were also suggested for *NBN* (*NBS1*), *BLM*, *RECQL*, *FANCM*, *BARD1* and several other genes, but, contrary to the evidence obtained for *ATM* and *CHEK2*, these observations have not been uniformly reproduced across distinct data sets[5,6,47,52-54]. *BRIP1* is the only known gene, which is associated with hereditary OC but not with hereditary BC[47]. There are no mechanistic explanations, why some genes predispose to BC, others to OC, and a few to both BC and OC.

Many "novel" BC/OC-predisposing loci were discovered by candidate gene approach, where genes with similar to *BRCA1/2* functions, *i.e.*, the participants of DNA repair pathways, were selected for DNA testing in case-control studies. These functional considerations also influenced the interpretation of whole-exome studies, *i.e.*, the priority was given to genes involved in the maintenance of cellular genome[55,56]. Overall, exome sequencing studies largely failed to reveal novel BC predisposing genes whose contribution to BC morbidity is comparable with the impact of *BRCA1/2, PALB2* or *CHEK2* germline PVs[53,57,58].

BC may arise as a part of multiorgan cancer syndrome. Germline *TP53* PVs predispose to Li-Fraumeni syndrome, which is manifested by a wide spectrum of tumors. *TP53* PVs are particularly common in very young patients with BC[59]. Recent large-scale next-generation sequencing (NGS) studies suggest that mutated *TP53* can be found in non-selected BC patients, which do not have personal or family history of non-breast tumors[60-63]. A rare BC subtype, lobular BC, is associated with *CDH1* germline PVs predisposing to diffuse stomach cancer[47,64].

There are convincing data indicating that patients with Lynch syndrome, *i.e.*, hereditary predisposition to colorectal and endometrial cancer, develop OC more often than in general population[46,65-69]. Unlike *BRCA1/2*-driven tumors, Lynch syndrome associated OCs often have non-serous histology [68]. Several other multiorgan cancer syndromes also render marginally increased OC risk[46,70].

Exome sequencing studies of OC families identified several promising OC-predisposing candidates, *e.g.*, *ANKRD11* and *POLE* genes[71]. Some data indicate that protein-truncating germline PVs in the *ERCC3* gene may confer increased OC risk[72]. Validation of these findings is complicated due to rarity of *BRCA1/2*-independent familial OC clustering.

Small cell carcinomas of the ovary, hypercalcemic type (SCCOHTs) constitute a rare variety of OC. SCCOHTs are associated with germline PV in the *SMARCA4* gene, which plays a role in chromatin remodeling[70].

Table 1 Health impact of major hereditary cancer genes: Frequency of pathogenic variants in non-selected subjects and oncological patients

Gene	Frequency of pathogenic variants in population	Contribution in cancer morbidity	Ref.
BRCA1	Approximately 0.1%; > 1% in some founder populations	Breast cancer: 1%-3%; High-grade serous ovarian cancer: 15%-30%	[5,6,45,230-233]
BRCA2	Approximately 0.3%; > 1% in some founder populations	Breast cancer: 1%-3%; High-grade serous ovarian cancer: 7%-12%; Prostate cancer: 2%-4%; Pancreatic cancer: 2%-3%	[5,6,45,99,102,112,232,233]
PALB2	Approximately 0.1%	Breast cancer: Approximately 0.5%-1%	[5,6,45]
CHEK2	0.5%-0.7%	Breast cancer: 0.5%-2%; Moderately elevated frequencies across several cancer types	[5,6,25,113,234,235]
ATM	0.3%-0.5%	Breast cancer: 0.5%-0.8%; Moderately elevated frequencies across several cancer types	[5,6,45,99,102,113]
MLH1, MSH2, MSH6, PMS2, EPCAM	0.02%-0.05% for *MLH1, MSH2, MSH6, EPCAM* each; approximately 0.1% for *PMS2*	Colorectal cancer: 1%-6%; Endometrial cancer: 2%-6%	[5,6,76,236-238]
CDH1	< 0.005%	Diffuse gastric cancer: 7%; Lobular breast cancer: 0.3%	[5,6,92]
TP53	< 0.01%	Breast cancer in women < 30 years old: 2%-6%; Pediatric cancers: 8%; Osteosarcoma: 4%	[161,239,240]
HOXB13	0.2%-0.4%	Prostate cancer: Approximately 1%	[112,117,241]

Colorectal tumors

The accumulation of multiple cases of colorectal cancer (CRC) in pedigrees was systematically described in 1967 by Lynch *et al*[73]. Lynch syndrome, also called hereditary non-polyposis colorectal cancer (HNPCC), is the best-known genetic cause of CRC predisposition. HNPCC is associated with heterozygous germline inactivation of genes involved in DNA mismatch repair (MMR), namely *MLH1*, *MSH2*, *MSH6* or *PMS2* (Table 1). In addition, some Lynch syndrome patients carry deletion of the last portion of epithelial cell adhesion molecule (*EPCAM*), a gene located upstream to the *MSH2* genomic segment. This deletion results in the loss of transcription of the termination polyadenylation signal at the end of *EPCAM* and consequent emergence of the read-through *EPCAM-MSH2* fusion RNA message; furthermore, cells expressing the *EPCAM-MSH2* chimera demonstrate methylation of the *MSH2* promoter and failure to produce functional MSH2 protein[74]. The genetic causes of Lynch syndrome are apparently limited to the germline inactivation of *MLH1*, *MSH2*, *MSH6* or *PMS2* genes, as attempts to link this disease with PVs in other participants of MMR were unsuccessful[4]. The lifetime risk of CRC for the carriers of pathogenic alleles falls within 40%–70% for *MLH1* and *MSH2* genes, however it reaches only 10%–20% for *MSH6* and *PMS2* heterozygous individuals. Lynch syndrome contributes approximately to 3% of CRC morbidity in Western countries, however this estimate is significantly lower in some other populations[3,4,75-79]. In addition to CRC, Lynch syndrome is associated with a highly elevated risk of endometrial cancer as well as increased susceptibility to gastric, small bowel, biliary, urothelial, ovarian, brain, and some other malignancies. The spectrum and the risk of extracolonic and extraendometrial cancers varies depending on the gene involved[4,77,80]. The development of tumors in Lynch syndrome patients involves somatic second-hit inactivation of the remaining copy of the disease-causing gene[4].

Malfunction of MMR in HNPCC-associated tumors results in a high tumor mutation burden (TMB). Short repetitive sequences, so-called microsatellites, are particularly prone to MMR defects. Consequently, Lynch syndrome tumors have high-level microsatellite instability (MSI-H) diagnosed by electrophoretic detection of multiple changes in the length of mononucleotide repeats. Electrophoretic equipment is not a component of the standard morphological laboratory; therefore, many hospitals chose to use immunohistochemical (IHC) detection of MMR deficiency (MMR-D). Indeed, tumors arising in carriers of *MLH1* PVs lack the expression of MLH1 and PMS2 proteins, while *MSH2*-related CRCs show concomitant loss of MSH2 and MSH6 staining. Germline heterozygosity for *MSH6* or *PMS2* genes is accompanied by tumor-specific IHC negativity for *MSH6* or *PMS2*, respectively[77,81]. Importantly, only a minority of tumors with MSI-H/MMR-D phenotype are hereditary cancers. MSI-H/MMR-D is also highly characteristic for sporadic colorectal, gastric and endometrial carcinomas, especially for malignancies occurring in elderly patients. Inactivation of MMR in sporadic tumors is usually attributed to the down-regulation of the *MLH1* gene *via* promoter hypermethylation[81]. For the time being, MSI-H/MMR-D screening is recommended for all patients with CRC[82]. The selection of patients with MSI-H/MMR-D phenotype for subsequent germline testing may include consideration of age, family history of cancer, tumor location, and, in some instances, molecular characteristics of cancer cells. For example, Lynch syndrome related CRCs usually do not have mutation in the *BRAF* oncogene and demonstrate lack of methylation in the *MLH1* gene promoter[81]. Increasing availability of NGS is

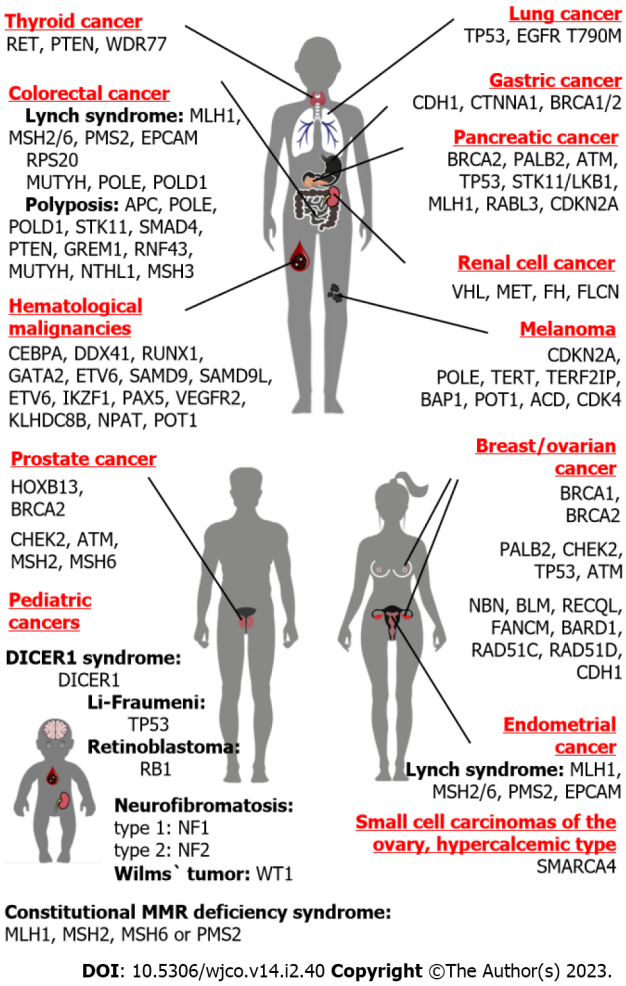


Figure 2 Main hereditary cancer genes and organs at risk. This figure illustrates major hereditary cancer types observed in females, males, adults of both genders, and children.

likely to result in the acceptance of uniform germline testing for all patients with microsatellite unstable colorectal and endometrial cancer, therefore the significance of procedures applied for the patient selection may diminish in the near future.

CRC familial clustering commonly occurs irrespective of MSI-H/MMR-D and Lynch syndrome. Surprisingly, the attempts to identify other than Lynch syndrome hereditary *CRC* genes were largely unsuccessful. Besides *MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM*, there is only one hereditary CRC gene with proven significance, *RPS20*. However, *RPS20* is altered only in a minority of multi-case CRC families and its impact is limited to a few selected populations[4,76,79].

Some germline PVs predispose to polyposis of gastrointestinal tract and increased risk of malignant transformation. There is a number of polyposis-related genes, which are associated with several scenarios of the disease development, *e.g.*, either the emergence of CRC in combination with the presence of multiple polyps, or, alternatively, the appearance of CRC in the absence of benign colon lesions. Some polyposis syndromes are transmitted by autosomal-dominant mode (*APC*, *POLE*, *POLD1*, *STK11*, *SMAD4*, *BMPR1A*, *PTEN*, *GREM1*, *RNF43*), while others involve recessive inheritance and biallelic gene inactivation in affected patients (*MUTYH*, *NTHL1*, *MSH3*, *MBD4*)[23,24,83].

The most known polyposis gene, adenomatous polyposis coli (*APC*), is associated with very severe impairment of gastrointestinal tract, although some hypomorphic *APC* variants cause an attenuated form of this disease. *APC* is a tumor suppressor gene, its inactivation results in up-regulation of the WNT signaling pathway. The incidence of APC is around 1:10000, and approximately 30% of detected *APC* PVs are de novo mutations. In addition to colon polyposis and CRC, there are some common extracolonic features of this disease, in particular, duodenal polyps and carcinomas, stomach polyps, osteomas, desmoid tumors and congenital hypertrophy of the retinal pigmented epithelium[84].

MUTYH-associated polyposis (MAP) has a somewhat lower incidence than APC, with estimates approaching approximately 1:20000. *MUTYH* gene is involved in base excision repair (BER), therefore its biallelic deficiency is associated with increased risk of accumulation of oncogenic mutations. MAP is usually characterized by a moderate number of polyps and relatively late disease onset. However, the probability of CRC development in MAP patients is high and approaches approximately 80%. *MUTYH*-

driven CRCs often contain *KRAS G12C* substitution. Approximately 5% of patients with *KRAS G12C*-mutated CRC are biallelic carriers of *MUTYH* pathogenic alleles, therefore somatic *KRAS* status may be used as an indicator for MAP screening in CRC patients. Extracolonic manifestations of MAP are relatively uncommon, with the exception of highly increased risk for kidney cancer[83]. Most patients of European ancestry with genetic MAP diagnosis are homozygotes or compound heterozygotes for founder *MUTYH* alleles, *Y165C* and/or *G382D*[3,84-86].

NTHL1-related polyposis is similar to MAP, as it is caused by germline biallelic inactivation of the gene involved in BER. It is exceptionally rare, with estimated incidence falling below 1:100000. Various extracolonic tumors are highly characteristic for this syndrome, with a particularly elevated risk for BC [24]. A recent study identified *MBD4*, another participant of BER pathway, as a genetic cause of polyposis and multiorgan cancer predisposition[83].

Heterozygous germline PVs in *POLE* and *POLD1* genes predispose to gastrointestinal polyposis, CRC, endometrial carcinomas and some other malignancies. Inactivation of these genes results in failure of proofreading activities of DNA polymerases, therefore tumors arising in carriers of *POLE* and *POLD1* pathogenic alleles contain ultrahigh number of somatic mutations[24,76,85,87].

Gastric cancer

Gastric cancer (GC) is among the most common malignancies worldwide. Its incidence is highly influenced by environmental and behavioral factors: GC risk is significantly associated with Helicobacter pylori infection, low hygienic standard, high consumption of salt, "Northern" diet, alcohol abuse, *etc.*[88]. Consequently, family clustering of GC is not necessarily attributed to genetic factors, but may also be observed due to sharing of some GC-predisposing attitudes.

Strong evidence for the role of heredity is obtained only for diffuse GC, a histological variety of GC characterized by poor differentiation and presence of signet-ring cells[9,89]. The causative gene, *CDH1*, was initially discovered in New Zealand Maori families characterized by an exceptionally high incidence of diffuse GC[90]. *CDH1* encodes E-cadherin, a protein involved in cell adhesion. *CDH1* germline PVs are uncommon in the majority of analyzed populations, with the frequency being around 1:5000–1:20000[5,6,91], while the proportion of *CDH1* heterozygotes in consecutive series of GC patients approaches approximately 7% for diffuse GC and 2% for non-selected GC[92]. A few hundred *CDH1*-related GC pedigrees have been described worldwide. Presence of *CDH1* germline PVs is also associated with high risk of lobular BC, a peculiar and relatively uncommon variety of BC disease. Family studies estimated penetrance of CDH1 PVs to be around 70% for GC and 40% for BC[9]. Unbiased NGS data sets revealed instances of *CDH1* germline PVs unrelated to clinically diagnosed diffuse GC, therefore, there are yet unknown factors modifying phenotypic consequences of *CDH1* heterozygosity[5,6,91]. Genetic analysis of *CDH1* PV-negative diffuse GC families led to the identification of subjects with inactivating PVs in *CTNNA1* gene, which encodes alpha-catenin and interacts with beta-catenin and E-cadherin[9].

There are studies suggesting the role of PVs in double-strand DNA repair genes in GC predisposition. For example, contribution of *PALB2* PVs has been suggested in some investigations[93,94], however the analysis of *PALB2*-related families did not confirm these findings[95]. GC is likely to be a part of *BRCA1/2* syndrome, as some GCs arise on *BRCA1/2*-mutated background and demonstrate somatic loss of the remaining allele of the involved gene[13,96,97]. Lynch syndrome and some hereditary polyposis syndromes may involve malignant transformation of stomach epithelia. The lifetime GC risk in carriers of *MLH1* or *MSH2* PVs approaches 7%–8%. Specific nucleotide substitutions located in the promoter 1B region of the *APC* gene cause a condition, which is called gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS). GAPPS is attributed to down-regulation of *APC* transcription in gastric mucosa; interestingly GAPPS patients do not have extensive involvement of the colon because *APC* expression in colonic epithelium is regulated by the promoter 1A[9,23,24,84,98].

Pancreatic cancer

Predisposition to pancreatic cancer (PanCa) is usually inherited as a part of multi-organ HCS. *BRCA2* is the best-established PanCa-predisposing gene (Table 1). PVs in *BRCA2* confer approximately 5%–10% lifetime risk of developing PanCa, which is an order of magnitude higher than in general population[99-102]. In contrast to *BRCA2*, the data on the contribution of *BRCA1* in PanCa morbidity are controversial [103]. It is safe to state that if *BRCA1* indeed plays a role in PanCa susceptibility, its penetrance towards this cancer type is significantly lower as compared to *BRCA2*[99-101].

The association of the *PALB2* gene with familial PanCa was initially demonstrated by exome sequencing analysis of a PanCa patient whose sister also suffered from this disease[104]. Family-based studies of *PALB2*-related pedigrees have confirmed this association, although the risk of PanCa associated with *PALB2* PVs is moderate[95]. Moderate-to-high elevation of PanCa risk is also characteristic for *ATM* heterozygotes[99,105-108].

PanCa may emerge as a part of Li-Fraumeni syndrome, a disease caused by *TP53* germline PVs, as well as a manifestation of Lynch syndrome[99,101,107]. Peutz-Jeghers syndrome (PJS) (attributed to PVs in *STK11/LKB1*) and *CDKN2A*-driven familial melanoma syndrome are associated with 20%–25% lifetime risk of PanCa[101,107,109].

Whole-genome sequencing study of a PanCa family revealed segregation of this disease with *RABL3* truncating PV[110]. *RABL3* is involved in the prenylation of KRAS protein. However, PVs in the *RABL3* gene appear to be exceptionally rare and are unlikely to significantly contribute to overall PanCa morbidity[111].

Prostate cancer

PVs in two genes, *HOXB13* and *BRCA2*, are associated with more than 5-fold elevation of prostate cancer (PrCa) risk, and, therefore, with almost 1:2 probability of developing this disease during lifetime. *HOXB13* is the only known gene specifically associated with PrCa (Table 1). It encodes a prostate-specific homeobox transcription factor. Its PVs are represented by several ethnicity-specific missense mutations, which affect the interaction between HOXB13 protein and MEIS homeobox cofactor. *HOXB13* PVs contribute to approximately 1% of PrCa incidence[112-114].

BRCA2 is apparently the most frequent cause of hereditary PrCa. Its penetrance towards PrCa in men is comparable to the risk estimates observed for BC in female *BRCA2* PV carriers[103,112,114,115]. Similar to pancreatic cancer, evidences regarding the contribution of *BRCA1* in PrCa morbidity are controversial, and associated risks are at best low-to-moderate[103,115,116]. The role of *ATM* PVs in PrCa predisposition is well established; *ATM*-heterozygous men have an approximately 2-fold elevation of the probability of PrCa development[112,114,117]. The impact of *PALB2* PVs has been suggested in some studies, although systematic investigations failed to validate these findings[95]. Lynch syndrome associated with PVs in *MSH2* and *MSH6* genes may also render an increased PrCa risk[118].

Renal cell cancer

Next-generation sequencing of DNA obtained from renal cell carcinoma (RCC) patients revealed an unexpectedly high frequency of germline PVs: Pathogenic or likely pathogenic alleles were detected in 41/254 (16%) analyzed subjects[119]. Approximately 5% of RCC incidence is associated with RCC-predisposing syndromes[119]. Von-Hippel-Lindau syndrome caused by germline PVs in the *von Hippel-Lindau* (*VHL*) gene renders approximately 30%–40% lifetime risk of RCC and is also associated with the development of pancreatic neuroendocrine tumors, pheochromocytomas and hemangioblastomas. PVs in the *fumarate hydratase* (*FH*) gene are responsible for hereditary leiomyomatosis and renal cell cancer. Germline PVs in *MET* receptor tyrosine kinase confer a fatal risk of papillary RCC. RCC is also characteristic for Birt-Hogg-Dubé syndrome, a disease caused by PVs in the *FLCN* gene and associated with slowly progressing renal lesions, skin fibrofolliculomas and lung cysts[120]. The risk of various types of RCC is increased in patients with tuberous sclerosis syndrome[121].

Lung cancer

Genuine hereditary lung cancer (LC) is an exceptionally rare disease. The best-described cause of familial LC is the inheritance of the *epidermal growth factor receptor (EGFR) T790M* variant[122,123]. *EGFR T790M* was initially discovered as a secondary somatic mutation acquired during the course of therapy by EGFR inhibitors[124,125]. Subsequent studies demonstrated that some subjects carry this missense substitution in germline. Inborn *EGFR T790M* allele is associated with the development of lung tumors, which contain tyrosine kinase inhibitor sensitizing mutations in exons 19 and 21 of the *EGFR* gene[126]. Only a few dozen subjects carrying germline *EGFR T790M* allele have been described worldwide[123]. The frequency of the *EGFR T790M* allele in consecutive LC series is vanishingly low[127,128]. In addition to *EGFR T790M*, a few unique LC families with other germline pathogenic *EGFR* variants have been described[123,128]. LC may also arise as a part of Li-Fraumeni syndrome, being attributed to germline *TP53* pathogenic allele[8,129].

Melanoma

Germline PVs in the *CDKN2A* gene have been detected in 20%–40% of families with multiple instances of cutaneous melanoma. *CDKN2A* PV carriers are at risk of development of other tumor types, particularly pancreatic cancer[130,131]. *CDKN2A* pathogenic alleles are associated with a more aggressive superficial spreading subtype, however there are controversial data with regard to their impact on melanoma-specific survival[132]. There are several described pedigrees where melanoma incidence is segregated with pathogenic alleles in *CDK4*, *POT1* or *TERT* genes[133].

Multiple endocrine neoplasia

Multiple endocrine neoplasia (MEN) type 1 affects parathyroid glands, pancreatic islet cells and the anterior pituitary. It is caused by heterozygous inactivation of the *MEN1* tumor suppressor gene, which encodes menin, a protein involved in regulation of a spectrum of biological processes. The prevalence of MEN1 syndrome is approximately 1:30000[22], although the population frequency of *MEN1* PVs may be slightly higher[5]. Most of MEN1 patients demonstrate primary hyperparathyroidism caused by parathyroid hyperplasia. This condition is accompanied by hypercalcemia with varying degrees of its consequences. Duodeno-pancreatic neuroendocrine tumors of pancreas are represented by gastrinomas, non-functioning tumors, insulinomas, glucagonomas and vasoactive intestinal peptide producing tumors. Anterior pituitary neoplasms include prolactinomas as well as somatropin-, adrenocorticotropic

hormone- and gonadotropin-secreting adenomas. In addition to the above three organs, MEN1 may manifest by adrenocortical, bronchopulmonary and thymic neuroendocrine tumors as well as by a number of non-endocrine neoplasms[134]. Unexpectedly, a strong association between *MEN1* heterozygosity and highly increased risk of acute pancreatitis has been demonstrated in a recent study[108]. Some patients, who have MEN1-related phenotype, but lack PVs in the *MEN1* gene, carry *CDNK1B* pathogenic alleles. *CDNK1B*-related MEN is now classified as MEN4 syndrome[22].

MEN2A (MEN2) and MEN2B (MEN3) syndromes are caused by activating PVs in *RET* receptor tyrosine kinase. Both these conditions are strongly associated with the development of medullary thyroid carcinoma (MTC). MTC is a relatively rare subtype of thyroid cancer, however germline *RET* pathogenic alleles make a very significant contribution to the incidence of this disease being detected in about a quarter of MTC patients. Besides MTC, approximately half of subjects with MEN2A syndrome develop pheochromocytomas, and up to a third of MEN2A cases are characterized by hyperparathyroidism. The prevalence of MEN2A is similar to the one for MEN1. MEN2A is caused by *RET* PVs in codon 634, or less, frequently, in codons 609, 611, 618, 620 or 630. These PVs, being located in the extracellular domain and resulting in replacements of cysteines, induce conformational changes in RET protein, which facilitate dimerization and cross-phosphorylation of this receptor. There are some other point mutations, which do not affect cysteines and generally cause a milder disease phenotype, *i.e.* the development of MTC in the absence of other endocrine tumors; isolated MTC may also be associated with cysteine mutations involving other than 634 codons of the *RET* oncogene. MEN2B (MEN3), being an order of magnitude less common than MEN2A, is a significantly more aggressive disease manifested in the first or second decade of life with highly metastatic and potentially fatal MTC. Patients with MEN2B also often develop pheochromocytomas as well as some non-endocrine features, *e.g.*, neuromas and musculoskeletal abnormalities. MEN2B is usually caused by *RET M918T* allele or, in less than 5% of cases, *A883F* substitution. These amino acid substitutions are located in the kinase domain and render dimerization-independent activation of RET receptor. Overall, the distinction between familial MTC, MEN2A and MEN2B may look counter-intuitive, as these maladies are all related to *RET* activating alleles and differ from each other mainly by the disease severity but not by underlying biological mechanisms[22,135,136].

Carney complex manifests with adrenocortical disease, pituitary adenomas, gonadal and thyroid tumors, spotty skin pigmentation, cardiac and cutaneous myxomas, and some other non-endocrine neoplasms. This condition is caused by *PRKAR1A* germline PVs[137]. There is a number of genes, associated with isolated endocrine cancers. Germline PVs in the *WDR77* gene have been recently shown to predispose to papillary subtype of thyroid cancer. *WDR77* is a component of a transmethylase complex responsible for posttranslational modification of histone H4[138]. Genetic susceptibility to pheochromocytoma and/or paraganglioma may be rendered by PVs affecting *SDHAF2*, *SDHB*, *SDHC*, *SDHD*, *MAX*, *TMEM127* or some other genes[139]. There are instances of familial pituitary adenoma associated with *AIP* germline PVs[140,141].

Li-Fraumeni syndrome

Li-Fraumeni syndrome is caused by PVs in the *TP53* gene. *TP53* is apparently the best-studied tumor suppressor gene, which is involved in the regulation of DNA damage response, programmed cell death, cell cycle and several other biological processes. Population occurrence of *TP53* germline heterozygosity is well below 1:10000, although some communities demonstrate a noticeable frequency of founder hypomorphic *TP53* variants[5,6,142]. Earlier family-based studies suggested nearly-fatal penetrance for *TP53* germline PVs, although recent data indicate that some carriers of *TP53* pathogenic alleles manage to achieve late adulthood without being affected by cancer disease[8].

TP53 PVs render a highly increased risk of childhood cancers. Li-Fraumeni syndrome-associated pediatric malignancies include adrenal cortical carcinomas, choroid plexus carcinomas, rhabdomyosarcomas and medulloblastomas. Adult cancers are mainly represented by very-young-onset BC in females as well as lung carcinomas, osteosarcomas, soft-tissue sarcomas and brain tumors[8,63,143]. Breast carcinomas arising in *TP53* PV carriers frequently carry *HER2* amplification[144]. Li-Fraumeni syndrome related lung carcinomas are characterized by an exceptionally high frequency of *EGFR* somatic mutations[129,145]. Carriers of *TP53* PVs also have highly elevated risk of hematological malignancies[146]. The analysis of specific groups of consecutive patients revealed that Li-Fraumeni syndrome is a significant contributor to the incidence of pediatric cancers, very-young-onset breast carcinomas and osteosarcomas[142,146-150].

PTEN hamartoma tumor syndrome

PTEN hamartoma tumor syndrome (PHTS) is manifested by multiple benign and malignant tumors affecting breast, thyroid, endometrium, skin, kidney, colon and some other organs[151-153]. It is caused by heterozygous inactivating PVs in the *PTEN* gene, which is involved in the negative regulation of phosphatidylinositol 3-kinase/AKT/mechanistic target of rapamycin (mTOR) pathway and plays a role in the regulation of cell survival, proliferation, apoptosis and various metabolic processes[152,154]. *PTEN*-related syndrome is commonly known as Cowden syndrome, however the PHTS is a more preferable definition as it includes some other *PTEN*-associated maladies, *e.g.*, Bannayan-Riley-Ruvalcaba syndrome and Lhermitte–Duclos disease[151,152]. Patients with PHTS often have a wide

range of skin and mucosal manifestations and frequently present with macrocephaly[151]. Based on clinical considerations, the reported frequency of PHTS is approximately 1:200000[154], although unbiased NGS studies suggest that approximately 1:10000 healthy people are *PTEN* heterozygotes[5,6]. Activating germline PVs in the *WWP1* gene, which encodes E3 ubiquitin ligase and negatively regulates PTEN, were detected in some *PTEN*-wild-type patients with PHTS-associated tumors[155].

PJS

PJS manifests *via* characteristic mucocutaneous pigmentations and various polyp-related complications. Multiple gastrointestinal hamartomatous polyps in the affected patients are located mainly in the small bowel. The disease is caused by heterozygous inactivating PVs in tumor suppressor kinase *STK11/LKB1*. *STK11/LKB1* is involved in the regulation of cell cycle, apoptosis and cell metabolism. Population occurrence of PJS is estimated to be within 1:50000–1:200000, however as many as 1 out of 10000 apparently healthy subjects may carry *STK11/LKB1* PVs[5,156]. *STK11/LKB1* is a highly-penetrant cancer-predisposing gene. This genetic condition is associated with highly elevated risk of breast, colon, stomach, pancreatic and some other malignancies[156]. In addition, there are rare tumor subtypes specifically linked to PJS, *e.g.*, so-called sex cord tumors with annular tubules affecting ovaries[157]. Clinical presentation of PJS may depend on the type of *STK11/LKB1* PVs[158].

Gorlin syndrome

Gorlin syndrome [nevoid basal cell carcinoma (BCC) syndrome] is characterized by the appearance of BCCs and the development of odontogenic keratocysts. This disease is also associated with increased risk of medulloblastoma. In addition, various developmental abnormalities are frequently seen in patients with this condition. Gorlin syndrome is a rare disease, being observed in approximately 1:30000–1:300000 subjects. The most frequent cause of Gorlin syndrome is a heterozygous inactivating PV in the *PTCH1* gene. *SUFU* or *PTCH2* pathogenic alleles have been identified in the affected subjects, who are mutation-negative for *PTCH1*. Tumor development in Gorlin syndrome patients involves upregulation of the Hedgehog signaling pathway due to loss of its negative regulation by *PTCH1*, *SUFU* or *PTCH2*[159]. BCC predisposition may also be rendered by heterozygous inactivating PVs in the *PTPN14* tumor suppressor gene[160].

Pediatric cancers

It is difficult to draw a strict distinction between "pediatric" and "adult" hereditary cancers because many HCSs may present with various manifestations both in childhood and in the middle of life. Relevant examples include Li-Fraumeni syndrome, Cowden syndrome, PJS, neurofibromatosis, *RET*-related malignancies, *etc.* Expectedly, NGS analysis of non-selected patients with pediatric cancers revealed elevated frequency of PVs in known cancer-predisposing genes[161,162].

Retinoblastoma was the first pediatric tumor for which the genetic origin was convincingly established and the causative gene was identified. Hereditary retinoblastoma is caused by germline inactivation of the *RB1* gene. *RB1*, being the first cloned tumor suppressor gene, is implicated in the negative regulation of the cell cycle[19]. *RB1* germline alterations are observed in all patients with familial and/or bilateral retinoblastoma as well as in 14% of subjects with sporadic unilateral appearance of this disease[163]. Retinoblastoma survivors are at high risk of developing other neoplasms, particularly sarcomas[164]. Spliceosome dysfunction has been recently shown to underlie the emergence of bone malignancies in *RB1* heterozygotes[165].

Wilms` tumor (nephroblastoma, WT) is a relatively common pediatric cancer. The most frequent genetic cause of WT is a mutation in the *WT1* gene, which can be associated either with isolated WT, or with its combination with aniridia, nephrotic syndrome and/or abnormal genitalia. WT can also be a part of so-called overgrowth syndromes (Beckwith-Wiedemann syndrome, Sotos syndrome, Simpson–Golabi–Behmel syndrome, Perlman syndrome) or several syndromes associated with a wide spectrum of cancers (Li-Fraumeni syndrome, Bloom syndrome, Fanconi anemia, *etc.*)[166].

Neurofibromatosis type 1 is caused by inactivating heterozygous PVs in the *NF1* gene. NF1 is a negative regulator of the RAS signaling pathway. *NF1* heterozygosity is estimated to occur in 1:3500 newborns and is manifested by *cafe au lait* spots, axillary freckles, Lisch nodules and neurofibromas. This syndrome is associated with a high risk of development of gliomas, hematological malignancies, pheochromocytomas and some other tumors. Neurofibromatosis type 2 is ten times less common than the type 1 disease. The *NF2* gene encodes merlin, its inactivation is associated with the development of schwannomas and meningiomas in adolescence or adulthood[167].

DICER1 syndrome has been described relatively recently[168]. It is associated with heterozygous germline inactivation of the *DICER1* gene. DICER1, a ribonuclease III family enzyme, is responsible for the maturation of microRNA. The pathogenesis of *DICER1*-related malignancies usually involves somatic alteration of the remaining gene allele. *DICER1* PVs are characterized by incomplete penetrance. Carriers of *DICER1* PVs are at risk of developing pleuropulmonary blastomas, gynandroblastomas, sarcomas, Sertoli-Leydig cell tumors and some other neoplasms[169,170].

PVs in the *SMARC* family genes, which regulate chromatin remodeling, are responsible for the rhabdoid tumor predisposition syndrome[171]. *SMARCB1* pathogenic alleles are associated with the

development of malignant rhabdoid tumors of the central nervous system and kidneys. Hypomorphic *SMARCB1* PVs are also implicated in familial schwannomatosis where the development of schwannomas involves concomitant down-regulation of both *SMARCB1* and *NF2* genes[172]. *SMARCE1* PVs predispose to the development of meningiomas. *SMARCA4* pathogenic alleles are associated with rhabdoid tumors as well as small-cell OC, hypercalcemic type[171].

Constitutional mismatch repair deficiency syndrome (CMMRD) is an autosomal-recessive disorder caused by biallelic inactivation of *MMR* genes[4]. This condition has characteristic cutaneous manifestations and renders a high probability of developing brain, gastrointestinal and hematological malignancies at a young age[173].

Hematological malignancies

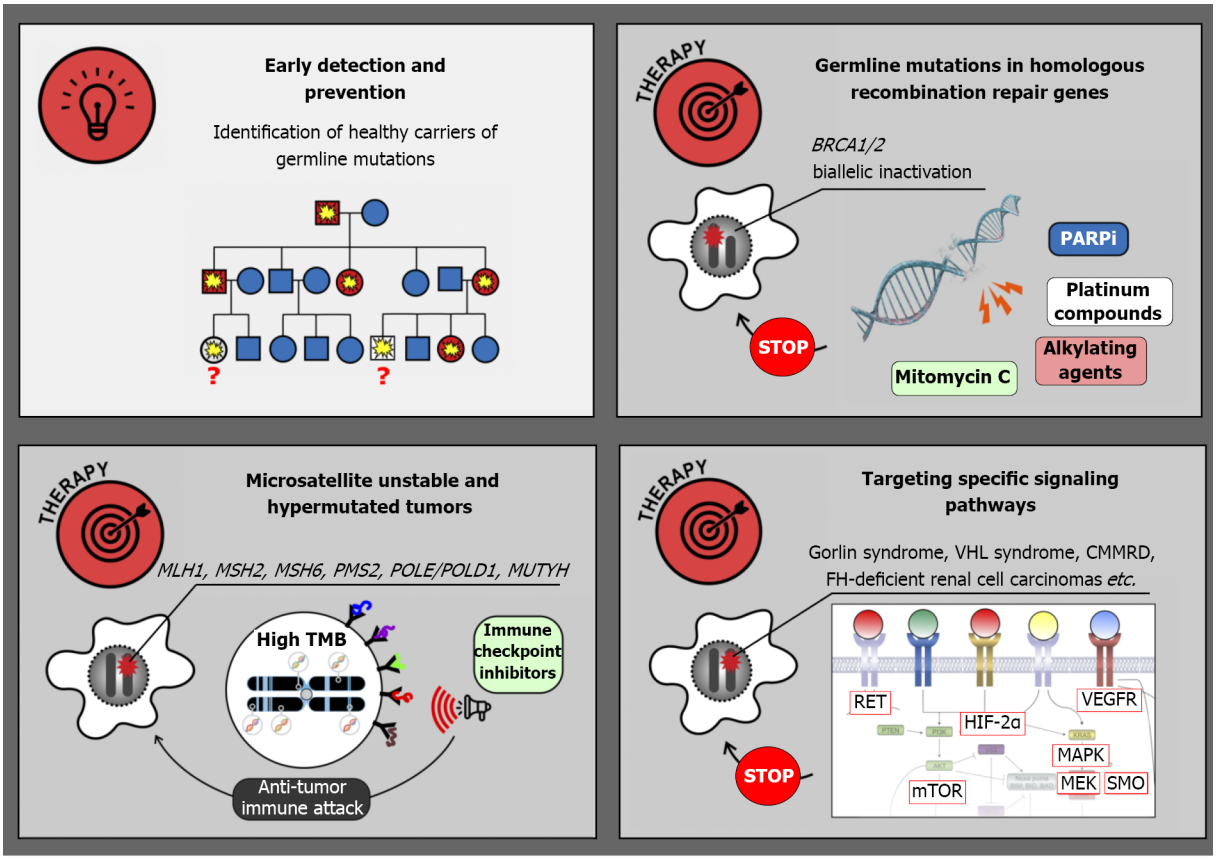
Hematological malignancies often manifest as a part of a syndromic condition. Various abnormalities of hematopoiesis resulting in the depletion of some cell lineages are frequently accompanied by myeloid-derived neoplasms. Immune deficiencies render an increased risk of development of lymphomas[174]. Familial clustering of acute myeloid leukemia may be attributed to germline PVs in *CEBPA*, *DDX41*, *RUNX1*, *GATA2*, *ETV6*, *SAMD9*, *SAMD9L* and some other genes. Hereditary acute lymphoblastic leukemia is related to germline PVs in *ETV6*, *IKZF1* or *PAX5* genes and may as well be a part of clinical manifestation of Li-Fraumeni syndrome[175]. Alterations in the *KDR* (vascular endothelial growth factor 2) receptor tyrosine kinase are the most frequent cause of hereditary Hodgkin lymphoma; high risk of this disease may also be rendered by germline PVs located in *KLHDC8B*, *NPAT* or *POT1* genes [176].

MANAGEMENT OF HEREDITARY TUMORS

Cancer detection and prevention

The research on HCSs was initially viewed mainly as a part of prophylactic medicine. Indeed, there is a strong emphasis on the identification of yet healthy people, who are carriers of tumor-predisposing PVs and may significantly benefit from early cancer detection and prevention (Figure 3). Diagnostic surveillance strategies have been articulated for all major cancer syndromes. For example, female carriers of *BRCA1*, *BRCA2* and some other pathogenic alleles are advised to start breast self-examination from 18 years old; regular clinical breast examination and magnetic resonance imaging are usually added beginning from 25 years, and they are supplemented by annual mammography in women aged 30–75 years. OC screening includes annual transvaginal ultrasound examination and CA-125 serum marker measurement starting at 30–35 years[84]. Clinical efficacy of surveillance is considerably higher in patients with Lynch syndrome. The adherence to colonoscopy performed every 1–2 years beginning from 20–25 years of age, upper endoscopy every 3–5 years starting at 30–35 years as well as endometrial cancer screening, significantly reduces individual risk of cancer death[84]. Effective surveillance is more complicated in subjects with multiorgan cancer predisposition. In particular, carriers of *TP53* germline PVs are advised to begin cancer screening in early childhood and, wherever possible, to abstain from potentially mutagenic diagnostic procedures, *e.g.*, X-ray examination[146]. The development of screening recommendations for subjects with HCSs is a continuous process, which is usually coordinated by international and national healthcare professional societies or initiative groups, involves interaction of a high number of experts working in different areas of medicine, requires significant research efforts aimed at collection of real-world data and is a subject of regular updates[84,146,177,178]. There is a multitude of published guidelines, which generally suggest similar diagnostic algorithms but differ from each other in many nuances. The detailed discussion on existing recommendations is beyond the scope of this review.

Prophylactic risk-reducing surgery has become a standard medical intervention, being particularly well investigated in subjects with the HBOC syndrome, hereditary diffuse GC, hereditary medullary thyroid cancer, *etc.*[9,22,146,179-181]. It is self-explanatory that surgical removal of the organ(s) at-risk may be applied only in situations when this procedure is not associated with life-threatening adverse effects or disproportional decrease of the quality of life, and only for syndromes with insufficient reliability of early cancer diagnosis. Carriers of highly-penetrant BC-predisposing PVs (*BRCA1*, *BRCA2*, *PALB2*, *TP53*, *etc.*) are encouraged to undergo risk-reducing breast surgery, given that even high compliance with diagnostic check-ups does not fully warrant cancer detection at early stage or good treatment outcome[182]. *BRCA1/2* heterozygous women are strongly recommended to opt for prophylactic salpingo-oophorectomy at the age of 35–45 years (or after the completion of childbearing)[177,178,183]. This procedure is justified by the poor clinical efficacy of OC screening and dispensability of ovaries for women entering their second half of life. Prophylactic gastrectomy in *CDH1* PV carriers is associated with severe impairment of the quality of life, however the abstinence from this procedure is associated with a significant risk of death due to diffuse GC[9]. Risk-reducing thyroidectomy followed by hormone replacement therapy is a standard option for carriers of *RET* high-risk PVs. This surgery is usually performed in childhood, and the recommended age for intervention varies depending on the type of *RET* PV[184,185].



DOI: 10.5306/wjco.v14.i2.40 **Copyright** ©The Author(s) 2023.

Figure 3 Management of hereditary cancer syndromes. PARPi: Poly (ADP-ribose) polymerase inhibitors; TMB: Tumor mutation burden; HIF-2α: Hypoxia inducible factor-2α; VHL: von Hippel-Lindau; mTOR: Mechanistic target of rapamycin; MAPK: Mitogen-activated protein kinase signaling pathway; MEK: Mitogen-activated protein kinase; VEGFR: Vascular endothelial growth factor receptor; SMO: Smoothened; CMMRD: Constitutional mismatch repair deficiency syndrome.

Benefit from risk-reducing surgeries has been confirmed by real-world data, however this experience is mainly limited to healthy relatives of cancer patients, who were found to be heterozygous for a highly-penetrant pathogenic allele[184,186,187]. Recent large-scale genetic investigations have identified some carriers of tumor-predisposing variants, who do not have a family history of cancers associated with their genetic findings[5,6]. Apparently, these individuals should be advised to undergo full-scale diagnostic surveillance, whereas great caution must be taken while considering prophylactic surgical interventions in subjects with favorable pedigree data[23].

Advances in cytotoxic and targeted therapy

Despite substantial advances in early detection and prevention of malignant diseases, cancer genetics remained an "exotic" discipline for many practicing oncologists until the second decade of this century. This was due to relative rarity of familial tumors and limited impact of germline DNA testing on the treatment strategies. Several discoveries, which were made within the past 10–15 years and resulted in the recognition of specific drug vulnerabilities in hereditary cancers, have moved familial cancer studies to the frontline of medical oncology[188,189].

BRCA1/2-driven breast and ovarian carcinomas arise due to somatic inactivation of the remaining allele of the involved gene (Figure 3 and Table 2). Consequently, these tumors are deficient in DNA double-strand break repair and demonstrate pronounced sensitivity to platinum compounds, mitomycin C, bifunctional alkylating agents and poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi). Several clinical studies involving cisplatin or carboplatin suggested that platinum-based regimens are highly effective in women with breast or ovarian *BRCA1/2*-associated cancer[190-192]. Combined administration of cisplatin and mitomycin C resulted in a remarkable improvement of treatment outcomes in patients with *BRCA1*-mutated carcinomas[193,194]. There are a number of successful clinical investigations, which resulted in the approval of PARPi for the treatment of hereditary breast, ovarian, pancreatic and prostate malignancies[195]. Interestingly, non-breast/ovarian carcinomas arising in *BRCA1/2* PV carriers often retain the second *BRCA1/2* allele and therefore do not have this drug vulnerability. Findings obtained on *BRCA1/2* PV carriers may or may not be applicable to other genes involved in homologous recombination, as not all of the latter trigger tumor development by the two-hit mechanism, and even biallelic defects in some genes, *e.g.*, *ATM* or *CHEK2*, are not

Table 2 Cytotoxic and targeted therapy for tumors arising in carriers of cancer-predisposing alleles

Tumor type	Target	Drugs	Ref.
BRCA1/2-driven carcinomas and their phenocopies	*BRCA1/2* inactivation resulting in the deficiency of DNA repair by homologous recombination	Platinum derivatives, Mitomycin C, Bifunctional alkylating agents, PARPi	[190-193, 195]
Hypermutated cancers (Lynch syndrome associated microsatellite unstable tumors; *POLD1/POLE*-deficient cancers; *MUTYH*-associated colorectal carcinomas; tumors in patients with CMMRD syndrome)	High tumor mutation burden resulting in excessive number of neoantigens	Immune checkpoint inhibitors	[199-206]
RET-associated malignancies	RET tyrosine kinase	RET inhibitors	[207-209]
Neurofibromatosis, type 1	Upregulation of RAS/RAF/MEK pathway due to NF1 inactivation	MEK inhibitors	[210, 211]
Basal cell carcinomas in patients with Gorlin syndrome	Hedgehog pathway	SMO inhibitors	[213]
Tumors arising in patients with tuberous sclerosis	mTOR pathway	mTOR inhibitors	[214, 215]
Renal cell carcinomas associated with von Hippel-Lindau syndrome	Up-regulation of HIF-2α due to *VHL* gene inactivation	HIF-2α inhibitors	[216]

HIF-2α: Hypoxia inducible factor-2α; PARPi: Poly (ADP-ribose) polymerase inhibitors; CMMRD: Constitutional mismatch repair deficiency syndrome; MEK: Mitogen-activated protein kinase; SMO: Smoothened; mTOR: Mechanistic target of rapamycin; VHL: von Hippel-Lindau.

necessarily associated with platinum or PARPi sensitivity[21,196-198].

Microsatellite-unstable cancers, including tumors arising due to Lynch syndrome, are characterized by an excessive number of somatic mutations, and, consequently, high tumor antigenicity. These malignancies can be managed by the administration of so-called immune checkpoint inhibitors, the drugs which antagonize immune suppressor molecules and restore proper antitumor immunity[199]. Clinical studies on microsatellite-unstable cancers involved both patients with Lynch syndrome and subjects with sporadic carcinomas. Pembrolizumab has been approved for the treatment of MSI-H tumors irrespective of their organ localization[200]. Interestingly, a small study comparing hereditary *vs* sporadic microsatellite-unstable endometrial carcinomas revealed that tumors associated with a germline pathogenic allele have higher TMB and are more responsive to this drug[201]. The results of available clinical trials support the use of pembrolizumab or a combination of nivolumab and ipilimumab in the first-line therapy of metastatic MSI-H CRC[199-202]. There are instances of successful utilization of immune checkpoint inhibitors for the treatment of *POLE/POLD1*- and *MUTYH*-related malignancies[203,204]. Several case studies reported clinical benefit from immune therapy in patients with CMMRD-associated tumors[205,206].

Some hereditary cancers are associated with the upregulation of specific signaling pathways. A multikinase inhibitor vandetanib, which has activity towards RET and several other tyrosine kinases, has demonstrated significant clinical activity in patients with hereditary MTCs[207]. Clinical studies on selective RET inhibitors, selpercatinib and pralsetinib, included subjects with both hereditary and sporadic *RET*-driven thyroid tumors, and demonstrated remarkable benefit from these drugs[136,208, 209].

Tumors arising in patients with neurofibromatosis type 1 are characterized by inactivation of *NF1* gene, which is a negative regulator of RAS/RAF/MEK pathway. Consequently, these malignancies are potentially sensitive to MEK inhibition[210,211]. MEK inhibitor selumetinib has been evaluated in 25 children with recurrent, refractory, or progressive pediatric low-grade *NF1*-related gliomas, which failed at least one prior therapy. Objective response was documented in 10 (40%) cases, and 24 (96%) patients experienced no progression of the disease within 2 years[210]. Another study included children with *NF1*-associated symptomatic inoperable plexiform neurofibromas. Objective responses were observed in 37/50 (70%) patients, with 28 instances of response lasting more than 1 year[211]. Activating mutations in RAS/RAF/MEK pathway are also characteristic for hypermutated cancers arising in CMMRD patients. Pronounced efficacy of selumetinib or trametinib has been demonstrated in several patients with heavily pretreated CMMRD-related brain tumors[212].

Gorlin syndrome related BCCs can be managed by down-regulation of G-protein coupled receptor smoothened (SMO), which is involved in the activation of the Hedgehog pathway. Vismodegib, a selective SMO inhibitor, has been evaluated in placebo-controlled trial involving 46 patients, who had at least ten tumors each. All subjects receiving this drug experienced the decrease of existing tumor burden. Furthermore, the use of vismodegib slowed the emergence of new cancer lesions in patients with Gorlin syndrome[213].

Cancers associated with tuberous sclerosis are responsive to mTOR targeted drugs. Clinical efficacy of everolimus has been repeatedly demonstrated in angiomyolipomas and subependymal giant cell astrocytomas associated with this syndorme[214,215]. There are promising results of the treatment of

VHL syndrome related tumors by hypoxia-inducible factor-2α inhibitor belzutifan[216]. FH-deficient RCCs often respond to the combination of anti-vascular endothelial growth factor therapy and mTOR antagonists or to multitargeted tyrosine kinase inhibitors[217,218].

Drug vulnerabilities detected in hereditary cancer often have clinical relevance to their sporadic phenocopies. For example, platinum/PARPi sensitivity was initially described in *BRCA1/2*-driven carcinomas, but subsequent research revealed that tumors with *BRCA1/2*-like (BRCAness) properties, *e.g.*, a specific pattern of chromosomal instability, are also sensitive to these compounds[219,220].

CONCLUSION

Increasing involvement of healthy people in whole exome or multigene sequencing will certainly identify a huge number of subjects, who have a potentially severe disease according to a genetic test, but continue to remain unaffected until the elderly age. We are already witnessing that virtually all updated penetrance estimates are significantly lower than the ones observed by earlier studies, and, *vice versa*, the population frequency of some presumably "fatal" germline PVs is manifold higher than the observed incidence of corresponding genetic diseases[4-6,8,9]. The distinction between genetic health and disease is likely to be reconsidered in the near future.

Earlier cancer genetic studies produced rather straightforward gene-disease interactions, where all relevant genes and associated diseases could be easily presented in a table-like format. Systematic large-scale investigations carried out in the last decade revealed substantial promiscuity in genotype-phenotype interactions, thus complicating the clinical diagnosis of HCSs and interpretation of genetic findings[4,17,103,108,119,149,161,162,221]. The unbiased cataloging of patient data may help to account for the diversity of HCS manifestations.

Most of the known non-cancer genetic diseases are recessive, while most of the already identified cancer predisposition syndromes are dominant. This difference is unlikely to be related to genuine biological reasons, but is rather attributed to difficulties in the genetic studies of common cancer types. Virtually all "classic" genetic pathologies are orphan maladies (*e.g.*, cystic fibrosis or phenylketonuria), so the appearance of even 2-3 patients with a unique phenotype in the same family/pedigree, or in the same neighborhood, is immediately recognizable by practicing physicians or clinical investigators. However, if we consider a recessive mechanism for say, conventional breast, lung, or colorectal carcinomas, *i.e.*, the situation when both parents are asymptomatic heterozygous carriers of a recessive tumor-predisposing allele, and the disease is manifested only in subjects with biallelic gene involvement, there is little if any chance to distinguish these subjects from sporadic phenocopies[222]. Indeed, already known recessive tumor-predisposing syndromes include mainly rare diseases with very characteristic phenotypic manifestation, *e.g.*, some hereditary polyposis syndromes[84]. Systematic germline sequencing of cancer patients and the analysis of accumulated "big data" may eventually identify some examples of recessive predisposition to common cancer types. Focus on large communities with pronounced founder effect may facilitate the research in this direction.

The critical mass of advances in clinical genetics, including studies on HCSs, has been achieved due to efforts of scientists working mainly in North America, Western Europe, Japan, and several other parts of the world distinguished by the combination of an exceptionally high level of technological development and strong dedication to biomedical research. Consequently, current knowledge on pathogenic alleles and corresponding familial diseases mainly reflects the genetic background of Western European populations and some Eastern Asian communities. It is self-explanatory that each ethnic group has its own ancestors, who have a unique composition of pathogenic gene variants. Consequently, the distribution of genetic diseases is a subject of major interethnic variations, with a number of maladies observed only in selected populations. It is important to encourage ethnicity-specific cataloging of pathogenic alleles and corresponding phenotypes in order to support proper practical implementation of gene-based tests. Furthermore, analysis of "novel" populations is likely to result in the discovery of new medically relevant genes and corresponding genetic diseases[36,223-226].

Most of cancer studies rely mainly on the identification of protein-truncating variants. The clarification of functional/pathogenic significance for missense mutations is complicated, and there is a need for robust bioinformatic and laboratory pipelines supporting the distinction between disease-causing and neutral amino acid substitutions[227,228]. Current research is mainly focused on the coding regions of the genome; however other genetic loci, to be studied by whole genome cataloging, are also very likely to be a source of disease-predisposing variations[229].

Identification of cancer-predisposing genes is an example of triumph of translational medicine. The development of methods of non-surgical prevention of tumor progression in carriers of disease-associated pathogenic alleles is an obvious priority for future studies in this field.

FOOTNOTES

Author contributions: Imyanitov EN and Kuligina ES wrote the article draft; Kuligina ES prepared the illustration; Sokolenko AP, Suspitsin EN, Yanus GA, Iyevleva AG, Ivantsov AO, Aleksakhina SN acquired the literature data and analyzed the results; All authors approved the final version of the article.

Supported by The Russian Science Foundation, No. 17-75-30027.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Russia

ORCID number: Evgeny N Imyanitov 0000-0003-4529-7891; Ekaterina S Kuligina 0000-0002-2396-6540; Anna P Sokolenko 0000-0001-6304-1609; Grigoriy A Yanus 0000-0002-9844-4536; Svetlana N Aleksakhina 0000-0002-2149-7728.

S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

REFERENCES

1 **Walsh MF**, Chang VY, Kohlmann WK, Scott HS, Cunniff C, Bourdeaut F, Molenaar JJ, Porter CC, Sandlund JT, Plon SE, Wang LL, Savage SA. Recommendations for Childhood Cancer Screening and Surveillance in DNA Repair Disorders. *Clin Cancer Res* 2017; **23**: e23-e31 [PMID: 28572264 DOI: 10.1158/1078-0432.CCR-17-0465]
2 **Sharma R**, Lewis S, Wlodarski MW. DNA Repair Syndromes and Cancer: Insights Into Genetics and Phenotype Patterns. *Front Pediatr* 2020; **8**: 570084 [PMID: 33194896 DOI: 10.3389/fped.2020.570084]
3 **Valle L**, de Voer RM, Goldberg Y, Sjursen W, Försti A, Ruiz-Ponte C, Caldés T, Garré P, Olsen MF, Nordling M, Castellvi-Bel S, Hemminki K. Update on genetic predisposition to colorectal cancer and polyposis. *Mol Aspects Med* 2019; **69**: 10-26 [PMID: 30862463 DOI: 10.1016/j.mam.2019.03.001]
4 **Peltomäki P**, Olkinuora A, Nieminen TT. Updates in the field of hereditary nonpolyposis colorectal cancer. *Expert Rev Gastroenterol Hepatol* 2020; **14**: 707-720 [PMID: 32755332 DOI: 10.1080/17474124.2020.1782187]
5 **Breast Cancer Association Consortium**, Dorling L, Carvalho S, Allen J, González-Neira A, Luccarini C, Wahlström C, Pooley KA, Parsons MT, Fortuno C, Wang Q, Bolla MK, Dennis J, Keeman R, Alonso MR, Álvarez N, Herraez B, Fernandez V, Núñez-Torres R, Osorio A, Valcich J, Li M, Törngren T, Harrington PA, Baynes C, Conroy DM, Decker B, Fachal L, Mavaddat N, Ahearn T, Aittomäki K, Antonenkova NN, Arnold N, Arveux P, Ausems MGEM, Auvinen P, Becher H, Beckmann MW, Behrens S, Bermisheva M, Białkowska K, Blomqvist C, Bogdanova NV, Bogdanova-Markov N, Bojesen SE, Bonanni B, Børresen-Dale AL, Brauch H, Bremer M, Briceno I, Brüning T, Burwinkel B, Cameron DA, Camp NJ, Campbell A, Carracedo A, Castelao JE, Cessna MH, Chanock SJ, Christiansen H, Collée JM, Cordina-Duverger E, Cornelissen S, Czene K, Dörk T, Ekici AB, Engel C, Eriksson M, Fasching PA, Figueroa J, Flyger H, Försti A, Gabrielson M, Gago-Dominguez M, Georgoulias V, Gil F, Giles GG, Glendon G, Garcia EBG, Alnæs GIG, Guénel P, Hadjisavvas A, Haeberle L, Hahnen E, Hall P, Hamann U, Harkness EF, Hartikainen JM, Hartman M, He W, Heemskerk-Gerritsen BAM, Hillemanns P, Hogervorst FBL, Hollestelle A, Ho WK, Hooning MJ, Howell A, Humphreys K, Idris F, Jakubowska A, Jung A, Kapoor PM, Kerin MJ, Khusnutdinova E, Kim SW, Ko YD, Kosma VM, Kristensen VN, Kyriacou K, Lakeman IMM, Lee JW, Lee MH, Li J, Lindblom A, Lo WY, Loizidou MA, Lophatananon A, Lubiński J, MacInnis RJ, Madsen MJ, Mannermaa A, Manoochehri M, Manoukian S, Margolin S, Martinez ME, Maurer T, Mavroudis D, McLean C, Meindl A, Mensenkamp AR, Michailidou K, Miller N, Mohd Taib NA, Muir K, Mulligan AM, Nevanlinna H, Newman WG, Nordestgaard BG, Ng PS, Oosterwijk JC, Park SK, Park-Simon TW, Perez JIA, Peterlongo P, Porteous DJ, Prajzendanc K, Prokofyeva D, Radice P, Rashid MU, Rhenius V, Rookus MA, Rüdiger T, Saloustros E, Sawyer EJ, Schmutzler RK, Schneeweiss A, Schürmann P, Shah M, Sohn C, Southey MC, Surowy H, Suvanto M, Thanasitthichai S, Tomlinson I, Torres D, Truong T, Tzardi M, Valova Y, van Asperen CJ, Van Dam RM, van den Ouweland AMW, van der Kolk LE, van Veen EM, Wendt C, Williams JA, Yang XR, Yoon SY, Zamora MP, Evans DG, de la Hoya M, Simard J, Antoniou AC, Borg Å, Andrulis IL, Chang-Claude J, García-Closas M, Chenevix-Trench G, Milne RL, Pharoah PDP, Schmidt MK, Spurdle AB, Vreeswijk MPG, Benitez J, Dunning AM, Kvist A, Teo SH, Devilee P, Easton DF. Breast Cancer Risk Genes - Association Analysis in More than 113,000 Women. *N Engl J Med* 2021; **384**: 428-439 [PMID: 33471991 DOI: 10.1056/NEJMoa1913948]
6 **Hu C**, Hart SN, Gnanaolivu R, Huang H, Lee KY, Na J, Gao C, Lilyquist J, Yadav S, Boddicker NJ, Samara R, Klebba J, Ambrosone CB, Anton-Culver H, Auer P, Bandera EV, Bernstein L, Bertrand KA, Burnside ES, Carter BD, Eliassen H, Gapstur SM, Gaudet M, Haiman C, Hodge JM, Hunter DJ, Jacobs EJ, John EM, Kooperberg C, Kurian AW, Le Marchand L, Lindstroem S, Lindstrom T, Ma H, Neuhausen S, Newcomb PA, O'Brien KM, Olson JE, Ong IM, Pal T, Palmer JR, Patel AV, Reid S, Rosenberg L, Sandler DP, Scott C, Tamimi R, Taylor JA, Trentham-Dietz A, Vachon CM, Weinberg C, Yao S, Ziogas A, Weitzel JN, Goldgar DE, Domchek SM, Nathanson KL, Kraft P, Polley EC, Couch FJ. A Population-

Based Study of Genes Previously Implicated in Breast Cancer. *N Engl J Med* 2021; **384**: 440-451 [PMID: 33471974 DOI: 10.1056/NEJMoa2005936]

7 **Roa BB**, Boyd AA, Volcik K, Richards CS. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. *Nat Genet* 1996; **14**: 185-187 [PMID: 8841191 DOI: 10.1038/ng1096-185]

8 **Amadou A**, Achatz MIW, Hainaut P. Revisiting tumor patterns and penetrance in germline TP53 mutation carriers: temporal phases of Li-Fraumeni syndrome. *Curr Opin Oncol* 2018; **30**: 23-29 [PMID: 29076966 DOI: 10.1097/CCO.0000000000000423]

9 **van der Post RS**, Oliveira C, Guilford P, Carneiro F. Hereditary gastric cancer: what's new? *Fam Cancer* 2019; **18**: 363-367 [PMID: 30989426 DOI: 10.1007/s10689-019-00127-7]

10 **Reckamp KL**, Behrendt CE, Slavin TP, Gray SW, Castillo DK, Koczywas M, Cristea MC, Babski KM, Stearns D, Marcum CA, Rodriguez YP, Hass AJ, Vecchio MM, Mora P, Cervantes AE, Sand SR, Mejia RM, Tsou TC, Salgia R, Weitzel JN. Germline mutations and age at onset of lung adenocarcinoma. *Cancer* 2021; **127**: 2801-2806 [PMID: 33858029 DOI: 10.1002/cncr.33573]

11 **Oh M**, McBride A, Yun S, Bhattacharjee S, Slack M, Martin JR, Jeter J, Abraham I. BRCA1 and BRCA2 Gene Mutations and Colorectal Cancer Risk: Systematic Review and Meta-analysis. *J Natl Cancer Inst* 2018; **110**: 1178-1189 [PMID: 30380096 DOI: 10.1093/jnci/djy148]

12 **de Jonge MM**, Ritterhouse LL, de Kroon CD, Vreeswijk MPG, Segal JP, Puranik R, Hollema H, Rookus MA, van Asperen CJ, van Leeuwen FE, Smit VTHBM, Howitt BE, Bosse T; HEBON Group. Germline BRCA-Associated Endometrial Carcinoma Is a Distinct Clinicopathologic Entity. *Clin Cancer Res* 2019; **25**: 7517-7526 [PMID: 31492746 DOI: 10.1158/1078-0432.CCR-19-0848]

13 **Avanesyan AA**, Sokolenko AP, Ivantsov AO, Kleshchev MA, Maydin MA, Bizin IV, Raskin GA, Shelekhova KV, Gorodnova TV, Bessonov AA, Anisimova EI, Volynshchikova OA, Romanko AA, Ni VI, Broyde RV, Tkachenko OB, Whitehead AJ, Scherbakov AM, Imyanitov EN. Gastric Cancer in BRCA1 Germline Mutation Carriers: Results of Endoscopic Screening and Molecular Analysis of Tumor Tissues. *Pathobiology* 2020; **87**: 367-374 [PMID: 33161400 DOI: 10.1159/000511323]

14 **Kim H**, Choi DH, Park W, Im YH, Ahn JS, Park YH, Nam SJ, Kim SW, Lee JE, Yu JH, Lee SK, Jung BY. The association between non-breast and ovary cancers and BRCA mutation in first- and second-degree relatives of high-risk breast cancer patients: a large-scale study of Koreans. *Hered Cancer Clin Pract* 2019; **17**: 1 [PMID: 30622657 DOI: 10.1186/s13053-018-0103-3]

15 **Ko JM**, Ning L, Zhao XK, Chai AWY, Lei LC, Choi SSA, Tao L, Law S, Kwong A, Lee NP, Chan KT, Lo A, Song X, Chen PN, Chang YL, Wang LD, Lung ML. BRCA2 loss-of-function germline mutations are associated with esophageal squamous cell carcinoma risk in Chinese. *Int J Cancer* 2020; **146**: 1042-1051 [PMID: 31396961 DOI: 10.1002/ijc.32619]

16 **Maccaroni E**, Giampieri R, Lenci E, Scortichini L, Bianchi F, Belvederesi L, Brugiati C, Pagliaretta S, Ambrosini E, Berardi R. BRCA mutations and gastrointestinal cancers: When to expect the unexpected? *World J Clin Oncol* 2021; **12**: 565-580 [PMID: 34367929 DOI: 10.5306/wjco.v12.i7.565]

17 **Jonsson P**, Bandlamudi C, Cheng ML, Srinivasan P, Chavan SS, Friedman ND, Rosen EY, Richards AL, Bouvier N, Selcuklu SD, Bielski CM, Abida W, Mandelker D, Birsoy O, Zhang L, Zehir A, Donoghue MTA, Baselga J, Offit K, Scher HI, O'Reilly EM, Stadler ZK, Schultz N, Socci ND, Viale A, Ladanyi M, Robson ME, Hyman DM, Berger MF, Solit DB, Taylor BS. Tumour lineage shapes BRCA-mediated phenotypes. *Nature* 2019; **571**: 576-579 [PMID: 31292550 DOI: 10.1038/s41586-019-1382-1]

18 **Hodgson D**, Lai Z, Dearden S, Barrett JC, Harrington EA, Timms K, Lanchbury J, Wu W, Allen A, Senkus E, Domchek SM, Robson M. Analysis of mutation status and homologous recombination deficiency in tumors of patients with germline BRCA1 or BRCA2 mutations and metastatic breast cancer: OlympiAD. *Ann Oncol* 2021; **32**: 1582-1589 [PMID: 34500047 DOI: 10.1016/j.annonc.2021.08.2154]

19 **Martínez-Sánchez M**, Hernandez-Monge J, Rangel M, Olivares-Illana V. Retinoblastoma: from discovery to clinical management. *FEBS J* 2022; **289**: 4371-4382 [PMID: 34042282 DOI: 10.1111/febs.16035]

20 **Preobrazhenskaya EV**, Shleykina AU, Gorustovich OA, Martianov AS, Bizin IV, Anisimova EI, Sokolova TN, Chuinyshena SA, Kuligina ES, Togo AV, Belyaev AM, Ivantsov AO, Sokolenko AP, Imyanitov EN. Frequency and molecular characteristics of PALB2-associated cancers in Russian patients. *Int J Cancer* 2021; **148**: 203-210 [PMID: 32997802 DOI: 10.1002/ijc.33317]

21 **Iyevleva AG**, Aleksakhina SN, Sokolenko AP, Baskina SV, Venina AR, Anisimova EI, Bizin IV, Ivantsov AO, Belysheva YV, Chernyakova AP, Togo AV, Imyanitov EN. Somatic loss of the remaining allele occurs approximately in half of CHEK2-driven breast cancers and is accompanied by a border-line increase of chromosomal instability. *Breast Cancer Res Treat* 2022; **192**: 283-291 [PMID: 35020107 DOI: 10.1007/s10549-022-06517-3]

22 **McDonnell JE**, Gild ML, Clifton-Bligh RJ, Robinson BG. Multiple endocrine neoplasia: an update. *Intern Med J* 2019; **49**: 954-961 [PMID: 31387156 DOI: 10.1111/imj.14394]

23 **Powers JM**, Ebrahimzadeh JE, Katona BW. Genetic testing for hereditary gastrointestinal cancer syndromes: Interpreting results in today's practice. *Curr Treat Options Gastroenterol* 2019; **17**: 636-649 [PMID: 31761969 DOI: 10.1007/s11938-019-00253-2]

24 **Magrin L**, Fanale D, Brando C, Fiorino A, Corsini LR, Sciacchitano R, Filorizzo C, Dimino A, Russo A, Bazan V. POLE, POLD1, and NTHL1: the last but not the least hereditary cancer-predisposing genes. *Oncogene* 2021; **40**: 5893-5901 [PMID: 34363023 DOI: 10.1038/s41388-021-01984-2]

25 **Sokolenko AP**, Bogdanova N, Kluzniak W, Preobrazhenskaya EV, Kuligina ES, Iyevleva AG, Aleksakhina SN, Mitiushkina NV, Gorodnova TV, Bessonov AA, Togo AV, Lubiński J, Cybulski C, Jakubowska A, Dörk T, Imyanitov EN. Double heterozygotes among breast cancer patients analyzed for BRCA1, CHEK2, ATM, NBN/NBS1, and BLM germ-line mutations. *Breast Cancer Res Treat* 2014; **145**: 553-562 [PMID: 24800916 DOI: 10.1007/s10549-014-2971-1]

26 **Hilbers FS**, van 't Hof PJ, Meijers CM, Mei H, Michailidou K, Dennis J, Hogervorst FBL, Nederlof PM, van Asperen CJ, Devilee P. Clustering of known low and moderate risk alleles rather than a novel recessive high-risk gene in non-BRCA1/2 sib trios affected with breast cancer. *Int J Cancer* 2020; **147**: 2708-2716 [PMID: 32383162 DOI:

10.1002/ijc.33039]
27 **Schubert SA**, Ruano D, Tiersma Y, Drost M, de Wind N, Nielsen M, van Hest LP, Morreau H, de Miranda NFCC, van Wezel T. Digenic inheritance of MSH6 and MUTYH variants in familial colorectal cancer. *Genes Chromosomes Cancer* 2020; **59**: 697-701 [PMID: 32615015 DOI: 10.1002/gcc.22883]
28 **Gao C**, Polley EC, Hart SN, Huang H, Hu C, Gnanaolivu R, Lilyquist J, Boddicker NJ, Na J, Ambrosone CB, Auer PL, Bernstein L, Burnside ES, Eliassen AH, Gaudet MM, Haiman C, Hunter DJ, Jacobs EJ, John EM, Lindström S, Ma H, Neuhausen SL, Newcomb PA, O'Brien KM, Olson JE, Ong IM, Patel AV, Palmer JR, Sandler DP, Tamimi R, Taylor JA, Teras LR, Trentham-Dietz A, Vachon CM, Weinberg CR, Yao S, Weitzel JN, Goldgar DE, Domchek SM, Nathanson KL, Couch FJ, Kraft P. Risk of Breast Cancer Among Carriers of Pathogenic Variants in Breast Cancer Predisposition Genes Varies by Polygenic Risk Score. *J Clin Oncol* 2021; **39**: 2564-2573 [PMID: 34101481 DOI: 10.1200/JCO.20.01992]
29 **Brown GR**, Simon M, Wentling C, Spencer DM, Parker AN, Rogers CA. A review of inherited cancer susceptibility syndromes. *JAAPA* 2020; **33**: 10-16 [PMID: 33234888 DOI: 10.1097/01.JAA.0000721648.46099.2c]
30 **Whitworth J**, Smith PS, Martin JE, West H, Luchetti A, Rodger F, Clark G, Carss K, Stephens J, Stirrups K, Penkett C, Mapeta R, Ashford S, Megy K, Shakeel H, Ahmed M, Adlard J, Barwell J, Brewer C, Casey RT, Armstrong R, Cole T, Evans DG, Fostira F, Greenhalgh L, Hanson H, Henderson A, Hoffman J, Izatt L, Kumar A, Kwong A, Lalloo F, Ong KR, Paterson J, Park SM, Chen-Shtoyerman R, Searle C, Side L, Skytte AB, Snape K, Woodward ER; NIHR BioResource Rare Diseases Consortium, Tischkowitz MD, Maher ER. Comprehensive Cancer-Predisposition Gene Testing in an Adult Multiple Primary Tumor Series Shows a Broad Range of Deleterious Variants and Atypical Tumor Phenotypes. *Am J Hum Genet* 2018; **103**: 3-18 [PMID: 29909963 DOI: 10.1016/j.ajhg.2018.04.013]
31 **Holstege H**, Joosse SA, van Oostrom CT, Nederlof PM, de Vries A, Jonkers J. High incidence of protein-truncating TP53 mutations in BRCA1-related breast cancer. *Cancer Res* 2009; **69**: 3625-3633 [PMID: 19336573 DOI: 10.1158/0008-5472.CAN-08-3426]
32 **Mavaddat N**, Barrowdale D, Andrulis IL, Domchek SM, Eccles D, Nevanlinna H, Ramus SJ, Spurdle A, Robson M, Sherman M, Mulligan AM, Couch FJ, Engel C, McGuffog L, Healey S, Sinilnikova OM, Southey MC, Terry MB, Goldgar D, O'Malley F, John EM, Janavicius R, Tihomirova L, Hansen TV, Nielsen FC, Osorio A, Stavropoulou A, Benítez J, Manoukian S, Peissel B, Barile M, Volorio S, Pasini B, Dolcetti R, Putignano AL, Ottini L, Radice P, Hamann U, Rashid MU, Hogervorst FB, Kriege M, van der Luijt RB; HEBON, Peock S, Frost D, Evans DG, Brewer C, Walker L, Rogers MT, Side LE, Houghton C; EMBRACE, Weaver J, Godwin AK, Schmutzler RK, Wappenschmidt B, Meindl A, Kast K, Arnold N, Niederacher D, Sutter C, Deissler H, Gadzicki D, Preisler-Adams S, Varon-Mateeva R, Schönbuchner I, Gevensleben H, Stoppa-Lyonnet D, Belotti M, Barjhoux L; GEMO Study Collaborators, Isaacs C, Peshkin BN, Caldes T, de la Hoya M, Cañadas C, Heikkinen T, Heikkilä P, Aittomäki K, Blanco I, Lazaro C, Brunet J, Agnarsson BA, Arason A, Barkardottir RB, Dumont M, Simard J, Montagna M, Agata S, D'Andrea E, Yan M, Fox S; kConFab Investigators, Rebbeck TR, Rubinstein W, Tung N, Garber JE, Wang X, Fredericksen Z, Pankratz VS, Lindor NM, Szabo C, Offit K, Sakr R, Gaudet MM, Singer CF, Tea MK, Rappaport C, Mai PL, Greene MH, Sokolenko A, Imyanitov E, Toland AE, Senter L, Sweet K, Thomassen M, Gerdes AM, Kruse T, Caligo M, Aretini P, Rantala J, von Wachenfeld A, Henriksson K; SWE-BRCA Collaborators, Steele L, Neuhausen SL, Nussbaum R, Beattie M, Odunsi K, Sucheston L, Gayther SA, Nathanson K, Gross J, Walsh C, Karlan B, Chenevix-Trench G, Easton DF, Antoniou AC; Consortium of Investigators of Modifiers of BRCA1/2. Pathology of breast and OCs among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). *Cancer Epidemiol Biomarkers Prev* 2012; **21**: 134-147 [PMID: 22144499 DOI: 10.1158/1055-9965.EPI-11-0775]
33 **Hoyer J**, Vasileiou G, Uebe S, Wunderle M, Kraus C, Fasching PA, Thiel CT, Hartmann A, Beckmann MW, Lux MP, Reis A. Addition of triple negativity of breast cancer as an indicator for germline mutations in predisposing genes increases sensitivity of clinical selection criteria. *BMC Cancer* 2018; **18**: 926 [PMID: 30257646 DOI: 10.1186/s12885-018-4821-8]
34 **Breast Cancer Association Consortium**, Mavaddat N, Dorling L, Carvalho S, Allen J, González-Neira A, Keeman R, Bolla MK, Dennis J, Wang Q, Ahearn TU, Andrulis IL, Beckmann MW, Behrens S, Benitez J, Bermisheva M, Blomqvist C, Bogdanova NV, Bojesen SE, Briceno I, Brüning T, Camp NJ, Campbell A, Castelao JE, Chang-Claude J, Chanock SJ, Chenevix-Trench G, Christiansen H, Czene K, Dörk T, Eriksson M, Evans DG, Fasching PA, Figueroa JD, Flyger H, Gabrielson M, Gago-Dominguez M, Geisler J, Giles GG, Guénel P, Hadjisavvas A, Hahnen E, Hall P, Hamann U, Hartikainen JM, Hartman M, Hoppe R, Howell A, Jakubowska A, Jung A, Khusnutdinova EK, Kristensen VN, Li J, Lim SH, Lindblom A, Loizidou MA, Lophatananon A, Lubinski J, Madsen MJ, Mannermaa A, Manoochehri M, Margolin S, Mavroudis D, Milne RL, Mohd Taib NA, Morra A, Muir K, Obi N, Osorio A, Park-Simon TW, Peterlongo P, Radice P, Saloustros E, Sawyer EJ, Schmutzler RK, Shah M, Sim X, Southey MC, Thorne H, Tomlinson I, Torres D, Truong T, Yip CH, Spurdle AB, Vreeswijk MPG, Dunning AM, García-Closas M, Pharoah PDP, Kvist A, Muranen TA, Nevanlinna H, Teo SH, Devilee P, Schmidt MK, Easton DF. Pathology of Tumors Associated With Pathogenic Germline Variants in 9 Breast Cancer Susceptibility Genes. *JAMA Oncol* 2022; **8**: e216744 [PMID: 35084436 DOI: 10.1001/jamaoncol.2021.6744]
35 **Waks AG**, Winer EP. Breast Cancer Treatment: A Review. *JAMA* 2019; **321**: 288-300 [PMID: 30667505 DOI: 10.1001/jama.2018.19323]
36 **Fierheller CT**, Alenezi WM, Tonin PN. The Genetic Analyses of French Canadians of Quebec Facilitate the Characterization of New Cancer Predisposing Genes Implicated in Hereditary Breast and/or Ovarian Cancer Syndrome Families. *Cancers (Basel)* 2021; **13** [PMID: 34298626 DOI: 10.3390/cancers13143406]
37 **Jayson GC**, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. *Lancet* 2014; **384**: 1376-1388 [PMID: 24767708 DOI: 10.1016/S0140-6736(13)62146-7]
38 **Lheureux S**, Gourley C, Vergote I, Oza AM. Epithelial ovarian cancer. *Lancet* 2019; **393**: 1240-1253 [PMID: 30910306 DOI: 10.1016/S0140-6736(18)32552-2]
39 **Loibl S**, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. *Lancet* 2021; **397**: 1750-1769 [PMID: 33812473 DOI: 10.1016/S0140-6736(20)32381-3]
40 **Kuchenbaecker KB**, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, Jervis S, van Leeuwen FE, Milne

RL, Andrieu N, Goldgar DE, Terry MB, Rookus MA, Easton DF, Antoniou AC; BRCA1 and BRCA2 Cohort Consortium, McGuffog L, Evans DG, Barrowdale D, Frost D, Adlard J, Ong KR, Izatt L, Tischkowitz M, Eeles R, Davidson R, Hodgson S, Ellis S, Nogues C, Lasset C, Stoppa-Lyonnet D, Fricker JP, Faivre L, Berthet P, Hooning MJ, van der Kolk LE, Kets CM, Adank MA, John EM, Chung WK, Andrulis IL, Southey M, Daly MB, Buys SS, Osorio A, Engel C, Kast K, Schmutzler RK, Caldes T, Jakubowska A, Simard J, Friedlander ML, McLachlan SA, Machackova E, Foretova L, Tan YY, Singer CF, Olah E, Gerdes AM, Arver B, Olsson H. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *JAMA* 2017; **317**: 2402-2416 [PMID: 28632866 DOI: 10.1001/jama.2017.7112]

41 **Rebbeck TR**, Mitra N, Wan F, Sinilnikova OM, Healey S, McGuffog L, Mazoyer S, Chenevix-Trench G, Easton DF, Antoniou AC, Nathanson KL; CIMBA Consortium, Laitman Y, Kushnir A, Paluch-Shimon S, Berger R, Zidan J, Friedman E, Ehrencrona H, Stenmark-Askmalm M, Einbeigi Z, Loman N, Harbst K, Rantala J, Melin B, Huo D, Olopade OI, Seldon J, Ganz PA, Nussbaum RL, Chan SB, Odunsi K, Gayther SA, Domchek SM, Arun BK, Lu KH, Mitchell G, Karlan BY, Walsh C, Lester J, Godwin AK, Pathak H, Ross E, Daly MB, Whittemore AS, John EM, Miron A, Terry MB, Chung WK, Goldgar DE, Buys SS, Janavicius R, Tihomirova L, Tung N, Dorfling CM, van Rensburg EJ, Steele L, Neuhausen SL, Ding YC, Ejlertsen B, Gerdes AM, Hansen Tv, Ramón y Cajal T, Osorio A, Benitez J, Godino J, Tejada MI, Duran M, Weitzel JN, Bobolis KA, Sand SR, Fontaine A, Savarese A, Pasini B, Peissel B, Bonanni B, Zaffaroni D, Vignolo-Lutati F, Scuvera G, Giannini G, Bernard L, Genuardi M, Radice P, Dolcetti R, Manoukian S, Pensotti V, Gismondi V, Yannoukakos D, Fostira F, Garber J, Torres D, Rashid MU, Hamann U, Peock S, Frost D, Platte R, Evans DG, Eeles R, Davidson R, Eccles D, Cole T, Cook J, Brewer C, Hodgson S, Morrison PJ, Walker L, Porteous ME, Kennedy MJ, Izatt L, Adlard J, Donaldson A, Ellis S, Sharma P, Schmutzler RK, Wappenschmidt B, Becker A, Rhiem K, Hahnen E, Engel C, Meindl A, Engert S, Ditsch N, Arnold N, Plendl HJ, Mundhenke C, Niederacher D, Fleisch M, Sutter C, Bartram CR, Dikow N, Wang-Gohrke S, Gadzicki D, Steinemann D, Kast K, Beer M, Varon-Mateeva R, Gehrig A, Weber BH, Stoppa-Lyonnet D, Sinilnikova OM, Mazoyer S, Houdayer C, Belotti M, Gauthier-Villars M, Damiola F, Boutry-Kryza N, Lasset C, Sobol H, Peyrat JP, Muller D, Fricker JP, Collonge-Rame MA, Mortemousque I, Nogues C, Rouleau E, Isaacs C, De Paepe A, Poppe B, Claes K, De Leeneer K, Piedmonte M, Rodriguez G, Wakely K, Boggess J, Blank SV, Basil J, Azodi M, Phillips KA, Caldes T, de la Hoya M, Romero A, Nevanlinna H, Aittomäki K, van der Hout AH, Hogervorst FB, Verhoef S, Collée JM, Seynaeve C, Oosterwijk JC, Gille JJ, Wijnen JT, Gómez Garcia EB, Kets CM, Ausems MG, Aalfs CM, Devilee P, Mensenkamp AR, Kwong A, Olah E, Papp J, Diez O, Lazaro C, Darder E, Blanco I, Salinas M, Jakubowska A, Lubinski J, Gronwald J, Jaworska-Bieniek K, Durda K, Sukiennicki G, Huzarski T, Byrski T, Cybulski C, Toloczko-Grabarek A, Złowocka-Perłowska E, Menkiszak J, Arason A, Barkardottir RB, Simard J, Laframboise R, Montagna M, Agata S, Alducci E, Peixoto A, Teixeira MR, Spurdle AB, Lee MH, Park SK, Kim SW, Friebel TM, Couch FJ, Lindor NM, Pankratz VS, Guidugli L, Wang X, Tischkowitz M, Foretova L, Vijai J, Offit K, Robson M, Rau-Murthy R, Kauff N, Fink-Retter A, Singer CF, Rappaport C, Gschwantler-Kaulich D, Pfeiler G, Tea MK, Berger A, Greene MH, Mai PL, Imyanitov EN, Toland AE, Senter L, Bojesen A, Pedersen IS, Skytte AB, Sunde L, Thomassen M, Moeller ST, Kruse TA, Jensen UB, Caligo MA, Aretini P, Teo SH, Selkirk CG, Hulick PJ, Andrulis I. Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. *JAMA* 2015; **313**: 1347-1361 [PMID: 25849179 DOI: 10.1001/jama.2014.5985]

42 **Milne RL**, Antoniou AC. Modifiers of breast and ovarian cancer risks for BRCA1 and BRCA2 mutation carriers. *Endocr Relat Cancer* 2016; **23**: T69-T84 [PMID: 27528622 DOI: 10.1530/ERC-16-0277]

43 **Sun J**, Meng H, Yao L, Lv M, Bai J, Zhang J, Wang L, Ouyang T, Li J, Wang T, Fan Z, Fan T, Lin B, Xie Y. Germline Mutations in Cancer Susceptibility Genes in a Large Series of Unselected Breast Cancer Patients. *Clin Cancer Res* 2017; **23**: 6113-6119 [PMID: 28724667 DOI: 10.1158/1078-0432.CCR-16-3227]

44 **Gorodnova T**, Sokolenko A, Ni V, Ivantsov A, Kotiv K, Petrik S, Amelina I, Berlev I, Imyanitov E. BRCA1-associated and sporadic ovarian carcinomas: outcomes of primary cytoreductive surgery or neoadjuvant chemotherapy. *Int J Gynecol Cancer* 2019; **29**: 779-786 [PMID: 30839285 DOI: 10.1136/ijgc-2018-000175]

45 **Kurian AW**, Ward KC, Howlader N, Deapen D, Hamilton AS, Mariotto A, Miller D, Penberthy LS, Katz SJ. Genetic Testing and Results in a Population-Based Cohort of Breast Cancer Patients and Ovarian Cancer Patients. *J Clin Oncol* 2019; **37**: 1305-1315 [PMID: 30964716 DOI: 10.1200/JCO.18.01854]

46 **Pietragalla A**, Arcieri M, Marchetti C, Scambia G, Fagotti A. Ovarian cancer predisposition beyond BRCA1 and BRCA2 genes. *Int J Gynecol Cancer* 2020; **30**: 1803-1810 [PMID: 32895312 DOI: 10.1136/ijgc-2020-001556]

47 **Colas C**, Golmard L, de Pauw A, Caputo SM, Stoppa-Lyonnet D. "Decoding hereditary breast cancer" benefits and questions from multigene panel testing. *Breast* 2019; **45**: 29-35 [PMID: 30822622 DOI: 10.1016/j.breast.2019.01.002]

48 **Yang X**, Song H, Leslie G, Engel C, Hahnen E, Auber B, Horváth J, Kast K, Niederacher D, Turnbull C, Houlston R, Hanson H, Loveday C, Dolinsky JS, LaDuca H, Ramus SJ, Menon U, Rosenthal AN, Jacobs I, Gayther SA, Dicks E, Nevanlinna H, Aittomäki K, Pelttari LM, Ehrencrona H, Borg Å, Kvist A, Rivera B, Hansen TVO, Djursby M, Lee A, Dennis J, Bowtell DD, Traficante N, Diez O, Balmaña J, Gruber SB, Chenevix-Trench G, Investigators K, Jensen A, Kjær SK, Høgdall E, Castéra L, Garber J, Janavicius R, Osorio A, Golmard L, Vega A, Couch FJ, Robson M, Gronwald J, Domchek SM, Culver JO, de la Hoya M, Easton DF, Foulkes WD, Tischkowitz M, Meindl A, Schmutzler RK, Pharoah PDP, Antoniou AC. Ovarian and Breast Cancer Risks Associated With Pathogenic Variants in RAD51C and RAD51D. *J Natl Cancer Inst* 2020; **112**: 1242-1250 [PMID: 32107557 DOI: 10.1093/jnci/djaa030]

49 **Setton J**, Selenica P, Mukherjee S, Shah R, Pecorari I, McMillan B, Pei IX, Kemel Y, Ceyhan-Birsoy O, Sheehan M, Tkachuk K, Brown DN, Zhang L, Cadoo K, Powell S, Weigelt B, Robson M, Riaz N, Offit K, Reis-Filho JS, Mandelker D. Germline RAD51B variants confer susceptibility to breast and ovarian cancers deficient in homologous recombination. *NPJ Breast Cancer* 2021; **7**: 135 [PMID: 34635660 DOI: 10.1038/s41523-021-00339-0]

50 **Antoniou AC**, Casadei S, Heikkinen T, Barrowdale D, Pylkäs K, Roberts J, Lee A, Subramanian D, De Leeneer K, Fostira F, Tomiak E, Neuhausen SL, Teo ZL, Khan S, Aittomäki K, Moilanen JS, Turnbull C, Seal S, Mannermaa A, Kallioniemi A, Lindeman GJ, Buys SS, Andrulis IL, Radice P, Tondini C, Manoukian S, Toland AE, Miron P, Weitzel JN, Domchek SM, Poppe B, Claes KB, Yannoukakos D, Concannon P, Bernstein JL, James PA, Easton DF, Goldgar DE, Hopper JL, Rahman N, Peterlongo P, Nevanlinna H, King MC, Couch FJ, Southey MC, Winqvist R, Foulkes WD, Tischkowitz M. Breast-cancer risk in families with mutations in PALB2. *N Engl J Med* 2014; **371**: 497-506 [PMID:

25099575 DOI: 10.1056/NEJMoa1400382]

51 **Song H**, Dicks EM, Tyrer J, Intermaggio M, Chenevix-Trench G, Bowtell DD, Traficante N; Group A, Brenton J, Goranova T, Hosking K, Piskorz A, van Oudenhove E, Doherty J, Harris HR, Rossing MA, Duerst M, Dork T, Bogdanova NV, Modugno F, Moysich K, Odunsi K, Ness R, Karlan BY, Lester J, Jensen A, Krüger Kjaer S, Høgdall E, Campbell IG, Lázaro C, Pujara MA, Cunningham J, Vierkant R, Winham SJ, Hildebrandt M, Huff C, Li D, Wu X, Yu Y, Permuth JB, Levine DA, Schildkraut JM, Riggan MJ, Berchuck A, Webb PM; Group OS, Cybulski C, Gronwald J, Jakubowska A, Lubinski J, Alsop J, Harrington P, Chan I, Menon U, Pearce CL, Wu AH, de Fazio A, Kennedy CJ, Goode E, Ramus S, Gayther S, Pharoah P. Population-based targeted sequencing of 54 candidate genes identifies PALB2 as a susceptibility gene for high-grade serous ovarian cancer. *J Med Genet* 2021; **58**: 305-313 [PMID: 32546565 DOI: 10.1136/jmedgenet-2019-106739]

52 **Sokolenko AP**, Iyevleva AG, Preobrazhenskaya EV, Mitiushkina NV, Abysheva SN, Suspitsin EN, Kuligina ESh, Gorodnova TV, Pfeifer W, Togo AV, Turkevich EA, Ivantsov AO, Voskresenskiy DV, Dolmatov GD, Bit-Sava EM, Matsko DE, Semiglazov VF, Fichtner I, Larionov AA, Kuznetsov SG, Antoniou AC, Imyanitov EN. High prevalence and breast cancer predisposing role of the BLM c.1642 C>T (Q548X) mutation in Russia. *Int J Cancer* 2012; **130**: 2867-2873 [PMID: 21815139 DOI: 10.1002/ijc.26342]

53 **Cybulski C**, Carrot-Zhang J, Kluźniak W, Rivera B, Kashyap A, Wokołorczyk D, Giroux S, Nadaf J, Hamel N, Zhang S, Huzarski T, Gronwald J, Byrski T, Szwiec M, Jakubowska A, Rudnicka H, Lener M, Masojć B, Tonin PN, Rousseau F, Górski B, Dębniak T, Majewski J, Lubiński J, Foulkes WD, Narod SA, Akbari MR. Germline RECQL mutations are associated with breast cancer susceptibility. *Nat Genet* 2015; **47**: 643-646 [PMID: 25915596 DOI: 10.1038/ng.3284]

54 **Neidhardt G**, Hauke J, Ramser J, Groß E, Gehrig A, Müller CR, Kahlert AK, Hackmann K, Honisch E, Niederacher D, Heilmann-Heimbach S, Franke A, Lieb W, Thiele H, Altmüller J, Nürnberg P, Klaschik K, Ernst C, Ditsch N, Jessen F, Ramirez A, Wappenschmidt B, Engel C, Rhiem K, Meindl A, Schmutzler RK, Hahnen E. Association Between Loss-of-Function Mutations Within the FANCM Gene and Early-Onset Familial Breast Cancer. *JAMA Oncol* 2017; **3**: 1245-1248 [PMID: 28033443 DOI: 10.1001/jamaoncol.2016.5592]

55 **Thompson ER**, Doyle MA, Ryland GL, Rowley SM, Choong DY, Tothill RW, Thorne H; kConFab, Barnes DR, Li J, Ellul J, Philip GK, Antill YC, James PA, Trainer AH, Mitchell G, Campbell IG. Exome sequencing identifies rare deleterious mutations in DNA repair genes FANCC and BLM as potential breast cancer susceptibility alleles. *PLoS Genet* 2012; **8**: e1002894 [PMID: 23028338 DOI: 10.1371/journal.pgen.1002894]

56 **Felicio PS**, Grasel RS, Campacci N, de Paula AE, Galvão HCR, Torrezan GT, Sabato CS, Fernandes GC, Souza CP, Michelli RD, Andrade CE, Barros BDF, Matsushita MM, Revil T, Ragoussis J, Couch FJ, Hart SN, Reis RM, Melendez ME, Tonin PN, Carraro DM, Palmero EI. Whole-exome sequencing of non-BRCA1/BRCA2 mutation carrier cases at high-risk for hereditary breast/ovarian cancer. *Hum Mutat* 2021; **42**: 290-299 [PMID: 33326660 DOI: 10.1002/humu.24158]

57 **Kuligina ES**, Sokolenko AP, Bizin IV, Romanko AA, Zagorodnev KA, Anisimova MO, Krylova DD, Anisimova EI, Mantseva MA, Varma AK, Hasan SK, Ni VI, Koloskov AV, Suspitsin EN, Venina AR, Aleksakhina SN, Sokolova TN, Milanović AM, Schürmann P, Prokofyeva DS, Bermisheva MA, Khusnutdinova EK, Bogdanova N, Dörk T, Imyanitov EN. Exome sequencing study of Russian breast cancer patients suggests a predisposing role for USP39. *Breast Cancer Res Treat* 2020; **179**: 731-742 [PMID: 31754952 DOI: 10.1007/s10549-019-05492-6]

58 **Koivuluoma S**, Tervasmäki A, Kauppila S, Winqvist R, Kumpula T, Kuismin O, Moilanen J, Pylkäs K. Exome sequencing identifies a recurrent variant in SERPINA3 associating with hereditary susceptibility to breast cancer. *Eur J Cancer* 2021; **143**: 46-51 [PMID: 33279852 DOI: 10.1016/j.ejca.2020.10.033]

59 **Shin SJ**, Dodd-Eaton EB, Peng G, Bojadzieva J, Chen J, Amos CI, Frone MN, Khincha PP, Mai PL, Savage SA, Ballinger ML, Thomas DM, Yuan Y, Strong LC, Wang W. Penetrance of Different Cancer Types in Families with Li-Fraumeni Syndrome: A Validation Study Using Multicenter Cohorts. *Cancer Res* 2020; **80**: 354-360 [PMID: 31719101 DOI: 10.1158/0008-5472.CAN-19-0728]

60 **Lolas Hamameh S**, Renbaum P, Kamal L, Dweik D, Salahat M, Jaraysa T, Abu Rayyan A, Casadei S, Mandell JB, Gulsuner S, Lee MK, Walsh T, King MC, Levy-Lahad E, Kanaan M. Genomic analysis of inherited breast cancer among Palestinian women: Genetic heterogeneity and a founder mutation in TP53. *Int J Cancer* 2017; **141**: 750-756 [PMID: 28486781 DOI: 10.1002/ijc.30771]

61 **Fortuno C**, James PA, Spurdle AB. Current review of TP53 pathogenic germline variants in breast cancer patients outside Li-Fraumeni syndrome. *Hum Mutat* 2018; **39**: 1764-1773 [PMID: 30240537 DOI: 10.1002/humu.23656]

62 **Rana HQ**, Gelman R, LaDuca H, McFarland R, Dalton E, Thompson J, Speare V, Dolinsky JS, Chao EC, Garber JE. Differences in TP53 Mutation Carrier Phenotypes Emerge From Panel-Based Testing. *J Natl Cancer Inst* 2018; **110**: 863-870 [PMID: 29529297 DOI: 10.1093/jnci/djy001]

63 **Kratz CP**, Freycon C, Maxwell KN, Nichols KE, Schiffman JD, Evans DG, Achatz MI, Savage SA, Weitzel JN, Garber JE, Hainaut P, Malkin D. Analysis of the Li-Fraumeni Spectrum Based on an International Germline TP53 Variant Data Set: An International Agency for Research on Cancer TP53 Database Analysis. *JAMA Oncol* 2021; **7**: 1800-1805 [PMID: 34709361 DOI: 10.1001/jamaoncol.2021.4398]

64 **Corso G**, Montagna G, Figueiredo J, La Vecchia C, Fumagalli Romario U, Fernandes MS, Seixas S, Roviello F, Trovato C, Guerini-Rocco E, Fusco N, Pravettoni G, Petrocchi S, Rotili A, Massari G, Magnoni F, De Lorenzi F, Bottoni M, Galimberti V, Sanches JM, Calvello M, Seruca R, Bonanni B. Hereditary Gastric and Breast Cancer Syndromes Related to CDH1 Germline Mutation: A Multidisciplinary Clinical Review. *Cancers (Basel)* 2020; **12** [PMID: 32560361 DOI: 10.3390/cancers12061598]

65 **Bonadona V**, Bonaïti B, Olschwang S, Grandjouan S, Huiart L, Longy M, Guimbaud R, Buecher B, Bignon YJ, Caron O, Colas C, Noguès C, Lejeune-Dumoulin S, Olivier-Faivre L, Polycarpe-Osaer F, Nguyen TD, Desseigne F, Saurin JC, Berthet P, Leroux D, Duffour J, Manouvrier S, Frébourg T, Sobol H, Lasset C, Bonaïti-Pellié C; French Cancer Genetics Network. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA* 2011; **305**: 2304-2310 [PMID: 21642682 DOI: 10.1001/jama.2011.743]

66 **Therkildsen C**, Ladelund S, Smith-Hansen L, Lindberg LJ, Nilbert M. Towards gene- and gender-based risk estimates in

Lynch syndrome; age-specific incidences for 13 extra-colorectal cancer types. *Br J Cancer* 2017; **117**: 1702-1710 [PMID: 29065108 DOI: 10.1038/bjc.2017.348]

67 **Carnevali I**, Riva C, Chiaravalli AM, Sahnane N, Di Lauro E, Viel A, Rovera F, Formenti G, Ghezzi F, Sessa F, Tibiletti MG. Inherited cancer syndromes in 220 Italian ovarian cancer patients. *Cancer Genet* 2019; **237**: 55-62 [PMID: 31447066 DOI: 10.1016/j.cancergen.2019.06.005]

68 **Dominguez-Valentin M**, Sampson JR, Seppälä TT, Ten Broeke SW, Plazzer JP, Nakken S, Engel C, Aretz S, Jenkins MA, Sunde L, Bernstein I, Capella G, Balaguer F, Thomas H, Evans DG, Burn J, Greenblatt M, Hovig E, de Vos Tot Nederveen Cappel WH, Sijmons RH, Bertario L, Tibiletti MG, Cavestro GM, Lindblom A, Della Valle A, Lopez-Köstner F, Gluck N, Katz LH, Heinimann K, Vaccaro CA, Büttner R, Görgens H, Holinski-Feder E, Morak M, Holzapfel S, Hüneburg R, Knebel Doeberitz MV, Loeffler M, Rahner N, Schackert HK, Steinke-Lange V, Schmiegel W, Vangala D, Pylvänäinen K, Renkonen-Sinisalo L, Hopper JL, Win AK, Haile RW, Lindor NM, Gallinger S, Le Marchand L, Newcomb PA, Figueiredo JC, Thibodeau SN, Wadt K, Therkildsen C, Okkels H, Ketabi Z, Moreira L, Sánchez A, Serra-Burriel M, Pineda M, Navarro M, Blanco I, Green K, Lalloo F, Crosbie EJ, Hill J, Denton OG, Frayling IM, Rødland EA, Vasen H, Mints M, Neffa F, Esperon P, Alvarez K, Kariv R, Rosner G, Pinero TA, Gonzalez ML, Kalfayan P, Tjandra D, Winship IM, Macrae F, Möslein G, Mecklin JP, Nielsen M, Møller P. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. *Genet Med* 2020; **22**: 15-25 [PMID: 31337882 DOI: 10.1038/s41436-019-0596-9]

69 **Lerner-Ellis J**, Mighton C, Lazaro C, Watkins N, Di Gioacchino V, Wong A, Chang MC, Charames GS. Multigene panel testing for hereditary breast and ovarian cancer in the province of Ontario. *J Cancer Res Clin Oncol* 2021; **147**: 871-879 [PMID: 32885271 DOI: 10.1007/s00432-020-03377-6]

70 **Shanbhogue KP**, Prasad AS, Ucisik-Keser FE, Katabathina VS, Morani AC. Hereditary ovarian tumour syndromes: current update on genetics and imaging. *Clin Radiol* 2021; **76**: 313.e15-313.e26 [PMID: 33353730 DOI: 10.1016/j.crad.2020.11.116]

71 **Zhu Q**, Zhang J, Chen Y, Hu Q, Shen H, Huang RY, Liu Q, Kaur J, Long M, Battaglia S, Eng KH, Lele SB, Zsiros E, Villella J, Lugade A, Yao S, Liu S, Moysich K, Odunsi KO. Whole-exome sequencing of ovarian cancer families uncovers putative predisposition genes. *Int J Cancer* 2020; **146**: 2147-2155 [PMID: 31265121 DOI: 10.1002/ijc.32545]

72 **Stradella A**, Del Valle J, Rofes P, Vargas-Parra G, Salinas M, González S, Montes E, López-Doriga A, Gómez C, de Cid R, Darder E, Teulé A, Solanes A, Munté E, Capellà G, Pineda M, Feliubadaló L, Brunet J, Lázaro C. ERCC3, a new ovarian cancer susceptibility gene? *Eur J Cancer* 2020; **141**: 1-8 [PMID: 33125943 DOI: 10.1016/j.ejca.2020.09.023]

73 **Lynch HT**, Krush AJ, Larsen AL. Heredity and multiple primary malignant neoplasms: six cancer families. *Am J Med Sci* 1967; **254**: 322-329 [PMID: 6054534 DOI: 10.1097/00000441-196709000-00007]

74 **Ligtenberg MJ**, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, Lee TY, Bodmer D, Hoenselaar E, Hendriks-Cornelissen SJ, Tsui WY, Kong CK, Brunner HG, van Kessel AG, Yuen ST, van Krieken JH, Leung SY, Hoogerbrugge N. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet* 2009; **41**: 112-117 [PMID: 19098912 DOI: 10.1038/ng.283]

75 **Yanus GA**, Belyaeva AV, Ivantsov AO, Kuligina ESh, Suspitsin EN, Mitiushkina NV, Aleksakhina SN, Iyevleva AG, Zaitseva OA, Yatsuk OS, Gorodnova TV, Strelkova TN, Efremova SA, Lepenchuk AY, Ochir-Garyaev AN, Paneyah MB, Matsko DE, Togo AV, Imyanitov EN. Pattern of clinically relevant mutations in consecutive series of Russian colorectal cancer patients. *Med Oncol* 2013; **30**: 686 [PMID: 23943423 DOI: 10.1007/s12032-013-0686-5]

76 **Valle L**, Vilar E, Tavtigian SV, Stoffel EM. Genetic predisposition to colorectal cancer: syndromes, genes, classification of genetic variants and implications for precision medicine. *J Pathol* 2019; **247**: 574-588 [PMID: 30584801 DOI: 10.1002/path.5229]

77 **Cohen SA**, Pritchard CC, Jarvik GP. Lynch Syndrome: From Screening to Diagnosis to Treatment in the Era of Modern Molecular Oncology. *Annu Rev Genomics Hum Genet* 2019; **20**: 293-307 [PMID: 30848956 DOI: 10.1146/annurev-genom-083118-015406]

78 **Clark SK**. Management of genetically determined colorectal cancer. *Surgeon* 2019; **17**: 165-171 [PMID: 30935877 DOI: 10.1016/j.surge.2019.03.003]

79 **Terradas M**, Capellá G, Valle L. Dominantly Inherited Hereditary Nonpolyposis Colorectal Cancer Not Caused by MMR Genes. *J Clin Med* 2020; **9** [PMID: 32585810 DOI: 10.3390/jcm9061954]

80 **Latham A**, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, Middha S, Hechtman J, Zehir A, Dubard-Gault M, Tran C, Stewart C, Sheehan M, Penson A, DeLair D, Yaeger R, Vijai J, Mukherjee S, Galle J, Dickson MA, Janjigian Y, O'Reilly EM, Segal N, Saltz LB, Reidy-Lagunes D, Varghese AM, Bajorin D, Carlo MI, Cadoo K, Walsh MF, Weiser M, Aguilar JG, Klimstra DS, Diaz LA Jr, Baselga J, Zhang L, Ladanyi M, Hyman DM, Solit DB, Robson ME, Taylor BS, Offit K, Berger MF, Stadler ZK. Microsatellite Instability Is Associated With the Presence of Lynch Syndrome Pan-Cancer. *J Clin Oncol* 2019; **37**: 286-295 [PMID: 30376427 DOI: 10.1200/JCO.18.00283]

81 **Vilar E**, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. *Nat Rev Clin Oncol* 2010; **7**: 153-162 [PMID: 20142816 DOI: 10.1038/nrclinonc.2009.237]

82 **Cavazza A**, Radia C, Harlow C, Monahan KJ. Experience of the implementation and outcomes of universal testing for Lynch syndrome in the United Kingdom. *Colorectal Dis* 2019; **21**: 760-766 [PMID: 30815953 DOI: 10.1111/codi.14597]

83 **Palles C**, West HD, Chew E, Galavotti S, Flensburg C, Grolleman JE, Jansen EAM, Curley H, Chegwidden L, Arbe-Barnes EH, Lander N, Truscott R, Pagan J, Bajel A, Sherwood K, Martin L, Thomas H, Georgiou D, Fostira F, Goldberg Y, Adams DJ, van der Biezen SAM, Christie M, Clendenning M, Thomas LE, Deltas C, Dimovski AJ, Dymerska D, Lubinski J, Mahmood K, van der Post RS, Sanders M, Weitz J, Taylor JC, Turnbull C, Vreede L, van Wezel T, Whalley C, Arnedo-Pac C, Caravagna G, Cross W, Chubb D, Frangou A, Gruber AJ, Kinnersley B, Noyvert B, Church D, Graham T, Houlston R, Lopez-Bigas N, Sottoriva A, Wedge D; Genomics England Research Consortium; CORGI Consortium; WGS500 Consortium, Jenkins MA, Kuiper RP, Roberts AW, Cheadle JP, Ligtenberg MJL, Hoogerbrugge N, Koelzer VH, Rivas AD, Winship IM, Ponte CR, Buchanan DD, Power DG, Green A, Tomlinson IPM, Sampson JR, Majewski IJ, de Voer RM. Germline MBD4 deficiency causes a multi-tumor predisposition syndrome. *Am J Hum Genet* 2022; **109**: 953-960 [PMID: 35460607 DOI: 10.1016/j.ajhg.2022.03.018]

84 **Samadder NJ**, Baffy N, Giridhar KV, Couch FJ, Riegert-Johnson D. Hereditary Cancer Syndromes-A Primer on Diagnosis and Management, Part 2: Gastrointestinal Cancer Syndromes. *Mayo Clin Proc* 2019; **94**: 1099-1116 [PMID: 31171120 DOI: 10.1016/j.mayocp.2019.01.042]

85 **Daca Alvarez M**, Quintana I, Terradas M, Mur P, Balaguer F, Valle L. The Inherited and Familial Component of Early-Onset Colorectal Cancer. *Cells* 2021; **10** [PMID: 33806975 DOI: 10.3390/cells10030710]

86 **Yanus GA**, Akhapkina TA, Ivantsov AO, Preobrazhenskaya EV, Aleksakhina SN, Bizin IV, Sokolenko AP, Mitiushkina NV, Kuligina ES, Suspitsin EN, Venina AR, Holmatov MM, Zaitseva OA, Yatsuk OS, Pashkov DV, Belyaev AM, Togo AV, Imyanitov EN, Iyevleva AG. Spectrum of APC and MUTYH germ-line mutations in Russian patients with colorectal malignancies. *Clin Genet* 2018; **93**: 1015-1021 [PMID: 29406563 DOI: 10.1111/cge.13228]

87 **Zarkavelis G**, Boussios S, Papadaki A, Katsanos KH, Christodoulou DK, Pentheroudakis G. Current and future biomarkers in colorectal cancer. *Ann Gastroenterol* 2017; **30**: 613-621 [PMID: 29118555 DOI: 10.20524/aog.2017.0191]

88 **Smyth EC**, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet* 2020; **396**: 635-648 [PMID: 32861308 DOI: 10.1016/S0140-6736(20)31288-5]

89 **Cosma LS**, Schlosser S, Tews HC, Müller M, Kandulski A. Hereditary Diffuse Gastric Cancer: Molecular Genetics, Biological Mechanisms and Current Therapeutic Approaches. *Int J Mol Sci* 2022; **23** [PMID: 35887173 DOI: 10.3390/ijms23147821]

90 **Guilford P**, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE. E-cadherin germline mutations in familial gastric cancer. *Nature* 1998; **392**: 402-405 [PMID: 9537325 DOI: 10.1038/32918]

91 **Blair VR**, McLeod M, Carneiro F, Coit DG, D'Addario JL, van Dieren JM, Harris KL, Hoogerbrugge N, Oliveira C, van der Post RS, Arnold J, Benusiglio PR, Bisseling TM, Boussioutas A, Cats A, Charlton A, Schreiber KEC, Davis JL, Pietro MD, Fitzgerald RC, Ford JM, Gamet K, Gullo I, Hardwick RH, Huntsman DG, Kaurah P, Kupfer SS, Latchford A, Mansfield PF, Nakajima T, Parry S, Rossaak J, Sugimura H, Svrcek M, Tischkowitz M, Ushijima T, Yamada H, Yang HK, Claydon A, Figueiredo J, Paringatai K, Seruca R, Bougen-Zhukov N, Brew T, Busija S, Carneiro P, DeGregorio L, Fisher H, Gardner E, Godwin TD, Holm KN, Humar B, Lintott CJ, Monroe EC, Muller MD, Norero E, Nouri Y, Paredes J, Sanches JM, Schulpen E, Ribeiro AS, Sporle A, Whitworth J, Zhang L, Reeve AE, Guilford P. Hereditary diffuse gastric cancer: updated clinical practice guidelines. *Lancet Oncol* 2020; **21**: e386-e397 [PMID: 32758476 DOI: 10.1016/S1470-2045(20)30219-9]

92 **Adib E**, El Zarif T, Nassar AH, Akl EW, Abou Alaiwi S, Mouhieddine TH, Esplin ED, Hatchell K, Nielsen SM, Rana HQ, Choueiri TK, Kwiatkowski DJ, Sonpavde G. CDH1 germline variants are enriched in patients with colorectal cancer, gastric cancer, and breast cancer. *Br J Cancer* 2022; **126**: 797-803 [PMID: 34949788 DOI: 10.1038/s41416-021-01673-7]

93 **Sahasrabudhe R**, Lott P, Bohorquez M, Toal T, Estrada AP, Suarez JJ, Brea-Fernández A, Cameselle-Teijeiro J, Pinto C, Ramos I, Mantilla A, Prieto R, Corvalan A, Norero E, Alvarez C, Tapia T, Carvallo P, Gonzalez LM, Cock-Rada A, Solano A, Neffa F, Della Valle A, Yau C, Soares G, Borowsky A, Hu N, He LJ, Han XY; Latin American Gastric Cancer Genetics Collaborative Group, Taylor PR, Goldstein AM, Torres J, Echeverry M, Ruiz-Ponte C, Teixeira MR, Carvajal-Carmona LG. Germline Mutations in PALB2, BRCA1, and RAD51C, Which Regulate DNA Recombination Repair, in Patients With Gastric Cancer. *Gastroenterology* 2017; **152**: 983-986.e6 [PMID: 28024868 DOI: 10.1053/j.gastro.2016.12.010]

94 **Fewings E**, Larionov A, Redman J, Goldgraben MA, Scarth J, Richardson S, Brewer C, Davidson R, Ellis I, Evans DG, Halliday D, Izatt L, Marks P, McConnell V, Verbist L, Mayes R, Clark GR, Hadfield J, Chin SF, Teixeira MR, Giger OT, Hardwick R, di Pietro M, O'Donovan M, Pharoah P, Caldas C, Fitzgerald RC, Tischkowitz M. Germline pathogenic variants in PALB2 and other cancer-predisposing genes in families with hereditary diffuse gastric cancer without CDH1 mutation: a whole-exome sequencing study. *Lancet Gastroenterol Hepatol* 2018; **3**: 489-498 [PMID: 29706558 DOI: 10.1016/S2468-1253(18)30079-7]

95 **Yang X**, Leslie G, Doroszuk A, Schneider S, Allen J, Decker B, Dunning AM, Redman J, Scarth J, Plaskocinska I, Luccarini C, Shah M, Pooley K, Dorling L, Lee A, Adank MA, Adlard J, Aittomäki K, Andrulis IL, Ang P, Barwell J, Bernstein JL, Bobolis K, Borg Å, Blomqvist C, Claes KBM, Concannon P, Cuggia A, Culver JO, Damiola F, de Pauw A, Diez O, Dolinsky JS, Domchek SM, Engel C, Evans DG, Fostira F, Garber J, Golmard L, Goode EL, Gruber SB, Hahnen E, Hake C, Heikkinen T, Hurley JE, Janavicius R, Kleibl Z, Kleiblova P, Konstantopoulou I, Kvist A, Laduca H, Lee ASG, Lesueur F, Maher ER, Mannermaa A, Manoukian S, McFarland R, McKinnon W, Meindl A, Metcalfe K, Mohd Taib NA, Moilanen J, Nathanson KL, Neuhausen S, Ng PS, Nguyen-Dumont T, Nielsen SM, Obermair F, Offit K, Olopade OI, Ottini L, Penkert J, Pylkäs K, Radice P, Ramus SJ, Rudaitis V, Side L, Silva-Smith R, Silvestri V, Skytte AB, Slavin T, Soukupova J, Tondini C, Trainer AH, Unzeitig G, Usha L, van Overeem Hansen T, Whitworth J, Wood M, Yip CH, Yoon SY, Yussuf A, Zogopoulos G, Goldgar D, Hopper JL, Chenevix-Trench G, Pharoah P, George SHL, Balmaña J, Houdayer C, James P, El-Haffaf Z, Ehrencrona H, Janatova M, Peterlongo P, Nevanlinna H, Schmutzler R, Teo SH, Robson M, Pal T, Couch F, Weitzel JN, Elliott A, Southey M, Winqvist R, Easton DF, Foulkes WD, Antoniou AC, Tischkowitz M. Cancer Risks Associated With Germline PALB2 Pathogenic Variants: An International Study of 524 Families. *J Clin Oncol* 2020; **38**: 674-685 [PMID: 31841383 DOI: 10.1200/JCO.19.01907]

96 **Moiseyenko VM**, Volkov NM, Suspistin EN, Yanus GA, Iyevleva AG, Kuligina ESh, Togo AV, Kornilov AV, Ivantsov AO, Imyanitov EN. Evidence for predictive role of BRCA1 and bTUBIII in gastric cancer. *Med Oncol* 2013; **30**: 545 [PMID: 23532817 DOI: 10.1007/s12032-013-0545-4]

97 **Li S**, Silvestri V, Leslie G, Rebbeck TR, Neuhausen SL, Hopper JL, Nielsen HR, Lee A, Yang X, McGuffog L, Parsons MT, Andrulis IL, Arnold N, Belotti M, Borg Å, Buecher B, Buys SS, Caputo SM, Chung WK, Colas C, Colonna SV, Cook J, Daly MB, de la Hoya M, de Pauw A, Delhomelle H, Eason J, Engel C, Evans DG, Faust U, Fehm TN, Fostira F, Fountzilas G, Frone M, Garcia-Barberan V, Garre P, Gauthier-Villars M, Gehrig A, Glendon G, Goldgar DE, Golmard L, Greene MH, Hahnen E, Hamann U, Hanson H, Hassan T, Hentschel J, Horvath J, Izatt L, Janavicius R, Jiao Y, John EM, Karlan BY, Kim SW, Konstantopoulou I, Kwong A, Laugé A, Lee JW, Lesueur F, Mebirouk N, Meindl A, Mouret-Fourme E, Musgrave H, Ngeow Yuen Yie J, Niederacher D, Park SK, Pedersen IS, Ramser J, Ramus SJ, Rantala J, Rashid MU, Reichl F, Ritter J, Rump A, Santamariña M, Saule C, Schmidt G, Schmutzler RK, Senter L, Shariff S, Singer CF, Southey MC, Stoppa-Lyonnet D, Sutter C, Tan Y, Teo SH, Terry MB, Thomassen M, Tischkowitz M, Toland AE, Torres

D, Vega A, Wagner SA, Wang-Gohrke S, Wappenschmidt B, Weber BHF, Yannoukakos D, Spurdle AB, Easton DF, Chenevix-Trench G, Ottini L, Antoniou AC. Cancer Risks Associated With BRCA1 and BRCA2 Pathogenic Variants. *J Clin Oncol* 2022; **40**: 1529-1541 [PMID: 35077220 DOI: 10.1200/JCO.21.02112]

98 **Lerner BA**, Llor X. Genetic Gastric Cancer Risk Syndromes. *Curr Treat Options Gastroenterol* 2020; **18**: 604-615 [PMID: 33776403 DOI: 10.1007/s11938-020-00312-z]

99 **Hu C**, Hart SN, Polley EC, Gnanaolivu R, Shimelis H, Lee KY, Lilyquist J, Na J, Moore R, Antwi SO, Bamlet WR, Chaffee KG, DiCarlo J, Wu Z, Samara R, Kasi PM, McWilliams RR, Petersen GM, Couch FJ. Association Between Inherited Germline Mutations in Cancer Predisposition Genes and Risk of Pancreatic Cancer. *JAMA* 2018; **319**: 2401-2409 [PMID: 29922827 DOI: 10.1001/jama.2018.6228]

100 **Llach J**, Carballal S, Moreira L. Familial Pancreatic Cancer: Current Perspectives. *Cancer Manag Res* 2020; **12**: 743-758 [PMID: 32099470 DOI: 10.2147/CMAR.S172421]

101 **Abe K**, Kitago M, Kitagawa Y, Hirasawa A. Hereditary pancreatic cancer. *Int J Clin Oncol* 2021; **26**: 1784-1792 [PMID: 34476650 DOI: 10.1007/s10147-021-02015-6]

102 **Astiazaran-Symonds E**, Goldstein AM. A systematic review of the prevalence of germline pathogenic variants in patients with pancreatic cancer. *J Gastroenterol* 2021; **56**: 713-721 [PMID: 34255164 DOI: 10.1007/s00535-021-01806-y]

103 **Mersch J**, Jackson MA, Park M, Nebgen D, Peterson SK, Singletary C, Arun BK, Litton JK. Cancers associated with BRCA1 and BRCA2 mutations other than breast and ovarian. *Cancer* 2015; **121**: 269-275 [PMID: 25224030 DOI: 10.1002/cncr.29041]

104 **Jones S**, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, Lin JC, Palmisano E, Brune K, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Parmigiani G, Kern SE, Velculescu VE, Kinzler KW, Vogelstein B, Eshleman JR, Goggins M, Klein AP. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* 2009; **324**: 217 [PMID: 19264984 DOI: 10.1126/science.1171202]

105 **Roberts NJ**, Jiao Y, Yu J, Kopelovich L, Petersen GM, Bondy ML, Gallinger S, Schwartz AG, Syngal S, Cote ML, Axilbund J, Schulick R, Ali SZ, Eshleman JR, Velculescu VE, Goggins M, Vogelstein B, Papadopoulos N, Hruban RH, Kinzler KW, Klein AP. ATM mutations in patients with hereditary pancreatic cancer. *Cancer Discov* 2012; **2**: 41-46 [PMID: 22585167 DOI: 10.1158/2159-8290.CD-11-0194]

106 **Hsu FC**, Roberts NJ, Childs E, Porter N, Rabe KG, Borgida A, Ukaegbu C, Goggins MG, Hruban RH, Zogopoulos G, Syngal S, Gallinger S, Petersen GM, Klein AP. Risk of Pancreatic Cancer Among Individuals With Pathogenic Variants in the ATM Gene. *JAMA Oncol* 2021; **7**: 1664-1668 [PMID: 34529012 DOI: 10.1001/jamaoncol.2021.3701]

107 **Gardiner A**, Kidd J, Elias MC, Young K, Mabey B, Taherian N, Cummings S, Malafa M, Rosenthal E, Permuth JB. Pancreatic Ductal Carcinoma Risk Associated With Hereditary Cancer-Risk Genes. *J Natl Cancer Inst* 2022; **114**: 996-1002 [PMID: 35445726 DOI: 10.1093/jnci/djac069]

108 **Zeng C**, Bastarache LA, Tao R, Venner E, Hebbring S, Andujar JD, Bland ST, Crosslin DR, Pratap S, Cooley A, Pacheco JA, Christensen KD, Perez E, Zawatsky CLB, Witkowski L, Zouk H, Weng C, Leppig KA, Sleiman PMA, Hakonarson H, Williams MS, Luo Y, Jarvik GP, Green RC, Chung WK, Gharavi AG, Lennon NJ, Rehm HL, Gibbs RA, Peterson JF, Roden DM, Wiesner GL, Denny JC. Association of Pathogenic Variants in Hereditary Cancer Genes With Multiple Diseases. *JAMA Oncol* 2022; **8**: 835-844 [PMID: 35446370 DOI: 10.1001/jamaoncol.2022.0373]

109 **Chan SH**, Chiang J, Ngeow J. CDKN2A germline alterations and the relevance of genotype-phenotype associations in cancer predisposition. *Hered Cancer Clin Pract* 2021; **19**: 21 [PMID: 33766116 DOI: 10.1186/s13053-021-00178-x]

110 **Nissim S**, Leshchiner I, Mancias JD, Greenblatt MB, Maertens O, Cassa CA, Rosenfeld JA, Cox AG, Hedgepeth J, Wucherpfennig JI, Kim AJ, Henderson JE, Gonyo P, Brandt A, Lorimer E, Unger B, Prokop JW, Heidel JR, Wang XX, Ukaegbu CI, Jennings BC, Paulo JA, Gableske S, Fierke CA, Getz G, Sunyaev SR, Wade Harper J, Cichowski K, Kimmelman AC, Houvras Y, Syngal S, Williams C, Goessling W. Mutations in RABL3 alter KRAS prenylation and are associated with hereditary pancreatic cancer. *Nat Genet* 2019; **51**: 1308-1314 [PMID: 31406347 DOI: 10.1038/s41588-019-0475-y]

111 **Roberts NJ**, Grant RC, Gallinger S, Klein AP; Familial Pancreatic Cancer Genome Sequencing Project. Germline sequence analysis of RABL3 in a large series of pancreatic ductal adenocarcinoma patients reveals no evidence of deleterious variants. *Genes Chromosomes Cancer* 2021; **60**: 559-564 [PMID: 33724601 DOI: 10.1002/gcc.22947]

112 **Nicolosi P**, Ledet E, Yang S, Michalski S, Freschi B, O'Leary E, Esplin ED, Nussbaum RL, Sartor O. Prevalence of Germline Variants in Prostate Cancer and Implications for Current Genetic Testing Guidelines. *JAMA Oncol* 2019; **5**: 523-528 [PMID: 30730552 DOI: 10.1001/jamaoncol.2018.6760]

113 **Bree KK**, Hensley PJ, Pettaway CA. Germline Predisposition to Prostate Cancer in Diverse Populations. *Urol Clin North Am* 2021; **48**: 411-423 [PMID: 34210495 DOI: 10.1016/j.ucl.2021.03.008]

114 **Bhanji Y**, Isaacs WB, Xu J, Cooney KA. Prostate Cancer Predisposition. *Urol Clin North Am* 2021; **48**: 283-296 [PMID: 34210485 DOI: 10.1016/j.ucl.2021.03.001]

115 **Nyberg T**, Tischkowitz M, Antoniou AC. BRCA1 and BRCA2 pathogenic variants and prostate cancer risk: systematic review and meta-analysis. *Br J Cancer* 2022; **126**: 1067-1081 [PMID: 34963702 DOI: 10.1038/s41416-021-01675-5]

116 **Silvestri V**, Leslie G, Barnes DR; CIMBA Group, Agnarsson BA, Aittomäki K, Alducci E, Andrulis IL, Barkardottir RB, Barroso A, Barrowdale D, Benitez J, Bonanni B, Borg A, Buys SS, Caldés T, Caligo MA, Capalbo C, Campbell I, Chung WK, Claes KBM, Colonna SV, Cortesi L, Couch FJ, de la Hoya M, Diez O, Ding YC, Domchek S, Easton DF, Ejlertsen B, Engel C, Evans DG, Feliubadalò L, Foretova L, Fostira F, Géczi L, Gerdes AM, Glendon G, Godwin AK, Goldgar DE, Hahnen E, Hogervorst FBL, Hopper JL, Hulick PJ, Isaacs C, Izquierdo A, James PA, Janavicius R, Jensen UB, John EM, Joseph V, Konstantopoulou I, Kurian AW, Kwong A, Landucci E, Lesueur F, Loud JT, Machackova E, Mai PL, Majidzadeh-A K, Manoukian S, Montagna M, Moserle L, Mulligan AM, Nathanson KL, Nevanlinna H, Ngeow J, Nikitina-Zake L, Offit K, Olah E, Olopade OI, Osorio A, Papi L, Park SK, Pedersen IS, Perez-Segura P, Petersen AH, Pinto P, Porfirio B, Pujana MA, Radice P, Rantala J, Rashid MU, Rosenzweig B, Rossing M, Santamariña M, Schmutzler RK, Senter L, Simard J, Singer CF, Solano AR, Southey MC, Steele L, Steinsnyder Z, Stoppa-Lyonnet D, Tan YY, Teixeira MR, Teo SH, Terry MB, Thomassen M, Toland AE, Torres-Esquius S, Tung N, van Asperen CJ, Vega A, Viel A, Vierstraete J, Wappenschmidt B, Weitzel JN, Wieme G, Yoon SY, Zorn KK, McGuffog L, Parsons MT, Hamann U,

Greene MH, Kirk JA, Neuhausen SL, Rebbeck TR, Tischkowitz M, Chenevix-Trench G, Antoniou AC, Friedman E, Ottini L. Characterization of the Cancer Spectrum in Men With Germline BRCA1 and BRCA2 Pathogenic Variants: Results From the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). *JAMA Oncol* 2020; **6**: 1218-1230 [PMID: 32614418 DOI: 10.1001/jamaoncol.2020.2134]

117 **Momozawa Y**, Iwasaki Y, Hirata M, Liu X, Kamatani Y, Takahashi A, Sugano K, Yoshida T, Murakami Y, Matsuda K, Nakagawa H, Spurdle AB, Kubo M. Germline Pathogenic Variants in 7636 Japanese Patients With Prostate Cancer and 12 366 Controls. *J Natl Cancer Inst* 2020; **112**: 369-376 [PMID: 31214711 DOI: 10.1093/jnci/djz124]

118 **Bancroft EK**, Page EC, Brook MN, Thomas S, Taylor N, Pope J, McHugh J, Jones AB, Karlsson Q, Merson S, Ong KR, Hoffman J, Huber C, Maehle L, Grindedal EM, Stormorken A, Evans DG, Rothwell J, Lalloo F, Brady AF, Bartlett M, Snape K, Hanson H, James P, McKinley J, Mascarenhas L, Syngal S, Ukaegbu C, Side L, Thomas T, Barwell J, Teixeira MR, Izatt L, Suri M, Macrae FA, Poplawski N, Chen-Shtoyerman R, Ahmed M, Musgrave H, Nicolai N, Greenhalgh L, Brewer C, Pachter N, Spigelman AD, Azzabi A, Helfand BT, Halliday D, Buys S, Ramon Y Cajal T, Donaldson A, Cooney KA, Harris M, McGrath J, Davidson R, Taylor A, Cooke P, Myhill K, Hogben M, Aaronson NK, Ardern-Jones A, Bangma CH, Castro E, Dearnaley D, Dias A, Dudderidge T, Eccles DM, Green K, Eyfjord J, Falconer A, Foster CS, Gronberg H, Hamdy FC, Johannsson O, Khoo V, Lilja H, Lindeman GJ, Lubinski J, Axcrona K, Mikropoulos C, Mitra AV, Moynihan C, Ni Raghallaigh H, Rennert G, Collier R; IMPACT Study Collaborators, Offman J, Kote-Jarai Z, Eeles RA. A prospective prostate cancer screening programme for men with pathogenic variants in mismatch repair genes (IMPACT): initial results from an international prospective study. *Lancet Oncol* 2021; **22**: 1618-1631 [PMID: 34678156 DOI: 10.1016/S1470-2045(21)00522-2]

119 **Carlo MI**, Mukherjee S, Mandelker D, Vijai J, Kemel Y, Zhang L, Knezevic A, Patil S, Ceyhan-Birsoy O, Huang KC, Redzematovic A, Coskey DT, Stewart C, Pradhan N, Arnold AG, Hakimi AA, Chen YB, Coleman JA, Hyman DM, Ladanyi M, Cadoo KA, Walsh MF, Stadler ZK, Lee CH, Feldman DR, Voss MH, Robson M, Motzer RJ, Offit K. Prevalence of Germline Mutations in Cancer Susceptibility Genes in Patients With Advanced Renal Cell Carcinoma. *JAMA Oncol* 2018; **4**: 1228-1235 [PMID: 29978187 DOI: 10.1001/jamaoncol.2018.1986]

120 **Al-Shinnag M**, Marfan H, Susman R, Wakeling J, Gustafson S, Wood S, Mallett AJ. Birt-Hogg-Dubé Syndrome and Hereditary Leiomyomatosis and Renal Cell Carcinoma Syndrome: An Effective Multidisciplinary Approach to Hereditary Renal Cancer Predisposing Syndromes. *Front Oncol* 2021; **11**: 738822 [PMID: 34604083 DOI: 10.3389/fonc.2021.738822]

121 **Shuch B**, Zhang J. Genetic Predisposition to Renal Cell Carcinoma: Implications for Counseling, Testing, Screening, and Management. *J Clin Oncol* 2018; JCO2018792523 [PMID: 30372385 DOI: 10.1200/JCO.2018.79.2523]

122 **de Alencar VTL**, Formiga MN, de Lima VCC. Inherited lung cancer: a review. *Ecancermedicalscience* 2020; **14**: 1008 [PMID: 32104210 DOI: 10.3332/ecancer.2020.1008]

123 **Benusiglio PR**, Fallet V, Sanchis-Borja M, Coulet F, Cadranel J. Lung cancer is also a hereditary disease. *Eur Respir Rev* 2021; **30** [PMID: 34670806 DOI: 10.1183/16000617.0045-2021]

124 **Pao W**, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med* 2005; **2**: e73 [PMID: 15737014 DOI: 10.1371/journal.pmed.0020073]

125 **Kobayashi S**, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, Halmos B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005; **352**: 786-792 [PMID: 15728811 DOI: 10.1056/NEJMoa044238]

126 **Bell DW**, Gore I, Okimoto RA, Godin-Heymann N, Sordella R, Mulloy R, Sharma SV, Brannigan BW, Mohapatra G, Settleman J, Haber DA. Inherited susceptibility to lung cancer may be associated with the T790M drug resistance mutation in EGFR. *Nat Genet* 2005; **37**: 1315-1316 [PMID: 16258541 DOI: 10.1038/ng1671]

127 **Lavdovskaia ED**, Iyevleva AG, Sokolenko AP, Mitiushkina NV, Preobrazhenskaya EV, Tiurin VI, Ivantsov AO, Bizin IV, Stelmakh LV, Moiseyenko FV, Karaseva NA, Orlov SV, Moiseyenko VM, Korzhenevskaya MA, Zaitsev IA, Kozak AR, Chistyakov IV, Akopov AL, Volkov NM, Togo AV, Imyanitov EN. EGFR T790M Mutation in TKI-Naïve Clinical Samples: Frequency, Tissue Mosaicism, Predictive Value and Awareness on Artifacts. *Oncol Res Treat* 2018; **41**: 634-642 [PMID: 30145586 DOI: 10.1159/000491441]

128 **Lu S**, Yu Y, Li Z, Yu R, Wu X, Bao H, Ding Y, Shao YW, Jian H. EGFR and ERBB2 Germline Mutations in Chinese Lung Cancer Patients and Their Roles in Genetic Susceptibility to Cancer. *J Thorac Oncol* 2019; **14**: 732-736 [PMID: 30610926 DOI: 10.1016/j.jtho.2018.12.006]

129 **Mezquita L**, Jové M, Nadal E, Kfoury M, Morán T, Ricordel C, Dhooge M, Tlemsani C, Léna H, Teulé A, Álvarez JV, Raimbourg J, Hiret S, Lacroix L, Menéndez M, Saldaña J, Brunet J, Lianes P, Coupier I, Auclin E, Recondo G, Friboulet L, Adam J, Green E, Planchard D, Frébourg T, Capellà G, Rouleau E, Lázaro C, Caron O, Besse B. High Prevalence of Somatic Oncogenic Driver Alterations in Patients With NSCLC and Li-Fraumeni Syndrome. *J Thorac Oncol* 2020; **15**: 1232-1239 [PMID: 32179180 DOI: 10.1016/j.jtho.2020.03.005]

130 **Vasen HF**, Gruis NA, Frants RR, van Der Velden PA, Hille ET, Bergman W. Risk of developing pancreatic cancer in families with familial atypical multiple mole melanoma associated with a specific 19 deletion of p16 (p16-Leiden). *Int J Cancer* 2000; **87**: 809-811 [PMID: 10956390]

131 **Potjer TP**, van der Stoep N, Houwing-Duistermaat JJ, Konings IC, Aalfs CM, van den Akker PC, Ausems MG, Dommering CJ, van der Kolk LE, Maiburg MC, Spruijt L, Wagner A, Vasen HF, Hes FJ. Pancreatic cancer-associated gene polymorphisms in a nation-wide cohort of p16-Leiden germline mutation carriers; a case-control study. *BMC Res Notes* 2015; **8**: 264 [PMID: 26111702 DOI: 10.1186/s13104-015-1235-4]

132 **Revythis A**, Shah S, Kutka M, Moschetta M, Ozturk MA, Pappas-Gogos G, Ioannidou E, Sheriff M, Rassy E, Boussios S. Unraveling the Wide Spectrum of Melanoma Biomarkers. *Diagnostics (Basel)* 2021; **11** [PMID: 34441278 DOI: 10.3390/diagnostics11081341]

133 **Rossi M**, Pellegrini C, Cardelli L, Ciciarelli V, Di Nardo L, Fargnoli MC. Familial Melanoma: Diagnostic and Management Implications. *Dermatol Pract Concept* 2019; **9**: 10-16 [PMID: 30775140 DOI: 10.5826/dpc.0901a03]

134 **Al-Salameh A**, Cadiot G, Calender A, Goudet P, Chanson P. Clinical aspects of multiple endocrine neoplasia type 1. *Nat*

Rev Endocrinol 2021; **17**: 207-224 [PMID: 33564173 DOI: 10.1038/s41574-021-00468-3]
135 **Drilon A**, Hu ZI, Lai GGY, Tan DSW. Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. *Nat Rev Clin Oncol* 2018; **15**: 150 [PMID: 29182164 DOI: 10.1038/nrclinonc.2017.188]
136 **Subbiah V**, Yang D, Velcheti V, Drilon A, Meric-Bernstam F. State-of-the-Art Strategies for Targeting RET-Dependent Cancers. *J Clin Oncol* 2020; **38**: 1209-1221 [PMID: 32083997 DOI: 10.1200/JCO.19.02551]
137 **Bouys L**, Bertherat J. MANAGEMENT OF ENDOCRINE DISEASE: Carney complex: clinical and genetic update 20 years after the identification of the CNC1 (PRKAR1A) gene. *Eur J Endocrinol* 2021; **184**: R99-R109 [PMID: 33444222 DOI: 10.1530/EJE-20-1120]
138 **Zhao Y**, Yu T, Sun J, Wang F, Cheng C, He S, Chen L, Xie D, Fu L, Guan X, Yan A, Li Y, Miao G, Zhu X. Germ-line mutations in WDR77 predispose to familial papillary thyroid cancer. *Proc Natl Acad Sci U S A* 2021; **118** [PMID: 34326253 DOI: 10.1073/pnas.2026327118]
139 **Muth A**, Crona J, Gimm O, Elmgren A, Filipsson K, Stenmark Askmalm M, Sandstedt J, Tengvar M, Tham E. Genetic testing and surveillance guidelines in hereditary pheochromocytoma and paraganglioma. *J Intern Med* 2019; **285**: 187-204 [PMID: 30536464 DOI: 10.1111/joim.12869]
140 **Cazabat L**, Bouligand J, Salenave S, Bernier M, Gaillard S, Parker F, Young J, Guiochon-Mantel A, Chanson P. Germline AIP mutations in apparently sporadic pituitary adenomas: prevalence in a prospective single-center cohort of 443 patients. *J Clin Endocrinol Metab* 2012; **97**: E663-E670 [PMID: 22319033 DOI: 10.1210/jc.2011-2291]
141 **Chang M**, Yang C, Bao X, Wang R. Genetic and Epigenetic Causes of Pituitary Adenomas. *Front Endocrinol (Lausanne)* 2020; **11**: 596554 [PMID: 33574795 DOI: 10.3389/fendo.2020.596554]
142 **Hahn EC**, Bittar CM, Vianna FSL, Netto CBO, Biazús JV, Cericatto R, Cavalheiro JA, de Melo MP, Menke CH, Rabin E, Leistner-Segal S, Ashton-Prolla P. TP53 p.Arg337His germline mutation prevalence in Southern Brazil: Further evidence for mutation testing in young breast cancer patients. *PLoS One* 2018; **13**: e0209934 [PMID: 30596752 DOI: 10.1371/journal.pone.0209934]
143 **Rocca V**, Blandino G, D'Antona L, Iuliano R, Di Agostino S. Li-Fraumeni Syndrome: Mutation of TP53 Is a Biomarker of Hereditary Predisposition to Tumor: New Insights and Advances in the Treatment. *Cancers (Basel)* 2022; **14** [PMID: 35954327 DOI: 10.3390/cancers14153664]
144 **Melhem-Bertrandt A**, Bojadzieva J, Ready KJ, Obeid E, Liu DD, Gutierrez-Barrera AM, Litton JK, Olopade OI, Hortobagyi GN, Strong LC, Arun BK. Early onset HER2-positive breast cancer is associated with germline TP53 mutations. *Cancer* 2012; **118**: 908-913 [PMID: 21761402 DOI: 10.1002/cncr.26377]
145 **Sokolova TN**, Breder VV, Shumskaya IS, Suspitsin EN, Aleksakhina SN, Yanus GA, Tiurin VI, Ivantsov AO, Vona B, Raskin GA, Gamajunov SV, Imyanitov EN. Revisiting multiple erroneous genetic testing results and clinical misinterpretations in a patient with Li-Fraumeni syndrome: lessons for translational medicine. *Hered Cancer Clin Pract* 2021; **19**: 2 [PMID: 33407806 DOI: 10.1186/s13053-020-00157-8]
146 **Kratz CP**, Achatz MI, Brugières L, Frebourg T, Garber JE, Greer MC, Hansford JR, Janeway KA, Kohlmann WK, McGee R, Mullighan CG, Onel K, Pajtler KW, Pfister SM, Savage SA, Schiffman JD, Schneider KA, Strong LC, Evans DGR, Wasserman JD, Villani A, Malkin D. Cancer Screening Recommendations for Individuals with Li-Fraumeni Syndrome. *Clin Cancer Res* 2017; **23**: e38-e45 [PMID: 28572266 DOI: 10.1158/1078-0432.CCR-17-0408]
147 **Lin YH**, Jewell BE, Gingold J, Lu L, Zhao R, Wang LL, Lee DF. Osteosarcoma: Molecular Pathogenesis and iPSC Modeling. *Trends Mol Med* 2017; **23**: 737-755 [PMID: 28735817 DOI: 10.1016/j.molmed.2017.06.004]
148 **Evans DG**, Woodward ER, Bajalica-Lagercrantz S, Oliveira C, Frebourg T. Germline TP53 Testing in Breast Cancers: Why, When and How? *Cancers (Basel)* 2020; **12** [PMID: 33327514 DOI: 10.3390/cancers12123762]
149 **Mirabello L**, Zhu B, Koster R, Karlins E, Dean M, Yeager M, Gianferante M, Spector LG, Morton LM, Karyadi D, Robison LL, Armstrong GT, Bhatia S, Song L, Pankratz N, Pinheiro M, Gastier-Foster JM, Gorlick R, de Toledo SRC, Petrilli AS, Patino-Garcia A, Lecanda F, Gutierrez-Jimeno M, Serra M, Hattinger C, Picci P, Scotlandi K, Flanagan AM, Tirabosco R, Amary MF, Kurucu N, Ilhan IE, Ballinger ML, Thomas DM, Barkauskas DA, Mejia-Baltodano G, Valverde P, Hicks BD, Wang M, Hutchinson AA, Tucker M, Sampson J, Landi MT, Freedman ND, Gapstur S, Carter B, Hoover RN, Chanock SJ, Savage SA. Frequency of Pathogenic Germline Variants in Cancer-Susceptibility Genes in Patients With Osteosarcoma. *JAMA Oncol* 2020; **6**: 724-734 [PMID: 32191290 DOI: 10.1001/jamaoncol.2020.0197]
150 **Rogoża-Janiszewska E**, Malińska K, Górski B, Scott RJ, Cybulski C, Kluźniak W, Lener M, Jakubowska A, Gronwald J, Huzarski T, Lubiński J, Dębniak T. Prevalence of germline TP53 variants among early-onset breast cancer patients from Polish population. *Breast Cancer* 2021; **28**: 226-235 [PMID: 32888145 DOI: 10.1007/s12282-020-01151-7]
151 **Pilarski R**. PTEN Hamartoma Tumor Syndrome: A Clinical Overview. *Cancers (Basel)* 2019; **11** [PMID: 31216739 DOI: 10.3390/cancers11060844]
152 **Ngeow J**, Eng C. PTEN in Hereditary and Sporadic Cancer. *Cold Spring Harb Perspect Med* 2020; **10** [PMID: 31570378 DOI: 10.1101/cshperspect.a036087]
153 **Hendricks LAJ**, Hoogerbrugge N, Schuurs-Hoeijmakers JHM, Vos JR. A review on age-related cancer risks in PTEN hamartoma tumor syndrome. *Clin Genet* 2021; **99**: 219-225 [PMID: 33140411 DOI: 10.1111/cge.13875]
154 **Eissing M**, Ripken L, Schreibelt G, Westdorp H, Ligtenberg M, Netea-Maier R, Netea MG, de Vries IJM, Hoogerbrugge N. PTEN Hamartoma Tumor Syndrome and Immune Dysregulation. *Transl Oncol* 2019; **12**: 361-367 [PMID: 30504085 DOI: 10.1016/j.tranon.2018.11.003]
155 **Lee YR**, Yehia L, Kishikawa T, Ni Y, Leach B, Zhang J, Panch N, Liu J, Wei W, Eng C, Pandolfi PP. WWP1 Gain-of-Function Inactivation of PTEN in Cancer Predisposition. *N Engl J Med* 2020; **382**: 2103-2116 [PMID: 32459922 DOI: 10.1056/NEJMoa1914919]
156 **Klimkowski S**, Ibrahim M, Ibarra Rovira JJ, Elshikh M, Javadi S, Klekers AR, Abusaif AA, Moawad AW, Ali K, Elsayes KM. Peutz-Jeghers Syndrome and the Role of Imaging: Pathophysiology, Diagnosis, and Associated Cancers. *Cancers (Basel)* 2021; **13** [PMID: 34680270 DOI: 10.3390/cancers13205121]
157 **Meserve EE**, Nucci MR. Peutz-Jeghers Syndrome: Pathobiology, Pathologic Manifestations, and Suggestions for Recommending Genetic Testing in Pathology Reports. *Surg Pathol Clin* 2016; **9**: 243-268 [PMID: 27241107 DOI: 10.1016/j.path.2016.01.006]

158 **Daniell J**, Plazzer JP, Perera A, Macrae F. An exploration of genotype-phenotype link between Peutz-Jeghers syndrome and STK11: a review. *Fam Cancer* 2018; **17**: 421-427 [PMID: 28900777 DOI: 10.1007/s10689-017-0037-3]

159 **Onodera S**, Nakamura Y, Azuma T. Gorlin Syndrome: Recent Advances in Genetic Testing and Molecular and Cellular Biological Research. *Int J Mol Sci* 2020; **21** [PMID: 33066274 DOI: 10.3390/ijms21207559]

160 **Olafsdottir T**, Stacey SN, Sveinbjornsson G, Thorleifsson G, Norland K, Sigurgeirsson B, Thorisdottir K, Kristjansson AK, Tryggvadottir L, Sarin KY, Benediktsson R, Jonasson JG, Sigurdsson A, Jonasdottir A, Kristmundsdottir S, Jonsson H, Gylfason A, Oddsson A, Fridriksdottir R, Gudjonsson SA, Zink F, Lund SH, Rognvaldsson S, Melsted P, Steinthorsdottir V, Gudmundsson J, Mikaelsdottir E, Olason PI, Stefansdottir L, Eggertsson HP, Halldorsson BV, Thorsteinsdottir U, Agustsson TT, Olafsson K, Olafsson JH, Sulem P, Rafnar T, Gudbjartsson DF, Stefansson K. Loss-of-Function Variants in the Tumor-Suppressor Gene PTPN14 Confer Increased Cancer Risk. *Cancer Res* 2021; **81**: 1954-1964 [PMID: 33602785 DOI: 10.1158/0008-5472.CAN-20-3065]

161 **Zhang J**, Walsh MF, Wu G, Edmonson MN, Gruber TA, Easton J, Hedges D, Ma X, Zhou X, Yergeau DA, Wilkinson MR, Vadodaria B, Chen X, McGee RB, Hines-Dowell S, Nuccio R, Quinn E, Shurtleff SA, Rusch M, Patel A, Becksfort JB, Wang S, Weaver MS, Ding L, Mardis ER, Wilson RK, Gajjar A, Ellison DW, Pappo AS, Pui CH, Nichols KE, Downing JR. Germline Mutations in Predisposition Genes in Pediatric Cancer. *N Engl J Med* 2015; **373**: 2336-2346 [PMID: 26580448 DOI: 10.1056/NEJMoa1508054]

162 **Newman S**, Nakitandwe J, Kesserwan CA, Azzato EM, Wheeler DA, Rusch M, Shurtleff S, Hedges DJ, Hamilton KV, Foy SG, Edmonson MN, Thrasher A, Bahrami A, Orr BA, Klco JM, Gu J, Harrison LW, Wang L, Clay MR, Ouma A, Silkov A, Liu Y, Zhang Z, Brady SW, Zhou X, Chang TC, Pande M, Davis E, Becksfort J, Patel A, Wilkinson MR, Rahbarinia D, Kubal M, Maciaszek JL, Pastor V, Knight J, Gout AM, Wang J, Gu Z, Mullighan CG, McGee RB, Quinn EA, Nuccio R, Mostafavi R, Gerhardt EL, Taylor LM, Valdez JM, Hines-Dowell SJ, Pappo AS, Robinson G, Johnson LM, Pui CH, Ellison DW, Downing JR, Zhang J, Nichols KE. Genomes for Kids: The Scope of Pathogenic Mutations in Pediatric Cancer Revealed by Comprehensive DNA and RNA Sequencing. *Cancer Discov* 2021; **11**: 3008-3027 [PMID: 34301788 DOI: 10.1158/2159-8290.CD-20-1631]

163 **Kratz CP**, Jongmans MC, Cavé H, Wimmer K, Behjati S, Guerrini-Rousseau L, Milde T, Pajtler KW, Golmard L, Gauthier-Villars M, Jewell R, Duncan C, Maher ER, Brugieres L, Pritchard-Jones K, Bourdeaut F. Predisposition to cancer in children and adolescents. *Lancet Child Adolesc Health* 2021; **5**: 142-154 [PMID: 33484663 DOI: 10.1016/S2352-4642(20)30275-3]

164 **Fabius AWM**, van Hoefen Wijsard M, van Leeuwen FE, Moll AC. Subsequent Malignant Neoplasms in Retinoblastoma Survivors. *Cancers (Basel)* 2021; **13** [PMID: 33801943 DOI: 10.3390/cancers13061200]

165 **Tu J**, Huo Z, Yu Y, Zhu D, Xu A, Huang MF, Hu R, Wang R, Gingold JA, Chen YH, Tsai KL, Forcioli-Conti NR, Huang SXL, Webb TR, Su J, Bazer DA, Jia P, Yustein JT, Wang LL, Hung MC, Zhao Z, Huff CD, Shen J, Zhao R, Lee DF. Hereditary retinoblastoma iPSC model reveals aberrant spliceosome function driving bone malignancies. *Proc Natl Acad Sci U S A* 2022; **119**: e2117857119 [PMID: 35412907 DOI: 10.1073/pnas.2117857119]

166 **Maciaszek JL**, Oak N, Nichols KE. Recent advances in Wilms' tumor predisposition. *Hum Mol Genet* 2020; **29**: R138-R149 [PMID: 32412586 DOI: 10.1093/hmg/ddaa091]

167 **Sur ML**, Armat I, Sur G, Pop DC, Samasca G, Lupan I, Timis TL, Florian IA, Sur D. Neurofibromatosis in Children: Actually and Perspectives. *Children (Basel)* 2022; **9** [PMID: 35053664 DOI: 10.3390/children9010040]

168 **Hill DA**, Ivanovich J, Priest JR, Gurnett CA, Dehner LP, Desruisseau D, Jarzembowski JA, Wikenheiser-Brokamp KA, Suarez BK, Whelan AJ, Williams G, Bracamontes D, Messinger Y, Goodfellow PJ. DICER1 mutations in familial pleuropulmonary blastoma. *Science* 2009; **325**: 965 [PMID: 19556464 DOI: 10.1126/science.1174334]

169 **Guillerman RP**, Foulkes WD, Priest JR. Imaging of DICER1 syndrome. *Pediatr Radiol* 2019; **49**: 1488-1505 [PMID: 31620849 DOI: 10.1007/s00247-019-04429-x]

170 **Stewart DR**, Best AF, Williams GM, Harney LA, Carr AG, Harris AK, Kratz CP, Dehner LP, Messinger YH, Rosenberg PS, Hill DA, Schultz KAP. Neoplasm Risk Among Individuals With a Pathogenic Germline Variant in DICER1. *J Clin Oncol* 2019; **37**: 668-676 [PMID: 30715996 DOI: 10.1200/JCO.2018.78.4678]

171 **Plon SE**, Lupo PJ. Genetic Predisposition to Childhood Cancer in the Genomic Era. *Annu Rev Genomics Hum Genet* 2019; **20**: 241-263 [PMID: 31082280 DOI: 10.1146/annurev-genom-083118-015415]

172 **Sestini R**, Bacci C, Provenzano A, Genuardi M, Papi L. Evidence of a four-hit mechanism involving SMARCB1 and NF2 in schwannomatosis-associated schwannomas. *Hum Mutat* 2008; **29**: 227-231 [PMID: 18072270 DOI: 10.1002/humu.20679]

173 **Tabori U**, Hansford JR, Achatz MI, Kratz CP, Plon SE, Frebourg T, Brugières L. Clinical Management and Tumor Surveillance Recommendations of Inherited Mismatch Repair Deficiency in Childhood. *Clin Cancer Res* 2017; **23**: e32-e37 [PMID: 28572265 DOI: 10.1158/1078-0432.CCR-17-0574]

174 **Mangaonkar AA**, Patnaik MM. Hereditary Predisposition to Hematopoietic Neoplasms: When Bloodline Matters for Blood Cancers. *Mayo Clin Proc* 2020; **95**: 1482-1498 [PMID: 32571604 DOI: 10.1016/j.mayocp.2019.12.013]

175 **Klco JM**, Mullighan CG. Advances in germline predisposition to acute leukaemias and myeloid neoplasms. *Nat Rev Cancer* 2021; **21**: 122-137 [PMID: 33328584 DOI: 10.1038/s41568-020-00315-z]

176 **Szmyd B**, Mlynarski W, Pastorczak A. Genetic predisposition to lymphomas: Overview of rare syndromes and inherited familial variants. *Mutat Res Rev Mutat Res* 2021; **788**: 108386 [PMID: 34893151 DOI: 10.1016/j.mrrev.2021.108386]

177 **Samadder NJ**, Giridhar KV, Baffy N, Riegert-Johnson D, Couch FJ. Hereditary Cancer Syndromes-A Primer on Diagnosis and Management: Part 1: Breast-Ovarian Cancer Syndromes. *Mayo Clin Proc* 2019; **94**: 1084-1098 [PMID: 31171119 DOI: 10.1016/j.mayocp.2019.02.017]

178 **Marmolejo DH**, Wong MYZ, Bajalica-Lagercrantz S, Tischkowitz M, Balmaña J; extended ERN-GENTURIS Thematic Group 3. Overview of hereditary breast and ovarian cancer (HBOC) guidelines across Europe. *Eur J Med Genet* 2021; **64**: 104350 [PMID: 34606975 DOI: 10.1016/j.ejmg.2021.104350]

179 **Piombino C**, Cortesi L, Lambertini M, Punie K, Grandi G, Toss A. Secondary Prevention in Hereditary Breast and/or Ovarian Cancer Syndromes Other Than BRCA. *J Oncol* 2020; **2020**: 6384190 [PMID: 32733558 DOI: 10.1155/2020/6384190]

180 **Pouptsis A**, Swafe L, Patwardhan M, Stavraka C. Surgical and Systemic Treatment of Hereditary Breast Cancer: A Mini-Review With a Focus on BRCA1 and BRCA2 Mutations. *Front Oncol* 2020; **10**: 553080 [PMID: 33194613 DOI: 10.3389/fonc.2020.553080]

181 **Al-Sarhani H**, Gottumukkala RV, Grasparil ADS 2nd, Tung EL, Gee MS, Greer MC. Screening of cancer predisposition syndromes. *Pediatr Radiol* 2022; **52**: 401-417 [PMID: 33791839 DOI: 10.1007/s00247-021-05023-w]

182 **Møller P**, Stormorken A, Jonsrud C, Holmen MM, Hagen AI, Clark N, Vabø A, Sun P, Narod SA, Mæhle L. Survival of patients with BRCA1-associated breast cancer diagnosed in an MRI-based surveillance program. *Breast Cancer Res Treat* 2013; **139**: 155-161 [PMID: 23615785 DOI: 10.1007/s10549-013-2540-z]

183 **Jacobson M**, Coakley N, Bernardini M, Branco KA, Elit L, Ferguson S, Kim R. Risk reduction strategies for BRCA1/2 hereditary ovarian cancer syndromes: a clinical practice guideline. *Hered Cancer Clin Pract* 2021; **19**: 39 [PMID: 34565426 DOI: 10.1186/s13053-021-00196-9]

184 **Machens A**, Dralle H. Long-term outcome after DNA-based prophylactic neck surgery in children at risk of hereditary medullary thyroid cancer. *Best Pract Res Clin Endocrinol Metab* 2019; **33**: 101274 [PMID: 31043326 DOI: 10.1016/j.beem.2019.04.008]

185 **Mathiesen JS**, Effraimidis G, Rossing M, Rasmussen ÅK, Hoejberg L, Bastholt L, Godballe C, Oturai P, Feldt-Rasmussen U. Multiple endocrine neoplasia type 2: A review. *Semin Cancer Biol* 2022; **79**: 163-179 [PMID: 33812987 DOI: 10.1016/j.semcancer.2021.03.035]

186 **Eleje GU**, Eke AC, Ezebialu IU, Ikechebelu JI, Ugwu EO, Okonkwo OO. Risk-reducing bilateral salpingo-oophorectomy in women with BRCA1 or BRCA2 mutations. *Cochrane Database Syst Rev* 2018; **8**: CD012464 [PMID: 30141832 DOI: 10.1002/14651858.CD012464.pub2]

187 **Heemskerk-Gerritsen BAM**, Jager A, Koppert LB, Obdeijn AI, Collée M, Meijers-Heijboer HEJ, Jenner DJ, Oldenburg HSA, van Engelen K, de Vries J, van Asperen CJ, Devilee P, Blok MJ, Kets CM, Ausems MGEM, Seynaeve C, Rookus MA, Hooning MJ. Survival after bilateral risk-reducing mastectomy in healthy BRCA1 and BRCA2 mutation carriers. *Breast Cancer Res Treat* 2019; **177**: 723-733 [PMID: 31302855 DOI: 10.1007/s10549-019-05345-2]

188 **Iyevleva AG**, Imyanitov EN. Cytotoxic and targeted therapy for hereditary cancers. *Hered Cancer Clin Pract* 2016; **14**: 17 [PMID: 27555886 DOI: 10.1186/s13053-016-0057-2]

189 **Thavaneswaran S**, Rath E, Tucker K, Joshua AM, Hess D, Pinese M, Ballinger ML, Thomas DM. Therapeutic implications of germline genetic findings in cancer. *Nat Rev Clin Oncol* 2019; **16**: 386-396 [PMID: 30783251 DOI: 10.1038/s41571-019-0179-3]

190 **Byrski T**, Gronwald J, Huzarski T, Grzybowska E, Budryk M, Stawicka M, Mierzwa T, Szwiec M, Wisniowski R, Siolek M, Dent R, Lubinski J, Narod S. Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy. *J Clin Oncol* 2010; **28**: 375-379 [PMID: 20008645 DOI: 10.1200/JCO.2008.20.7019]

191 **Tutt A**, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P, Owen J, Abraham J, Barrett S, Barrett-Lee P, Brown R, Chan S, Dowsett M, Flanagan JM, Fox L, Grigoriadis A, Gutin A, Harper-Wynne C, Hatton MQ, Hoadley KA, Parikh J, Parker P, Perou CM, Roylance R, Shah V, Shaw A, Smith IE, Timms KM, Wardley AM, Wilson G, Gillett C, Lanchbury JS, Ashworth A, Rahman N, Harries M, Ellis P, Pinder SE, Bliss JM. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. *Nat Med* 2018; **24**: 628-637 [PMID: 29713086 DOI: 10.1038/s41591-018-0009-7]

192 **Imyanitov EN**. Cytotoxic and targeted therapy for BRCA1/2-driven cancers. *Hered Cancer Clin Pract* 2021; **19**: 36 [PMID: 34454564 DOI: 10.1186/s13053-021-00193-y]

193 **Gorodnova TV**, Kotiv KB, Ivantsov AO, Mikheyeva ON, Mikhailiuk GI, Lisyanskaya AS, Mikaya NA, Guseynov KD, Bondarev NE, Matveyeva NS, Nekrasova EA, Sidoruk AA, Roman LD, Manikhas GM, Belyaev AM, Sokolenko AP, Berlev IV, Imyanitov EN. Efficacy of Neoadjuvant Therapy With Cisplatin Plus Mitomycin C in BRCA1-Mutated Ovarian Cancer. *Int J Gynecol Cancer* 2018; **28**: 1498-1506 [PMID: 30247247 DOI: 10.1097/IGC.0000000000001352]

194 **Gorodnova TV**, Sokolenko AP, Kondratiev SV, Kotiv KB, Belyaev AM, Berlev IV, Imyanitov EN. Mitomycin C plus cisplatin for systemic treatment of recurrent BRCA1-associated ovarian cancer. *Invest New Drugs* 2020; **38**: 1872-1878 [PMID: 32591974 DOI: 10.1007/s10637-020-00965-8]

195 **Chan CY**, Tan KV, Cornelissen B. PARP Inhibitors in Cancer Diagnosis and Therapy. *Clin Cancer Res* 2021; **27**: 1585-1594 [PMID: 33082213 DOI: 10.1158/1078-0432.CCR-20-2766]

196 **Póti Á**, Gyergyák H, Németh E, Rusz O, Tóth S, Kovácsházi C, Chen D, Szikriszt B, Spisák S, Takeda S, Szakács G, Szallasi Z, Richardson AL, Szüts D. Correlation of homologous recombination deficiency induced mutational signatures with sensitivity to PARP inhibitors and cytotoxic agents. *Genome Biol* 2019; **20**: 240 [PMID: 31727117 DOI: 10.1186/s13059-019-1867-0]

197 **Stopsack KH**. Efficacy of PARP Inhibition in Metastatic Castration-resistant Prostate Cancer is Very Different with Non-BRCA DNA Repair Alterations: Reconstructing Prespecified Endpoints for Cohort B from the Phase 3 PROfound Trial of Olaparib. *Eur Urol* 2021; **79**: 442-445 [PMID: 33012578 DOI: 10.1016/j.eururo.2020.09.024]

198 **Westphalen CB**, Fine AD, André F, Ganesan S, Heinemann V, Rouleau E, Turnbull C, Garcia Palacios L, Lopez JA, Sokol ES, Mateo J. Pan-cancer Analysis of Homologous Recombination Repair-associated Gene Alterations and Genome-wide Loss-of-Heterozygosity Score. *Clin Cancer Res* 2022; **28**: 1412-1421 [PMID: 34740923 DOI: 10.1158/1078-0432.CCR-21-2096]

199 **André T**, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, Smith D, Garcia-Carbonero R, Benavides M, Gibbs P, de la Fouchardiere C, Rivera F, Elez E, Bendell J, Le DT, Yoshino T, Van Cutsem E, Yang P, Farooqui MZH, Marinello P, Diaz LA Jr; KEYNOTE-177 Investigators. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. *N Engl J Med* 2020; **383**: 2207-2218 [PMID: 33264544 DOI: 10.1056/NEJMoa2017699]

200 **Le DT**, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Greten TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH, Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA, Diaz LA Jr. Mismatch repair deficiency predicts response of solid tumors to PD-

1 blockade. *Science* 2017; **357**: 409-413 [PMID: 28596308 DOI: 10.1126/science.aan6733]

201 **Bellone S**, Roque DM, Siegel ER, Buza N, Hui P, Bonazzoli E, Guglielmi A, Zammataro L, Nagarkatti N, Zaidi S, Lee J, Silasi DA, Huang GS, Andikyan V, Damast S, Clark M, Azodi M, Schwartz PE, Tymon-Rosario JR, Harold JA, Mauricio D, Zeybek B, Menderes G, Altwerger G, Ratner E, Alexandrov LB, Iwasaki A, Kong Y, Song E, Dong W, Elvin JA, Choi J, Santin AD. A phase 2 evaluation of pembrolizumab for recurrent Lynch-like versus sporadic endometrial cancers with microsatellite instability. *Cancer* 2022; **128**: 1206-1218 [PMID: 34875107 DOI: 10.1002/cncr.34025]

202 **Lenz HJ**, Van Cutsem E, Luisa Limon M, Wong KYM, Hendlisz A, Aglietta M, García-Alfonso P, Neyns B, Luppi G, Cardin DB, Dragovich T, Shah U, Abdullaev S, Gricar J, Ledeine JM, Overman MJ, Lonardi S. First-Line Nivolumab Plus Low-Dose Ipilimumab for Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: The Phase II CheckMate 142 Study. *J Clin Oncol* 2022; **40**: 161-170 [PMID: 34637336 DOI: 10.1200/JCO.21.01015]

203 **Johanns TM**, Miller CA, Dorward IG, Tsien C, Chang E, Perry A, Uppaluri R, Ferguson C, Schmidt RE, Dahiya S, Ansstas G, Mardis ER, Dunn GP. Immunogenomics of Hypermutated Glioblastoma: A Patient with Germline POLE Deficiency Treated with Checkpoint Blockade Immunotherapy. *Cancer Discov* 2016; **6**: 1230-1236 [PMID: 27683556 DOI: 10.1158/2159-8290.CD-16-0575]

204 **Volkov NM**, Yanus GA, Ivantsov AO, Moiseenko FV, Matorina OG, Bizin IV, Moiseyenko VM, Imyanitov EN. Efficacy of immune checkpoint blockade in MUTYH-associated hereditary colorectal cancer. *Invest New Drugs* 2020; **38**: 894-898 [PMID: 31377904 DOI: 10.1007/s10637-019-00842-z]

205 **AlHarbi M**, Ali Mobark N, AlMubarak L, Aljelaify R, AlSaeed M, Almutairi A, Alqubaishi F, Hussain ME, Balbaid AAO, Said Marie A, AlSubaie L, AlShieban S, alTassan N, Ramkissoon SH, Abedalthagafi M. Durable Response to Nivolumab in a Pediatric Patient with Refractory Glioblastoma and Constitutional Biallelic Mismatch Repair Deficiency. *Oncologist* 2018; **23**: 1401-1406 [PMID: 30104292 DOI: 10.1634/theoncologist.2018-0163]

206 **Pavelka Z**, Zitterbart K, Nosková H, Bajčiová V, Slabý O, Štěrba J. Effective Immunotherapy of Glioblastoma in an Adolescent with Constitutional Mismatch Repair-Deficiency Syndrome. *Klin Onkol* 2019; **32**: 70-74 [PMID: 30764633 DOI: 10.14735/amko201970]

207 **Fox E**, Widemann BC, Chuk MK, Marcus L, Aikin A, Whitcomb PO, Merino MJ, Lodish M, Dombi E, Steinberg SM, Wells SA, Balis FM. Vandetanib in children and adolescents with multiple endocrine neoplasia type 2B associated medullary thyroid carcinoma. *Clin Cancer Res* 2013; **19**: 4239-4248 [PMID: 23766359 DOI: 10.1158/1078-0432.CCR-13-0071]

208 **Wirth LJ**, Sherman E, Robinson B, Solomon B, Kang H, Lorch J, Worden F, Brose M, Patel J, Leboulleux S, Godbert Y, Barlesi F, Morris JC, Owonikoko TK, Tan DSW, Gautschi O, Weiss J, de la Fouchardière C, Burkard ME, Laskin J, Taylor MH, Kroiss M, Medioni J, Goldman JW, Bauer TM, Levy B, Zhu VW, Lakhani N, Moreno V, Ebata K, Nguyen M, Heirich D, Zhu EY, Huang X, Yang L, Kherani J, Rothenberg SM, Drilon A, Subbiah V, Shah MH, Cabanillas ME. Efficacy of Selpercatinib in RET-Altered Thyroid Cancers. *N Engl J Med* 2020; **383**: 825-835 [PMID: 32846061 DOI: 10.1056/NEJMoa2005651]

209 **Zheng X**, Ji Q, Sun Y, Ge M, Zhang B, Cheng Y, Lei S, Shi F, Guo Y, Li L, Chen L, Shao J, Zhang W, Gao M. Efficacy and safety of selpercatinib in Chinese patients with advanced RET-altered thyroid cancers: results from the phase II LIBRETTO-321 study. *Ther Adv Med Oncol* 2022; **14**: 17588359221119318 [PMID: 36062046 DOI: 10.1177/17588359221119318]

210 **Fangusaro J**, Onar-Thomas A, Young Poussaint T, Wu S, Ligon AH, Lindeman N, Banerjee A, Packer RJ, Kilburn LB, Goldman S, Pollack IF, Qaddoumi I, Jakacki RI, Fisher PG, Dhall G, Baxter P, Kreissman SG, Stewart CF, Jones DTW, Pfister SM, Vezina G, Stern JS, Panigrahy A, Patay Z, Tamrazi B, Jones JY, Haque SS, Enterline DS, Cha S, Fisher MJ, Doyle LA, Smith M, Dunkel IJ, Fouladi M. Selumetinib in paediatric patients with BRAF-aberrant or neurofibromatosis type 1-associated recurrent, refractory, or progressive low-grade glioma: a multicentre, phase 2 trial. *Lancet Oncol* 2019; **20**: 1011-1022 [PMID: 31151904 DOI: 10.1016/S1470-2045(19)30277-3]

211 **Gross AM**, Wolters PL, Dombi E, Baldwin A, Whitcomb P, Fisher MJ, Weiss B, Kim A, Bornhorst M, Shah AC, Martin S, Roderick MC, Pichard DC, Carbonell A, Paul SM, Therrien J, Kapustina O, Heisey K, Clapp DW, Zhang C, Peer CJ, Figg WD, Smith M, Glod J, Blakeley JO, Steinberg SM, Venzon DJ, Doyle LA, Widemann BC. Selumetinib in Children with Inoperable Plexiform Neurofibromas. *N Engl J Med* 2020; **382**: 1430-1442 [PMID: 32187457 DOI: 10.1056/NEJMoa1912735]

212 **Campbell BB**, Galati MA, Stone SC, Riemenschneider AN, Edwards M, Sudhaman S, Siddaway R, Komosa M, Nunes NM, Nobre L, Morrissy AS, Zatzman M, Zapotocky M, Joksimovic L, Kalimuthu SN, Samuel D, Mason G, Bouffet E, Morgenstern DA, Aronson M, Durno C, Malkin D, Maris JM, Taylor MD, Shlien A, Pugh TJ, Ohashi PS, Hawkins CE, Tabori U. Mutations in the RAS/MAPK Pathway Drive Replication Repair-Deficient Hypermutated Tumors and Confer Sensitivity to MEK Inhibition. *Cancer Discov* 2021; **11**: 1454-1467 [PMID: 33563663 DOI: 10.1158/2159-8290.CD-20-1050]

213 **Tang JY**, Ally MS, Chanana AM, Mackay-Wiggan JM, Aszterbaum M, Lindgren JA, Ulerio G, Rezaee MR, Gildengorin G, Marji J, Clark C, Bickers DR, Epstein EH Jr. Inhibition of the hedgehog pathway in patients with basal-cell nevus syndrome: final results from the multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Oncol* 2016; **17**: 1720-1731 [PMID: 27838224 DOI: 10.1016/S1470-2045(16)30566-6]

214 **Franz DN**, Belousova E, Sparagana S, Bebin EM, Frost M, Kuperman R, Witt O, Kohrman MH, Flamini JR, Wu JY, Curatolo P, de Vries PJ, Berkowitz N, Anak O, Niolat J, Jozwiak S. Everolimus for subependymal giant cell astrocytoma in patients with tuberous sclerosis complex: 2-year open-label extension of the randomised EXIST-1 study. *Lancet Oncol* 2014; **15**: 1513-1520 [PMID: 25456370 DOI: 10.1016/S1470-2045(14)70489-9]

215 **Geynisman DM**, Kadow BT, Shuch BM, Boorjian SA, Matin SF, Rampersaud E, Milestone BN, Plimack ER, Zibelman MR, Kutikov A, Smaldone MC, Chen DY, Viterbo R, Joshi S, Greenberg RE, Malizzia L, McGowan T, Ross EA, Uzzo RG. Sporadic Angiomyolipomas Growth Kinetics While on Everolimus: Results of a Phase II Trial. *J Urol* 2020; **204**: 531-537 [PMID: 32250730 DOI: 10.1097/JU.0000000000001065]

216 **Jonasch E**, Donskov F, Iliopoulos O, Rathmell WK, Narayan VK, Maughan BL, Oudard S, Else T, Maranchie JK, Welsh SJ, Thamake S, Park EK, Perini RF, Linehan WM, Srinivasan R; MK-6482-004 Investigators. Belzutifan for Renal Cell

Carcinoma in von Hippel-Lindau Disease. *N Engl J Med* 2021; **385**: 2036-2046 [PMID: 34818478 DOI: 10.1056/NEJMoa2103425]

217 **Carril-Ajuria L**, Colomba E, Cerbone L, Romero-Ferreiro C, Crouzet L, Laguerre B, Thibault C, Vicier C, de Velasco G, Fléchon A, Saldana C, Benusiglio PR, Bressac-de Paillerets B, Guillaud-Bataille M, Gaignard P, Scoazec JY, Richard S, Caron O, Escudier B, Albiges L. Response to systemic therapy in fumarate hydratase-deficient renal cell carcinoma. *Eur J Cancer* 2021; **151**: 106-114 [PMID: 33975058 DOI: 10.1016/j.ejca.2021.04.009]

218 **Gleeson JP**, Nikolovski I, Dinatale R, Zucker M, Knezevic A, Patil S, Ged Y, Kotecha RR, Shapnik N, Murray S, Russo P, Coleman J, Lee CH, Stadler ZK, Hakimi AA, Feldman DR, Motzer RJ, Reznik E, Voss MH, Chen YB, Carlo MI. Comprehensive Molecular Characterization and Response to Therapy in Fumarate Hydratase-Deficient Renal Cell Carcinoma. *Clin Cancer Res* 2021; **27**: 2910-2919 [PMID: 33658299 DOI: 10.1158/1078-0432.CCR-20-4367]

219 **Telli ML**, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, Szallasi Z, Barry WT, Winer EP, Tung NM, Isakoff SJ, Ryan PD, Greene-Colozzi A, Gutin A, Sangale Z, Iliev D, Neff C, Abkevich V, Jones JT, Lanchbury JS, Hartman AR, Garber JE, Ford JM, Silver DP, Richardson AL. Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer. *Clin Cancer Res* 2016; **22**: 3764-3773 [PMID: 26957554 DOI: 10.1158/1078-0432.CCR-15-2477]

220 **Sokolenko AP**, Gorodnova TV, Bizin IV, Kuligina ES, Kotiv KB, Romanko AA, Ermachenkova TI, Ivantsov AO, Preobrazhenskaya EV, Sokolova TN, Broyde RV, Imyanitov EN. Molecular predictors of the outcome of paclitaxel plus carboplatin neoadjuvant therapy in high-grade serous ovarian cancer patients. *Cancer Chemother Pharmacol* 2021; **88**: 439-450 [PMID: 34080040 DOI: 10.1007/s00280-021-04301-6]

221 **Mighton C**, Lerner-Ellis JP. Principles of molecular testing for hereditary cancer. *Genes Chromosomes Cancer* 2022; **61**: 356-381 [PMID: 35436018 DOI: 10.1002/gcc.23048]

222 **Kuligina ESh**, Sokolenko AP, Mitiushkina NV, Abysheva SN, Preobrazhenskaya EV, Gorodnova TV, Yanus GA, Togo AV, Cherdyntseva NV, Bekhtereva SA, Dixon JM, Larionov AA, Kuznetsov SG, Imyanitov EN. Value of bilateral breast cancer for identification of rare recessive at-risk alleles: evidence for the role of homozygous GEN1 c.2515_2519delAAGTT mutation. *Fam Cancer* 2013; **12**: 129-132 [PMID: 23104382 DOI: 10.1007/s10689-012-9575-x]

223 **Kurian AW**. BRCA1 and BRCA2 mutations across race and ethnicity: distribution and clinical implications. *Curr Opin Obstet Gynecol* 2010; **22**: 72-78 [PMID: 19841585 DOI: 10.1097/GCO.0b013e328332dca3]

224 **AlHarthi FS**, Qari A, Edress A, Abedalthagafi M. Familial/inherited cancer syndrome: a focus on the highly consanguineous Arab population. *NPJ Genom Med* 2020; **5**: 3 [PMID: 32025336 DOI: 10.1038/s41525-019-0110-y]

225 **Palmer JR**, Polley EC, Hu C, John EM, Haiman C, Hart SN, Gaudet M, Pal T, Anton-Culver H, Trentham-Dietz A, Bernstein L, Ambrosone CB, Bandera EV, Bertrand KA, Bethea TN, Gao C, Gnanaolivu RD, Huang H, Lee KY, LeMarchand L, Na J, Sandler DP, Shah PD, Yadav S, Yang W, Weitzel JN, Domchek SM, Goldgar DE, Nathanson KL, Kraft P, Yao S, Couch FJ. Contribution of Germline Predisposition Gene Mutations to Breast Cancer Risk in African American Women. *J Natl Cancer Inst* 2020; **112**: 1213-1221 [PMID: 32427313 DOI: 10.1093/jnci/djaa040]

226 **Yadav S**, LaDuca H, Polley EC, Hu C, Niguidula N, Shimelis H, Lilyquist J, Na J, Lee KY, Gutierrez S, Yussuf A, Hart SN, Davis BT, Chao EC, Pesaran T, Goldgar DE, Dolinsky JS, Couch FJ. Racial and Ethnic Differences in Multigene Hereditary Cancer Panel Test Results for Women With Breast Cancer. *J Natl Cancer Inst* 2021; **113**: 1429-1433 [PMID: 33146377 DOI: 10.1093/jnci/djaa167]

227 **Monteiro AN**, Bouwman P, Kousholt AN, Eccles DM, Millot GA, Masson JY, Schmidt MK, Sharan SK, Scully R, Wiesmüller L, Couch F, Vreeswijk MPG. Variants of uncertain clinical significance in hereditary breast and ovarian cancer genes: best practices in functional analysis for clinical annotation. *J Med Genet* 2020; **57**: 509-518 [PMID: 32152249 DOI: 10.1136/jmedgenet-2019-106368]

228 **Boonen RACM**, Wiegant WW, Celosse N, Vroling B, Heijl S, Kote-Jarai Z, Mijuskovic M, Cristea S, Solleveld-Westerink N, van Wezel T, Beerenwinkel N, Eeles R, Devilee P, Vreeswijk MPG, Marra G, van Attikum H. Functional Analysis Identifies Damaging CHEK2 Missense Variants Associated with Increased Cancer Risk. *Cancer Res* 2022; **82**: 615-631 [PMID: 34903604 DOI: 10.1158/0008-5472.CAN-21-1845]

229 **Evans DGR**, van Veen EM, Byers HJ, Wallace AJ, Ellingford JM, Beaman G, Santoyo-Lopez J, Aitman TJ, Eccles DM, Lalloo FI, Smith MJ, Newman WG. A Dominantly Inherited 5' UTR Variant Causing Methylation-Associated Silencing of BRCA1 as a Cause of Breast and Ovarian Cancer. *Am J Hum Genet* 2018; **103**: 213-220 [PMID: 30075112 DOI: 10.1016/j.ajhg.2018.07.002]

230 **Hansen TV**, Ejlertsen B, Albrechtsen A, Bergsten E, Bjerregaard P, Hansen T, Myrhøj T, Nielsen PB, Timmermans-Wielenga V, Andersen MK, Jønson L, Nielsen FC. A common Greenlandic Inuit BRCA1 RING domain founder mutation. *Breast Cancer Res Treat* 2009; **115**: 69-76 [PMID: 18500671 DOI: 10.1007/s10549-008-0060-z]

231 **Harboe TL**, Eiberg H, Kern P, Ejlertsen B, Nedergaard L, Timmermans-Wielenga V, Nielsen IM, Bisgaard ML. A high frequent BRCA1 founder mutation identified in the Greenlandic population. *Fam Cancer* 2009; **8**: 413-419 [PMID: 19504351 DOI: 10.1007/s10689-009-9257-5]

232 **Marchetti C**, De Leo R, Musella A, D'Indinosante M, Capoluongo E, Minucci A, Benedetti Panici P, Scambia G, Fagotti A. BRCA Mutation Status to Personalize Management of Recurrent Ovarian Cancer: A Multicenter Study. *Ann Surg Oncol* 2018; **25**: 3701-3708 [PMID: 30128899 DOI: 10.1245/s10434-018-6700-6]

233 **Morgan RD**, Burghel GJ, Flaum N, Bulman M, Clamp AR, Hasan J, Mitchell CL, Schlecht H, Woodward ER, Lallo FI, Crosbie EJ, Edmondson RJ, Wallace AJ, Jayson GC, Evans DGR. Prevalence of germline pathogenic BRCA1/2 variants in sequential epithelial ovarian cancer cases. *J Med Genet* 2019; **56**: 301-307 [PMID: 30683677 DOI: 10.1136/jmedgenet-2018-105792]

234 **AlDubayan SH**, Pyle LC, Gamulin M, Kulis T, Moore ND, Taylor-Weiner A, Hamid AA, Reardon B, Wubbenhorst B, Godse R, Vaughn DJ, Jacobs LA, Meien S, Grgic M, Kastelan Z, Markt SC, Damrauer SM, Rader DJ, Kember RL, Loud JT, Kanetsky PA, Greene MH, Sweeney CJ, Kubisch C, Nathanson KL, Van Allen EM, Stewart DR, Lessel D; Regeneron Genetics Center (RGC) Research Team. Association of Inherited Pathogenic Variants in Checkpoint Kinase 2 (CHEK2) With Susceptibility to Testicular Germ Cell Tumors. *JAMA Oncol* 2019; **5**: 514-522 [PMID: 30676620 DOI: 10.1001/jamaoncol.2018.6477]

235 **Ni VI**, Ivantsov AO, Kotkova MA, Baskina SV, Ponomareva EV, Orlova RV, Topuzov EE, Kryukov KK, Shelekhova KV, Aleksakhina SN, Sokolenko AP, Imyanitov EN. Small fraction of testicular cancer cases may be causatively related to CHEK2 inactivating germ-line mutations: evidence for somatic loss of the remaining CHEK2 allele in the tumor tissue. *Fam Cancer* 2021; **20**: 49-53 [PMID: 32451744 DOI: 10.1007/s10689-020-00190-5]

236 **Ring KL**, Bruegl AS, Allen BA, Elkin EP, Singh N, Hartman AR, Daniels MS, Broaddus RR. Germline multi-gene hereditary cancer panel testing in an unselected endometrial cancer cohort. *Mod Pathol* 2016; **29**: 1381-1389 [PMID: 27443514 DOI: 10.1038/modpathol.2016.135]

237 **Cameron A**, Chiarella-Redfern H, Chu P, Perrier R, Duggan MA. Universal Testing to Identify Lynch Syndrome Among Women With Newly Diagnosed Endometrial Carcinoma. *J Obstet Gynaecol Can* 2020; **42**: 137-143 [PMID: 31679916 DOI: 10.1016/j.jogc.2019.06.018]

238 **Pearlman R**, Frankel WL, Swanson BJ, Jones D, Zhao W, Yilmaz A, Miller K, Bacher J, Bigley C, Nelsen L, Goodfellow PJ, Goldberg RM, Paskett E, Shields PG, Freudenheim JL, Stanich PP, Lattimer I, Arnold M, Prior TW, Haut M, Kalady MF, Heald B, Paquette I, Draper DJ, Brell JM, Mahesh S, Weeman K, Bastola S, Zangmeister J, Gowda A, Kencana F, Malcolm A, Liu Y, Cole S, Bane C, Li C, Rehmus E, Pritchard CC, Shirts BH, Jacobson A, Cummings SA, de la Chapelle A, Hampel H. Prospective Statewide Study of Universal Screening for Hereditary Colorectal Cancer: The Ohio Colorectal Cancer Prevention Initiative. *JCO Precis Oncol* 2021; **5** [PMID: 34250417 DOI: 10.1200/PO.20.00525]

239 **Bakhuizen JJ**, Hogervorst FB, Velthuizen ME, Ruijs MW, van Engelen K, van Os TA, Gille JJ, Collée M, van den Ouweland AM, van Asperen CJ, Kets CM, Mensenkamp AR, Leter EM, Blok MJ, de Jong MM, Ausems MG. TP53 germline mutation testing in early-onset breast cancer: findings from a nationwide cohort. *Fam Cancer* 2019; **18**: 273-280 [PMID: 30607672 DOI: 10.1007/s10689-018-00118-0]

240 **Evans DG**, van Veen EM, Byers HJ, Evans SJ, Burghel GJ, Woodward ER, Harkness EF, Eccles DM, Greville-Haygate SL, Ellingford JM, Bowers NL, Pereira M, Wallace AJ, Howell SJ, Howell A, Lalloo F, Newman WG, Smith MJ. High likelihood of actionable pathogenic variant detection in breast cancer genes in women with very early onset breast cancer. *J Med Genet* 2022; **59**: 115-121 [PMID: 33758026 DOI: 10.1136/jmedgenet-2020-107347]

241 **Beebe-Dimmer JL**, Hathcock M, Yee C, Okoth LA, Ewing CM, Isaacs WB, Cooney KA, Thibodeau SN. The HOXB13 G84E Mutation Is Associated with an Increased Risk for Prostate Cancer and Other Malignancies. *Cancer Epidemiol Biomarkers Prev* 2015; **24**: 1366-1372 [PMID: 26108461 DOI: 10.1158/1055-9965.EPI-15-0247]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 February 24; 14(2): 69-80

DOI: 10.5306/wjco.v14.i2.69

ISSN 2218-4333 (online)

REVIEW

Significance of music therapy in treating depression and anxiety disorders among people with cancer

Chiedu Eseadi, Millicent O Ngwu

Specialty type: Behavioral sciences

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Liu Z, China; Lu H, China

Received: December 29, 2022
Peer-review started: December 29, 2022
First decision: January 9, 2023
Revised: January 16, 2023
Accepted: February 7, 2023
Article in press: February 7, 2023
Published online: February 24, 2023

Chiedu Eseadi, Department of Educational Psychology, University of Johannesburg, Gauteng 2006, South Africa

Millicent O Ngwu, Department of Sociology and Anthropology, University of Nigeria, Nsukka 41001, Enugu, Nigeria

Corresponding author: Chiedu Eseadi, PhD, Senior Postdoctoral Fellow, Department of Educational Psychology, University of Johannesburg, Gauteng 2006, South Africa. chiedu.eseadi@unn.edu.ng

Abstract

Globally, cancer cases and mortality have recently escalated and have attracted global concern. The clinical diagnosis and manifestation of cancer can result in significant mental health issues like depression and anxiety disorders. The tendency of people with cancer to suffer from psychological disorders such as anxiety and depression is usually high. A significant number of deaths related to cancer may likely not be from the killer disease but from psychological disorders associated with the illness. The utilization of music as a remedial approach to healing mental disorders cannot be overstated. Thus, identifying the impacts of music therapy in dealing with depression and anxiety disorders among people with cancer is relevant, as the majority of methods used in treating cancer have some side effects which may trigger psychological disorders in cancer patients. Ultimately, this study explored the significance of music therapy in treating depression and anxiety disorders among people with cancer. To achieve the aim of this study, the authors employed a narrative literature review to investigate the significance of music therapy in addressing depression and anxiety disorders among people with cancer. The type of literature review employed in this study is to provide an understanding of the selected research papers. The review found that music therapy significantly reduces depression and anxiety disorders among breast cancer, lung cancer, prostate cancer, and colorectal cancer patients. It is needful for healthcare providers to incorporate music therapy interventions while treating people with cancer. This will help reduce cancer deaths resulting from psychological disorders rather than the killer disease, cancer. However, the standardized procedures and evaluation criteria for applying music-based intervention strategies in oncology medicine still need to be further established and improved.

Key Words: Anxiety disorders; Breast cancer; Cancer; Cancer patients; Colorectal cancer;

Depression; Lung cancer; Music therapy; Prostate cancer

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: People diagnosed with cancer and receiving treatment experience anxiety and depression, which may influence their healing process. Most cancer patients may die from depression, anxiety, and other psychological disorders. The mental health of cancer patients is important as their physical health. Therefore, addressing the psychological needs of people with cancer is necessary to improve their health status. In this review, we demonstrate music therapy as a significant treatment approach for reducing depression and anxiety disorders among patients with breast cancer, lung cancer, prostate cancer, and colorectal cancer.

Citation: Eseadi C, Ngwu MO. Significance of music therapy in treating depression and anxiety disorders among people with cancer. *World J Clin Oncol* 2023; 14(2): 69-80
URL: https://www.wjgnet.com/2218-4333/full/v14/i2/69.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i2.69

INTRODUCTION

A health condition known as cancer occurs when a small percentage of the cells in the body develop abnormally and disseminate to other bodily regions. Cancer can develop from any body part that accommodates trillions of cells[1]. The dissemination of cancerous cells to other parts of the body system is known as metastasizing, which mainly results in death from cancer. Whereas the 21st century has witnessed an improved performance of public nutrition, social behavior, and personal hygiene, as well as the methods adopted in preventing, controlling, and treating infectious diseases, which have all contributed to the current rise in the average lifespan of people, there is still an upsurge of cancer-related cases and deaths[2]. Globally, cancer has had a remarkable impact on the physical and psychological well-being and finances of individuals, family relations, communities, and the healthcare sector. Most of the healthcare system in resource-limited areas is poorly equipped. Thus, many cancer patients in these locations lack access to prompt, effective detection and subsequent medical therapy. The availability of early detection, effective therapy, and post-treatment care in nations with robust health systems have improved the survival rates of many different malignancies[3,4]. However, cancer prevalence and its common occurrence in emerging economies have continued to vary globally. Thus, in 1975, limited-resource areas accounted for over half (51%) of all cancer cases globally. By 2007, this number had risen to 55%, it has been predicted to rise to 61% by 2050[5,6]. Lung, breast, colorectal, and prostate cancers primarily associated with advanced economics have become a global concern. Diagnosing cancer induces mental stress, which may result in depression, fear, and anxiety[7,8].

Approximately 50% to 85% of cancer patients experiencing late-stage treatment report anxiety and depression[7]. Depression and anxiety negatively influence all aspects of cancer malignancy and its development, the efficacy of given therapy, and the patient's quality of life[9]. The practice of using music to aid recovery and improve the quality of life by medical and music experts is known as music therapy. Music therapy can have several advantages when used in conjunction with traditional cancer treatment[10]. Music therapy is referred to as the most widely used supportive and creative method of treating psychosocial-related impacts of cancer disease[11]. Music therapy is further defined as a systematic method of using music as a therapeutic means of rejuvenating, maintaining, and improving individuals' psychological and physical well-being[12]. Music therapy aims to reduce or eliminate psychological discomfort and improve the health status of individuals suffering from cancer-related health issues. Studies on people living with chronic cancer cases and those in palliative care have revealed that music therapy has drawn massive attention in research and medical treatment[13,14].

Further, the utilization of music as a remedial approach to healing mental disorders dates back to the olden days. Music is referred to as a healer as it aids in the reduction of anxiety and improves relaxation in patients suffering from chronic illness[15]. In the same vein, the realization of music as a contemporary psychotherapeutic approach in medical practice started after the second world war in the 20th century, which witnessed the introduction of courses and training sections and the establishment of national bodies around the globe[16]. The primary objective of this study is to examine depression and anxiety disorders' reports in cancer patients, give a narrative review of related studies and assess the significance of music interventions or music therapy in addressing depression and anxiety disorders among people suffering from the most common types of cancer diseases such as cancer of the breast, lung, prostate, and colorectal cancer[17]. In other words, the study focuses on the need to address the mental health of cancer patients using music therapy. Furthermore, the study aimed to enhance

awareness among medical practitioners providing oncological support and care regarding the significance of music therapy in treating the psychiatric conditions that may emanate from cancer diagnosis.

METHODOLOGY

This study employed a narrative literature review of studies to ascertain the significance of music therapy in treating depression and anxiety disorders among people with cancer. Since the study is a narrative literature review, ethical approval and informed consent are not required. Through a literature search on electronic databases, the authors could access published reviews and research articles, which were analysed using narrative syntheses. A narrative literature review provides informative educational materials as it draws ideas from a variety of papers and transforms these ideas into a readable resource. Thus, a narrative review provides a broader perspective on the topic of discussion. The authors conducted an extensive literature search and were able to find papers on depression and anxiety disorders in relation to people with various cancer malignancies, music therapy in caring for patients with cancer, and the effects of music therapy in treating depression and other psychosocial disorders from varieties of electronic databases such as Google Scholar, Lens, DOAJ, Scilit, Reference Citation Analysis, Dimensions, Scopus, PubMed Central, and SciElo. Also, searches through the references of the articles retrieved for the study were conducted to access more resources, peer-reviewed papers, and authoritative texts. The literature search terms were limited to depression and anxiety disorders, music therapy, people with cancer or cancer patients, music intervention, colorectal cancer, breast cancer, lung cancer, prostate cancer, psychological disorders, and cancer treatment. This study included relevant qualitative and quantitative research and review papers published in English.

DISCUSSION

Depression and anxiety disorders among people with cancer

Recently, cancer has been considered the primary cause of death, resulting in about a 10 million mortality rate in 2020, accounting for 1 in 6 deaths globally. The most prevalent cancer affecting the human race include breast cancer which accounts for 2.26 million cancer manifestations. This is followed by lung cancer with about 2.21 million recorded victims, colon and rectum with 1.93 million, and prostate with about 1.20 million cases. Lung cancer led to about 1.80 million deaths, while colorectal and breast cancer accounted for 916000 and 685000, respectively, in 2020[5]. Hence, the mortality rate from chronic diseases such as cancer has recently increased. The most significant psychological disorders affecting people with this chronic disease are depression and anxiety disorders. Depression is characterized by sadness, emptiness, or irritating moods, along with mental and physical abnormalities that negatively impact an individual's functioning, which may be attributed to environmental factors [18]. Anxiety disorder is an emotional state associated with excessive fear of uncertainty, lack of concentration, insomnia, and restlessness[19]. Globally, about 154 million people are affected by this disorder, and it is ranked as the most common cause of the severe impact of the disease. Depression is predicted to surpass all other causes by 2030[20]. According to estimates, the likelihood of early death will be higher for 40% to 60% of those with this condition than for the general population[21]. According to a research finding, about 75% of cancer patients are reported to develop depression and anxiety disorders. At the same time, 50% and 85% of cancer patients suffer acute depression and anxiety simultaneously[7, 22]. Depression and anxiety disorders are common psychiatric conditions that are often disregarded. These neglected psychiatric conditions are some of the impacts of cancer that affect the physical and mental well-being, adherence to therapy, the rate of surviving cancer, and cost of care among people with cancer[23].

Depression and anxiety disorders resulting from an individual being diagnosed with cancer at the initial stage is further exacerbated during therapeutic cancer management, which may have an unfavourable impact on the cancer patient. Studies revealed that a significant number of people living with cancer and receiving treatment have suffered from psychological distress, such as depression and anxiety disorders; between 15% and 54% of cancer patients experience these psychological conditions [24-27]. There are perhaps some variables that may be attributed to having caused depression and anxiety among cancer patients. Some of the variables that may lead to depression and anxiety at the individual level include demographic variables like age, sex, location, gender, and religion. Also, social and economic barriers like the inability to secure paid employment, low-level educational attainment, and inadequate social support are contributing factors[27]. The interaction between two or more of these factors often results in some mental health conditions among people with cancer[26].

Cancer management has a significant financial burden on patients. Thus, a psychological disorder affecting people with cancer can be linked to structural-level indicators such as availability, access, and utilization of healthcare services for the treatment of cancer, as well as the provision of welfare packages

for people with cancer[28,29]. This is because of the potential financial consequences of cancer. Psychological factors such as distinctiveness of the severe mental illness have been identified as one of the variables. Studies have also revealed that people with a high tendency to mental illness occurrence and subsequently diagnosed with cancer constitute the more significant percentage of cancer deaths. This can also be due to the severity of the cancer disease, late clinical diagnosis, poor therapeutic procedures, and a substantial decline in good healthcare-seeking behavior[30,31]. Also, people with cancer tend to suffer from depression and anxiety disorders due to factors such as a lack of adequate coping skills and neuroticism[32]. Another risk associated with cancer patients is suicide. People with cancer have a high probability of committing suicide when compared to the general population. Individuals who had suicidal thoughts in the past are more vulnerable, especially in the first six months of being diagnosed with such malignancy[33,34]. Another factor worthy of mention is how people with cancer deal with a cancer diagnosis using psychological coping strategies. An individual diagnosed with cancer may be susceptible to grief which could, in turn, affect how well the individual accepts their condition, especially if the diagnosis was delayed and the cancer cells have developed to a large extent[35].

However, despair, helplessness, and uncertainty about survival and death may also have detrimental effects on the mental health of people diagnosed with cancer. In addition, the distress associated with receiving a positive cancer diagnosis can interfere with sleep, which reduces the ability to concentrate, thereby increasing the risk of depression and anxiety[36]. People living with cancer may suffer from feelings of guilt and shame which often results from the stigma associated with being mentally ill and having some cancers, such as lung cancer. Depression and anxiety may be triggered by this event[35]. For example, women who develop cervical cancer due to promiscuity may blame themselves for their health condition and tend to feel isolated if they remember the activity that led to the manifestation of cervical cancer.

Additionally, during cancer management, the development of depression and anxiety disorders can also be attributed to the following variables-type of cancer an individual is diagnosed with, the stage of cancer, and the future outcome of the malignancy. The inability to recognize depression and anxiety among individuals with cancer can also potentially impair their quality of life. The treatment of cancers using chemotherapy, corticosteroids, and immunotherapy can also cause depression and anxiety among people with cancers as it involves biochemical procedures which result in inflammatory cytokines. Further, most of the medication used during chemotherapeutic procedures causes nausea, affecting dopamine receptors' neurotransmitter process. This action gives rise to depressive feelings among people with cancer[37]. Research has demonstrated that steroid treatment and androgen deprivation therapy is associated with depression and an increased risk of anxiety disorders in patients with cancer [38,39]. Among people with prostate cancer, depression may also be exacerbated by the clinical manifestations of some malignancies, such as leakage and erectile problems linked to prostate cancer [40]. Research on the psychological condition of people with cancer and the stages or survival rate has recently become a significant and expanding clinical research focus. Research has shown that many variables, including the kind and stage of the cancer malignancy, contribute to the declining mental health of people with cancer. In comparison with the general population, cancer patients are more likely to suffer from anxiety and depression[26].

Treatment of depression and anxiety disorders among people with cancer using music therapy

Music has psychological and physiological impacts and, as such, provides support in improving the mental and physical health of people with depression and anxiety disorders[21]. Musical stimuli are associated with the large production of endorphins and hormones secreted in the brain and nervous system. This hormone has many psychological functions. It activates enthusiasm, vital energy, excitement, and confidence in individuals. Therefore, the endorphins produced during musical display aid in lessening pain perception, stress, depression, and anxiety and increasing well-being[41]. Furthermore, music therapy (MT) is a significant contributor to the psychological well-being of cancer patients at all stages of their treatment[12].

Music therapy research indicates that people with cancer have benefitted from music expression and experience[42]. Music therapy is a simple, affordable, effective, and convenient method of treating depression and anxiety disorders[22,43]. As such, music therapy is highly recommended to be incorporated into healthcare services for people with cancer. The use of music therapy among cancer patients is helpful and supportive in ameliorating the depressive symptoms and anxiety exhibited by people with cancer[44].

Depression and anxiety disorders are often linked to non-compliance with proper treatment and poor cancer survival and health outcome among people with cancer. It is pertinent to note that cancer patients have an elevated risk of committing suicide[45,46]. Thus, adequate attention is required to address the resulting psychological disorders among cancer patients. Treating depression and anxiety disorders using music therapy involves qualified music therapists employing music as a supplementary or holistic therapeutic solution to help cancer patients cope with their sickness and reduce symptoms related to their condition or treatment procedures[47]. There is growing evidence that supports the use of MT in cancer patients. According to studies, music therapy aids in reducing anxiety levels in cancer patients undergoing major surgery[48,49]. Also, MT reduces depression, as revealed by studies[22,50]. Thus, cancer patients who undergo music therapy have been shown to benefit from its treatment of

depression and anxiety disorders.

The result of a meta-analysis on the effectiveness of music therapy for addressing psychological disorders among cancer patients shows that, compared to other conventional treatments, music therapy is more efficient in addressing depression and anxiety disorders[51]. The study found that music therapy can significantly improve the mental health of patients suffering from depression and anxiety disorders. Cancer patients are recommended to receive music therapy sessions for 1-2 mo to improve their quality of life[51]. Chen *et al*[52] stated that cancer treatment using music therapy reduces depressed mood, neuroticism, despair, and hopelessness. The therapeutic use of music therapy in managing depression and anxiety disorders and treatments carried out in surgery departments, and medical oncology should be encouraged among healthcare professionals[53]. Literature indicate that in addition to music interventions, it is also crucial for patients to receive social support, exercise, and relaxation interventions to minimize the mental health problems associated with a cancer diagnosis and the financial and emotional consequences of the disease[54-69].

Significance of music therapy in managing depression and anxiety disorders in people with various kinds of cancer

This section is dedicated to reviewing papers that examined the significant results of applying music therapy in treating mental health issue in patients with breast cancer, lung cancer, prostate cancer, and colorectal cancer (see also Table 1).

Breast cancer

Women are more likely to develop breast cancer than any other cancer malignancy. There are approximately 685000 cancer deaths in women worldwide caused by this type of cancer[54], resulting in the largest share of all cancer deaths in women[3,54]. Globally, about 2261419 new breast cancer occurrences were recorded in 2020, constituting 12.5% of all cancer recorded in 2020[55]. Despite the medical advancement in cancer treatment and prevention, which has resulted in an increased survival rate, breast cancer has a long-term negative mental and physical impacts[56]. Breast cancer patients often express a worse quality of life, experience cancer-related tiredness, and struggle to manage their condition and therapeutic tasks[57-59].

Women diagnosed with breast cancer may experience severe psychological and physical trauma, including altered body views, sleeplessness, exhaustion, discomfort, sadness, and other distressful feelings[60]. Depression and anxiety disorders are regarded to be most prevalent at the acute stage of cancer therapy[61]. The decision of people with cancer to receive cancer treatment may be influenced by depressive symptoms, including feelings of helplessness. It is estimated that approximately half (50%) of all breast cancer patients suffer from depression or anxiety. There is a possibility of experiencing severe depression during conventional chemotherapy, particularly with taxane-based chemotherapies. This condition may last for as long as 18 mo following the conclusion of the chemotherapy treatment[62,63].

Improving the depression and anxiety conditions of women diagnosed with breast cancer involves many interventions ranging from muscle relaxation training, music therapy, exercise, and laughter therapy[64-67]. Research has shown that they might also have unforeseen consequences and adverse implications that could affect breast cancer patients' mental health conditions[68]. Also, the chemotherapeutic session has been reported to be stressful and may negatively impact the mental state of breast cancer patients. Integrating music therapy and emotional expression could help reduce the negative psychological consequences of the treatment[69]. Music therapy is a distraction tool aimed at managing emotions and diverting an individual's attention from an unpleasant condition to a more pleasant and happy moment thereby reducing the risk of mental stress associated with an unpleasant or life-threatening health condition like breast cancer. This distraction method involves the breast cancer patient listening to music regulated by the music therapist[70,71].

Additionally, adopting music therapy in the treatment of depression and anxiety disorders among female patients with breast cancer fosters the reduction of the psychiatric consequences of cancer during and after an oncology treatment session[72]. Kievisiene *et al*[73] stated that music therapy helps reduce adverse psychological effects resulting from the clinical manifestation and treatment of breast cancer. Similarly, music therapy intervention could assist people with breast cancer to ease the cardiotoxicity pain resulting from chemotherapy treatment consisting of anthracycline[74]. Thus, music therapy is efficient and recommended for the treatment of psychological disorders like depression and anxiety as well in people with breast cancer.

Lung cancer

Lung cancer is among the most prevalent type of cancer affecting people, with an estimated 1.8 million recorded cases as of 2012 and 2.21 million new cases, according to recent reports[3,75]. Patients with severe lung cancer experience excruciating pain. About 75% and 80% of these patients reported that pain management is inefficient in reducing the painful consequence of lung cancer[76]. Lung cancer is majorly treated using a chemotherapeutic approach which also has side effects. About 25% of 50% of people with small cell lung cancer were reported to experience psychological distress after chemotherapy[77]. The physical pains and psychological trauma associated with post-surgery and

Table 1 Results on the significance of music therapy on people with the most common types of cancer

Ref.	Objective of the study	Research designs/Methods	Findings/Results
Romito *et al* [69]	To measure the effects of music therapy and emotional expression on the reduction of negative emotions in patients undergoing chemotherapy for breast cancer	62 breast cancer patients were randomly recruited into the experimental and control group	The combination of music therapy and emotional expression was identified to help reduce anger and depression that impacts the mental health of women with breast cancer
Zhou *et al* [72]	To examine the effects of music therapy and muscle relaxation training on depression and anxiety, as well as the length of hospitalization	An intervention group of 170 patients was randomly selected and assigned to the study; a randomized controlled trial was conducted	Depression and anxiety level reduction using music therapy
Kievisiene *et al*[73]	To explore the available reports on the effects of music therapy and art therapy interventions among breast cancer patients	A systematic literature search was conducted in PubMed, EBSCO, and the Cochrane Central database. A total of 20 randomized controlled trials were systematically reviewed	Music therapy is commonly used for anxiety reduction during and after oncological treatment sessions
Wang *et al* [83]	To examine the effect on hemodynamics and analgesia of postoperative intravenous sufentanil combined with music therapy in patients with lung cancer in comparison to sufentanil alone	60 lung cancer patients were randomly distributed to a music therapy group and a control group	After lung cancer surgery, patients in the music therapy group were reported to have significantly low anxiety rate, heart rate, blood pressure *etc.* which would have resulted in a psychological disorder
Mou *et al* [84]	To examine the effects of passive music therapy on patients with lung cancer during the initial peripherally implanted central catheter implantation operation on their anxiety levels and vital signs	304 lung cancer patients participated in the randomized controlled trial	Blood pressure, heart rate, and anxiety decreased significantly among lung cancer patients in the experimental group. The findings indicate that music therapy is beneficial for lung cancer patients when they are undergoing central catheter insertion
Tang *et al* [86]	To determine if six-step music therapy is effective in reducing pain and anxiety in patients with lung cancer receiving platinum-based chemotherapy and whether it improves sleep	Two groups-music treatment and a control group-each consisting of 100 patients with small cell lung cancer, were chosen at random	Patients with lung cancer who receive music therapy report less discomfort, less worry, and better sleep
Mishra *et al* [93]	To explore how music therapy affects patients having a RALP after surgery	18 yr and older men (40 patients) undergoing RALP were randomly assigned to music and control group	Music facilitates the comfort and reduction of narcotic usage among prostate cancer patients
Yung *et al* [94]	To ascertain how music therapy affects Chinese males having transurethral prostate resections in terms of pre-operative anxiety	A quasi-experimental design involving 30 patients with TURP	Music intervention is associated with a significant reduction in anxiety levels
Smolen *et al* [99]	To investigate the impact of music therapy on physiological and self-reported indicators of anxiety	32 adult patients scheduled for ambulatory colonoscopy were involved in the study	Patients who are having colonoscopies benefit from music therapy as it reduces the level of anxiety
Tanriverdi *et al*[100]	To determine how music therapy affects patients with early-stage colorectal cancer in terms of anxiety and chemotherapy-related nausea	A randomized controlled trial involving 62 patients	Music therapy was identified to be associated with a decrease in anxiety levels
Li *et al*[101]	To investigate the effects of music therapy on patients suffering from breast cancer in terms of their mental and physical state	25 to 65 years old female patients with breast cancer and receiving mastectomy were grouped into intervention and control groups	Music therapy was found to be useful and significant in improving the mental and physical health of women with breast cancer
Chirico *et al* [102]	To access the efficacy of virtual reality and compare its effects with music therapy	30 breast cancer patients were recruited into VR and MT groups respectively and 34 who were receiving standard chemotherapeutic care were assigned to a control group	Music intervention was discovered to be useful in addressing anxiety and facilitating the mental well-being of breast cancer patients

RALP: Robotically assisted laparoscopic prostatectomy; TURP: Transurethral resection of the prostate.

chemotherapy often harm the physical and psychosocial well-being of the lung cancer patient. While it is pertinent to provide adequate pain relief therapy for patients with lung cancer after surgery[78,79], the analgesia known as opioids which are commonly used to provide relief, has adverse side effects[80].

Music therapy is employed as an alternative intervention in managing pains associated with lung cancer that could lead to psychological distress, such as depression and anxiety disorders[81,82]. The aim is to enhance the patient's quality of life, promote longevity, and maintain the patient's mental health. The combination of music therapy with other care given to lung cancer patients after surgery helps reduce blood pressure, stress, anxiety disorder, and other psychological problems associated with lung cancer pain and trauma[83] and improves the general well-being of people living with lung cancer.

Music therapy is highly suggested for lung cancer patients undergoing any invasive clinical surgery[84] as it aids in improving the psychological issues resulting from both preoperative and postoperative surgery interventions. Studies have revealed that music therapy efficiently reduces adverse physical and psychological effects associated with terminal illnesses like cancer[77,85,86]. Music therapy, according to Tang *et al*[86], helps treat anxiety among patients with lung cancer. It is an efficient form of cancer care support that can be employed as a therapeutic means of improving lung cancer patients' psychological well-being[86] during chemotherapy or other treatment procedures.

Prostate cancer

Prostate cancer is the leading cause of death for men in 48 countries and the most common cancer affecting men in 112 countries[55]. Similarly, prostate cancer is among men's most prevalent diagnosed cancer in 2022, accounting for 27% of diagnosed cases[17]. Death resulting from this type of cancer malignancy accounts for 37.5 per 100000 and 11.3 per 100000 in higher and lower Human Development Index countries, respectively[87]. Prostate cancer is treated using radical prostatectomy[88]. Although the use of robotic-assisted laparoscopic prostatectomy has contributed to the reduction of postoperative pain among prostate cancer patients, there is a need for further advancement in managing pain and other psychological issues associated with prostate cancer[89]. During the perioperative stage, anxiety and pain are commonly associated with cancer patients. According to Kühlmann *et al*[90], about 75% of surgery patients experience anxiety, increasing postoperative pain. Prostate cancer patients often develop severe anxiety due to concern over the diagnosed cancer and its impact on their sexual life[91, 92]. Music therapy has been identified as a helpful approach in supporting prostate cancer patients and reducing anxiety[93]. Also, for prostate cancer patients undergoing transurethral resection of the prostate (that is, a surgical procedure aimed at treating an enlarged prostate-related urinary issues), music therapy intervention efficiently reduces preoperative anxiety during surgery[94]. Therefore, research evidence has revealed that music therapy can be used to treat psychological problems such as anxiety disorder and depression associated with prostate cancer diagnosis and should be introduced during preoperative and postoperative care.

Colorectal cancer

Men are highly susceptible to colorectal cancer, one of the leading causes of cancer death. In 2020, about 1.9 million people had colorectal cancer, and 935000 deaths were predicted to occur. Thus, accounting for one-tenth of all cancer cases and mortalities[55]. Colorectal cancer ranks second for death and third for incidence in men and accounts for 29 per 100000 on the higher Human Development Index (that is, an indicator of a country's performance in three of the major aspects of human development, namely health, education, and standard of living)[87,95,96]. Music therapy is used in addressing the psychological problems of colorectal cancer patients. A review of related research shows that a colorectal cancer patient listening to preferred music while having a sigmoidoscopy significantly lowers anxiety and increases comfort during surgery[97,98]. When music therapy was used, according to Palakanis *et al* [98], the patient's preferred music led to a decrease in the level of anxiety during sigmoidoscopy operations. Music therapy can potentially reduce anxiety and other indicators of psychological disorders among colorectal cancer patients undergoing colonoscopy[99]. Music therapy also reduces anxiety levels among patients with colorectal cancer during chemotherapy sessions[100]. Thus, music therapy intervention is efficient in supporting patients with colorectal cancer to adjust to psychological issues like depression and anxiety associated with a cancer diagnosis. Conversely, research on the effectiveness of using music as a kind of therapy to help people with colorectal cancer cope better with procedures like sigmoidoscopy or colonoscopy and to lessen their anxiety has been fragmentary.

RESEARCH IMPLICATIONS AND RECOMMENDATIONS

Music therapy improves the mental and physical state of people with cancer[101,102]. While cancer diagnosis and treatment procedures are linked to substantial financial costs[103], music therapy's cheap costs, absence of side effects, and significant benefits in reducing stress are crucial for the prevention and treatment of psychological issues caused by cancer and its diagnosis[104]. People with various kinds of cancer tend to suffer from psychological disorders like depression and anxiety, as stated earlier in this paper. Most cancer patients, as well as their families, music therapists, and medical experts feel optimistic about the remedies provided by music therapy[105]. Because the use of music therapy significantly lessens anxiety and depressive symptoms associated with cancer[106], it is vital for practitioners to continue to examine the significance of music therapy in addressing these psychiatric disorders among patients with various types of cancer malignancy.

A large percentage of cancer mortality could be attributed to depression and anxiety disorders resulting from cancer diagnosis and treatment. However, accessing the psychological state of people with cancer before and after clinical diagnosis and treatment is essential to reducing cancer death. The findings of this review are essential to medical practice and policy concerning oncological disease and treatment procedures. It is, therefore, pertinent to investigate and address the psychological disorders

observed in people with cancer. Because music therapy has been found to be significant in treating depression and anxiety in people with cancer, music therapists should be among the medical team treating cancer patients.

Medical practitioners who provide medical care to people with cancer should endeavor to examine the psychological health of cancer patients under their care. Given the significance of music therapy in reducing anxiety levels and treating depression in people with breast cancer, lung cancer, prostate cancer and colorectal cancer, it is therefore pertinent for the oncologist collaborate with qualified music professionals in order employ music therapy during the treatment of cancer patients. Music therapy should also be incorporated into medical, radiation, and surgical oncology curriculum. A further empirical study should be conducted to obtain more research on this issue.

CONCLUSION

Identifying the symptoms of mental illness in cancer patients is essential for managing their mental health. Cancer deaths may occur due to the inability to address the psychological disorders associated with cancer diagnosis among people with any of the most common cancer types. Music therapy has been identified to be significant in treating psychological issues like depression and anxiety that many cancer patients experience. It is needful for healthcare providers to incorporate music therapy interventions while treating people with cancer. This will help reduce cancer deaths resulting from psychological disorders rather than the killer disease, cancer. However, the standardized procedures and evaluation criteria for applying music-based intervention strategies in oncology medicine still need to be further established and improved.

FOOTNOTES

Author contributions: Eseadi C and Ngwu MO conceived the study; Eseadi C and Ngwu MO designed the study, conducted the literature review and were all responsible for the analysis, drafting, editing, and approval of the final version of this manuscript.

Conflict-of-interest statement: All the author declare no conflict of interests for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: South Africa

ORCID number: Chiedu Eseadi 0000-0003-1711-7558; Millicent O Ngwu 0000-0002-9125-1037.

S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

REFERENCES

1 **National Cancer Institute**. Understanding Cancer. What is cancer ? 2021. Available from: https://www.cancer.gov/about-cancer/understanding/what-is-cancer
2 **Rahmani A**, Ferguson C, Jabarzadeh F, Mohammadpoorasl A, Moradi N, Pakpour V. Supportive care needs of Iranian cancer patients. *Indian J Palliat Care* 2014; **20**: 224-228 [PMID: 25191012 DOI: 10.4103/0973-1075.138400]
3 **World Health Organization [WHO]**. Cancer Reports. Heal. Top. 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer#:~:text=The problem-, Cancer is a leading cause of death worldwide%2C accounting for,lung (2.21 million cases)%3B
4 **Shah SC**, Kayamba V, Peek RM Jr, Heimburger D. Cancer Control in Low- and Middle-Income Countries: Is It Time to Consider Screening? *J Glob Oncol* 2019; **5**: 1-8 [PMID: 30908147 DOI: 10.1200/JGO.18.00200]
5 **Ferlay J**, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. *Int J Cancer* 2021 [PMID: 33818764 DOI: 10.1002/ijc.33588]
6 **Bray F**, Møller B. Predicting the future burden of cancer. *Nat Rev Cancer* 2006; **6**: 63-74 [PMID: 16372017 DOI: 10.1038/nrc1781]
7 **Zabora J**, BrintzenhofeSzoc K, Curbow B, Hooker C, Piantadosi S. The prevalence of psychological distress by cancer site. *Psychooncology* 2001; **10**: 19-28 [PMID: 11180574 DOI:

10.1002/1099-1611(200101/02)10:1<19::AID-PON501>3.0.CO;2-6]
8 **Zaza C**, Sellick SM, Hillier LM. Coping with cancer: what do patients do. *J Psychosoc Oncol* 2005; **23**: 55-73 [PMID: 16492644 DOI: 10.1300/J077v23n01_04]
9 **Priscilla D**, Hamidin A, Azhar MZ, Noorjan KO, Salmiah MS, Bahariah K. Assessment of depression and anxiety in haematological cancer patients and their relationship with quality of life. *East Asian Arch Psychiatry* 2011; **21**: 108-114 [PMID: 21921304]
10 **O'Callaghan CC**, McDermott F, Reid P, Michael N, Hudson P, Zalcberg JR, Edwards J. Music's Relevance for People Affected by Cancer: A Meta-Ethnography and Implications for Music Therapists. *J Music Ther* 2016; **53**: 398-429 [PMID: 27980035 DOI: 10.1093/jmt/thw013]
11 **Bro ML**, Jespersen KV, Hansen JB, Vuust P, Abildgaard N, Gram J, Johansen C. Kind of blue: A systematic review and meta-analysis of music interventions in cancer treatment. *Psychooncology* 2018; **27**: 386-400 [PMID: 28626867 DOI: 10.1002/pon.4470]
12 **Köhler F**, Martin ZS, Hertrampf RS, Gäbel C, Kessler J, Ditzen B, Warth M. Music Therapy in the Psychosocial Treatment of Adult Cancer Patients: A Systematic Review and Meta-Analysis. *Front Psychol* 2020; **11**: 651 [PMID: 32373019 DOI: 10.3389/fpsyg.2020.00651]
13 **Warth M**, Kessler J, Koehler F, Aguilar-Raab C, Bardenheuer HJ, Ditzen B. Brief psychosocial interventions improve quality of life of patients receiving palliative care: A systematic review and meta-analysis. *Palliat Med* 2019; **33**: 332-345 [PMID: 30648926 DOI: 10.1177/0269216318818011]
14 **Yang T**, Wang S, Wang R, Wei Y, Kang Y, Liu Y, Zhang C. Effectiveness of five-element music therapy in cancer patients: A systematic review and meta-analysis. *Complement Ther Clin Pract* 2021; **44**: 101416. [PMID: 34020291 DOI: 10.1016/j.ctcp.2021.101416]
15 **Umbrello M**, Sorrenti T, Mistraletti G, Formenti P, Chiumello D, Terzoni S. Music therapy reduces stress and anxiety in critically ill patients: a systematic review of randomized clinical trials. *Minerva Anestesiol* 2019; **85**: 886-898 [PMID: 30947484 DOI: 10.23736/S0375-9393.19.13526-2]
16 **Edwards J**. The Use of Music in Healthcare Contexts: A Select Review of Writings From the 1890s to the 1940s. *Voices A World Forum Music Ther* 2008; **8** [DOI: 10.15845/voices.v8i2.428]
17 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* 2022; **72**: 7-33 [PMID: 35020204 DOI: 10.3322/caac.21708]
18 **Spitzer RL**, Gibbon ME, Skodol AE, Williams JB, First MB. DSM-IV-TR Casebook: A Learning Companion to the Diagnostic and Statistical Manual of Mental Disorders. *APA* [DOI: 10.1176/appi.books.9781585622665]
19 **American Psychiatric Association**. Diagnostic and statistical manual of mental disorders, 4th edn. Washington DC: American Psychiatric Publishing Inc, 1994. Available from: https://psycnet.apa.org/record/1994-97698-000
20 **Malhi GS,** Mann JJ. Depression. *Lancet* 2018; **392**: 2299-2312 [PMID: 30396512 DOI: 10.1016/S0140-6736(18)31948-2]
21 **Antonio E**, Marchant E, Angeles M, Amparo M. Effects of music therapy in depression and anxiety disorder. *Life Res* 2019; **2**: 64 [DOI: 10.53388/life2019-0425-003]
22 **Jasemi M**, Aazami S, Zabihi RE. The Effects of Music Therapy on Anxiety and Depression of Cancer Patients. *Indian J Palliat Care* 2016; **22**: 455-458 [PMID: 27803568 DOI: 10.4103/0973-1075.191823]
23 **Hage MP**, Azar ST. The Link between Thyroid Function and Depression. *J Thyroid Res* 2012; **2012**: 590648 [PMID: 22220285 DOI: 10.1155/2012/590648]
24 **Soqia J**, Al-Shafie M, Agha LY, Alameer MB, Alhomsi D, Saadoun R, Saifo M. Depression, anxiety and related factors among Syrian breast cancer patients: a cross-sectional study. *BMC Psychiatry* 2022; **22**: 796 [PMID: 36528568 DOI: 10.1186/s12888-022-04469-y]
25 **Lin MF**, Hsieh YJ, Hsu YY, Fetzer S, Hsu MC. A randomised controlled trial of the effect of music therapy and verbal relaxation on chemotherapy-induced anxiety. *J Clin Nurs* 2011; **20**: 988-999 [PMID: 21385249 DOI: 10.1111/j.1365-2702.2010.03525.x]
26 **Niedzwiedz CL**, Knifton L, Robb KA, Katikireddi SV, Smith DJ. Depression and anxiety among people living with and beyond cancer: a growing clinical and research priority. *BMC Cancer* 2019; **19**: 943 [PMID: 31604468 DOI: 10.1186/s12885-019-6181-4]
27 **Wen S**, Xiao H, Yang Y. The risk factors for depression in cancer patients undergoing chemotherapy: a systematic review. *Support Care Cancer* 2019; **27**: 57-67 [PMID: 30225571 DOI: 10.1007/s00520-018-4466-9]
28 **Gilligan AM**, Alberts DS, Roe DJ, Skrepnek GH. Death or Debt? *Am J Med* 2018; **131**: 1187-1199.e5 [PMID: 29906429 DOI: 10.1016/j.amjmed.2018.05.020]
29 **Lu L**, O'Sullivan E, Sharp L. Cancer-related financial hardship among head and neck cancer survivors: Risk factors and associations with health-related quality of life. *Psychooncology* 2019; **28**: 863-871 [PMID: 30779397 DOI: 10.1002/pon.5034]
30 **Klaassen Z**, Wallis CJD, Goldberg H, Chandrasekar T, Sayyid RK, Williams SB, Moses KA, Terris MK, Nam RK, Urbach D, Austin PC, Kurdyak P, Kulkarni GS. The impact of psychiatric utilisation prior to cancer diagnosis on survival of solid organ malignancies. *Br J Cancer* 2019; **120**: 840-847 [PMID: 30837680 DOI: 10.1038/s41416-019-0390-0]
31 **Musuuza JS**, Sherman ME, Knudsen KJ, Sweeney HA, Tyler CV, Koroukian SM. Analyzing excess mortality from cancer among individuals with mental illness. *Cancer* 2013; **119**: 2469-2476 [PMID: 23585241 DOI: 10.1002/cncr.28091]
32 **Cook SA**, Salmon P, Hayes G, Byrne A, Fisher PL. Predictors of emotional distress a year or more after diagnosis of cancer: A systematic review of the literature. *Psychooncology* 2018; **27**: 791-801 [PMID: 29318702 DOI: 10.1002/pon.4601]
33 **Henson KE**, Brock R, Charnock J, Wickramasinghe B, Will O, Pitman A. Risk of Suicide After Cancer Diagnosis in England. *JAMA Psychiatry* 2019; **76**: 51-60 [PMID: 30476945 DOI: 10.1001/jamapsychiatry.2018.3181]
34 **Wang SM**, Chang JC, Weng SC, Yeh MK, Lee CS. Risk of suicide within 1 year of cancer diagnosis. *Int J Cancer* 2018; **142**: 1986-1993 [PMID: 29250783 DOI: 10.1002/ijc.31224]
35 **Ball H**, Moore S, Leary A. A systematic literature review comparing the psychological care needs of patients with

mesothelioma and advanced lung cancer. *Eur J Oncol Nurs* 2016; **25**: 62-67 [PMID: 27865254 DOI: 10.1016/j.ejon.2016.09.007]

36 **Howell D**, Oliver TK, Keller-Olaman S, Davidson JR, Garland S, Samuels C, Savard J, Harris C, Aubin M, Olson K, Sussman J, MacFarlane J, Taylor C. Sleep disturbance in adults with cancer: a systematic review of evidence for best practices in assessment and management for clinical practice. *Ann Oncol* 2014; **25**: 791-800 [PMID: 24287882 DOI: 10.1093/annonc/mdt506]

37 **Smith HR**. Depression in cancer patients: Pathogenesis, implications and treatment (Review). *Oncol Lett* 2015; **9**: 1509-1514 [PMID: 25788991 DOI: 10.3892/ol.2015.2944]

38 **Ismail MF**, Lavelle C, Cassidy EM. Steroid-induced mental disorders in cancer patients: a systematic review. *Future Oncol* 2017; **13**: 2719-2731 [PMID: 29186986 DOI: 10.2217/fon-2017-0306]

39 **Nead KT**, Sinha S, Yang DD, Nguyen PL. Association of androgen deprivation therapy and depression in the treatment of prostate cancer: A systematic review and meta-analysis. *Urol Oncol* 2017; **35**: 664.e1-664.e9 [PMID: 28803700 DOI: 10.1016/j.urolonc.2017.07.016]

40 **De Sousa A**, Sonavane S, Mehta J. Psychological aspects of prostate cancer: a clinical review. *Prostate Cancer Prostatic Dis* 2012; **15**: 120-127 [PMID: 22212706 DOI: 10.1038/pcan.2011.66]

41 **Schulkin J**, Raglan GB. The evolution of music and human social capability. *Front Neurosci* 2014; **8**: 292 [DOI: 10.3389/fnins.2014.00292]

42 **Stanczyk MM**. Music therapy in supportive cancer care. *Rep Pract Oncol Radiother* 2011; **16**: 170-172 [PMID: 24376975 DOI: 10.1016/j.rpor.2011.04.005]

43 **Jiménez-Jiménez M**, García-Escalona A, Martín-López A, De Vera-Vera R, De Haro J. Intraoperative stress and anxiety reduction with music therapy: a controlled randomized clinical trial of efficacy and safety. *J Vasc Nurs* 2013; **31**: 101-106 [PMID: 23953858 DOI: 10.1016/j.jvn.2012.10.002]

44 **Bradt J**, Potvin N, Kesslick A, Shim M, Radl D, Schriver E, Gracely EJ, Komarnicky-Kocher LT. The impact of music therapy versus music medicine on psychological outcomes and pain in cancer patients: a mixed methods study. *Support Care Cancer* 2015; **23**: 1261-1271 [PMID: 25322972 DOI: 10.1007/s00520-014-2478-7]

45 **DiMatteo MR**, Haskard-Zolnierek KB. Impact of depression on treatment adherence and survival from cancer. Depression and cancer. Depress Cancer 2011; 101–124 [DOI: 10.1002/9780470972533.ch5]

46 **Robson A**, Scrutton F, Wilkinson L, MacLeod F. The risk of suicide in cancer patients: a review of the literature. *Psychooncology* 2010; **19**: 1250-1258 [PMID: 20213857 DOI: 10.1002/pon.1717]

47 **Kruse J**. Music Therapy in United States Cancer Settings: Recent Trends in Practice. *Ther Perspect* 2003; **21**: 89-98 [DOI: 10.1093/mtp/21.2.89]

48 **Clements-Cortés A**. Singing and Vocal Interventions in Palliative and Cancer Care: Music Therapists' Perceptions of Usage. *J Music Ther* 2017; **54**: 336-361 [PMID: 28992349 DOI: 10.1093/jmt/thx010]

49 **Mirbagher M**, Aghajani N. Comparing the Effect of Holy Quran Recitation and Music on Patient Anxiety and Vital Signs before Abdominal Surgeries. *Islam Lifestyle Cent Heal* 2012; **1:** 66–84. Available from: http://islamiclifej.com/browse.php?a_id=105&sid=1&slc_lang=fa

50 **Zhou KN**, Li XM, Yan H, Dang SN, Wang DL. Effects of music therapy on depression and duration of hospital stay of breast cancer patients after radical mastectomy. *Chin Med J (Engl)* 2011; **124**: 2321-2327 [PMID: 21933563 DOI: 10.3760/cma.j.issn.0366-6999.2011.15.014]

51 **Li Y**, Xing X, Shi X, Yan P, Chen Y, Li M, Zhang W, Li X, Yang K. The effectiveness of music therapy for patients with cancer: A systematic review and meta-analysis. *J Adv Nurs* 2020; **76**: 1111-1123 [PMID: 32017183 DOI: 10.1111/jan.14313]

52 **Chen SC**, Chou CC, Chang HJ, Lin MF. Comparison of group vs self-directed music interventions to reduce chemotherapy-related distress and cognitive appraisal: an exploratory study. *Support Care Cancer* 2018; **26**: 461-469 [PMID: 28799076 DOI: 10.1007/s00520-017-3850-1]

53 **Gramaglia C**, Gambaro E, Vecchi C, Licandro D, Raina G, Pisani C, Burgio V, Farruggio S, Rolla R, Deantonio L, Grossini E, Krengli M, Zeppegno P. Outcomes of music therapy interventions in cancer patients-A review of the literature. *Crit Rev Oncol Hematol* 2019; **138**: 241-254 [PMID: 31121392 DOI: 10.1016/j.critrevonc.2019.04.004]

54 **Kaye EC**, Snaman JM, Johnson L, Levine D, Powell B, Love A, Smith J, Ehrentraut JH, Lyman J, Cunningham M, Baker JN. Communication with children with cancer and their families throughout the illness journey and at the end of life. In: Wolfe J, Jones BL, Kreicbergs U, Jankovic M, editor. Pediatric Oncology. 2018: 55–93. [DOI: 10.1007/978-3-319-61391-8_4]

55 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

56 **Bodai BI**, Tuso P. Breast cancer survivorship: a comprehensive review of long-term medical issues and lifestyle recommendations. *Perm J* 2015; **19**: 48-79 [PMID: 25902343 DOI: 10.7812/TPP/14-241]

57 **Triberti S**, Savioni L, Sebri V, Pravettoni G. eHealth for improving quality of life in breast cancer patients: A systematic review. *Cancer Treat Rev* 2019; **74**: 1-14 [PMID: 30658289 DOI: 10.1016/j.ctrv.2019.01.003]

58 **Berger AM**, Mooney K, Alvarez-Perez A, Breitbart WS, Carpenter KM, Cella D, Cleeland C, Dotan E, Eisenberger MA, Escalante CP, Jacobsen PB, Jankowski C, LeBlanc T, Ligibel JA, Loggers ET, Mandrell B, Murphy BA, Palesh O, Pirl WF, Plaxe SC, Riba MB, Rugo HS, Salvador C, Wagner LI, Wagner-Johnston ND, Zachariah FJ, Bergman MA, Smith C; National comprehensive cancer network. Cancer-Related Fatigue, Version 2.2015. *J Natl Compr Canc Netw* 2015; **13**: 1012-1039 [PMID: 26285247 DOI: 10.6004/jnccn.2015.0122]

59 **Abrahams HJG**, Gielissen MFM, Verhagen CAHHVM, Knoop H. The relationship of fatigue in breast cancer survivors with quality of life and factors to address in psychological interventions: A systematic review. *Clin Psychol Rev* 2018; **63**: 1-11 [PMID: 29852324 DOI: 10.1016/j.cpr.2018.05.004]

60 **Shinden Y**, Kijima Y, Hirata M, Nakajo A, Tanoue K, Arigami T, Kurahara H, Maemura K, Natsugoe S. Clinical characteristics of breast cancer patients with mental disorders. *Breast* 2017; **36**: 39-43 [PMID: 28942099 DOI:

10.1016/j.breast.2017.08.010]
61 **Krebber AM**, Buffart LM, Kleijn G, Riepma IC, de Bree R, Leemans CR, Becker A, Brug J, van Straten A, Cuijpers P, Verdonck-de Leeuw IM. Prevalence of depression in cancer patients: a meta-analysis of diagnostic interviews and self-report instruments. *Psychooncology* 2014; **23**: 121-130 [PMID: 24105788 DOI: 10.1002/pon.3409]
62 **Burgess C**, Cornelius V, Love S, Graham J, Richards M, Ramirez A. Depression and anxiety in women with early breast cancer: five year observational cohort study. *BMJ* 2005; **330**: 702 [PMID: 15695497 DOI: 10.1136/bmj.38343.670868.D3]
63 **Thornton LM**, Carson WE 3rd, Shapiro CL, Farrar WB, Andersen BL. Delayed emotional recovery after taxane-based chemotherapy. *Cancer* 2008; **113**: 638-647 [PMID: 18521922 DOI: 10.1002/cncr.23589]
64 **Li XM**, Zhou KN, Yan H, Wang DL, Zhang YP. Effects of music therapy on anxiety of patients with breast cancer after radical mastectomy: a randomized clinical trial. *J Adv Nurs* 2012; **68**: 1145-1155 [PMID: 21978087 DOI: 10.1111/j.1365-2648.2011.05824.x]
65 **Kashani F**, Babaee S, Bahrami M, Valiani M. The effects of relaxation on reducing depression, anxiety and stress in women who underwent mastectomy for breast cancer. *Iran J Nurs Midwifery Res* 2012; **17**: 30-33 [PMID: 23493112]
66 **Ergun M**, Eyigor S, Karaca B, Kisim A, Uslu R. Effects of exercise on angiogenesis and apoptosis-related molecules, quality of life, fatigue and depression in breast cancer patients. *Eur J Cancer Care (Engl)* 2013; **22**: 626-637 [PMID: 23731173 DOI: 10.1111/ecc.12068]
67 **Cho EA**, Oh HE. [Effects of laughter therapy on depression, quality of life, resilience and immune responses in breast cancer survivors]. *J Korean Acad Nurs* 2011; **41**: 285-293 [PMID: 21804337 DOI: 10.4040/jkan.2011.41.3.285]
68 **Stanton AL**, Bower JE. Psychological Adjustment in Breast Cancer Survivors. *Adv Exp Med Biol* 2015; **862**: 231-242 [PMID: 26059939 DOI: 10.1007/978-3-319-16366-6_15]
69 **Romito F**, Lagattolla F, Costanzo C, Giotta F, Mattioli V. Music therapy and emotional expression during chemotherapy. How do breast cancer patients feel? Eur J Integr Med 2013; 5: 438–442 [DOI: 10.1016/j.eujim.2013.04.001]
70 **Atiwannapat P**, Thaipisuttikul P, Poopityastaporn P, Katekaew W. Active versus receptive group music therapy for major depressive disorder-A pilot study. *Complement Ther Med* 2016; **26**: 141-145 [PMID: 27261995 DOI: 10.1016/j.ctim.2016.03.015]
71 **Bruscia KE**. A working definition of Music Therapy (3rd Edition, 2014). 3rd ed. Available from: https://www.researchgate.net/publication/325204109_A_working_definition_of_Music_Therapy_3rd_Edition_2014
72 **Zhou K**, Li X, Li J, Liu M, Dang S, Wang D, Xin X. A clinical randomized controlled trial of music therapy and progressive muscle relaxation training in female breast cancer patients after radical mastectomy: results on depression, anxiety and length of hospital stay. *Eur J Oncol Nurs* 2015; **19**: 54-59 [PMID: 25181938 DOI: 10.1016/j.ejon.2014.07.010]
73 **Kievisiene J**, Jautakyte R, Rauckiene-Michaelsson A, Fatkulina N, Agostinis-Sobrinho C. The Effect of Art Therapy and Music Therapy on Breast Cancer Patients: What We Know and What We Need to Find out - A Systematic Review. *Evidence-based Complement Altern Med* 2020; **2020**: 1-14 [DOI: 10.1155/2020/7390321]
74 **Chuang CY**, Han WR, Li PC, Song MY, Young ST. Effect of long-term music therapy intervention on autonomic function in anthracycline-treated breast cancer patients. *Integr Cancer Ther* 2011; **10**: 312-316 [PMID: 21382955 DOI: 10.1177/1534735411400311]
75 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]
76 **Tagányi K**. [Management of lung cancer-related pain]. *Orv Hetil* 2011; **152**: 1184-1191 [PMID: 21733791 DOI: 10.1556/OH.2011.29136]
77 **Wintner LM**, Giesinger JM, Zabernigg A, Sztankay M, Meraner V, Pall G, Hilbe W, Holzner B. Quality of life during chemotherapy in lung cancer patients: results across different treatment lines. *Br J Cancer* 2013; **109**: 2301-2308 [PMID: 24091620 DOI: 10.1038/bjc.2013.585]
78 **Magill L**. The use of music therapy to address the suffering in advanced cancer pain. *J Palliat Care* 2001; **17**: 167-172 [PMID: 11816757]
79 **Wu CL**, Raja SN. Treatment of acute postoperative pain. *Lancet* 2011; **377**: 2215-2225 [PMID: 21704871 DOI: 10.1016/S0140-6736(11)60245-6]
80 **Raehal KM**, Schmid CL, Groer CE, Bohn LM. Functional selectivity at the μ-opioid receptor: implications for understanding opioid analgesia and tolerance. *Pharmacol Rev* 2011; **63**: 1001-1019 [PMID: 21873412 DOI: 10.1124/pr.111.004598]
81 **Li XM**, Yan H, Zhou KN, Dang SN, Wang DL, Zhang YP. Effects of music therapy on pain among female breast cancer patients after radical mastectomy: results from a randomized controlled trial. *Breast Cancer Res Treat* 2011; **128**: 411-419 [PMID: 21537935 DOI: 10.1007/s10549-011-1533-z]
82 **Archie P**, Bruera E, Cohen L. Music-based interventions in palliative cancer care: a review of quantitative studies and neurobiological literature. *Support Care Cancer* 2013; **21**: 2609-2624 [PMID: 23715815 DOI: 10.1007/s00520-013-1841-4]
83 **Wang Y**, Tang H, Guo Q, Liu J, Liu X, Luo J, Yang W. Effects of Intravenous Patient-Controlled Sufentanil Analgesia and Music Therapy on Pain and Hemodynamics After Surgery for Lung Cancer: A Randomized Parallel Study. *J Altern Complement Med* 2015; **21**: 667-672 [PMID: 26331434 DOI: 10.1089/acm.2014.0310]
84 **Mou Q**, Wang X, Xu H, Liu X, Li J. Effects of passive music therapy on anxiety and vital signs in lung cancer patients undergoing peripherally inserted central catheter placement procedure. *J Vasc Access* 2020; **21**: 875-882 [PMID: 32141365 DOI: 10.1177/1129729820908088]
85 **Samakouri M**, Bouhos G, Kadoglou M, Giantzelidou A, Tsolaki K, Livaditis M. [Standardization of the Greek version of Zung's Self-rating Anxiety Scale (SAS)]. *Psychiatriki* 2012; **23**: 212-220 [PMID: 23073544]
86 **Tang H**, Chen L, Wang Y, Zhang Y, Yang N. The efficacy of music therapy to relieve pain, anxiety, and promote sleep quality, in patients with small cell lung cancer receiving platinum-based chemotherapy. *Support Care Cancer* 2021; **29**:

7299-7306 [PMID: 34041615 DOI: 10.1007/s00520-021-06152-6]
87 **Arnold M**, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 2017; **66**: 683-691 [PMID: 26818619 DOI: 10.1136/gutjnl-2015-310912]
88 **Lowrance WT**, Eastham JA, Savage C, Maschino AC, Laudone VP, Dechet CB, Stephenson RA, Scardino PT, Sandhu JS. Contemporary open and robotic radical prostatectomy practice patterns among urologists in the United States. *J Urol* 2012; **187**: 2087-2092 [PMID: 22498227 DOI: 10.1016/j.juro.2012.01.061]
89 **Batley SE**, Prasad V, Vasdev N, Mohan-S G. Post-Operative Pain Management in Patients Undergoing Robotic Urological Surgery. *Curr Urol* 2016; **9**: 5-11 [PMID: 26989364 DOI: 10.1159/000442843]
90 **Kühlmann AYR**, de Rooij A, Kroese LF, van Dijk M, Hunink MGM, Jeekel J. Meta-analysis evaluating music interventions for anxiety and pain in surgery. *Br J Surg* 2018; **105**: 773-783 [PMID: 29665028 DOI: 10.1002/bjs.10853]
91 **Arslan S**, Özer N, Özyurt F. Effect of music on preoperative anxiety in men undergoing urogenital surgery. *Aust J Adv Nursing* 2008; **26:** 46–54. Available from: https://www.ajan.com.au/archive/Vol26/26-2_Ozer.pdf
92 **Yadav N**, Singhal S, Bharti D. Effect of music on preoperative anxiety in patients undergoing laparoscopic cholecystectomy. *Bali J Anesthesiol* 2020; **4**: 90-94 [DOI: 10.4103/BJOA.BJOA_19_20]
93 **Mishra K**, Jesse E, Bukavina L, Sopko E, Arojo I, Fernstrum A, Ray A 3rd, Mahran A, Calaway A, Block S, Ponsky L. Impact of Music on Postoperative Pain, Anxiety, and Narcotic Use After Robotic Prostatectomy: A Randomized Controlled Trial. *J Adv Pract Oncol* 2022; **13**: 121-126 [PMID: 35369398 DOI: 10.6004/jadpro.2022.13.2.3]
94 **Yung PM**, Chui-Kam S, French P, Chan TM. A controlled trial of music and pre-operative anxiety in Chinese men undergoing transurethral resection of the prostate. *J Adv Nurs* 2002; **39**: 352-359 [PMID: 12139647 DOI: 10.1046/j.1365-2648.2002.02296.x]
95 **Bray F**. Transitions in human development and the global cancer burden. *World cancer Rep* 2014; **4:** 54-68. Available from: http://scholar.google.com.sci-hub.io/scholar?q=Transitions in human development and the global cancer burden In Wild CP Stewart B Eds World Cancer Report#0
96 **Fidler MM**, Soerjomataram I, Bray F. A global view on cancer incidence and national levels of the human development index. *Int J Cancer* 2016; **139**: 2436-2446 [PMID: 27522007 DOI: 10.1002/ijc.30382]
97 **Bampton P**, Draper B. Effect of relaxation music on patient tolerance of gastrointestinal endoscopic procedures. *J Clin Gastroenterol* 1997; **25**: 343-345 [PMID: 9412917 DOI: 10.1097/00004836-199707000-00010]
98 **Palakanis KC**, DeNobile JW, Sweeney WB, Blankenship CL. Effect of music therapy on state anxiety in patients undergoing flexible sigmoidoscopy. *Dis Colon Rectum* 1994; **37**: 478-481 [PMID: 8181411 DOI: 10.1007/BF02076195]
99 **Smolen D**, Topp R, Singer L. The effect of self-selected music during colonoscopy on anxiety, heart rate, and blood pressure. *Appl Nurs Res* 2002; **15**: 126-136 [PMID: 12173164 DOI: 10.1053/apnr.2002.34140]
100 **Tanriverdi O**, Karaoglu T, Aydemir NF. 1855P Music therapy can reduce both anxiety and chemotherapy-related nausea and vomiting in patients with early stage colorectal cancer treated with adjuvant infusion chemotherapy: A controlled, randomized study (PEGASUS study). *Ann Oncol* 2020; **31**: S1061-S1062 [DOI: 10.1016/j.annonc.2020.08.1502]
101 **Li X**, Du G, Liu W, Wang F. Music intervention improves the physical and mental status for patients with breast cancer: A protocol of randomized controlled trial. *Medicine (Baltimore)* 2020; **99**: e23461 [PMID: 33285746 DOI: 10.1097/MD.0000000000023461]
102 **Chirico A**, Maiorano P, Indovina P, Milanese C, Giordano GG, Alivernini F, Iodice G, Gallo L, De Pietro G, Lucidi F, Botti G, De Laurentiis M, Giordano A. Virtual reality and music therapy as distraction interventions to alleviate anxiety and improve mood states in breast cancer patients during chemotherapy. *J Cell Physiol* 2020; **235**: 5353-5362 [PMID: 31957873 DOI: 10.1002/jcp.29422]
103 **Carrera PM**, Kantarjian HM, Blinder VS. The financial burden and distress of patients with cancer: Understanding and stepping-up action on the financial toxicity of cancer treatment. *Cancer J Clin* 2018; **68**: 153-165 [PMID: 29338071 DOI: 10.3322/caac.21443]
104 **de Witte M**, Pinho ADS, Stams GJ, Moonen X, Bos AER, van Hooren S. Music therapy for stress reduction: a systematic review and meta-analysis. *Health Psychol Rev* 2022; **16**: 134-159 [PMID: 33176590 DOI: 10.1080/17437199.2020.1846580]
105 **Rodríguez-Rodríguez RC**, Noreña-Peña A, Chafer-Bixquert T, Lorenzo Vásquez A, González de Dios J, Solano Ruiz C. The relevance of music therapy in paediatric and adolescent cancer patients: a scoping review. *Glob Health Action* 2022; **15**: 2116774 [PMID: 36174131 DOI: 10.1080/16549716.2022.2116774]
106 **Zang L**, Cheng C, Zhou Y, Liu X. Music therapy effect on anxiety reduction among patients with cancer: A meta-analysis. *Front Psychol* 2022; **13**: 1028934 [PMID: 36687940 DOI: 10.3389/fpsyg.2022.1028934]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 February 24; 14(2): 81-88

DOI: 10.5306/wjco.v14.i2.81

ISSN 2218-4333 (online)

MINIREVIEWS

Therapeutic challenge for immunotherapy targeting cold colorectal cancer: A narrative review

Shi-Xun Ma, Li Li, Hui Cai, Tian-Kang Guo, Lei-Sheng Zhang

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Cabezuelo AS, Spain; Govindarajan KK, India; Jeong KY, South Korea

Received: September 20, 2022
Peer-review started: September 20, 2022
First decision: November 28, 2022
Revised: December 13, 2022
Accepted: February 7, 2023
Article in press: February 7, 2023
Published online: February 24, 2023

Shi-Xun Ma, Hui Cai, Tian-Kang Guo, Lei-Sheng Zhang, Department of General Surgery, Gansu Provincial Hospital, Lanzhou 73000, Gansu Province, China

Li Li, Scientific Research Division, Gansu Provincial Hospital, Lanzhou 730000, Gansu Province, China

Lei-Sheng Zhang, Key Laboratory of Radiation Technology and Biophysics, Hefei Institute of Physical Science, Chinese Academy of Sciences, Hefei 230031, Anhui Province, China

Corresponding author: Lei-Sheng Zhang, PhD, Professor, Department of General Surgery, Gansu Provincial Hospital, No. 204 West Donggang RD, Lanzhou 730000, Gansu Province, China. leisheng_zhang@163.com

Abstract

Cold colorectal tumors are not likely to trigger a robust immune response and tend to suppress the immune response. There may be three reasons. First, the complex tumor microenvironment of cold colorectal cancer (CRC) leads to tolerance and clearance of immunotherapy. Second, the modification and concealment of tumor-specific targets in cold CRC cause immune escape and immune response interruption. Finally, the difference in number and function of immune cell subsets in patients with cold CRC makes them respond poorly to immunotherapy. Therefore, we can only overcome the challenges in immunotherapy of cold CRC through in-depth research and understanding the changes and mechanisms in the above three aspects of cold CRC.

Key Words: Cold colorectal cancer; Immunotherapy; Tumor microenvironment; Immune targets; Immune cells

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Advanced colorectal tumors are poorly treated, and immunotherapy has improved these patients' outcomes. However, cold colorectal tumors are less likely to trigger a robust immune response and tend to suppress it. To address this phenomenon, we discuss the role of the tumor microenvironment, immune targets, and immune cells in the treatment of cold colorectal tumors.

Citation: Ma SX, Li L, Cai H, Guo TK, Zhang LS. Therapeutic challenge for immunotherapy targeting cold colorectal cancer: A narrative review. *World J Clin Oncol* 2023; 14(2): 81-88
URL: https://www.wjgnet.com/2218-4333/full/v14/i2/81.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i2.81

INTRODUCTION

Colorectal cancer (CRC) has the third highest incidence and fourth mortality (after lung cancer, hepatic carcinoma, and stomach cancer) worldwide, which also serves as a biological and genetic paradigm for dissecting the evolutionary paths of solid tumors[1]. The risk factors of CRC are advanced age, dietary habits, obesity, lack of physical activity, constipation, chronic enteritis, intestinal polyps, alcohol consumption, and smoking[2]. With the robust advancement of fundamental research and medical technology, the treatment options for CRC have gradually formed a personalized and comprehensive treatment schedule led by surgery (*e.g.*, manual surgery, robotic surgery)[3]. Current treatment options include local endoscopic resection, radical surgical resection, local radiotherapy, systemic chemotherapy, palliative surgery, radiofrequency ablation of metastases, targeted therapy, and immunotherapy[4]. Of note, the survival benefit of patients with various tumors has increased significantly due to the rapid development of immunotherapy and the combined utilization with surgery, chemotherapy, radiotherapy, and targeted therapy. Generally, cancer immunotherapy can be divided into monoclonal antibodies, cytokines, immune checkpoint inhibitors (ICIs), tumor vaccines, and immune cells (*e.g.*, natural killer cells, tumor-infiltrating cells, T lymphocytes)[5]. Despite the increase in overall survival of patients with advanced CRC, new challenges have continuously emerged in treating "cold" CRC due to the current strategies in triggering a robust immune response and suppressing cancer[6].

To manage this phenomenon, we discuss the role of the tumor microenvironment (TME), immune targets, and immune cells in treating colorectal tumors.

LITERATURE SEARCH AND REVIEW

For the purpose, we primarily searched the literature on CRC immunotherapy published in the last 5 years through PubMed and Google Scholar databases. After importing them into the literature management software EndNote and de-duplicating them, we double-checked their titles, abstracts, and texts one-by-one to screen out the literature related to cold CRC treatment. The article was written according to a pre-planned framework, and the references were added by selecting the National Library of Medicine mode.

IMMUNOLOGICAL SIGNATURE-BASED CRC CLASSIFICATION

Accurate monomolecular typing is essential to screen CRC patients who may benefit from immunotherapy and whose TME needs reprogramming for beneficial immune-mediated responses[5]. Based on the degree of immune infiltration, tumors can be classified as "hot tumors" with high infiltration, "variable tumors" with rejection and immunosuppression, and "cold tumors" without infiltration[7]. Overall, the subsets of the aforementioned cancers have variations in pathological features, genetic mutations, immune cell composition, immune phenotypes, cytokines, clinical outcomes, and responses to immunotherapy[5]. CRC patients with a resistant "cold" phenotype are extremely challenging to treat with immunotherapy due to the low tumor mutation rate and lack of immune cell infiltration[5]. Approximately 80%-85% of CRC patients are considered to have "cold" tumors with microsatellite stability (MSS) or low microsatellite instability (MSI-L) (referred to as MSS/MSI-L CRC), which lack response to ICIs[8-10]. Immunosubtype classification can identify altered immune microenvironments in CRC patients. In addition, immune subtyping can guide personalized CRC immunotherapy and tumor prognosis[11-15].

RELATED STUDIES BASED ON THE TME

CRC is a highly heterogeneous disease, and mutant gene polymorphisms create a diversity of tumor subtypes and their corresponding TME. Sobral *et al*[16] demonstrated, in a study of genetic and microenvironmental intra-tumor heterogeneity affecting the evolution and metastatic development of CRC, that the diversity of CRC is caused by asynchronous forms of molecular alterations in which

mutations and chromosomal instability collectively contribute to the genetic and microenvironmental intra-tumor heterogeneity. Studies have shown that the greater the genetic mutation and TME differences, the lower the ability of tumors to metastasize. By contrast, advanced tumor gene mutations exploit tumor proliferation and metastasis. Wang *et al*[17] employed methionine enkephalin to inhibit colorectal carcinogenesis by reshaping the immune status of the TME. It has been shown that methionine enkephalin promotes antitumor immune responses, remodels the immune state of the tumor immune microenvironment in CRC, inhibits tumor development, and is a potential therapeutic agent for CRC, especially useful for improving the efficacy of immunotherapy. Chen *et al*[18] further proposed that metabolic changes in the TME were closely related to the development of CRC. In details, tumor cells secrete carriers beneficially utilized by surrounding cells in the TME to induce metabolic changes and cancer transformation. At the same time, tumor cells secrete pages that provide energy for their proliferation, metastasis, and drug resistance.

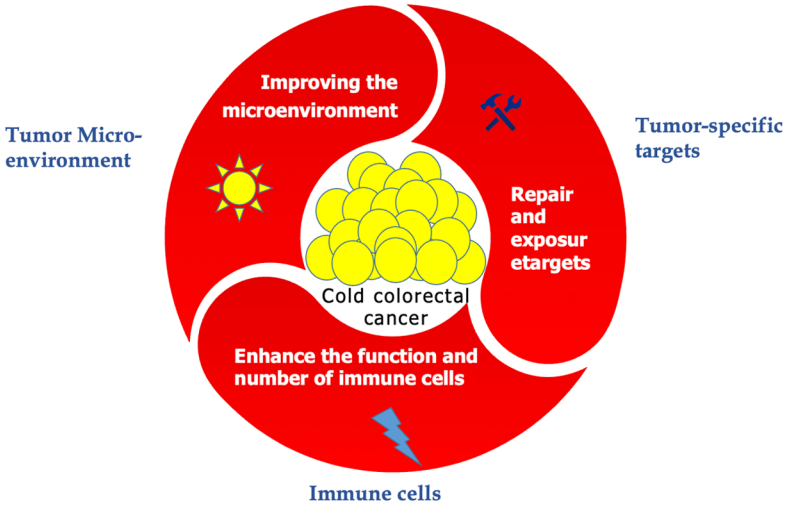
The tumor immune microenvironment is highly variable and extremely complex, and many immunosuppressive pathways have been identified in microsatellite-stabilized CRC[19]. Regorafenib, a tyrosine kinase inhibitor, is one of two drugs approved for treating MSS CRC[20]. The REGONIVO study showed a 36% response rate for regorafenib in metastatic MSS CRC[23]. Cabozantinib is another drug being investigated for the treatment of MSS CRC. Toll-like receptor (TLR) modulators are a new class of immunomodulatory drugs[24]. REVEAL is a phase 2 trial investigating TLR7/8 agonists in combination with nivolumab against tumors. Keynote-559 is a phase 1/2 trial investigating C-X-C motif chemokine ligand 12 (CXCL12) antagonists in combination with pembrolizumab for mCRC and metastatic pancreatic cancer. The chemokine CXCL12 promotes tumor proliferation, metastasis and angiogenesis by inducing signals, which can recruit B cells, plasma cells, and regulatory T cells to induce an immunosuppressive environment[25]. Investigators are devoted to developing multidisciplinary approaches to increase immune-mediated responses, improve the TME, and convert "cold" tumors into "hot" tumors to promote immunotherapy[15].

RELATED STUDIES BASED ON IMMUNE TARGETS

ICIs typically respond to CRCs with defective mismatch repair (dMMR) or high MSI (MSI-H). Approximately 85% of CRCs do not respond to immunotherapy or eventually become resistant due to MMR resistance or MSS[10]. MMR/MSS CRCs typically have low tumor mutational load, low chemotherapy response rates, low tumor-infiltrating lymphocytes, and poor prognosis compared to dMMR/MSI CRCs. Ros *et al*[26] verified that inhibition of transforming growth factor beta (TGF-β) could play a vital role in the development and metastasis of CRC by enhancing T-cell action. He *et al*[27] used *in situ*-forming albumin corpuscles to target liposomes and reshape the "cold" tumor immune microenvironment through epigenetic-based therapy. It was found that *in situ*-forming albumin corpuscles further enhanced tumor-targeted delivery, and that targeted liposome treatment effectively inhibited the effects between tumor metabolism and immune evasion by inhibiting glycolysis and immune normalization. Janssen *et al*[28] explained the available evidence for the potential impact of RAS mutations on the microenvironment of CRC in a study of mutated RAS and TME as dual therapeutic targets in advanced CRC[29]. Takahashi *et al*[30] showed that the combination of stromal programmed death ligand 1 (PD-L1)+ immune cells and nuclear β-catenin$^+$ tumor budding might contribute to tumor progression in CRC and resistance to neoadjuvant chemotherapy in locally advanced rectal cancer. Dmitrieva-Posocco *et al*[31] found that the ketogenic diet exhibited strong tumor suppressive effects. The ketone body β-hydroxybutyric acid reduced colonic crypt cells proliferation and effectively inhibited intestinal tumor growth. It is suggested that oral or systemic interventions using a single metabolite could complement current CRC prevention and treatment strategies. High PD-L1 expression in tumors is a sign of poor prognosis, which also shows good responsiveness to ICIs and immunomodulatory drugs such as C-X-C motif chemokine receptor 4, poly (ADP-ribose) polymerase or TGF-β inhibitors in combination[6]. Li *et al*[32] investigated the relationship between genetic changes in CRC and intercellular transformation in cancer cell biology and TME. Key advances in the development of effective therapeutic approaches for this cancer were analyzed from immunological and single-cell perspectives[33]. Long-noncoding RNAs (lncRNAs) are important regulators of microRNA expression in CRC and might be promising biomarkers and potential therapeutic targets in CRC research. For example, Lv *et al*[34] provided insights into the pathogenesis, diagnosis, and development of therapeutic strategies for CRC by studying lncRNAs.

RELATED STUDIES BASED ON IMMUNE CELLS

The current therapeutic strategies have limited efficacy in CRC[35-38]. Approximately one-quarter of CRC patients are diagnosed with a combination of distant metastases[39-41], and of these, another one-quarter recurs or metastasizes within 5 years. The 5-year survival rate for CRC patients with combined metastases is approximately 15%[42-44]. Therefore, there is an urgent need for new approaches to treat



DOI: 10.5306/wjco.v14.i2.81 **Copyright** ©The Author(s) 2023.

Figure 1 Pattern of immunotherapy strategies for cold colorectal cancer.

CRC using immunotherapy[28,45]. The current cancer classification is based on the American Joint Committee on Cancer/Union for International Cancer Control - Tumor Node Metastasis (TNM) system, and the prediction of the effect of immunotherapy cannot be assessed[35]. Relevant evidence suggests that the prognosis of CRC patients correlates with the type, density, and function of immune cells within the tumor[46]. Galon *et al*[35] developed an immunohistochemical and digital pathology-based assay named Immunoscore, which quantified two tumor regions (core and invasive margin of the tumor) in two T-cell subsets (cluster of differentiation 3 [CD3] and [CD8]). Immunoscore is an immune function-based scoring system that is more valuable than the traditional TNM score in determining the predictive value of patients with CRC[47-50]. Relative studies have also demonstrated the predictive value of Immunoscore for the prognosis of patients with colon cancer[51-53], which is conducive to classify tumors and guide clinical decisions[54-58]. Tumor lysis virus is a novel antitumor agent that both lyses tumor cells and modulates the TME, which can convert "cold" tumors into "hot" tumors and thus allows ICIs to work. For example, Ren *et al*[36] recently investigated the status of tumor lysing viruses and ICIs for treating CRC. The feasibility of combining tumor lysis virus with ICIs for treating CRC will be discussed in terms of the mechanism of action of tumor lysis virus for tumor treatment.

FUTURE DIRECTIONS

For cold CRC, immunotherapy strategies focus on converting "cold" tumors to "hot" tumors through various approaches[6,59-62]. Various immunotherapies or chemotherapy can be used to modulate the patient's immune status[63-66]. Regulation of the number and function of *Escherichia coli* in the patient's intestine can improve the role of the patient's immune microenvironment[67-69]. Therapies that enhance the operation and number of immune cells may also improve treatment outcomes[70-72]. Further functional and mechanistic studies of mutated genes could identify new targets for cold CRC therapy [73-75].

CONCLUSION

In summary, the fundamental reasons for the challenge of immunotherapy for cold CRC are the low tumor mutational load and lack of immune cell infiltration. To conquer this phenomenon, we should conduct comprehensive research on the TME, immune targets and immune cells to warm up CRC (Figure 1). Meanwhile, we should also combine the aforementioned cancer immunotherapy with traditional tumor treatment remedies such as surgery, radiotherapy, and chemotherapy. Only personalized, comprehensive treatment plans for CRC, and a good prognosis for patients are the ultimate goals we pursue.

FOOTNOTES

Author contributions: Ma SX, and Li L wrote the paper; Zhang LS, Cai H, and Guo TK performed the data collection; All authors have read and approved the final manuscript.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Shi-Xun Ma 0000-0002-4208-9248; Hui Cai 0000-0001-5857-1744; Tian-Kang Guo https://orcid.org/0000-0003-1035-7348; Lei-Sheng Zhang 0000-0001-6540-0943.

S-Editor: Liu GL
L-Editor: Filipodia
P-Editor: Liu GL

REFERENCES

1 **Baidoun F**, Elshiwy K, Elkeraie Y, Merjaneh Z, Khoudari G, Sarmini MT, Gad M, Al-Husseini M, Saad A. Colorectal Cancer Epidemiology: Recent Trends and Impact on Outcomes. *Curr Drug Targets* 2021; **22**: 998-1009 [PMID: 33208072 DOI: 10.2174/1389450121999201117115717]
2 **Dekker E**, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet* 2019; **394**: 1467-1480 [PMID: 31631858 DOI: 10.1016/S0140-6736(19)32319-0]
3 **Fleming-de-Moraes CD**, Rocha MR, Tessmann JW, de Araujo WM, Morgado-Diaz JA. Crosstalk between PI3K/Akt and Wnt/β-catenin pathways promote colorectal cancer progression regardless of mutational status. *Cancer Biol Ther* 2022; **23**: 1-13 [PMID: 35944058 DOI: 10.1080/15384047.2022.2108690]
4 **Paty PB**, Garcia-Aguilar J. Colorectal cancer. *J Surg Oncol* 2022; **126**: 881-887 [PMID: 36087081 DOI: 10.1002/jso.27079]
5 **Xu M**, Chang J, Wang W, Wang X, Weng W, Tan C, Zhang M, Ni S, Wang L, Huang Z, Deng Z, Li W, Huang D, Sheng W. Classification of colon adenocarcinoma based on immunological characterizations: Implications for prognosis and immunotherapy. *Front Immunol* 2022; **13**: 934083 [PMID: 35967414 DOI: 10.3389/fimmu.2022.934083]
6 **Majidpoor J**, Mortezaee K. The efficacy of PD-1/PD-L1 blockade in cold cancers and future perspectives. *Clin Immunol* 2021; **226**: 108707 [PMID: 33662590 DOI: 10.1016/j.clim.2021.108707]
7 **Galon J**, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov* 2019; **18**: 197-218 [PMID: 30610226 DOI: 10.1038/s41573-018-0007-y]
8 **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255 DOI: 10.1056/NEJMoa1500596]
9 **Chen DS**, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature* 2017; **541**: 321-330 [PMID: 28102259 DOI: 10.1038/nature21349]
10 **Lizardo DY**, Kuang C, Hao S, Yu J, Huang Y, Zhang L. Immunotherapy efficacy on mismatch repair-deficient colorectal cancer: From bench to bedside. *Biochim Biophys Acta Rev Cancer* 2020; **1874**: 188447 [PMID: 33035640 DOI: 10.1016/j.bbcan.2020.188447]
11 **Goodman AM**, Kato S, Bazhenova L, Patel SP, Frampton GM, Miller V, Stephens PJ, Daniels GA, Kurzrock R. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Mol Cancer Ther* 2017; **16**: 2598-2608 [PMID: 28835386 DOI: 10.1158/1535-7163.MCT-17-0386]
12 **Goodman AM**, Sokol ES, Frampton GM, Lippman SM, Kurzrock R. Microsatellite-Stable Tumors with High Mutational Burden Benefit from Immunotherapy. *Cancer Immunol Res* 2019; **7**: 1570-1573 [PMID: 31405947 DOI: 10.1158/2326-6066.CIR-19-0149]
13 **Wang W**, Green M, Choi JE, Gijón M, Kennedy PD, Johnson JK, Liao P, Lang X, Kryczek I, Sell A, Xia H, Zhou J, Li G, Li J, Li W, Wei S, Vatan L, Zhang H, Szeliga W, Gu W, Liu R, Lawrence TS, Lamb C, Tanno Y, Cieslik M, Stone E, Georgiou G, Chan TA, Chinnaiyan A, Zou W. CD8(+) T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature* 2019; **569**: 270-274 [PMID: 31043744 DOI: 10.1038/s41586-019-1170-y]
14 **Golstein P**, Griffiths GM. An early history of T cell-mediated cytotoxicity. *Nat Rev Immunol* 2018; **18**: 527-535 [PMID: 29662120 DOI: 10.1038/s41577-018-0009-3]
15 **Newman JH**, Chesson CB, Herzog NL, Bommareddy PK, Aspromonte SM, Pepe R, Estupinian R, Aboelatta MM, Buddhadev S, Tarabichi S, Lee M, Li S, Medina DJ, Giurini EF, Gupta KH, Guevara-Aleman G, Rossi M, Nowicki C, Abed A, Goldufsky JW, Broucek JR, Redondo RE, Rotter D, Jhawar SR, Wang SJ, Kohlhapp FJ, Kaufman HL, Thomas

PG, Gupta V, Kuzel TM, Reiser J, Paras J, Kane MP, Singer EA, Malhotra J, Denzin LK, Sant'Angelo DB, Rabson AB, Lee LY, Lasfar A, Langenfeld J, Schenkel JM, Fidler MJ, Ruiz ES, Marzo AL, Rudra JS, Silk AW, Zloza A. Intratumoral injection of the seasonal flu shot converts immunologically cold tumors to hot and serves as an immunotherapy for cancer. *Proc Natl Acad Sci U S A* 2020; **117**: 1119-1128 [PMID: 31888983 DOI: 10.1073/pnas.1904022116]

16 **Sobral D**, Martins M, Kaplan S, Golkaram M, Salmans M, Khan N, Vijayaraghavan R, Casimiro S, Fernandes A, Borralho P, Ferreira C, Pinto R, Abreu C, Costa AL, Zhang S, Pawlowski T, Godsey J, Mansinho A, Macedo D, Lobo-Martins S, Filipe P, Esteves R, Coutinho J, Costa PM, Ramires A, Aldeia F, Quintela A, So A, Liu L, Grosso AR, Costa L. Genetic and microenvironmental intra-tumor heterogeneity impacts colorectal cancer evolution and metastatic development. *Commun Biol* 2022; **5**: 937 [PMID: 36085309 DOI: 10.1038/s42003-022-03884-x]

17 **Wang X**, Li S, Yan S, Shan Y, Wang X, Jingbo Z, Wang Y, Shan F, Griffin N, Sun X. Methionine enkephalin inhibits colorectal cancer by remodeling the immune status of the tumor microenvironment. *Int Immunopharmacol* 2022; **111**: 109125 [PMID: 35988519 DOI: 10.1016/j.intimp.2022.109125]

18 **Chen J**, Zhu H, Yin Y, Jia S, Luo X. Colorectal cancer: Metabolic interactions reshape the tumor microenvironment. *Biochim Biophys Acta Rev Cancer* 2022; **1877**: 188797 [PMID: 36100193 DOI: 10.1016/j.bbcan.2022.188797]

19 **Lazarus J**, Maj T, Smith JJ, Perusina Lanfranca M, Rao A, D'Angelica MI, Delrosario L, Girgis A, Schukow C, Shia J, Kryczek I, Shi J, Wasserman I, Crawford H, Nathan H, Pasca Di Magliano M, Zou W, Frankel TL. Spatial and phenotypic immune profiling of metastatic colon cancer. *JCI Insight* 2018; **3** [PMID: 30429368 DOI: 10.1172/jci.insight.121932]

20 **Grothey A**, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D; CORRECT Study Group. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013; **381**: 303-312 [PMID: 23177514 DOI: 10.1016/S0140-6736(12)61900-X]

21 **Fondevila F**, Méndez-Blanco C, Fernández-Palanca P, González-Gallego J, Mauriz JL. Anti-tumoral activity of single and combined regorafenib treatments in preclinical models of liver and gastrointestinal cancers. *Exp Mol Med* 2019; **51**: 1-15 [PMID: 31551425 DOI: 10.1038/s12276-019-0308-1]

22 **Abou-Elkacem L**, Arns S, Brix G, Gremse F, Zopf D, Kiessling F, Lederle W. Regorafenib inhibits growth, angiogenesis, and metastasis in a highly aggressive, orthotopic colon cancer model. *Mol Cancer Ther* 2013; **12**: 1322-1331 [PMID: 23619301 DOI: 10.1158/1535-7163.MCT-12-1162]

23 **Fukuoka S**, Hara H, Takahashi N, Kojima T, Kawazoe A, Asayama M, Yoshii T, Kotani D, Tamura H, Mikamoto Y, Hirano N, Wakabayashi M, Nomura S, Sato A, Kuwata T, Togashi Y, Nishikawa H, Shitara K. Regorafenib Plus Nivolumab in Patients With Advanced Gastric or Colorectal Cancer: An Open-Label, Dose-Escalation, and Dose-Expansion Phase Ib Trial (REGONIVO, EPOC1603). *J Clin Oncol* 2020; **38**: 2053-2061 [PMID: 32343640 DOI: 10.1200/JCO.19.03296]

24 **Urban-Wojciuk Z**, Khan MM, Oyler BL, Fåhraeus R, Marek-Trzonkowska N, Nita-Lazar A, Hupp TR, Goodlett DR. The Role of TLRs in Anti-cancer Immunity and Tumor Rejection. *Front Immunol* 2019; **10**: 2388 [PMID: 31695691 DOI: 10.3389/fimmu.2019.02388]

25 **Nagarsheth N**, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol* 2017; **17**: 559-572 [PMID: 28555670 DOI: 10.1038/nri.2017.49]

26 **Ros XR**, Vermeulen L. Turning Cold Tumors Hot by Blocking TGF-β. *Trends Cancer* 2018; **4**: 335-337 [PMID: 29709256 DOI: 10.1016/j.trecan.2018.03.005]

27 **He Y**, Fang Y, Zhang M, Zhao Y, Tu B, Shi M, Muhitdinov B, Asrorov A, Xu Q, Huang Y. Remodeling "cold" tumor immune microenvironment *via* epigenetic-based therapy using targeted liposomes with in situ formed albumin corona. *Acta Pharm Sin B* 2022; **12**: 2057-2073 [PMID: 35847495 DOI: 10.1016/j.apsb.2021.09.022]

28 **Janssen JBE**, Medema JP, Gootjes EC, Tauriello DVF, Verheul HMW. Mutant RAS and the tumor microenvironment as dual therapeutic targets for advanced colorectal cancer. *Cancer Treat Rev* 2022; **109**: 102433 [PMID: 35905558 DOI: 10.1016/j.ctrv.2022.102433]

29 **Sun Y**, Li Z, Wang W, Zhang X, Li W, Du G, Yin J, Xiao W, Yang H. Identification and verification of YBX3 and its regulatory gene HEIH as an oncogenic system: A multidimensional analysis in colon cancer. *Front Immunol* 2022; **13**: 957865 [PMID: 36059530 DOI: 10.3389/fimmu.2022.957865]

30 **Takahashi H**, Watanabe H, Hashimura M, Matsumoto T, Yokoi A, Nakagawa M, Ishibashi Y, Ito T, Ohhigata K, Saegusa M. A combination of stromal PD-L1 and tumoral nuclear β-catenin expression as an indicator of colorectal carcinoma progression and resistance to chemoradiotherapy in locally advanced rectal carcinoma. *J Pathol Clin Res* 2022; **8**: 458-469 [PMID: 35762092 DOI: 10.1002/cjp2.285]

31 **Dmitrieva-Posocco O**, Wong AC, Lundgren P, Golos AM, Descamps HC, Dohnalová L, Cramer Z, Tian Y, Yueh B, Eskiocak O, Egervari G, Lan Y, Liu J, Fan J, Kim J, Madhu B, Schneider KM, Khoziainova S, Andreeva N, Wang Q, Li N, Furth EE, Bailis W, Kelsen JR, Hamilton KE, Kaestner KH, Berger SL, Epstein JA, Jain R, Li M, Beyaz S, Lengner CJ, Katona BW, Grivennikov SI, Thaiss CA, Levy M. β-Hydroxybutyrate suppresses colorectal cancer. *Nature* 2022; **605**: 160-165 [PMID: 35477756 DOI: 10.1038/s41586-022-04649-6]

32 **Li J**, Ma X, Chakravarti D, Shalapour S, DePinho RA. Genetic and biological hallmarks of colorectal cancer. *Genes Dev* 2021; **35**: 787-820 [PMID: 34074695 DOI: 10.1101/gad.348226.120]

33 **Zhao EY**, Jones M, Jones SJM. Whole-Genome Sequencing in Cancer. *Cold Spring Harb Perspect Med* 2019; **9** [PMID: 29844223 DOI: 10.1101/cshperspect.a034579]

34 **Lv Y**, Wang Y, Zhang Z, Bao J, Su H. Potentials of long non-coding RNAs as biomarkers of colorectal cancer. *Clin Transl Oncol* 2022; **24**: 1715-1731 [PMID: 35581419 DOI: 10.1007/s12094-022-02834-7]

35 **Galon J**, Lanzi A. Immunoscore and its introduction in clinical practice. *Q J Nucl Med Mol Imaging* 2020; **64**: 152-161 [PMID: 32107902 DOI: 10.23736/S1824-4785.20.03249-5]

36 **Ren Y**, Miao JM, Wang YY, Fan Z, Kong XB, Yang L, Cheng G. Oncolytic viruses combined with immune checkpoint therapy for colorectal cancer is a promising treatment option. *Front Immunol* 2022; **13**: 961796 [PMID: 35911673 DOI: 10.3389/fimmu.2022.961796]

37 **Lian W**, Wang Z, Ma Y, Tong Y, Zhang X, Jin H, Zhao S, Yu R, Ju S, Guo X, Huang T, Ding X, Peng M. FABP6 Expression Correlates with Immune Infiltration and Immunogenicity in Colorectal Cancer Cells. *J Immunol Res* 2022; **2022**: 3129765 [PMID: 36033394 DOI: 10.1155/2022/3129765]

38 **Bolzacchini E**, Libera L, Church SE, Sahnane N, Bombelli R, Digiacomo N, Giordano M, Petracco G, Sessa F, Capella C, Furlan D. Tumor Antigenicity and a Pre-Existing Adaptive Immune Response in Advanced BRAF Mutant Colorectal Cancers. *Cancers (Basel)* 2022; **14** [PMID: 36010943 DOI: 10.3390/cancers14163951]

39 **Collienne M**, Loghmani H, Heineman TC, Arnold D. GOBLET: a phase I/II study of pelareorep and atezolizumab +/- chemo in advanced or metastatic gastrointestinal cancers. *Future Oncol* 2022; **18**: 2871-2878 [PMID: 35796248 DOI: 10.2217/fon-2022-0453]

40 **Wu JY**, Song QY, Huang CZ, Shao Y, Wang ZL, Zhang HQ, Fu Z. N7-methylguanosine-related lncRNAs: Predicting the prognosis and diagnosis of colorectal cancer in the cold and hot tumors. *Front Genet* 2022; **13**: 952836 [PMID: 35937987 DOI: 10.3389/fgene.2022.952836]

41 **Li S**, Na R, Li X, Zhang Y, Zheng T. Targeting interleukin-17 enhances tumor response to immune checkpoint inhibitors in colorectal cancer. *Biochim Biophys Acta Rev Cancer* 2022; **1877**: 188758 [PMID: 35809762 DOI: 10.1016/j.bbcan.2022.188758]

42 **Hubbard JM**, Tőke ER, Moretto R, Graham RP, Youssoufian H, Lőrincz O, Molnár L, Csiszovszki Z, Mitchell JL, Wessling J, Tóth J, Cremolini C. Safety and Activity of PolyPEPI1018 Combined with Maintenance Therapy in Metastatic Colorectal Cancer: an Open-Label, Multicenter, Phase Ib Study. *Clin Cancer Res* 2022; **28**: 2818-2829 [PMID: 35472243 DOI: 10.1158/1078-0432.CCR-22-0112]

43 **Li C**, Li T, Niu K, Xiao Z, Huang J, Pan X, Sun Y, Wang Y, Ma D, Xie P, Shuai X, Meng X. Mild phototherapy mediated by manganese dioxide-loaded mesoporous polydopamine enhances immunotherapy against colorectal cancer. *Biomater Sci* 2022; **10**: 3647-3656 [PMID: 35670464 DOI: 10.1039/d2bm00505k]

44 **Yu G**, Wang W, He X, Xu J, Xu R, Wan T, Wu Y. Synergistic Therapeutic Effects of Low Dose Decitabine and NY-ESO-1 Specific TCR-T Cells for the Colorectal Cancer With Microsatellite Stability. *Front Oncol* 2022; **12**: 895103 [PMID: 35774131 DOI: 10.3389/fonc.2022.895103]

45 **Gatenbee CD**, Baker AM, Schenck RO, Strobl M, West J, Neves MP, Hasan SY, Lakatos E, Martinez P, Cross WCH, Jansen M, Rodriguez-Justo M, Whelan CJ, Sottoriva A, Leedham S, Robertson-Tessi M, Graham TA, Anderson ARA. Immunosuppressive niche engineering at the onset of human colorectal cancer. *Nat Commun* 2022; **13**: 1798 [PMID: 35379804 DOI: 10.1038/s41467-022-29027-8]

46 **Qiao G**, Kone LB, Phillips EH, Lee SS, Brown GE, Khetani SR, Thakur A, Lum LG, Prabhakar BS, Maker AV. LIGHT enhanced bispecific antibody armed T-cells to treat immunotherapy resistant colon cancer. *Oncogene* 2022; **41**: 2054-2068 [PMID: 35177811 DOI: 10.1038/s41388-022-02209-w]

47 **Wen H**, Li F, Bukhari I, Mi Y, Guo C, Liu B, Zheng P, Liu S. Comprehensive Analysis of Colorectal Cancer Immunity and Identification of Immune-Related Prognostic Targets. *Dis Markers* 2022; **2022**: 7932655 [PMID: 35401882 DOI: 10.1155/2022/7932655]

48 **Zheng Y**, Fu Y, Wang PP, Ding ZY. Neoantigen: A Promising Target for the Immunotherapy of Colorectal Cancer. *Dis Markers* 2022; **2022**: 8270305 [PMID: 35211210 DOI: 10.1155/2022/8270305]

49 **Di Guida R**, Casillo A, Stellavato A, Kawai S, Ogawa T, Di Meo C, Kawamoto J, Kurihara T, Schiraldi C, Corsaro MM. Capsular polysaccharide from a fish-gut bacterium induces/promotes apoptosis of colon cancer cells *in vitro* through Caspases' pathway activation. *Carbohydr Polym* 2022; **278**: 118908 [PMID: 34973729 DOI: 10.1016/j.carbpol.2021.118908]

50 **Wang Z**, Moresco P, Yan R, Li J, Gao Y, Biasci D, Yao M, Pearson J, Hechtman JF, Janowitz T, Zaidi RM, Weiss MJ, Fearon DT. Carcinomas assemble a filamentous CXCL12-keratin-19 coating that suppresses T cell-mediated immune attack. *Proc Natl Acad Sci U S A* 2022; **119** [PMID: 35046049 DOI: 10.1073/pnas.2119463119]

51 **Kristensen LK**, Christensen C, Alfsen MZ, Cold S, Nielsen CH, Kjaer A. Monitoring CD8a(+) T Cell Responses to Radiotherapy and CTLA-4 Blockade Using [(64)Cu]NOTA-CD8a PET Imaging. *Mol Imaging Biol* 2020; **22**: 1021-1030 [PMID: 32086762 DOI: 10.1007/s11307-020-01481-0]

52 **Martinez NW**, Sánchez A, Diaz P, Broekhuizen R, Godoy J, Mondaca S, Catenaccio A, Macanas P, Nervi B, Calvo M, Court FA. Metformin protects from oxaliplatin induced peripheral neuropathy in rats. *Neurobiol Pain* 2020; **8**: 100048 [PMID: 32490289 DOI: 10.1016/j.ynpai.2020.100048]

53 **Taylor K**, Loo Yau H, Chakravarthy A, Wang B, Shen SY, Ettayebi I, Ishak CA, Bedard PL, Abdul Razak A, R Hansen A, Spreafico A, Cescon D, Butler MO, Oza AM, Lheureux S, Stjepanovic N, Van As B, Boross-Harmer S, Wang L, Pugh TJ, Ohashi PS, Siu LL, De Carvalho DD. An open-label, phase II multicohort study of an oral hypomethylating agent CC-486 and durvalumab in advanced solid tumors. *J Immunother Cancer* 2020; **8** [PMID: 32753546 DOI: 10.1136/jitc-2020-000883]

54 **Yu W**, Sun J, Liu F, Yu S, Hu J, Zhao Y, Wang X, Liu X. Treating Immunologically Cold Tumors by Precise Cancer Photoimmunotherapy with an Extendable Nanoplatform. *ACS Appl Mater Interfaces* 2020; **12**: 40002-40012 [PMID: 32805869 DOI: 10.1021/acsami.0c09469]

55 **Janji B**, Hasmim M, Parpal S, De Milito A, Berchem G, Noman MZ. Lighting up the fire in cold tumors to improve cancer immunotherapy by blocking the activity of the autophagy-related protein PIK3C3/VPS34. *Autophagy* 2020; **16**: 2110-2111 [PMID: 32892693 DOI: 10.1080/15548627.2020.1815439]

56 **Kleeman SO**, Leedham SJ. Not All Wnt Activation Is Equal: Ligand-Dependent versus Ligand-Independent Wnt Activation in Colorectal Cancer. *Cancers (Basel)* 2020; **12** [PMID: 33202731 DOI: 10.3390/cancers12113355]

57 **Biasci D**, Smoragiewicz M, Connell CM, Wang Z, Gao Y, Thaventhiran JED, Basu B, Magiera L, Johnson TI, Bax L, Gopinathan A, Isherwood C, Gallagher FA, Pawula M, Hudecova I, Gale D, Rosenfeld N, Barmpounakis P, Popa EC, Brais R, Godfrey E, Mir F, Richards FM, Fearon DT, Janowitz T, Jodrell DI. CXCR4 inhibition in human pancreatic and colorectal cancers induces an integrated immune response. *Proc Natl Acad Sci U S A* 2020; **117**: 28960-28970 [PMID: 33127761 DOI: 10.1073/pnas.2013644117]

58 **Matsumoto T**, Okayama H, Nakajima S, Saito K, Nakano H, Endo E, Kase K, Ito M, Yamauchi N, Yamada L, Kanke Y,

Onozawa H, Fujita S, Sakamoto W, Saito M, Saze Z, Momma T, Mimura K, Kono K. Tn Antigen Expression Defines an Immune Cold Subset of Mismatch-Repair Deficient Colorectal Cancer. *Int J Mol Sci* 2020; **21** [PMID: 33260328 DOI: 10.3390/ijms21239081]

59 **Ren J**, Xu M, Chen J, Ding J, Wang P, Huo L, Li F, Liu Z. PET imaging facilitates antibody screening for synergistic radioimmunotherapy with a (177)Lu-labeled αPD-L1 antibody. *Theranostics* 2021; **11**: 304-315 [PMID: 33391476 DOI: 10.7150/thno.45540]

60 **Angelova A**, Ferreira T, Bretscher C, Rommelaere J, Marchini A. Parvovirus-Based Combinatorial Immunotherapy: A Reinforced Therapeutic Strategy against Poor-Prognosis Solid Cancers. *Cancers (Basel)* 2021; **13** [PMID: 33477757 DOI: 10.3390/cancers13020342]

61 **Fabian KP**, Malamas AS, Padget MR, Solocinski K, Wolfson B, Fujii R, Abdul Sater H, Schlom J, Hodge JW. Therapy of Established Tumors with Rationally Designed Multiple Agents Targeting Diverse Immune-Tumor Interactions: Engage, Expand, Enable. *Cancer Immunol Res* 2021; **9**: 239-252 [PMID: 33355290 DOI: 10.1158/2326-6066.CIR-20-0638]

62 **Fabian KP**, Padget MR, Fujii R, Schlom J, Hodge JW. Differential combination immunotherapy requirements for inflamed (warm) tumors versus T cell excluded (cool) tumors: engage, expand, enable, and evolve. *J Immunother Cancer* 2021; **9** [PMID: 33602696 DOI: 10.1136/jitc-2020-001691]

63 **Fathi M**, Pustokhina I, Kuznetsov SV, Khayrullin M, Hojjat-Farsangi M, Karpisheh V, Jalili A, Jadidi-Niaragh F. T-cell immunoglobulin and ITIM domain, as a potential immune checkpoint target for immunotherapy of colorectal cancer. *IUBMB Life* 2021; **73**: 726-738 [PMID: 33686787 DOI: 10.1002/iub.2461]

64 **Chen L**, Chen H, Ye J, Ge Y, Wang H, Dai E, Ren J, Liu W, Ma C, Ju S, Guo ZS, Liu Z, Bartlett DL. Intratumoral expression of interleukin 23 variants using oncolytic vaccinia virus elicit potent antitumor effects on multiple tumor models *via* tumor microenvironment modulation. *Theranostics* 2021; **11**: 6668-6681 [PMID: 34093846 DOI: 10.7150/thno.56494]

65 **Fang Y**, He Y, Wu C, Zhang M, Gu Z, Zhang J, Liu E, Xu Q, Asrorov AM, Huang Y. Magnetism-mediated targeting hyperthermia-immunotherapy in "cold" tumor with CSF1R inhibitor. *Theranostics* 2021; **11**: 6860-6872 [PMID: 34093858 DOI: 10.7150/thno.57511]

66 **Lee SJ**, Yang H, Kim WR, Lee YS, Lee WS, Kong SJ, Lee HJ, Kim JH, Cheon J, Kang B, Chon HJ, Kim C. STING activation normalizes the intraperitoneal vascular-immune microenvironment and suppresses peritoneal carcinomatosis of colon cancer. *J Immunother Cancer* 2021; **9** [PMID: 34145029 DOI: 10.1136/jitc-2020-002195]

67 **Chen D**, Bao X, Zhang R, Ding Y, Zhang M, Li B, Zhang H, Li X, Tong Z, Liu L, Zhou X, Wang S, Cheng X, Zheng Y, Ruan J, Fang W, Zhao P. Depiction of the genomic and genetic landscape identifies CCL5 as a protective factor in colorectal neuroendocrine carcinoma. *Br J Cancer* 2021; **125**: 994-1002 [PMID: 34331023 DOI: 10.1038/s41416-021-01501-y]

68 **Huis In 't Veld RV**, Da Silva CG, Jager MJ, Cruz LJ, Ossendorp F. Combining Photodynamic Therapy with Immunostimulatory Nanoparticles Elicits Effective Anti-Tumor Immune Responses in Preclinical Murine Models. *Pharmaceutics* 2021; **13** [PMID: 34575546 DOI: 10.3390/pharmaceutics13091470]

69 **Liu N**, Shan F, Ma M. Strategic enhancement of immune checkpoint inhibition in refractory Colorectal Cancer: Trends and future prospective. *Int Immunopharmacol* 2021; **99**: 108017 [PMID: 34352568 DOI: 10.1016/j.intimp.2021.108017]

70 **Wang Z**, Little N, Chen J, Lambesis KT, Le KT, Han W, Scott AJ, Lu J. Immunogenic camptothesome nanovesicles comprising sphingomyelin-derived camptothecin bilayers for safe and synergistic cancer immunochemotherapy. *Nat Nanotechnol* 2021; **16**: 1130-1140 [PMID: 34385682 DOI: 10.1038/s41565-021-00950-z]

71 **Matsumoto T**, Okayama H, Nakano H, Ito M, Nakajima S, Saito M, Saze Z, Momma T, Mimura K, Kono K. [Immunotherapy Targeting Tumor-Associated Carbohydrate Antigens in Deficient Mismatch Repair Colorectal Cancer]. *Gan To Kagaku Ryoho* 2021; **48**: 1275-1277 [PMID: 34657062]

72 **Baraibar I**, Mirallas O, Saoudi N, Ros J, Salvà F, Tabernero J, Élez E. Combined Treatment with Immunotherapy-Based Strategies for MSS Metastatic Colorectal Cancer. *Cancers (Basel)* 2021; **13** [PMID: 34944931 DOI: 10.3390/cancers13246311]

73 **Gao Y**, Päivinen P, Tripathi S, Domènech-Moreno E, Wong IPL, Vaahtomeri K, Nagaraj AS, Talwelkar SS, Foretz M, Verschuren EW, Viollet B, Yan Y, Mäkelä TP. Inactivation of AMPK Leads to Attenuation of Antigen Presentation and Immune Evasion in Lung Adenocarcinoma. *Clin Cancer Res* 2022; **28**: 227-237 [PMID: 34667030 DOI: 10.1158/1078-0432.CCR-21-2049]

74 **Shang S**, Yang YW, Chen F, Yu L, Shen SH, Li K, Cui B, Lv XX, Zhang C, Yang C, Liu J, Yu JJ, Zhang XW, Li PP, Zhu ST, Zhang HZ, Hua F. TRIB3 reduces CD8(+) T cell infiltration and induces immune evasion by repressing the STAT1-CXCL10 axis in colorectal cancer. *Sci Transl Med* 2022; **14**: eabf0992 [PMID: 34985967 DOI: 10.1126/scitranslmed.abf0992]

75 **Liu Z**, Guo Y, Yang X, Chen C, Fan D, Wu X, Si C, Xu Y, Shao B, Chen Z, Dang Q, Cui W, Han X, Ji Z, Sun Z. Immune Landscape Refines the Classification of Colorectal Cancer With Heterogeneous Prognosis, Tumor Microenvironment and Distinct Sensitivity to Frontline Therapies. *Front Cell Dev Biol* 2021; **9**: 784199 [PMID: 35083217 DOI: 10.3389/fcell.2021.784199]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 February 24; 14(2): 89-98

DOI: 10.5306/wjco.v14.i2.89

ISSN 2218-4333 (online)

MINIREVIEWS

New trends in the surgical management of soft tissue sarcoma: The role of preoperative biopsy

Efstathios T Pavlidis, Theodoros E Pavlidis

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Elkady N, Egypt; Wiemer EA, Netherlands

Received: October 21, 2022
Peer-review started: October 21, 2022
First decision: December 26, 2022
Revised: December 26, 2022
Accepted: January 9, 2023
Article in press: January 9, 2023
Published online: February 24, 2023

Efstathios T Pavlidis, Theodoros E Pavlidis, 2nd Propedeutic Department of Surgery, Hippocration Hospital, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki 54642, Greece

Corresponding author: Theodoros E Pavlidis, Doctor, PhD, Full Professor, Surgeon, 2nd Propedeutic Department of Surgery, Hippocration Hospital, School of Medicine, Aristotle University of Thessaloniki, Konstantinoupoleos 49, Thessaloniki 54642, Greece.
pavlidth@auth.gr

Abstract

Soft tissue sarcoma (STS) accounts for 1% of all malignant neoplasms in adults. Their diagnosis and management constitute a challenging target. They originate from the mesenchyme, and 50 subtypes with various cytogenetic profiles concerning soft tissue and bones have been recognized. These tumors mainly affect middle-aged adults but may be present at any age. Half of the patients have metastatic disease at the time of diagnosis and require systemic therapy. Tumors above 3-5 cm in size must be suspected of potential malignancy. A thorough history, clinical examination and imaging that must precede biopsy are necessary. Modern imaging techniques include ultrasound, computed tomography (CT), new magnetic resonance imaging (MRI), and positron emission tomography/CT. MRI findings may distinguish low-grade from high-grade STS based on a diagnostic score (tumor heterogeneity, intratumoral and peritumoral enhancement). A score ≥ 2 indicates a high-grade lesion, and a score ≤ 1 indicates a low-grade lesion. For disease staging, abdominal imaging is recommended to detect early abdominal or retroperitoneal metastases. Liquid biopsy by detecting genomic material in serum is a novel diagnostic tool. A preoperative biopsy is necessary for diagnosis, prognosis and optimal planning of surgical intervention. Core needle biopsy is the most indicative and effective. Its correct performance influences surgical management. An unsuccessful biopsy means the dissemination of cancer cells into healthy anatomical structures that ultimately affect resectability and survival. Complete therapeutic excision (R0) with an acceptable resection margin of 1 cm is the method of choice. However, near significant structures, *i.e.*, vessels, nerves, an R2 resection (macroscopic margin involvement) preserving functionality but having a risk of local recurrence can be an acceptable choice, after informing the patient, to prevent an unavoidable amputation. For borderline resectability of the tumor, neoadjuvant chemo/radiotherapy has a place. Likewise, after surgical excision, adjuvant therapy is indicated, but chemotherapy in nonmetastatic disease is still debatable. The five-year survival

rate reaches up to 55%. Reresection is considered after positive or uncertain resection margins. Current strategies are based on novel chemotherapeutic agents, improved radiotherapy applications to limit local side effects and targeted biological therapy or immunotherapy, including vaccines. Young age is a risk factor for distant metastasis within 6 mo following primary tumor resection. Neoadjuvant radiotherapy lasting 5-6 wk and surgical resection are indicated for high-grade STS (grade 2 or 3). Wide surgical excision alone may be acceptable for patients older than 70 years. However, locally advanced disease requires a multidisciplinary task of decision-making for amputation or limb salvage.

Key Words: Soft tissue sarcoma; Soft tissue tumors; Sarcomas; Oncology; Preoperative biopsy; Surgical management

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The diagnosis and treatment of soft tissue sarcoma are multidisciplinary tasks, and wide surgical resection is an absolute necessity. Modern imaging, especially magnetic resonance imaging, is valuable, and preoperative core needle biopsy is the most indicated and effective diagnostic tool. Its correct planning affects surgical management because the opposite means dissemination of cancer cells into healthy anatomical structures influencing resectability and survival. New therapeutic modalities, including chemoradiation, biological agents and immunotherapy, can improve the outcomes of the main surgical management. In any case, the management policy is personalized.

Citation: Pavlidis ET, Pavlidis TE. New trends in the surgical management of soft tissue sarcoma: The role of preoperative biopsy. *World J Clin Oncol* 2023; 14(2): 89-98
URL: https://www.wjgnet.com/2218-4333/full/v14/i2/89.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i2.89

INTRODUCTION

Soft tissue sarcomas (STSs) are rare tumors that originate from the mesenchyme (embryonic mesoderm) and affect children more often than adults[1]. They represent aggressive lesions accounting for approximately 1% of all adult malignancies and 7% of pediatric neoplasms[2,3]. Their incidence is calculated to affect 4-5 individuals *per* 100000 *per* year in Europe[4]; annually in the United States, there have been approximately 10000 new cases of soft tissue and bone sarcomas[5]. Likewise, in 2019, approximately 13000 new cases of ST and bone sarcomas were recognized in the United States with a main location (60%) in the limbs and trunk[6]. A French nationwide registry showed a continuing increase in incidence that is higher than reported and varies among different countries; however, the pathology evaluation should be made by sarcoma experts to avoid misdiagnosis which can occur in up to 30% of cases[7].

Limb STS has a rather better prognosis than retroperitoneal or pelvic STS. The most predominant pathologic type of STS is liposarcoma and leiomyosarcoma in adults and rhabdomyosarcoma in children[4]. Overall, 50 histopathologic subtypes with various cytogenetic profiles concerning soft tissue and bones have been recognized. The location in the vast majority concerns limbs, trunk, head and less often retro peritoneum and abdominal cavity[2]. These tumors mainly affect middle-aged adults but may be present at any age. Half of the patients have metastatic disease (first in the lungs and second in the liver) and intermediate-high grade STS at the time of diagnosis and require systemic therapy. The 5-year overall survival is approximately 55%[7-9].

Tumors above 3-5 cm in size, fast growing, deeply located, solid, cumbersome, possibly accompanied by palpable lymph nodes and causing or not causing pain must be suspected of potential malignancy. Then, an imaging evaluation [ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI)] is required and must precede biopsy[10]. Preoperative biopsy (percutaneous core needle, preferably) is a crucial diagnostic tool since there has been progress in planning multimodality management, which ensures improved outcomes[6,11]. The Ki-67 proliferation index has been proposed as a prognostic biomarker that, in addition to survival prediction, may determine the indication for lung or liver metastasectomy in carefully selected patients, improving the treatment[12].

Surgical intervention constitutes the cornerstone of management aiming at therapeutic wide excision with adequate margins[2]. Recurrence occurs in up to 50% of cases after surgery, mainly in the lungs[6]. Any effort must be made for limb salvage to avoid amputation. Neoadjuvant or adjuvant radiotherapy or chemotherapy has contributed to current progress[2]. Likewise, immunotherapy is a promising novel

therapeutic option[1]. Young age is the only known risk factor for distant metastasis within 6 mo following curative resection[13]. Diabetes mellitus has a negative influence on the clinical outcome after therapeutic excision of STS[14].

Modern imaging, including positron emission tomography (PET)/CT and regular follow-up (every 3 mo for the first 3 years, every 6 mo for the following 2 years and then once every year for the next 10 years), nomograms and artificial intelligence for local recurrence or distant metastasis after surgery, have assisted further and improved the outcome[4,12].

In this narrative review, we highlight the current data on the diagnosis and treatment of STS, providing comprehensive, complete and modern knowledge to manage them.

DIAGNOSIS

A thorough history, clinical examination and imaging are necessary requirements. Modern imaging techniques include US, CT, new MRI, and PET/CT. Imaging findings of limb STS correlate with the histopathologic findings[15]. According to the United Kingdom guidelines for the management of STS, any soft tissue lump more than 5 cm in size and, most importantly, increasing rapidly in size or painful must be considered malignant until assessed otherwise on imaging. Therefore, immediate US is mandatory. If the lesion seems to be benign, then the investigation will be terminated. Otherwise, a CT will follow and then MRI if it is indicated. When positive for malignancy or equivocal imaging findings exist, a preoperative biopsy will always be performed to confirm the diagnosis of STS[8], as described in detail below.

However, MRI is currently the method of choice. It provides accurate location, architecture, and vascularization of the tumor and determines the relationships with neighboring vital anatomic structures to plan the operative strategy and the extent of resection[16]. MRI findings may also distinguish low-grade from high-grade STS based on a diagnostic score (tumor heterogeneity, intratumoral and peritumoral enhancement). A score of 2 or 3 indicates a high-grade lesion, and a score of 0 or 1 indicates a low-grade lesion[16] . MRI radiomics and machine learning may accurately predict the tumor grade[17]. MRI is useful not only because it can guide preoperative biopsy[6] but also because high-grade sarcomas need neoadjuvant chemoradiation therapy[16]. It is known that preoperative biopsy may underassess the real grade of the definite complete specimen pathology due to the heterogeneity of STS[17]. Additionally, novel multiparametric MRI has provided promising results for the selection of patients who need neoadjuvant radiotherapy[9]. Preoperative imaging assessment of margin infiltration degree is essential for STS prognosis. MRI using the radiomics mode is a novel promising tool[18]. In addition, MRI using a deep learning radiomics nomogram can accurately predict preoperative lung metastases[19].

Preoperative imaging, pathologic subtypes and molecular findings are crucial. Mutations in the tumor suppressor genes, *i.e.*, the *Rb1* gene (retinoblastoma 1) and *TP53* gene (tumor protein 53) can exist [12]. Liquid biopsy by detecting genomic material in serum is a novel diagnostic, prognostic and staging tool. Genetic material mainly from blood but also from other body fluids (cerebrospinal fluid, saliva, urine, or feces) may be useful for the discovery of circulating tumor cells, cell-free DNA, exosomes, or metabolites[2]. These biomarkers provide valuable information regarding the tumor genetic profile and the status of the disease to ensure optimal monitoring and to identify the mechanisms implicating treatment resistance. The preliminary results are promising despite the technical difficulties, and liquid biopsy could replace invasive tissue biopsy in the future[20]. The heterogeneity of sarcomas poses further prognostic limitations. Furthermore, circulating tumor noncoding RNAs are promising biomarkers. However, all the above research efforts are in the preclinical stage for sarcomas[21]. In addition, the genomic profile may determine the adjuvant treatment choices[22].

For disease staging, the following imaging is necessary: (1) CT chest to detect lung metastases, since they are the most common metastatic involvement; and (2) CT abdomen to detect early hepatic or pelvic metastasis, particularly for the lower limb location of the primary focus to detect retroperitoneal lymph node involvement[4,8]. Based on relevant indications, the following imaging is recommended: (1) Whole-body scintigraphy for possible bone metastases; (2) CT or preferably MRI brain for possible metastases; (3) Whole-body MRI may be useful for occult metastases[8]; and (4) Likewise, for this reason, 18F-Fluoro-2-deoxyglucose PET/CT is currently more often in use[3]. However, it is absolutely indicated before making decisions for amputation or after postoperative recurrence[8]. After neoadjuvant radiation therapy, approximately 20% of patients with limb and trunk STS require a change in the management strategy because of distant lung metastases. The followed scheme includes a total dose of 50 Gy in 25 sessions of 2 Gy within a period of five weeks and then surgical intervention after an elapse of approximately ten weeks. Therefore, chest CT is reasonable for restaging after such a long time of 15 wk[23].

Histopathologic diagnosis is based on morphological, immunohistochemical and molecular pathologic features[10]. It should be made according to the latest World Health Organization classification of soft tissue tumors. Liposarcoma, leiomyosarcoma, myxofibrosarcoma, pleomorphic undifferentiated sarcoma and synovial sarcoma constitute 75% of all STSs[15]. A second opinion of a

pathologist expert may be valuable. There are three malignancy grades based on differentiation, necrosis and mitotic rate according to the Federation of the French Cancer Centres histological grading criteria for STS[8,10]. These parameters are scored 1 to 3 for differentiation and mitotic index and 0 to 2 for necrosis. A 3-grade system is obtained by summing the scores obtained for each of these 3 parameters, as shown in Table 1[8]. The Ki-67 proliferation index grading system may be useful for the evaluation of the histological grade of STS[24]. The staging of STS is based on the Tumor-Node-Metastasis classification system according to the American Joint Committee on Cancer 8th edition, as shown in Table 2[25].

PREOPERATIVE BIOPSY

Diagnosis and management of STS should be performed by experienced centers[26]. A preoperative biopsy is necessary to establish the diagnosis after the imaging evaluation. Imaging should be performed first to avoid any interference with the anatomical integrity of the region by the biopsy manipulations. The biopsy ensures diagnosis of histological type and staging, predicts the biological behavior of the tumor, indicates the need for preoperative (neoadjuvant) or even intraoperative radiation treatment, and neoadjuvant systemic chemotherapy, determines the best planning of the operative strategy and offers better patient information (reassuring) by weighing the risks and expectations[8,10,11,27]. A preoperative frozen section for immediate diagnosis is not recommended. It has no practical value since the regular review of a core needle biopsy (CNB) will safely establish the diagnosis [10].

The primary method of the first choice is CNB with needles of 14-18 gauges. Several needle samples (4-10) are required to increase the maximum chance of a correct diagnosis[6,8,10,28]. It was performed under imaging guidance (US, CT) and achieved adequate specimens for complete histopathologic evaluation along with immunohistochemical assays. Most cases are performed under local anesthesia, but sedation may be required in some cases. The complications (hemorrhage or infection) are minimal [11]. A large series from Royal Marsden Hospital United Kingdom including 530 cases of CNB performed under local anesthesia showed that it was diagnostic in 93% of cases, needed to be repeated in 7% of cases, had a complication rate of 0.4%, had a diagnostic accuracy rate of 97.6% in distinguishing STS from benign lesions (sensitivity of 96.3%, specificity of 99.4%, positive predictive value of 99.5%, negative predictive value of 95.1%) and had a grade accuracy rate of 86.3%[29]. Adequate tissue samples must be obtained in different directions within the tumor through a single skin incision; to avoid rare needle tract recurrence, the selection of the biopsy site should be planned so that it is included in the subsequent resection, if required[29].

Preoperative CT-guided CNB is accurate and valuable for intraabdominal and retroperitoneal sarcomas[30]. A recent study from the United States based on the National Cancer Database including 2620 patients who underwent surgery for nonmetastatic retroperitoneal sarcoma showed that preoperative biopsy (performed in 42.4% of cases) was proven useful with better outcomes and improved survival[31].

Fine needle aspiration does not provide tissue samples and offers cytologic rather than histologic information. Its utility is limited only to recurrence cases of an already known STS[11]. Open biopsy techniques include incisional biopsy by removing a small part of the tumor. It is associated with a 2% possibility of complications (inflammation, hematoma) but most importantly dissemination of malignant cells and delay in the treatment. Its rare indication is limited to failure of CNB. Excisional biopsy by whole tumor removal does not have any place in suspected STS but only in superficial small soft tissue tumors (less than 2 cm in size), which have minimal malignant potential. The basic principles of open biopsy are meticulous hemostasis and avoidance of drain placement[8,11].

MANAGEMENT

The management of abdominal STS at an experienced center with a multidisciplinary approach provides improved outcomes and better prognosis[32]. The initial referral, even based on suspicion, to such a center is of great importance to ensure the optimal chance in accurate diagnosis and proper management[22]. Surgery is the standard treatment and must be performed by an experienced surgeon. Wide excision with adequate margins at least 1 cm or even 2 cm, free of involvement, constitutes the operative target to achieve a residual zero (R0) resection[2,33-35]. However, vital neighboring anatomical structures may sometimes restrict the resection margin, and microscopic infiltration may be found within it (R1 resection). Further treatment is needed for positive resection margins to restrict recurrence[36]. It has been reported that high-grade tumors have a negative effect on overall survival, but resection margins do not. The 5-year overall survival was 71.1% for R0 resection and 70.2% for R1 resection[37]. Lymph node metastases are rare in STS, and sentinel lymph node biopsy and lymphadenectomy are limited and debatable[38]. Neoadjuvant radiotherapy and wide excision have been widely used but are associated with wound complications[39], reaching up to 39%[40]. This rate is limited to

Table 1 Federation of the French Cancer Centres histological grading criteria

Differentiation (score)	Necrosis (score)	Mitotic count (score)
Well (1)	Absent (0)	$n < 10^1$ (1)
Moderate (2)	< 50% (1)	$n = 10–19^1$ (2)
Poor (anaplastic) (3)	≥ 50% (2)	$n \geq 20^1$ (3)

[1]Number of mitoses *per* 10 high power fields.
After summing the three scores, grade 1 is defined as a total score of 2 or 3; grade 2 as a total score of 4 or 5; and grade 3 as a total score of 6 to 8.

Table 2 American Joint Committee on Cancer classification and staging for soft tissue sarcoma, 8th Edition

TNM classification	Stage
T1: Tumor ≤ 5 cm	IA: T1; N0; M0; G1
T2: Tumor > 5 cm and ≤ 10 cm	IB: T2, T3, T4; N0; M0; G1
T3: Tumor > 10 cm and ≤ 15 cm	II: T1; N0; M0; G2/3
T4: Tumor > 15 cm	IIIA: T2; N0; M0; G2/3
N0: No regional lymph node metastasis or unknown lymph node status	IIIB: T3, T4; N0; M0; G2/3
N1: Regional lymph node metastasis	IV: Any T; N1; M0; any G Any T; any N; M1; any G
M0: No distant metastasis	
M1: Distant metastasis	

G expresses the histological grading sum score. TNM: Tumor-Node-Metastasis.

half at experienced centers[40]. Neoadjuvant radiotherapy tends to replace adjuvant radiotherapy and is strongly recommended[41,42]. Concurrent neoadjuvant chemoradiation therapy increases the chance of R0 resection[43]. For high-grade deep tumors, T2 or more (stage II or III), wide excision and adjuvant radiation therapy (external beam 60-76 Gy) for local control is the indicated policy[2,10,44,45]. Limb sparing surgery combined with radiotherapy is the current preferable method for such tumors of limbs [46]. It must precede preoperative traditional fractioned radiotherapy of 50-50.4 Gy with a daily dose of 1.8-2 Gy over 5-6 wk[47-49]. Generally, a daily dose > 2.2 Gy is usually hypofractionated radiotherapy [47]. Novel techniques for radiotherapy, including intensity-modulated radiation therapy, proton beam therapy, intraoperative electron radiotherapy and postoperative brachytherapy (*via* catheters in the surgical field), promise to decrease the side effects of standard radiotherapy while achieving better local control[2,8,10,50].

Chemotherapy with doxorubicin alone or in combination with ifosfamide is the basic scheme as a neo-adjuvant or adjuvant[2]. However, there have been conflicting aspects for adjuvant chemotherapy after R0 resection[51]. Tyrosine-kinase inhibitors (pazopanib, sunitinib, imatinib) have been indicated in some specific types[2]. In advanced metastatic cases, gemcitabine has been used in combination with docetaxel, vinorelbine, or dacarbazine, but with limited results[2]. Isolated hyperthermic limb perfusion (IHLP) with tumor necrosis factor-alpha and melphalan is another proposed option for limb STS[2,52]. A recent nationwide multicenter study from the Netherlands showed that in unresectable limb STS, preoperative IHLP or neoadjuvant radiotherapy avoided both amputations with acceptable oncological outcomes[53]. Wide surgical excision alone without neoadjuvant or adjuvant chemotherapy may be acceptable for patients over 70 years of age, providing comparable survival[54]. Frail very elderly patients (more than 80 years old) can tolerate an operative intervention for limb STS well[55,56].

Overall, unplanned excisions were 18.2% among 2187 primary operations for STS in the Netherlands Cancer Registry database[57]. It is known that unplanned surgical excision is related to an increased risk of local recurrence despite any adjuvant oncologic therapy[58]. For this reason, reresection is an option after positive or uncertain resection margins, but it is associated with increased morbidity and residual disease, which requires complete information for the patient[59]. However, a recent large study from Japan including 4483 operations (4128 planned excisions and 355 unplanned excisions) for limb STS showed that additional excision after unplanned excision was not associated with increased mortality and local recurrence compared to planned excision[60]. Furthermore, in the case of R1 or even R2 resection, reresection in combination with perioperative radiotherapy must be considered[61]. Surgical resection of lung metastases has improved overall survival (49 mo median and 42% 5-year). However, R1 resection of the primary tumor and ≥ 2 metastases decrease it[62]. Pulmonary metastasectomy

improves the prognosis compared to conservative treatment[63].

The comprehensive assessment of recurrence risk has led to an increasing number of personalized management tools[64], including surgical operation, radiotherapy, novel promising targeted biological agents and immunotherapy (monoclonal antibodies, cellular therapies with modified T cells and natural killer cells, or vaccines)[1,2,12,65,66]. For retroperitoneal STS, aggressive surgical management has been recommended, since it showed satisfactory results for primary tumors but not for recurrence[67-70]. Likewise, for abdominal STS, surgery is the standard treatment[71]. Operative intervention and radiotherapy maximize local control[72]. For abdominal wall STS, extensive surgery is indicated for local control despite the rate but acceptability of incisional hernia[73]. For metastatic STS, systemic therapy and local control by surgical resection, usually or recently by stereotactic body radiation therapy, have been recommended[74]. MRI-guided radiotherapy is another recent alternative modality [75]. For advanced retroperitoneal liposarcoma, the most common subtype of retroperitoneal STS, treatment based on targetable molecular pathways may be the future perspective[76].

A recent systematic review showed that patients with hepatic, abdominal or retroperitoneal metastasis undergoing metastasectomy have a survival benefit for a long period of time compared with those undergoing chemotherapy[4]. A multicenter retrospective cohort study from the United States using the National Cancer Database including 8953 cases showed that younger adult patients under 40 years old had a notable proportion (14.3%) of limb STS and more challenging management. They received chemotherapy more often than radiotherapy *vs* older patients[77]. A study including 1124 patients with distant metastases at diagnosis, stage IV STS, from the United States National Cancer Database showed that metastasectomy after resection of the primary site increased survival[78]. In any case, regardless of the subsequent kind of metastasis management, primary tumor resection is necessary to improve survival[79].

Visceral obesity is common in retroperitoneal and trunk sarcoma, and it has a negative effect on surgical results but not on oncologic outcomes[80]. A recent international multicenter study using clinical data as prognostic factors of 493 patients with STS found that increased modified Glasgow prognostic score (used in various malignancies and based on preoperative C-reactive protein and albumin levels to calculate a score from 0 to 2), tumor size, grade, neutrophil/lymphocyte ratio, and recurrence were associated with reduced survival[81]. Likewise, another study found a predictive effect on survival of retroperitoneal STS using body mass index, total protein serum levels and blood white cell count by performing prognostic models[82].

The 5-year survival for limb and trunk STS was found to be 71.6% in local recurrence-free patients, 75.7% in metastasis-free and 84.7% in disease-specific[83]. The 3-year overall survival for head and neck STS was 68%, for disease specific 71% and recurrence free 61%. Higher tumor grade and tumor size greater than 5 cm were associated with reduced disease-specific survival[26].

CONCLUSION

Any suspected soft tissue lump above 5 cm in size must be investigated thoroughly, first by US and CT. For further detailed information, if needed, modern MRI prevails among the imaging modalities and constitutes the method of the first option. Preoperative CNB, always after imaging, is essential in confirming the diagnosis and determining the staging with prognosis and the optimal planning of the management policy. Liquid biopsy and genomic profiling will likely be useful in diagnosis, prognosis and treatment. A multidisciplinary approach is valuable and mandatory. Wide surgical excision with an acceptable healthy margin of 1 cm is the method of choice in management. In locally borderline tumors affecting limb vessels or nerves, modern neoadjuvant or adjuvant chemoradiation therapy may ensure limb savings by downstaging the tumor, thus avoiding amputation. Additionally, this therapy in the advanced metastatic stage improves surgical outcomes after mandatory primary tumor excision. Novel targeted biological agents and immunotherapy may contribute further. Detailed follow-up for a long time is recommended because of the outstanding possibility of recurrence, in which the chance of reresection or stereotactic radiotherapy exists. However, in any case, the management of STS should be personalized and performed by an expert team.

FOOTNOTES

Author contributions: Pavlidis TE designed research, contributed new analytic tools, analyzed data and review; Pavlidis ET performed research, analyzed data review and wrote the paper.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license

their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Greece

ORCID number: Efstathios T Pavlidis 0000-0002-7282-8101; Theodoros E Pavlidis 0000-0002-8141-1412.

S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

REFERENCES

1 **Ghosn M**, El Rassy E, Kourie HR. Immunotherapies in sarcoma: Updates and future perspectives. *World J Clin Oncol* 2017; **8**: 145-150 [PMID: 28439495 DOI: 10.5306/wjco.v8.i2.145]
2 **Gómez J**, Tsagozis P. Multidisciplinary treatment of soft tissue sarcomas: An update. *World J Clin Oncol* 2020; **11**: 180-189 [PMID: 32355640 DOI: 10.5306/wjco.v11.i4.180]
3 **Benz MR**, Crompton JG, Harder D. PET/CT Variants and Pitfalls in Bone and Soft Tissue Sarcoma. *Semin Nucl Med* 2021; **51**: 584-592 [PMID: 34238508 DOI: 10.1053/j.semnuclmed.2021.06.009]
4 **Smolle MA**, Leithner A, Bernhardt GA. Abdominal metastases of primary extremity soft tissue sarcoma: A systematic review. *World J Clin Oncol* 2020; **11**: 74-82 [PMID: 32133276 DOI: 10.5306/wjco.v11.i2.74]
5 **Blank AT**, Larson BM, Shaw S, Wakefield CJ, King T, Jones KB, Randall RL. National Comprehensive Cancer Network guidelines compliance of a sarcoma service: A retrospective review. *World J Clin Oncol* 2020; **11**: 389-396 [PMID: 32874952 DOI: 10.5306/wjco.v11.i6.389]
6 **Roland CL**. Soft Tissue Tumors of the Extremity. *Surg Clin North Am* 2020; **100**: 669-680 [PMID: 32402308 DOI: 10.1016/j.suc.2020.02.015]
7 **de Pinieux G**, Karanian M, Le Loarer F, Le Guellec S, Chabaud S, Terrier P, Bouvier C, Batistella M, Neuville A, Robin YM, Emile JF, Moreau A, Larousserie F, Leroux A, Stock N, Lae M, Collin F, Weinbreck N, Aubert S, Mishellany F, Charon-Barra C, Croce S, Doucet L, Quintin-Rouet I, Chateau MC, Bazille C, Valo I, Chetaille B, Ortonne N, Brouchet A, Rochaix P, Demuret A, Ghnassia JP, Mescam L, Macagno N, Birtwisle-Peyrottes I, Delfour C, Angot E, Pommepuy I, Ranchere D, Chemin-Airiau C, Jean-Denis M, Fayet Y, Courrèges JB, Mesli N, Berchoud J, Toulmonde M, Italiano A, Le Cesne A, Penel N, Ducimetiere F, Gouin F, Coindre JM, Blay JY; NetSarc/RePPS/ResSos and French Sarcoma Group-Groupe d'Etude des Tumeurs Osseuses (GSF-GETO) networks. Nationwide incidence of sarcomas and connective tissue tumors of intermediate malignancy over four years using an expert pathology review network. *PLoS One* 2021; **16**: e0246958 [PMID: 33630918 DOI: 10.1371/journal.pone.0246958]
8 **Dangoor A**, Seddon B, Gerrand C, Grimer R, Whelan J, Judson I. UK guidelines for the management of soft tissue sarcomas. *Clin Sarcoma Res* 2016; **6**: 20 [PMID: 27891213 DOI: 10.1186/s13569-016-0060-4]
9 **Kershaw L**, Forker L, Roberts D, Sanderson B, Shenjere P, Wylie J, Coyle C, Kochhar R, Manoharan P, Choudhury A. Feasibility of a multiparametric MRI protocol for imaging biomarkers associated with neoadjuvant radiotherapy for soft tissue sarcoma. *BJR Open* 2021; **3**: 20200061 [PMID: 35707756 DOI: 10.1259/bjro.20200061]
10 **Casali PG**, Blay JY; ESMO/CONTICANET/EUROBONET Consensus Panel of experts. Soft tissue sarcomas: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; **21** Suppl 5: v198-v203 [PMID: 20555081 DOI: 10.1093/annonc/mdq209]
11 **Tuttle R**, Kane JM 3rd. Biopsy techniques for soft tissue and bowel sarcomas. *J Surg Oncol* 2015; **111**: 504-512 [PMID: 25663366 DOI: 10.1002/jso.23870]
12 **Brennan MF**, Singer S. Five decades of sarcoma care at Memorial Sloan Kettering Cancer Center. *J Surg Oncol* 2022; **126**: 896-901 [PMID: 36087086 DOI: 10.1002/jso.27032]
13 **Tsuchie H**, Emori M, Nagasawa H, Murahashi Y, Mizushima E, Shimizu J, Yamashita T, Miyakoshi N. Risk Factors of Early Distant Metastasis After Primary Tumor Treatment in Soft Tissue Sarcoma. *In Vivo* 2022; **36**: 2260-2264 [PMID: 36099122 DOI: 10.21873/invivo.12954]
14 **Stelzl A**, Aziz F, Riedl JM, Posch F, Smolle MA, Stojakovic T, Terbuch A, Pichler M, Bergovec M, Leithner A, Liegl-Atzwanger B, Stotz M, Gerger A, Sourij H, Szkandera J. Diabetes mellitus is independently associated with adverse clinical outcome in soft tissue sarcoma patients. *Sci Rep* 2020; **10**: 12438 [PMID: 32709908 DOI: 10.1038/s41598-020-69237-y]
15 **Ramakrishnan K**, Levy N, Goldbach A, Nagarathinam R, Ali S, Ling S, Jonnalagadda P. Imaging of soft tissue sarcomas of the extremities with radiologic-pathologic correlation. *Curr Probl Diagn Radiol* 2022; **51**: 868-877 [PMID: 35654634 DOI: 10.1067/j.cpradiol.2022.04.005]
16 **Boudabbous S**, Hamard M, Saiji E, Gorican K, Poletti PA, Becker M, Neroladaki A. What morphological MRI features enable differentiation of low-grade from high-grade soft tissue sarcoma? *BJR Open* 2022; **4**: 20210081 [PMID: 36105415 DOI: 10.1259/bjro.20210081]
17 **Xu W**, Hao D, Hou F, Zhang D, Wang H. Soft Tissue Sarcoma: Preoperative MRI-Based Radiomics and Machine Learning May Be Accurate Predictors of Histopathologic Grade. *AJR Am J Roentgenol* 2020; **215**: 963-969 [PMID: 32755226 DOI: 10.2214/AJR.19.22147]
18 **Lee S**, Jung JY, Nam Y, Jung CK, Lee SY, Lee J, Shin SH, Chung YG. Diagnosis of Marginal Infiltration in Soft Tissue Sarcoma by Radiomics Approach Using T2-Weighted Dixon Sequence. *J Magn Reson Imaging* 2022 [PMID: 35808915 DOI: 10.1002/jmri.28331]
19 **Liang HY**, Yang SF, Zou HM, Hou F, Duan LS, Huang CC, Xu JX, Liu SL, Hao DP, Wang HX. Deep Learning

Radiomics Nomogram to Predict Lung Metastasis in Soft-Tissue Sarcoma: A Multi-Center Study. *Front Oncol* 2022; **12**: 897676 [PMID: 35814362 DOI: 10.3389/fonc.2022.897676]

20 **van der Laan P**, van Houdt WJ, van den Broek D, Steeghs N, van der Graaf WTA. Liquid Biopsies in Sarcoma Clinical Practice: Where Do We Stand? *Biomedicines* 2021; **9** [PMID: 34680432 DOI: 10.3390/biomedicines9101315]

21 **Wei J**, Liu X, Li T, Xing P, Zhang C, Yang J. The new horizon of liquid biopsy in sarcoma: the potential utility of circulating tumor nucleic acids. *J Cancer* 2020; **11**: 5293-5308 [PMID: 32742476 DOI: 10.7150/jca.42816]

22 **Kirchberg J**, Blum SFU, Pablik J, Herold S, Hoffmann RT, Baretton G, Weitz J. [Preoperative diagnostics and typing of abdominal soft tissue sarcomas]. *Chirurg* 2022; **93**: 5-15 [PMID: 34757436 DOI: 10.1007/s00104-021-01528-0]

23 **Acem I**, Schultze BTA, Schoonbeek A, van Houdt WJ, van de Sande MAJ, Visser JJ, Grünhagen DJ, Verhoef C. The added value of chest imaging after neoadjuvant radiotherapy for soft tissue sarcoma of the extremities and trunk wall: A retrospective cohort study. *Eur J Surg Oncol* 2022; **48**: 1543-1549 [PMID: 35393169 DOI: 10.1016/j.ejso.2022.03.231]

24 **Tanaka K**, Hasegawa T, Nojima T, Oda Y, Mizusawa J, Fukuda H, Iwamoto Y. Prospective evaluation of Ki-67 system in histological grading of soft tissue sarcomas in the Japan Clinical Oncology Group Study JCOG0304. *World J Surg Oncol* 2016; **14**: 110 [PMID: 27091124 DOI: 10.1186/s12957-016-0869-6]

25 **Cates JMM**. The AJCC 8th Edition Staging System for Soft Tissue Sarcoma of the Extremities or Trunk: A Cohort Study of the SEER Database. *J Natl Compr Canc Netw* 2018; **16**: 144-152 [PMID: 29439175 DOI: 10.6004/jnccn.2017.7042]

26 **Roos JH**, Mäkitie AA, Tarkkanen J, Ilmarinen TT. Pretreatment tumor sampling and prognostic factors in patients with soft-tissue sarcoma of the head and neck. *Eur Arch Otorhinolaryngol* 2022; **279**: 3147-3155 [PMID: 34773167 DOI: 10.1007/s00405-021-07162-0]

27 **MacGillis KJ**, Heaberlin J, Mejia A. Clinical Decision Making for a Soft Tissue Hand Mass: When and How to Biopsy. *J Hand Surg Am* 2018; **43**: 1123-1129 [PMID: 29908925 DOI: 10.1016/j.jhsa.2018.03.032]

28 **Young R**, Snow H, Hendry S, Mitchell C, Slavin J, Schlicht S, Na L, Hofman MS, Gyorki DE. Correlation between percutaneous biopsy and final histopathology for retroperitoneal sarcoma: a single-centre study. *ANZ J Surg* 2020; **90**: 497-502 [PMID: 32064728 DOI: 10.1111/ans.15723]

29 **Strauss DC**, Qureshi YA, Hayes AJ, Thway K, Fisher C, Thomas JM. The role of core needle biopsy in the diagnosis of suspected soft tissue tumours. *J Surg Oncol* 2010; **102**: 523-529 [PMID: 20872955 DOI: 10.1002/jso.21600]

30 **Luca Alatzides G**, Luisa Steinberg H, Schildhaus HU, Hamacher R, Kaths M, Grueneisen J, Treckmann J, Bauer S, Umutlu L, Schaarschmidt B. Is preoperative CT-guided biopsy a valuable tool in the diagnostic workup of patients with visceral and retroperitoneal sarcoma? *Eur J Radiol* 2022; **155**: 110470 [PMID: 35985092 DOI: 10.1016/j.ejrad.2022.110470]

31 **Straker RJ 3rd**, Song Y, Shannon AB, Marcinak CT, Miura JT, Fraker DL, Karakousis GC. Preoperative Biopsy in Patients with Retroperitoneal Sarcoma: Usage and Outcomes in a National Cohort. *Ann Surg Oncol* 2021; **28**: 6868-6879 [PMID: 33591480 DOI: 10.1245/s10434-021-09691-8]

32 **Acem I**, Smit MM, Verhoef C, van Houdt WJ, Haas RL, van der Hage JA, Grünhagen DJ, van de Sande MAJ. Management of Soft Tissue Sarcomas in Extremities: Variation in Treatment Recommendations and Surveillance According to Specialty and Continent. *Ann Surg Oncol* 2021; **28**: 7923-7936 [PMID: 33970372 DOI: 10.1245/s10434-021-09946-4]

33 **Patel A**, Kane JM 3rd. Wide Resection of Extremity/Truncal Soft Tissue Sarcomas. *Surg Clin North Am* 2022; **102**: 551-565 [PMID: 35952687 DOI: 10.1016/j.suc.2022.05.002]

34 **Kheiran A**, Eastley NC, Hanson JR, McCulloch TA, Allen PE, Ashford RU. The importance of the early appropriate management of foot and ankle soft tissue sarcomas - experiences of a regional sarcoma service. *Foot (Edinb)* 2022; **50**: 101866 [PMID: 35219135 DOI: 10.1016/j.foot.2021.101866]

35 **Gronchi A**. Surgery in soft tissue sarcoma: the thin line between a surgical or more conservative approach. *Future Oncol* 2021; **17**: 3-6 [PMID: 34107729 DOI: 10.2217/fon-2021-0449]

36 **Matheron G**, Ardakani A, Nasir A, Gikas P. Positive Margins Following Excision of Primary Bone & Soft Tissue Tumours in a Tertiary Centre and the Impact on Patient Outcomes. *Cureus* 2022; **14**: e21235 [PMID: 35186531 DOI: 10.7759/cureus.21235]

37 **Shafiq MB Sr**, Rafi I, Shoaib A, Ali S, Iqbal F, Latif T, Mushtaq U. The Outcome of Extremity Soft Tissue Sarcomas in Terms of Resection Margins: A Study From a Cancer Dedicated Center. *Cureus* 2022; **14**: e26086 [PMID: 35875266 DOI: 10.7759/cureus.26086]

38 **Witt RG**, Cope B, Erstad DJ, Chiang YJ, Nassif EF, Scally CP, Torres KE, Hunt KK, Feig BW, Roland CL, Keung EZ. Sentinel Lymph Node Biopsy and Formal Lymphadenectomy for Soft Tissue Sarcoma: A Single Center Experience of 86 Consecutive Cases. *Ann Surg Oncol* 2022; **29**: 7092-7100 [PMID: 35501583 DOI: 10.1245/s10434-022-11803-x]

39 **Kungwengwe G**, Clancy R, Vass J, Slade R, Sandhar S, Dobbs TD, Bragg TWH. Preoperative versus Post-operative Radiotherapy for Extremity Soft tissue Sarcoma: a Systematic Review and Meta-analysis of Long-term Survival. *J Plast Reconstr Aesthet Surg* 2021; **74**: 2443-2457 [PMID: 34266806 DOI: 10.1016/j.bjps.2021.05.043]

40 **Ellison C**, King DM, Neilson JC, Wooldridge A, Charlson JA, Hackbarth DA, Johnstone C, Bedi M. Preoperative Radiation Performed at a Nonsarcoma Center May Lead to Increased Wound Complications Following Resection in Patients With Soft Tissue Sarcomas. *Am J Clin Oncol* 2021; **44**: 619-623 [PMID: 34753886 DOI: 10.1097/COC.0000000000000870]

41 **Van Meekeren M**, Fiocco M, Ho VKY, Bovée JVMG, Gelderblom H, Haas RL. Patterns of Perioperative Treatment and Survival of Localized, Resected, Intermediate- or High-Grade Soft Tissue Sarcoma: A 2000-2017 Netherlands Cancer Registry Database Analysis. *Sarcoma* 2021; **2021**: 9976122 [PMID: 34335076 DOI: 10.1155/2021/9976122]

42 **Salerno KE**, Alektiar KM, Baldini EH, Bedi M, Bishop AJ, Bradfield L, Chung P, DeLaney TF, Folpe A, Kane JM, Li XA, Petersen I, Powell J, Stolten M, Thorpe S, Trent JC, Voermans M, Guadagnolo BA. Radiation Therapy for Treatment of Soft Tissue Sarcoma in Adults: Executive Summary of an ASTRO Clinical Practice Guideline. *Pract Radiat Oncol* 2021; **11**: 339-351 [PMID: 34326023 DOI: 10.1016/j.prro.2021.04.005]

43 **Attal J**, Cabarrou B, Valentin T, Nesseler JP, Stoeckle E, Ducassou A, Filleron T, Le Guellec S, Boulet B, Vogin G, Ferron G, Moyal EC, Delannes M, Chevreau C. Evaluation of two modalities of perioperative treatment in the management

of extremity and truncal soft tissue sarcomas: neoadjuvant concurrent chemoradiotherapy and sequential treatment. *Strahlenther Onkol* 2021; **197**: 1051-1062 [PMID: 34673991 DOI: 10.1007/s00066-021-01847-5]

44 **Lawrenz JM**, Johnson SR, Zhu K, McKeon M, Moran CP, Vega J, Hajdu KS, Norris JP, Luo LY, Shinohara ET, Cates JMM, Rubin BP, Reith JD, Halpern JL, Mesko NW, Schwartz HS, Nystrom LM, Holt GE. Adjuvant Radiation after Primary Resection of Atypical Lipomatous Tumors of the Extremity Reduces Local Recurrence but Increases Complications: A Multicenter Evaluation. *Sarcoma* 2022; **2022**: 2091677 [PMID: 36046749 DOI: 10.1155/2022/2091677]

45 **Levy A**, Honoré C, Dumont S, Bourdais R, Cavalcanti A, Faron M, Ngo C, Haddag-Miliani L, Le Cesne A, Mir O, Le Péchoux C. [Preoperative versus postoperative radiotherapy in soft tissue sarcomas: State of the art and perspectives]. *Bull Cancer* 2021; **108**: 868-876 [PMID: 34246458 DOI: 10.1016/j.bulcan.2021.03.012]

46 **Cammelli S**, Cortesi A, Buwenge M, Zamagni A, Ferioli M, Ghigi G, Romeo A, Morganti AG. The role of radiotherapy in adult soft tissues sarcoma of the extremities. *Eur J Orthop Surg Traumatol* 2021; **31**: 1583-1596 [PMID: 33954820 DOI: 10.1007/s00590-021-02990-6]

47 **Roohani S**, Ehret F, Kobus M, Flörcken A, Märdian S, Striefler JK, Rau D, Öllinger R, Jarosch A, Budach V, Kaul D. Preoperative hypofractionated radiotherapy for soft tissue sarcomas: a systematic review. *Radiat Oncol* 2022; **17**: 159 [PMID: 36104789 DOI: 10.1186/s13014-022-02072-9]

48 **Kaiser D**, Schelm M, Gerber C, Brown ML, Müller DA. The effect of preoperative radiotherapy on surgical resectability, tumor volume and the necrosis rate of soft tissue sarcomas: A retrospective single-center analysis. *Surg Oncol* 2021; **39**: 101668 [PMID: 34653769 DOI: 10.1016/j.suronc.2021.101668]

49 **Dei Tos AP**, Bonvalot S, Haas R. Evolution in the management of soft tissue sarcoma: classification, surgery and use of radiotherapy. *Expert Rev Anticancer Ther* 2020; **20**: 3-13 [PMID: 32293209 DOI: 10.1080/14737140.2020.1753509]

50 **Laughlin BS**, Golafshar MA, Ahmed S, Prince M, Anderson JD, Vern-Gross T, Seetharam M, Goulding K, Petersen I, DeWees T, Ashman JB. Early Experience Using Proton Beam Therapy for Extremity Soft Tissue Sarcoma: A Multicenter Study. *Int J Part Ther* 2022; **9**: 1-11 [PMID: 35774491 DOI: 10.14338/IJPT-21-00037.1]

51 **Eraslan E**, Ilhan Gulesen A, Yildiz F, Tufan G, Yalcintas Arslan U, Alkis N. The effect of adjuvant chemotherapy on the survival of patients with high-risk soft tissue sarcomas: Single center experience. *J BUON* 2021; **26**: 2169-2175 [PMID: 34761631]

52 **Lindner LH**, Blay JY, Eggermont AMM, Issels RD. Perioperative chemotherapy and regional hyperthermia for high-risk adult-type soft tissue sarcomas. *Eur J Cancer* 2021; **147**: 164-169 [PMID: 33684876 DOI: 10.1016/j.ejca.2021.02.002]

53 **van Praag VM**, Fiocco M, Bleckman RF, van Houdt WJ, Haas RLM, Verhoef C, Grunhagen DJ, van Ginkel RJ, Bonenkamp JJ, van de Sande MAJ. The oncological outcomes of isolated limb perfusion and neo-adjuvant radiotherapy in soft tissue sarcoma patients - A nationwide multicenter study. *Eur J Surg Oncol* 2022 [PMID: 36085118 DOI: 10.1016/j.ejso.2022.08.018]

54 **Aoki Y**, Tome Y, Oshiro H, Katsuki R, Tamaki T, Wada N, Karube K, Nishida K. Wide excision alone for elderly patients aged > 70 years old with soft tissue sarcomas. *Medicine (Baltimore)* 2022; **101**: e30127 [PMID: 36086737 DOI: 10.1097/MD.0000000000030127]

55 **Sivarajah G**, Davies E, Hurley A, Strauss DC, Smith MJF, Hayes AJ. Frailty in Very Elderly Patients is Not Associated with Adverse Surgical or Oncological Outcomes in Extremity Surgery for Soft Tissue Sarcoma. *Ann Surg Oncol* 2022; **29**: 3982-3990 [PMID: 35118523 DOI: 10.1245/s10434-021-11292-4]

56 **Hartley LJ**, AlAqeel M, Kurisunkal VJ, Evans S. Outcomes of soft-tissue sarcoma in nonagenarians. *Bone Joint J* 2022; **104-B**: 177-182 [PMID: 34969278 DOI: 10.1302/0301-620X.104B1.BJJ-2021-0761.R1]

57 **Melis AS**, Vos M, Schuurman MS, van Dalen T, van Houdt WJ, van der Hage JA, Schrage YM, Been LB, Bonenkamp JB, Bemelmans MHA, Grünhagen DJ, Verhoef C, Ho VKY; Dutch Sarcoma Group (DSG). Incidence of unplanned excisions of soft tissue sarcomas in the Netherlands: A population-based study. *Eur J Surg Oncol* 2022; **48**: 994-1000 [PMID: 34848102 DOI: 10.1016/j.ejso.2021.11.123]

58 **Grignol VP**, Lopez-Aguiar AG. The Implications of an Unplanned Sarcoma Excision (the "Whoops" Operation). *Surg Clin North Am* 2022; **102**: 529-538 [PMID: 35952685 DOI: 10.1016/j.suc.2022.04.002]

59 **Traweek RS**, Martin AN, Rajkot NF, Guadagnolo BA, Bishop AJ, Lazar AJ, Keung EZ, Torres KE, Hunt KK, Feig BW, Roland CL, Scally CP. Re-excision After Unplanned Excision of Soft Tissue Sarcoma is Associated with High Morbidity and Limited Pathologic Identification of Residual Disease. *Ann Surg Oncol* 2023; **30**: 480-489 [PMID: 36085392 DOI: 10.1245/s10434-022-12359-6]

60 **Nakamura T**, Kawai A, Hagi T, Asanuma K, Sudo A. A comparison of clinical outcomes between additional excision after unplanned and planned excisions in patients with soft-tissue sarcoma of the limb : a propensity matching cohort study. *Bone Joint J* 2021; **103-B**: 1809-1814 [PMID: 34847719 DOI: 10.1302/0301-620X.103B12.BJJ-2021-0037.R1]

61 **Le Péchoux C**, Llacer C, Sargos P, Moureau-Zabotto L, Ducassou A, Sunyach MP, Biston MC, Thariat J. Conformal radiotherapy in management of soft tissue sarcoma in adults. *Cancer Radiother* 2022; **26**: 377-387 [PMID: 34961656 DOI: 10.1016/j.canrad.2021.11.023]

62 **Lee RM**, Ethun CG, Gamboa AC, Turgeon MK, Tran T, Poultsides G, Grignol V, Bedi M, Mogal H, Clarke CN, Tseng J, Roggin KK, Chouliaras K, Votanopoulos K, Krasnick BA, Fields RC, Oskouei SV, Monson DK, Reimer NB, Maithel SK, Pickens A, Cardona K. A novel preoperative risk score to guide patient selection for resection of soft tissue sarcoma lung metastases: An analysis from the United States Sarcoma Collaborative. *J Surg Oncol* 2021; **124**: 1477-1484 [PMID: 34374088 DOI: 10.1002/jso.26635]

63 **Gusho CA**, Seder CW, Lopez-Hisijos N, Blank AT, Batus M. Pulmonary metastasectomy in bone and soft tissue sarcoma with metastasis to the lung. *Interact Cardiovasc Thorac Surg* 2021; **33**: 879-884 [PMID: 34516633 DOI: 10.1093/icvts/ivab178]

64 **Acem I**, van de Sande MAJ. Prediction tools for the personalized management of soft-tissue sarcomas of the extremity. *Bone Joint J* 2022; **104-B**: 1011-1016 [PMID: 36047022 DOI: 10.1302/0301-620X.104B9.BJJ-2022-0647]

65 **Banks LB**, D'Angelo SP. The Role of Immunotherapy in the Management of Soft Tissue Sarcomas: Current Landscape and Future Outlook. *J Natl Compr Canc Netw* 2022; **20**: 834-844 [PMID: 35830892 DOI: 10.6004/jnccn.2022.7027]

66 **Yuan J**, Li X, Yu S. Molecular targeted therapy for advanced or metastatic soft tissue sarcoma. *Cancer Control* 2021; **28**:

10732748211038424 [PMID: 34844463 DOI: 10.1177/10732748211038424]
67 **Lv A**, Li Y, Li ZW, Mao LL, Tian XY, Hao CY. Treatment algorithm and surgical outcome for primary and recurrent retroperitoneal sarcomas: A long-term single-center experience of 242 cases. *J Surg Oncol* 2022; **126**: 1288-1298 [PMID: 35943840 DOI: 10.1002/jso.27040]
68 **Siew CCH**, Cardona K, van Houdt WJ. Management of recurrent retroperitoneal sarcomas. *Eur J Surg Oncol* 2022 [PMID: 35810040 DOI: 10.1016/j.ejso.2022.06.008]
69 **Danieli M**, Swallow CJ, Gronchi A. How to treat liposarcomas located in retroperitoneum. *Eur J Surg Oncol* 2022 [PMID: 35623985 DOI: 10.1016/j.ejso.2022.04.020]
70 **Willis F**, Schneider M. [Retroperitoneal soft tissue sarcoma: surgical management]. *Chirurg* 2022; **93**: 16-26 [PMID: 34596706 DOI: 10.1007/s00104-021-01506-6]
71 **Djafarrian R**, Zingg T, Digklia A, Becce F, Özsahin EM, La Rosa S, Demartines N, Matter M. [Management of abdominal soft tissue sarcomas]. *Rev Med Suisse* 2021; **17**: 1172-1176 [PMID: 34133095]
72 **Rust DJ**, Kato T, Yoon SS. Treatment for local control of retroperitoneal and pelvis sarcomas: A review of the literature. *Surg Oncol* 2022; **43**: 101814 [PMID: 35834940 DOI: 10.1016/j.suronc.2022.101814]
73 **Neuberg M**, Mir O, Levy A, Sourrouille I, Dumont S, Haddag-Miliani L, Ngo C, Mihoubi F, Rimareix F, Le Péchoux C, Adam J, Honart JF, Ceribelli C, Le Cesne A, Leymarie N, Faron M, Honoré C. Surgical management of soft tissue tumors of the abdominal wall: A retrospective study in a high-volume sarcoma center. *J Surg Oncol* 2021; **124**: 679-686 [PMID: 34120344 DOI: 10.1002/jso.26566]
74 **Gutkin PM**, von Eyben R, Chin A, Donaldson SS, Oh J, Jiang A, Ganjoo KN, Avedian RS, Bruzoni M, Steffner RJ, Moding EJ, Hiniker SM. Local Control Outcomes Using Stereotactic Body Radiation Therapy or Surgical Resection for Metastatic Sarcoma. *Int J Radiat Oncol Biol Phys* 2022; **114**: 771-779 [PMID: 35643255 DOI: 10.1016/j.ijrobp.2022.05.017]
75 **Blitzer GC**, Yadav P, Morris ZS. The Role of MRI-Guided Radiotherapy for Soft Tissue Sarcomas. *J Clin Med* 2022; **11** [PMID: 35207317 DOI: 10.3390/jcm11041042]
76 **Casadei L**, de Faria FCC, Lopez-Aguiar A, Pollock RE, Grignol V. Targetable Pathways in the Treatment of Retroperitoneal Liposarcoma. *Cancers (Basel)* 2022; **14** [PMID: 35326514 DOI: 10.3390/cancers14061362]
77 **Seldon C**, Shrivastava G, Al-Awady A, Asher D, Ramey S, Fernandez M, Dooley S, Kwon D, Zhao W, Goel N, Diwanji T, Subhawong T, Trent J, Yechieli R. Variation in Management of Extremity Soft-Tissue Sarcoma in Younger vs Older Adults. *JAMA Netw Open* 2021; **4**: e2120951 [PMID: 34415314 DOI: 10.1001/jamanetworkopen.2021.20951]
78 **Abugideiri M**, Janopaul-Naylor J, Switchenko J, Tian S, Read W, Press R, Oskouei S, Reimer N, Ferris M, Cassidy RJ, Behera M, Monson D, Landry J, Godette KD, Patel PR. Impact of Metastasectomy and Aggressive Local Therapy in Newly Diagnosed Metastatic Soft Tissue Sarcoma: An Analysis of the NCDB. *Ann Surg Oncol* 2022; **29**: 649-659 [PMID: 34272614 DOI: 10.1245/s10434-021-10466-4]
79 **Matsuoka M**, Onodera T, Yokota I, Iwasaki K, Matsubara S, Hishimura R, Kondo E, Iwasaki N. Surgical resection of primary tumor in the extremities improves survival for metastatic soft-tissue sarcoma patients: a population-based study of the SEER database. *Clin Transl Oncol* 2021; **23**: 2474-2481 [PMID: 34060011 DOI: 10.1007/s12094-021-02646-1]
80 **Boyle EA**, Elliott JA, McIntyre TV, Barnes ME, Donlon NE, Umair M, Gillis AE, Ridgway PF. Body composition is associated with operative and oncologic outcomes in the management of retroperitoneal and trunk soft tissue sarcoma. *Am J Surg* 2022; **223**: 729-737 [PMID: 34389158 DOI: 10.1016/j.amjsurg.2021.08.005]
81 **Spence S**, Doonan J, Farhan-Alanie OM, Chan CD, Tong D, Cho HS, Sahu MA, Traub F, Gupta S; The MPGS Study Group. Does the modified Glasgow Prognostic Score aid in the management of patients undergoing surgery for a soft-tissue sarcoma? *Bone Joint J* 2022; **104-B**: 168-176 [PMID: 34969280 DOI: 10.1302/0301-620X.104B1.BJJ-2021-0874.R1]
82 **Yao Y**, Wang Z, Yong L, Yao Q, Tian X, Wang T, Yang Q, Hao C, Zhou T. Longitudinal and time-to-event modeling for prognostic implications of radical surgery in retroperitoneal sarcoma. *CPT Pharmacometrics Syst Pharmacol* 2022; **11**: 1170-1182 [PMID: 35758865 DOI: 10.1002/psp4.12835]
83 **Morii T**, Anazawa U, Sato C, Iwata S, Nakagawa M, Endo M, Nakamura T, Ikuta K, Nishida Y, Nakayama R, Udaka T, Kawamoto T, Kito M, Sato K, Imanishi J, Akiyama T, Kobayashi H, Nagano A, Outani H, Toki S, Nishisho T, Sasa K, Suehara Y, Kawano H, Ueda T, Morioka H. Dedifferentiated liposarcoma in the extremity and trunk wall: A multi-institutional study of 132 cases by the Japanese Musculoskeletal Oncology Group (JMOG). *Eur J Surg Oncol* 2022 [PMID: 36088237 DOI: 10.1016/j.ejso.2022.08.024]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 March 24; 14(3): 99-137

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 3 March 24, 2023

REVIEW

99 Budd-Chiari syndrome in myeloproliferative neoplasms: A review of literature

Găman MA, Cozma MA, Manan MR, Srichawla BS, Dhali A, Ali S, Nahian A, Elton AC, Simhachalam Kutikuppala LV, Suteja RC, Diebel S, Găman AM, Diaconu CC

MINIREVIEWS

117 Immune microenvironment of medulloblastoma: The association between its molecular subgroups and potential targeted immunotherapeutic receptors

Kurdi M, Mulla N, Malibary H, Bamaga AK, Fadul MM, Faizo E, Hakamy S, Baeesa S

CASE REPORT

131 Unusual breast metastasis of gastrointestinal stromal tumor: A case report and literature review

Filonenko D, Karnaukhov N, Kvetenadze G, Zhukova L

World Journal of Clinical Oncology

Monthly Volume 14 Number 3 March 24, 2023

Contents

ABOUT COVER

Peer Reviewer of *World Journal of Clinical Oncology*, Zheng Wang, MD, PhD, Attending Surgeon, Senior Researcher, Assistant Professor, Comprehensive Breast Health Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, No. 197 Ruijin Second Road, Shanghai 200025, China. zhengwang@shsmu.edu.cn

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 edition of Journal Citation Reports® cites the 2021 Journal Citation Indicator (JCI) for *WJCO* as 0.35.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Yu-Jie Ma*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
March 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 March 24; 14(3): 99-116

DOI: 10.5306/wjco.v14.i3.99

ISSN 2218-4333 (online)

REVIEW

Budd-Chiari syndrome in myeloproliferative neoplasms: A review of literature

Mihnea-Alexandru Găman, Matei-Alexandru Cozma, Muhammad Romail Manan, Bahadar S Srichawla, Arkadeep Dhali, Sajjad Ali, Ahmed Nahian, Andrew C Elton, L V Simhachalam Kutikuppala, Richard Christian Suteja, Sebastian Diebel, Amelia Maria Găman, Camelia Cristina Diaconu

Specialty type: Hematology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Guo X, China; Manesis EK, Greece; Qi XS, China

Received: November 20, 2022
Peer-review started: November 20, 2022
First decision: January 3, 2023
Revised: February 10, 2023
Accepted: March 1, 2023
Article in press: March 1, 2023
Published online: March 24, 2023

Mihnea-Alexandru Găman, Matei-Alexandru Cozma, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest 050474, Romania

Mihnea-Alexandru Găman, Department of Hematology, Center of Hematology and Bone Marrow Transplantation, Fundeni Clinical Institute, Bucharest 022328, Romania

Muhammad Romail Manan, Services Institute of Medical Sciences, Lahore 54660, Pakistan

Bahadar S Srichawla, Department of Neurology, University of Massachusetts Chan Medical School, Worcester, Massachusetts 01655, United States

Arkadeep Dhali, Department of Internal Medicine, Nottingham University Hospitals NHS Trust, Nottingham NG7 2UH, United Kingdom

Sajjad Ali, Department of Medicine, Ziauddin University, Karachi 75600, Pakistan

Ahmed Nahian, California Baptist University-Lake Erie College of Osteopathic Medicine, Riverside, CA 92504, United States

Andrew C Elton, University of Minnesota Medical School, Minneapolis, MN 55455, United States

L V Simhachalam Kutikuppala, Department of General Surgery, Dr NTR University of Health Sciences, Andhra Pradesh, Vijayawada 520008, India

Richard Christian Suteja, Department of Undergraduate Medicine, Faculty of Medicine, Udayana University Denpasar, Bali 80232, Indonesia

Sebastian Diebel, Department of Family Medicine, Northern Ontario School of Medicine Timmins, Ontario 91762, Canada

Amelia Maria Găman, Department of Pathophysiology, University of Medicine and Pharmacy of Craiova, Romania & Clinic of Hematology, Filantropia City Hospital, Craiova 200143, Romania

Camelia Cristina Diaconu, Department of Internal Medicine, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Romania & Internal Medicine Clinic, Clinical Emergency Hospital of Bucharest, Bucharest 105402, Romania

Corresponding author: Mihnea-Alexandru Găman, Doctor, MD, PhD, Research Fellow, Researcher, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, 8 Eroii Sanitari Blvd, Bucharest 050474, Romania. mihneagaman@yahoo.com

Abstract

Myeloproliferative neoplasms (MPNs) are defined as clonal disorders of the hematopoietic stem cell in which an exaggerated production of terminally differentiated myeloid cells occurs. Classical, Philadelphia-negative MPNs, *i.e.*, polycythemia vera, essential thrombocythemia and primary myelofibrosis, exhibit a propensity towards the development of thrombotic complications that can occur in unusual sites, *e.g.*, portal, splanchnic or hepatic veins, the placenta or cerebral sinuses. The pathogenesis of thrombotic events in MPNs is complex and requires an intricate mechanism involving endothelial injury, stasis, elevated leukocyte adhesion, integrins, neutrophil extracellular traps, somatic mutations (*e.g.*, the V617F point mutation in the *JAK2* gene), microparticles, circulating endothelial cells, and other factors, to name a few. Herein, we review the available data on Budd-Chiari syndrome in Philadelphia-negative MPNs, with a particular focus on its epidemiology, pathogenesis, histopathology, risk factors, classification, clinical presentation, diagnosis, and management.

Key Words: Myeloproliferative neoplasms; Budd-Chiari syndrome; Thrombosis; Polycythemia vera; Essential thrombocythemia; Primary myelofibrosis

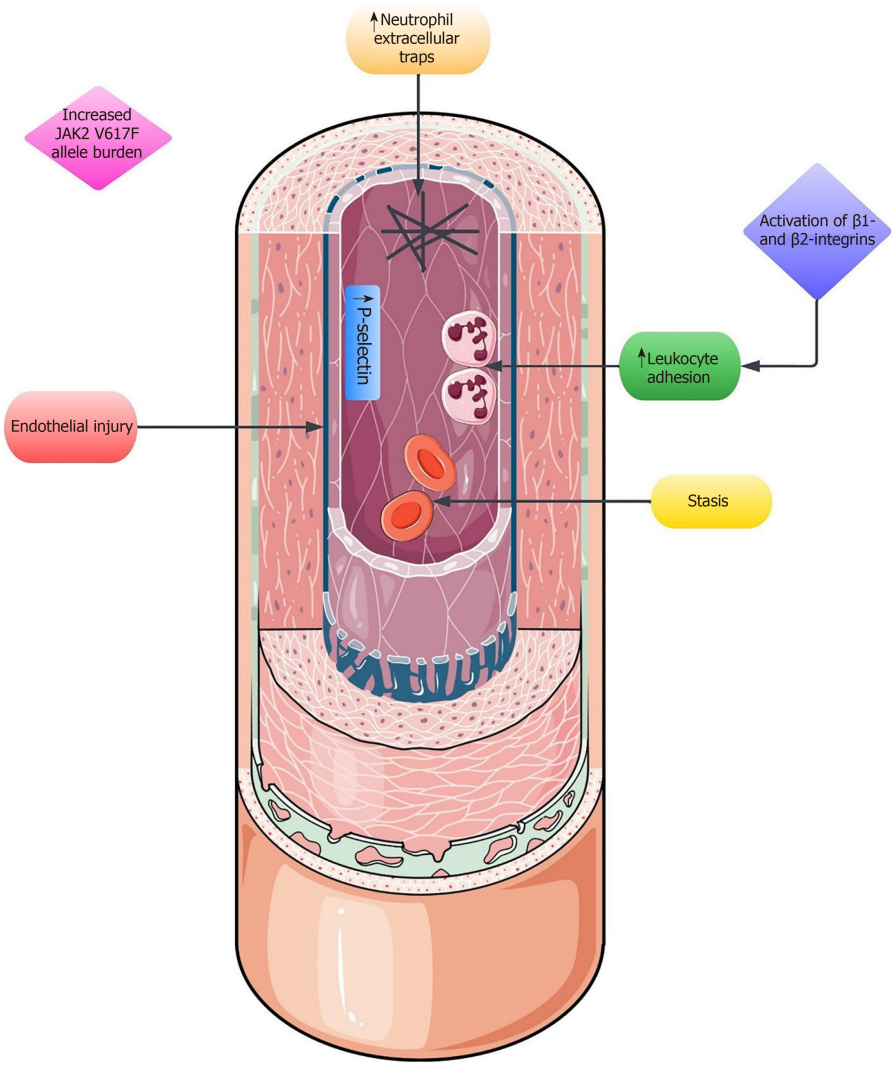
©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Myeloproliferative neoplasms (MPNs) are defined as clonal disorders of the hematopoietic stem cell in which an exaggerated production of terminally differentiated myeloid cells occurs. MPNs are characterized by a propensity towards the development of thrombotic complications, including Budd-Chiari syndrome (BCS). Herein, we review the available data on BCS in MPNs, with a particular focus on its epidemiology, pathogenesis, histopathology, risk factors, classification, clinical presentation, diagnosis, and management.

Citation: Găman MA, Cozma MA, Manan MR, Srichawla BS, Dhali A, Ali S, Nahian A, Elton AC, Simhachalam Kutikuppala LV, Suteja RC, Diebel S, Găman AM, Diaconu CC. Budd-Chiari syndrome in myeloproliferative neoplasms: A review of literature. *World J Clin Oncol* 2023; 14(3): 99-116
URL: https://www.wjgnet.com/2218-4333/full/v14/i3/99.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i3.99

INTRODUCTION

Myeloproliferative neoplasms (MPNs) are defined as clonal disorders of the hematopoietic stem cell in which an exaggerated production of terminally differentiated myeloid cells occurs[1]. Classical, Philadelphia-negative MPNs include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis, whereas chronic myeloid leukemia (CML) is the hallmark Philadelphia-positive MPN[1,2]. Philadelphia-negative MPNs exhibit a propensity towards the development of thrombotic complications[2,3]. In MPNs, thrombosis can occur in unusual sites, *e.g.*, portal, splanchnic or hepatic veins, the placenta or cerebral sinuses[4]. The pathogenesis of thrombotic events in MPNs is complex and requires an intricate mechanism involving endothelial injury, stasis, elevated leukocyte adhesion, integrins, neutrophil extracellular traps (NETs), somatic mutations (*e.g.*, the V617F point mutation in the *JAK2* gene), microparticles, circulating endothelial cells, and other factors, to name a few (Figure 1)[4,5]. Herein, we review the available data on Budd-Chiari syndrome (BCS) in Philadelphia-negative MPNs, with a particular focus on its epidemiology, pathogenesis, histopathology, risk factors, classification, clinical presentation, diagnosis, and management. MPNs lead to an increased risk of thrombosis through various mechanisms. This includes increased P-selectin expression, activation of integrins causing leukocyte adhesion, and the novel mechanism of NETs formation.



DOI: 10.5306/wjco.v14.i3.99 **Copyright** ©The Author(s) 2023.

Figure 1 Myeloproliferative neoplasms lead to an increased risk of thrombosis through various mechanisms. This includes increased *P*-selectin expression, activation of integrins causing leukocyte adhesion, and the novel mechanism of neutrophil extracellular traps formation.

BRIEF OVERVIEW OF BUDD-CHIARI SYNDROME

BCS is a heterogeneous group of disorders characterized by hepatic venous outflow tract obstruction, ranging from the small hepatic veins, the three suprahepatic veins and all the way to the junction of the inferior vena cava (IVC) and right atrium. This classification eliminates hepatic blood flow impairments caused by cardiac illness, pericardial disease, or sinusoidal obstruction syndrome (porto-sinusoidal vascular disorder)[6-9]. Primary BCS is the obstruction due to a predominantly venous process (thrombosis or phlebitis), whereas secondary BCS denominates the compression or invasion of the hepatic veins and/or IVC by a lesion that originates from outside of the vein (most commonly malignancy, abscess, or lymphadenopathy)[7,10,11]. It is a typical example of post-sinusoidal portal hypertension[6,8].

EPIDEMIOLOGY AND PATHOGENESIS OF BUDD-CHIARI SYNDROME IN MYELOPROLIFERATIVE NEOPLASMS

BCS is a rare condition across the globe. Expectedly, there is limited epidemiologic data on this entity. A recent meta-analysis involving data from Asian and European studies highlighted a pooled incidence of BCS at 1 case per million people and a prevalence of 11 cases per million people[12]. This report found significant heterogeneity among the analyzed assessments due to differences in study designs (diagnostic criteria, population characteristics, population sizes), as well as generally limited data on the topic from the Americas or Africa. The investigations were conducted on populations from Japan, South

Korea, Denmark, Sweden, Italy, and France. Earlier studies from China depicted the incidence of BCS at 0.88 per million and a prevalence ranging from 6.40 to 7.69 per million[13,14]. It is important to note, though, that most of these epidemiologic studies included both primary and secondary BCS. One study from France reported a median age of patients with primary BCS at 46.9 years. In their cohort, 30.6% were male and 69.4% female. Oral contraceptive use and pregnancy are gender-specific risk factors of BCS which may contribute to the female predominance[15]. In fact, hepatic vein thrombosis leading to BCS was more common in females, whereas obstruction of both the hepatic veins and IVC was more common in males[16]. There seems to be a geographic distribution of MPNs-related BCS cases. For example, Qi *et al*[17] reported that of their cohort of 246 cases of BCS diagnosed over nearly 12 years in China, only 5 cases were attributable to MPNs.

BCS, by definition, is the obstruction of the hepatic veins and/or outflow tract into the IVC. This entity is distinguished from portal vein thrombosis (PVT) and splanchnic vein thrombosis (SVT), which can coexist with BCS though are distinct pathologic processes[18]. Prior literature even reports presence of PVT in BCS at 10%-20%, which suggests a relatively poor prognosis[19]. Generally, the etiology of BCS is categorized as thrombotic or non-thrombotic. Thrombotic obstruction is the most common cause of BCS, and this is referred to as primary BCS. There are numerous conditions associated with primary BCS, including inherited thrombophilia, thalassemia, paroxysmal nocturnal hemoglobinuria, MPNs, pregnancy, oral contraceptive use, or even inflammatory conditions, *e.g.*, Behcet's disease, celiac disease, and ulcerative colitis[20-22]. Non-thrombotic causes of BCS, referred to as secondary BCS, typically involve a mass lesion involving the hepatic veins or compression by adjacent structures. There are many case reports describing unique causes of secondary BCS, *e.g.*, polycystic kidney disease, liver abscess, hydatid cysts, and cardiac myxoma; however, this etiology is uncommon[23-26]. MPNs are the most common cause of BCS, and the prevalence of BCS in the setting of MPN ranges from 32.9% up to 49.5% [27,28]. MPNs represent a malignant proliferation of myeloid cell lines, with the most common blood cancers classified in this category being CML, PV, ET and PMF. Thrombotic complications, such as BCS, are of particular concern in the setting of PV and ET, as these conditions carry significantly greater risk of such thrombotic processes[28]. A meta-analysis found that PV was the most common of MPNs to be diagnosed in the setting of BCS, even more than in subjects with PVT[28]. There is also evidence pointing towards increased thrombotic risk in MPNs carrying *JAK2* gene mutations[29] and about 41% of individuals with BCS exhibit genetic changes in the aforementioned gene[30,31].

HISTOPATHOLOGY OF BUDD-CHIARI SYNDROME

Histopathological features of BCS are studied in great detail and the histopathology of BCS is well established in the existing body of evidence[32-35]. Sinusoidal dilatation and congestion, centrilobular inflammation and necrosis, regenerative hyperplasia, macrovesicular steatosis, cholestasis, glycogenated nuclei, and perivenular fibrosis are the common histological features seen in this condition. Regenerative nodules, even though seen in both BCS and cardiac cirrhosis, is more common in BCS. Sinusoidal dilatation is the hallmark microscopic finding which can be appreciated in the initial stages of the disease[36]. However, this finding is not unique to BCS and can be seen in other conditions[37]. Having said that, in the presence of prominent sinusoidal dilatation, hepatic outflow obstruction should be ruled out as an important differential. Centrilobular necrosis is another important pathological feature that is more commonly seen in BCS compared to cardiac cirrhosis. This is attributed to the fact that hepatic hypoxia preferentially affects the centrilobular hepatocytes[38,39]. To the best of our knowledge, there is no literature on variation of the histopathological patterns in BCS secondary to MPNs. Moreover, a prognostic grading system for the same also does not exist and is a potential area for further studies.

RISK FACTORS OF BUDD-CHIARI SYNDROME IN MYELOPROLIFERATIVE NEOPLASMS

Smalberg *et al*[28] have depicted in their meta-analysis a strong relation between MPNs and SVT. This was confirmed by the high prevalence of *JAK2V617F* in BCS. MPNs and *JAK2V617F* are more commonly associated with BCS compared to PVT. This may be due to focal inflammatory insult to the portal venous system which is required for PVT[40]. There is a considerable difference between PVT and BCS with regard to the subtypes of MPNs. PV is more commonly associated with BCS than with PVT. There is a high pro-thrombotic effect as the hematocrit increases. In these situations, low-shear venous circulation is impacted more by the increased blood viscosity[41-43]. The interaction between adhesion molecules and red cells may be responsible for this mechanism. However, it was observed that PVT is more common in PMF compared to BCS[28]. This may be due to the fact that splenomegaly in PMF causes compression of the portal system leading to stasis of blood. In the same study, *JAK2V617F*-positive MPNs were found to be associated with PVT more frequently than BCS[28]. However, the exact reason is not known. In terms of *CALR* gene mutations, Li *et al*[45] highlighted that 1.41% of BCS cases exhibit genetic alterations in the *CALR* gene. In *JAK2V617F*-negative MPNs-related BCS cases, *CALR*

gene mutations were detected in 17.22% of the examined individuals [44].Mutations in other genes, *i.e.*, *MPL* or *TET2*, have rarely been depicted in BCS. However, the detection of a somatic gene mutation and especially of *JAK2V617F* in BCS should alert the clinician to screen for MPNs, including at follow-up if the diagnosis of overt MPNs is not established. In addition, work-up for hereditary thrombophilia should be performed as part of the molecular-driven diagnosis of BCS.

CLASSIFICATION OF BUDD-CHIARI SYNDROME

BCS can be classified based on three factors: (1) Origin of obstructive lesion (endoluminal/primary and extraluminal/secondary); (2) Site of obstruction; (3) Onset of disease pathology (fulminant, subacute, acute, and chronic) (Table 1). Primary BCS refers to occlusion resulting from endoluminal venous pathologies such as thrombosis, stenosis, endophlebitis, and webs, while secondary BCS is extraluminal in origin with compression being caused extrinsically by neighboring structures, *i.e.*, cysts, abscess, hyperplastic nodules, or invasive tumors[18]. Three classifications can be presented based on the site of the obstructive lesion (Table 2). As highlighted by Patil *et al* veno-occlusive disease (type III presented by Chaubal *et al*[46]) results when sinusoidal endothelial cells are primarily injured, and so it may be regarded as a separate entity known as the sinusoidal obstruction syndrome[46-48].

CLINICAL PRESENTATION OF BUDD-CHIARI SYNDROME

The clinical presentation of BCS varies widely depending upon the extent as well as the site and rapidity of hepatic venous outflow obstruction. This causes varied degrees of liver involvement, resulting in about 20% of the patients having little to no symptoms at all[49,50]. Owing to the development of intrahepatic, extrahepatic, or portosystemic collaterals, these patients do not show any discernible signs of venous obstruction. In contrast, patients with symptomatic hepatic vein obstruction present with symptoms of portal hypertension such as ascites, upper gastrointestinal bleeding, and hepatic encephalopathy with right upper quadrant abdominal pain. Abdominal examination may further reveal a tender hepatomegaly with splenomegaly. Therefore, a classical triad of abdominal pain, ascites, and hepatomegaly should raise a clinical suspicion of BCS. In patients with obstruction of the IVC, the signs and symptoms greatly vary. As a result, some authors refer to the hepatic complications of IVC obstruction as "obliterative hepatocavopathy"[51]. These patients may have signs of caval obstruction such as pedal edema, varicocele, lower limb ulcers, and/or dilated subcutaneous veins in the abdomen, chest, and back[52]. The rapidity of venous obstruction may give rise to varied degrees and forms of presentation, *i.e.*, fulminant, acute, subacute, and chronic. Patients with fulminant disease have a hyperacute onset of disease pathology (≤ 2 month), which is manifested as acute hepatic failure with ascites, hyperbilirubinemia, tender hepatomegaly, and renal failure secondary to renal outflow compromise resulting from hepatic vein obstruction[8,53]. Particularly, the development of hepatic encephalopathy within 2 months of onset of jaundice is regarded as fulminant disease[54]. Fulminant disease requires acute obstruction of all three hepatic veins and so its recorded incidence is quite low [55]. Acute BCS has a short duration of onset which is usually within a month while the onset of subacute ranges from one to six months[54]. Interestingly, there is data to suggest geographical variation in the incidence of various types of BCS based on onset of pathology. In the eastern geographic regions, chronic presentations are more prevalent with onset ranging from 6 months to 30 years[54,56]. In the western geographic region, acute presentation is encountered relatively more frequently[56]. Esophageal bleeding, ascites, and hepatic necrosis may be absent in patients of subacute BCS[8]. Finally, the chronic form may take more than 6 months to develop and is characterized by progressive abdominal distention without jaundice. These patients may have signs of portal hypertension including variceal bleeding as well as splenomegaly. Renal impairment may not be seen in 50% of these patients with chronic BCS[57]. These symptoms may or may not be accompanied by a wide range of nonspecific symptoms. Though a plethora of differential diagnosis may be present at this point-and though it is true that BCS is generally a rare disease-clinicians must not exclude the possibility of BCS and as discussed by Aydinli and Bayraktar, clinical suspicion of BCS should escalate in the following scenarios: Acute onset ascites with tender hepatomegaly, massive ascites with relatively preserved liver functions, fulminant hepatic failure associated with hepatomegaly and ascites, unexplained chronic liver disease, liver disease with thrombogenic disorder, and sinusoidal dilation on liver biopsy without heart disease [18].

Acute liver failure is a sequelae of BCS that is infrequent in its occurrence. According to two case series, BCS accounts for 0.9% to 15% of the cases of acute liver failure. Majority of the patients, in the larger case series reporting 20 cases of BCS in 2344 patients of acute liver failure, were middle aged Caucasian women with PV. Acute-on-chronic manifestation, however, is relatively more frequent as compared to acute liver failure. To direct appropriate management, the Asian Pacific Association for the Study of the Liver has further classified the acute-on-chronic manifestation of BCS into three types based on underlying liver disease and acute insult (Table 2)[56]. Table 3 depicts the clinical character-

Table 1 Classification of Budd-Chiari syndrome

Ref.	Type	Site of obstruction
Chaubal *et al*[46]	I	Obstruction of IVC with or without secondary hepatic vein occlusion
	II	Obstruction of major hepatic veins
	III	Obstruction of small centrilobular veins (considered by some as veno-occlusive disease)
Patil *et al*[48]	I	Lesions of the IVC
	IIa	Short segment (< 4 cm) lesion of the hepatic vein
	IIb	Diffuse lesion of the hepatic vein
	III	Mixed type with lesions of IVC and the hepatic vein
Bansal *et al*[47]	I	Hepatic vein obstruction or thrombosis without IVC obstruction or compression
	II	Hepatic vein obstruction or thrombosis with IVC obstruction or thrombosis
	III	Isolated hepatic venous webs
	IV	Isolated IVC webs

IVC: Inferior vena cava.

Table 2 Acute-on-chronic manifestation of Budd-Chiari syndrome classification

Type	Description of pathology
A	Acute hepatic vein thrombosis or stent block precipitates ACLF in a BCS
B	Non-thrombotic acute insult precipitates ACLF in a chronic BCS
C	Acute hepatic vein thrombosis precipitates ACLF in a non-vascular chronic
	Liver disease

ACLF: Acute-on-chronic liver failure; BCS: Budd-Chiari syndrome.

istics of BCS based on geographic region of the studies population.

DIAGNOSIS OF BUDD-CHIARI SYNDROME

Although BCS is considered a rare disease, it has the potential to rapidly deteriorate a patient's health. Therefore, the need to obtain a correct diagnosis, followed by rapid specific treatment is urgent and extremely important.

Medical history

As BCS may be classified as primary (endoluminal lesion-like thrombosis) or secondary (extra-venous system causes), assessment of medical history plays a key role in identifying predisposing factors towards BCS[95]. Knowledge about these key points might be suggestive, not sufficient, of BCS diagnosis. Treatments administered by clinicians must put into account not only the obstruction by itself, but also its possible underlying causes: MPNs, *e.g.*, PV, ET, PMF[63]; History of hereditary or acquired thrombogenic disorders[64]; Use of oral contraceptives[65,66]; Paroxysmal nocturnal hemoglobinuria[67]; Status and history of recent pregnancy[68,69]; History of hepatocellular carcinoma [70]; Chronic liver disease, remained unexplained after exclusion of alcoholism, chronic viral hepatitis B or C, autoimmunity, iron overload, Wilson's disease and alpha-1 antitrypsin deficiency[71]; and other possible risk factors towards thrombosis or obstruction.

Physical examination

Majority of BCS patients present with the classic triad of abdominal pain especially in the upper right quadrant presenting in 61% of cases, ascites presenting in 83% of cases, and hepatomegaly presenting in 67% of cases[6,72]. The classic triad may form suddenly in acute cases (< 6 months), or progressively in chronic cases (> 6 months)[73]. These symptoms may or may not be accompanied by a wide range of nonspecific symptoms. Though a plethora of differential diagnosis may be present at this point-and

Table 3 Clinical characteristics of Budd-Chiari syndrome based on geographic region of the studied population[15,58-62]

Clinical characteristics	Percentage in various geographic regions						
	Egypt	France	Sweden	China	Japan	United States	Algeria
Ascites/Abdominal distension	82	74.4	88	51.9	31.2	29.9	74.8
Abdominal fullness	-	-	-	-	26.1	-	-
Abdominal discomfort	-	-	-	-	17.8	-	-
Abdominal pain	96	72.4	81	-	2.5	-	42.6
Cirrhosis	-	-	-	-	-	18.6	-
Hepatomegaly	50	70.1	72	-	54.7	-	-
Splenomegaly	42	49.1	66	-	-	-	-
Esophageal varices	-	54.8	67	-	-	7.4	-
Hematemesis/Variceal bleed	36	-	9	16	8.3	3.2	-
Melena	-	-	-	-	2.5	-	-
Jaundice	16	20.3	29	-	5.7	-	13.9
Fever	-	20.1	27	-	-	-	15.7
Hydrothorax	-	13.0	-	-	-	-	-
Acute kidney injury	-	-	-	-	-	18.8	-
History of recurrent abortions	6	-	-	-	-	-	-
History of previous thrombosis	10	-	-	-	-	7	-
Recurrent orogenital ulcers	4	-	-	-	-	-	-
Leg ulcers	2	-	-	-	3.8	-	-
Lower limb edema	68	-	-	58.9	31.8	-	13
Dilated abdominal veins	40	-	-	57	27.3	-	-
Hepatic encephalopathy	36	-	12	0.7	-	9.5	5.2
Ileus	-	-	21	-	-	-	-
General malaise	-	-	-	-	12.1	-	-
Acute respiratory failure	-	-	-	-	-	7	-

though it is true that BCS is generally a rare disease – clinicians must not exclude the possibility of BCS.

General supporting examination

Routine laboratory examinations might help aid clinicians to further pursue the presumptive diagnosis of BCS. Results that suggest the possibility of BCS include[6,73]: Diagnosis of PV, ET or PMF; normal or increase in liver function tests (alanine aminotransferase; aspartate aminotransferase); or findings which indicate thrombosis.

Specific supporting examination: imaging modalities

If a patient presents with the suspected risk factors, clinical presentation, and general supporting examination, clinicians must continue the diagnostic work-up with a high index of suspicion towards BCS. Specific supporting examination lies all around imaging modalities. These are the investigation methods useful towards diagnosing BCS, in an arranged order from the first line to the last[71]: Doppler ultrasonography; Magnetic resonance imaging (MRI) or computed tomography (CT) scan; Venography; Liver biopsy. Doppler ultrasonography is regarded as the initial technique of choice, offering a pooled sensitivity of 89% and specificity of 68% across multiple studies[74]. Doppler ultrasonography will show findings of no venous flow, retrograde venous flow, no visualization of the vein (possibly due to venous collapse) in the affected areas[75]. These findings might indicate BCS, though still overlapped by advanced cirrhosis[71]. However, in conditions where sonographic examination is inadequate to evaluate BCS, or in conditions where the distinct characteristics of BCS were not found, imaging through MRI or CT scan may be used in place to evaluate the presumptive diagnosis of BCS. MRI scan offers a pooled sensitivity of 93% and specificity of 55%, while CT scan offers a pooled sensitivity of 89% and specificity of 72%[74]. These modalities usually offer a clear diagnosis, though there might be

uncertainty in patients with advanced cirrhosis[71]. Venography, and especially liver biopsy might be the last, most invasive, yet the gold standard of diagnosis. However, biopsy provides the best explanation towards the damage and specific etiologies contributing to the disease.

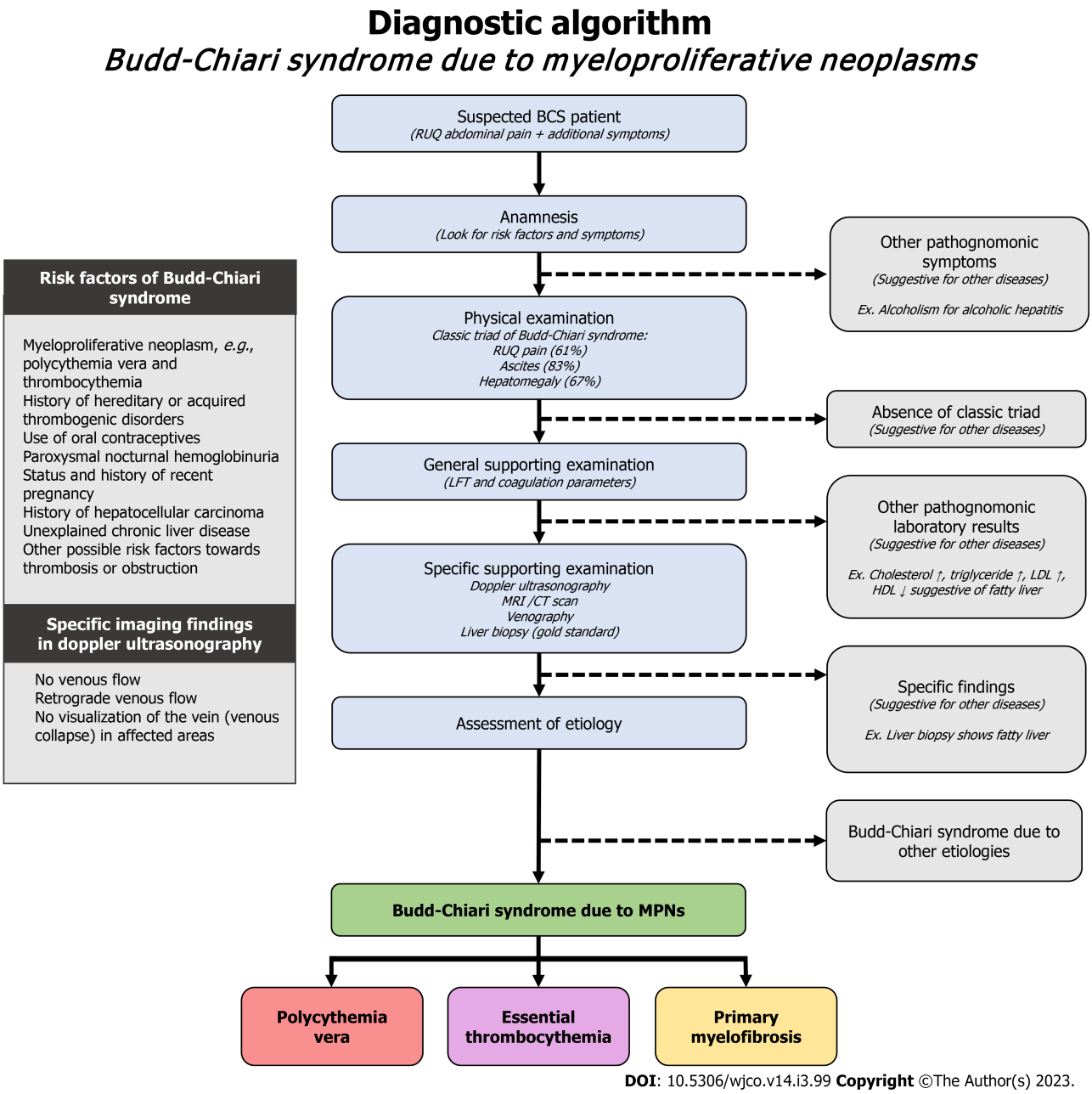
Assessment of etiology

The assessment of BCS etiology should definitely actively search for potential MPNs. A meta-analysis found that distribution of MPN subtypes in BCS were as follows: PV (52.9%), ET (24.6%), PMF (6.7%), and unclassifiable MPNs (17.0%)[28]. A presumption about the etiology of BCS might be estimated from the results of general supporting examination. We propose a diagnostic algorithm for this instance as reported in Figure 2.

MANAGEMENT OF BUDD-CHIARI SYNDROME IN MYELOPROLIFERATIVE NEOPLASMS

The treatment of BCS in MPNs requires a stepwise approach that may require a complete interdisciplinary team to adequately manage[54]. The goals of treatment aim at relieving obstruction, correcting the underlying conditions, and lastly to monitor for any liver deterioration[72]. The level of liver dysfunction can affect the coagulopathy of the patient and make anticoagulation difficult to predict in patients with BCS[54]. Despite this, the first line treatment for BCS due to MPNs still consists of anticoagulation therapy in order to relieve any obstruction. Furthermore, it is important to note that antiplatelet therapy should be initiated in patients as soon as possible once a diagnosis is established. Currently, the consensus when it comes to anticoagulation therapy is to treat with low molecular weight heparin (LMWH) and target an international normalized ratio (INR). In addition to the LMWH, patients should be started on an oral vitamin K antagonists (VKAs) (*i.e.*, warfarin). Once the INR is between 2 and 3, the LMWH can be discontinued, however, the oral vitamin K antagonist should be continued lifelong[6]. Although anticoagulation is the first line therapy for patients with BCS in MPNs, unfortunately, only 15%-20% of patients will respond to anticoagulation, as a result, other interventions may also need to be implemented in 80-85% of cases[54]. When looking at acute BCS it is important to consider methods that will restore patency of the thrombosed veins[76]. Treatment may start with thrombolytic therapy in select patients who have had symptoms for a few weeks and who have a well-established clot[54]. However, it is important to note that pharmacological thrombolysis has only been demonstrated to be effective in patients presenting with acute (less than a few weeks) BCS, therefore, at times other management options must also be considered. In addition, publications on thrombolytic agents in this particular setting have been limited to case reports and small case series[77,78]. It is also important to note that thrombolytic agents should not be considered when managing chronic BCS due to the fact that these clots have been demonstrated to be resistant to thrombolytic agents and may increase the bleeding risk for the patient far beyond the potential therapeutic benefit[78]. Another management option that is worth considering in acute or subacute BCS in MPNs include angioplasty and stenting [13]. Both angioplasty and stenting can be considered in patients that have a demonstrated obstruction that is appreciated on radiological findings[13]. In addition, this intervention is typically reserved for patients who are symptomatic[71,79]. It is also important to note that performing angioplasty can be done in combination with thrombolytic therapy for patients who have an acute obstruction and may benefit from both interventions[79].

Although angioplasty and stenting has started to play a major role and is a staple in the treatment of BCS it is not without risks. The primary risk associated with stenting is the risk of reocclusion. One intervention that has shown some promise in preventing reocclusion is the placement of a metal stent following angioplasty. However, it is important to also note that increased outcomes for this particular method is limited to some small case studies[40]. For BCS patients, inclusive of the individuals waiting for orthotopic liver transplantation, transjugular intrahepatic portosystemic shunt (TIPS) is suggested as a safer and very successful treatment strategy. TIPS is one of the major treatment options available for the treatment of BCS[81]. The Baveno IV consensus has resulted in a fairly uniform course of care for the patients of BCS, with prior consideration for the medical therapy alongside anticoagulation among all patients with no contraindications. This has been the case for more than 20 years of TIPS usage in BCS [82]. Because of the technical difficulty in sustaining venous patency for a longer period of time, TIPS should be specifically considered for the patients with Rotterdam class III, acute liver failure, or in those individuals who have failed medical therapy, diffuse hepatic vein thrombosis or prior hepatic venous stenting. Ascites is typically the most prevalent symptom, followed by variceal or gastrointestinal hemorrhage, with ascites rates in the trials under review reaching 100% and variceal bleeding rates reaching up to 30.9%. Since it has not been found as a potential risk factor in the occurrence of post-TIPS hepatic encephalopathy, prior hepatic encephalopathy need not be regarded as a contraindication to TIPS in BCS[81]. Additionally, pre-procedure jaundice is not regarded as a contraindication for TIPS in BCS patients, despite the fact that this is the case in patients with end-stage liver disease due to the higher mortality in that cohort, with the reason for the difference being hypothesized to be related to the absence of hepatocyte necrosis in the BCS patients[83]. Although there is no universal agreement across the studies regarding the ideal timing to perform TIPS, patients presenting with refractory ascites,



DOI: 10.5306/wjco.v14.i3.99 **Copyright** ©The Author(s) 2023.

Figure 2 Diagnostic algorithm of Budd-Chiari syndrome due to myeloproliferative neoplasms.

hepatic failure, or the gastrointestinal hemorrhage should have access to this right away[84]. BCS patients have a greater rate of shunt dysfunction than other cirrhotic patients receiving TIPS (about 50% *vs* 80% within 1 year), which is likely related to the higher incidence of underlying thrombophilia[85]. Development of stents coated with polytetrafluoroethylene to be used during TIPS in the management of BCS has improved patients' prognosis, mainly due to the decrease in the need for re-interventions and due to the tripling of the shunt patency[86]. Hence, TIPS could be a safer and effective treatment option for managing BCS in MPNs.

Over time, transplantation results have significantly improved and liver transplant outcomes are not harmed by prior TIPS. For patients with BCS, establishing venous outflow after liver transplantation is very essential and necessitates a variety of surgical procedures. These patients' outcomes exhibit various issues, such as vascular thrombosis and biliary difficulties[87]. Anticoagulation therapy, angioplasty, and TIPS fail in the 10% to 20% of BCS patients treated with a step-by-step management method, either due to technical failure or due to subpar clinical outcomes of a technically successful procedure necessitating rescue transplantation. Additionally, patients with fulminant liver failure and those with extremely advanced liver cirrhosis may benefit most from liver transplantation as treatment[88]. In the case that a medical therapy does not succeed, interventional revascularization and TIPS are recommended. The only guaranteed alternative for treating BCS is liver transplantation, if the presumptive medications and procedures are ineffective. Liver transplantation may be recommended as

a last resort or in fulminant situations, with promising and favorable outcomes. Ibach *et al*[89] analyzed 46 cases of BCS of whom 22 suffered from MPNs and reported that individuals with BCS who were subjected to liver transplantation experienced a median survival of approximately 24 years. Mortality rates were higher in patients with BCS and MPNs (RR=3.44, P = 0.05), however, two patients diagnosed with these blood cancers died due to secondary acute myeloid leukemia and extramedullary hematopoiesis in the spleen with consequent organ rupture, events which can occur during disease evolution irrespective of the use of liver transplantation in these cases. Considering 5-year survival rates for PV and ET are of about 80% and of about 50% for PMF, the use of liver transplantation for MPNs-linked BCS is satisfactory in terms of survival prolongation[90]. Patients with BCS and Philadelphia-negative MPNs receive the same care as those without MPNs during the acute phase. LMWH or unfractionated heparin should be administered as soon as possible, followed by VKAs. It is advised to proceed gradually. A second-line approach based on invasive treatments, such as angioplasty with or without stenting, TIPS, or surgical portosystemic shunt, should be taken into consideration in the event that clinical deterioration persists despite anticoagulation [14,40,59]. While catheter-directed thrombolysis may be helpful for the treatment of acute and partially occlusive thrombosis, systemic thrombolytic therapy with tissue plasminogen activator is not very successful[78,91,92]. TIPS has recently been suggested as the preferred course of care for individuals with BCS who exhibit symptoms of portal hypertension. If TIPS is ineffective or inappropriate, angioplasty/stenting should be the second line of treatment for the subset of individuals. When TIPS and angioplasty/stenting are ineffective or inappropriate, surgical shunts ought to be the first line of treatment[93]. Consider liver transplantation as a curative measure[3,40,93,94].

Long-term antithrombotic treatment

An improved prognosis was introduced in the 1980s with the systematic use of VKAs in BCS patients [71,95], while the impact of oral anticoagulation on the survival of the most severe patients is debatable [6]. Although the ideal time frame for VKA is uncertain, lifelong medication is generally advised for BCS[40,94,96]. Only 5 (8%) of the 163 patients in the comprehensive survey-of whom the majority (86%) were getting VKA-experienced non-fatal variceal hemorrhage[97]. The rate of both recurrent thrombosis and bleeding complications was 11% in a different study on patients with BCS who underwent liver transplantation and received VKAs afterwards, but the mortality rate related to recurrence is higher than that related to bleeding (4.4% and 0.8% of patients, respectively)[98]. There are few specific studies on the effectiveness and safety of VKAs treatment in individuals with MPN-related BCS, and the majority of the studies refer to SVT as a whole. In total, 49 of the 604 patients with SVT in the aforementioned multicenter prospective cohort (55 had BCS) had MPNs and had a 9-fold increased risk of recurrent thrombosis during follow-up[99]. The presence of *JAK2* gene mutations was substantially correlated with liver-related thrombotic problems in a series of 36 BCS patients with recurrent thrombosis following liver transplantation in 42% of instances (15/36). Moreover, 11 of the 12 patients who experienced post-transplant thrombotic events and 10 of the 24 patients who did not (P = 0.005) both exhibited *JAK2V617F*. Additionally, an increased incidence of thrombosis at any site was linked to a *JAK2* gene mutation (14/15 *vs* 7/21, P = 0.005). Liver-related thrombotic problems were more common in people with overt MPNs (9/12 *vs* 8/24, P = 0.03)[100]. An investigation of 181 patients suffering from MPNs who had their first episode of SVT was conducted retrospectively. In total, 31 (17.1%) and 109 (60.3%) patients, respectively, had BCS and extra-hepatic portal vein obstruction diagnosis; isolated thrombosis of the mesenteric or splenic veins was found in 18 and 23 cases, respectively. Following this index occurrence, the subjects were observed for 735 patient-years, and during that time, 31 recurrences occurred, representing an incidence rate of 4.2 per 100 patient-years. The recurrence rate was 3.9 per 100 patient-years in the 85% of patients who received VKAs, compared to 7.2 per 100 patient-years in the small portion (15%) of patients who did not. Compared to those who had thrombosis at the portal or other abdominal sites, patients with BCS had an incidence rate of new events that was significantly higher at 8.0 per 100 patient-years. In contrast, there was no difference in the rate of new arterial thrombosis between the two groups. Of note, patients with BCS had a 3-fold higher risk of recurrent SVT than those with other index SVT[101]. This difference was caused by an increased rate of venous events in BCS patients. Nine individuals with BCS (4 without and 5 with liver cirrhosis) were included in a survey on the use of direct oral anticoagulants (DOACs) in 94 patients with SVT, but no information was provided regarding the presence of MPNs as the underlying cause of SVT[102]. Anecdotally, it has been mentioned that a patient with PV and BCS used the direct factor Xa oral inhibitor rivaroxaban [103]. Semmler *et al*[104] analyzed the potential efficacy of DOACs, specifically edoxaban, apixaban, rivaroxaban and dabigatran, in the management of BCS. Their sample size consisted of 47 BCS subjects: 22 (of whom 10 had MPNs) were put on DOACs, whereas 21 (of whom 9 had MPNs) received LMWHs or VKAs. Complete response was noted in >60% of the BCS subjects who were prescribed DOACs. Complications during DOAC use included major spontaneous or surgery-related hemorrhage (n = 4 and n = 1, respectively) and minor hemorrhages (n = 7), whereas transplant-free survival at 5 years exceeded 90% and at 10 years exceeded 80%. *JAK2V617F*-negative MPNs experienced better treatment responses to DOACs as compared to *JAK2V617F*-positive individuals. Nevertheless, further research needs to assess the benefits of DOAC use in MPN-related BCS as the sample of the aforementioned investigation was too small to draw pertinent conclusions.

Cytoreductive therapy

Cytoreduction is necessary in MPN patients who have experienced thrombosis in the past[105]. It is unknown whether it is appropriate to administer cytoreduction to SVT patients with *JAK2V617F* but without an explicit MPN diagnosis in accordance with the WHO criteria. Given the lack of evidence, care must be taken when prescribing cytoreductive regimens to *JAK2V617F*-positive SVT patients because approximately half of them will not develop MPN during the follow-up[106]. On the other hand, the *JAK2V617F* mutation increases the incidence of recurrent thrombosis in both BCS patients who have undergone liver transplantation[100] and SVT patients generally[107,108]. Therefore, it seems sensible to utilize medications to slow the growth of the mutant clone. Only one of the 17 MPN patients with BCS in a small retrospective cohort who received hydroxyurea and aspirin after liver donation developed recurrent extrahepatic portal vein obstruction (EHPVO)[109]. The rate of recurrence was 22% (4/18) in another small series of 18 MPN individuals with BCS; all new thrombotic events occurred in patients who were not receiving cytoreductive therapy[110]. In a pooled cohort of 1500 patients with MPNs and thrombosis, the multivariable analysis limited to the patients with first arterial thrombosis showed that recurrent arterial thrombosis was prevented by antiplatelet agents and by hydroxyurea and only partially by VKAs; on the contrary, in patients with the first venous thrombosis, venous recurrences were more prevented by VKAs than by antiplatelet agents or hydroxyurea. Notably, after adjusting for age, sex, antiplatelet treatment, VKA treatment, and cytoreductive drugs other than hydroxyurea in 218 patients with SVT (38 with BCS), it was verified that hydroxyurea had no significant impact on the rate of either recurrent thrombosis or recurrent VTE[111]. The cause of this finding is unclear; however, it may be hypothesized that since hypercytemia is less common in SVT patients[112], cytoreduction may not be as important as it might be in other circumstances.

Orthotopic liver transplantation

Patients with BCS who experience failure of the aforementioned therapies are candidates for orthotopic liver transplantation in the range of 10 to 20 percent of cases[6]. Following liver transplantation, the 1-year and 5-year survival rates in a group of 36 BCS patients were 84% and 69%, respectively; the presence of a molecular characteristic for MPNs had no bearing on these survival rates[100]. The mortality rate following liver transplantation in a different series of 25 BCS patients was comparable in MPN (3/18, 16.7%) and non-MPN patients (1/7, 14.3%)[110]. In a retrospective cohort of 78 BCS patients, the 5-year survival was 78% *vs* 76%, and the 10-year survival was 68% *vs* 73%, respectively. Long-term survival following liver transplantation was similar in MPN (n = 41) and non-MPN patients (n = 37), with P values of 0.81 and 0.66, respectively. Twelve of the 41 MPN patients (or 29%) passed away within the first three years following liver transplantation, but only one death with recurrent BCS was attributable to the hematologic condition[146]. Following liver transplantation, progression to myelofibrosis or acute leukemia was not noted in 17 cases with a follow-up period of up to 20 years [109], nor in 78 cases in a mean follow-up period of 12.4 years (range 3-28.4 years)[113] in two series of BCS patients. While there are many treatment options available for BCS, the availability of many creates obstacles in maintaining a standard treatment plan. It is usual for clinicians to use anticoagulation as the first line of treatment for tackling BCS[114]; however, in cases when the condition cannot be controlled by medical treatment alone, several trials have shown encouraging outcomes with the use of TIPS in BCS patients as an alternative to shunt surgery or liver transplantation[115,116].

Anticoagulant therapy has been an accepted standard of treatment in BCS[117]; however, this standard remains controversial amidst clinicians. Emergent anticoagulation may not significantly improve clinical outcomes for individuals with acute ischemic stroke, according to several clinical investigations[118-120]. The current mode of treatment also selects LT as a de-facto last resort when it requires for a complex venous outflow reconstruction that would be difficult to acquire in medically underserved areas[6,121]. We have previously established that TIPS is the equivocally accepted and proven form of treatment for most BCS patients; in fact, the 10-year survival of the procedure is 69% [122]. It naturally becomes a concern if the current chronology requires a deeper revision. For instance, if anticoagulants truly seem to have no significant impact on the pathophysiology of the condition, could we derive a better medicinal first line of treatment for the condition? Could TIPS become the first mode of treatment if more non-invasive mechanisms are innovated to perform it? BCS is a rare disease, and it is important to remember that time is an invaluable resource in situations as such. Resorting to ineffective treatment not only delays medical management, but it deters the patient's condition. If the first line of treatment leads a patient to the second line in the longer run, maybe it is a scope for physicians to rethink the chronology of treatment. Constant revisions of guidelines will allow us to not only discard what is counterintuitive, but it will promote clinicians to adapt to newer and more effective modes of treatment.

DIFFERENTIAL DIAGNOSIS, PROGNOSIS AND COMPLICATIONS OF BUDD-CHIARI SYNDROME IN MYELOPROLIFERATIVE NEOPLASMS

Hepatic vein obstruction in BCS may lead to abdominal pain, ascites, jaundice, and hepatomegaly. Given the significant overlap of these symptoms with other hepatic pathologies the differential diagnosis remains broad. PVT is distinct from BCS and directly involves the liver vasculature. MPNs are the most common cause of noncirrhotic, nonmalignant PVT[28]. Given the similar presentation of both PVT and BCS and its high prevalence in MPNs, diagnostic tests and imaging modalities should be utilized in differentiating both conditions. In BCS, Doppler ultrasound is effective in visualizing occlusion of the hepatic vein[123]. The absence of hepatic vein thrombosis and presence of reduced or absent flow in the portal veins with duplex ultrasound points towards PVT as the primary differential diagnosis[10]. Other differential diagnoses that should be given consideration include granulomatous liver disease, hemochromatosis, and alcoholic liver disease. A retrospective study across three centers in Europe studied the prognostic factors associated with BCS in MPNs. Their results indicated poorer baseline prognostic features, earlier hepatic decompression procedures, but no effect on 5-year survival. However, the presence of MPNs was associated with event free survival in BCS[124]. Generally, the determinants of prognosis in BCS are age, serum creatinine, Child-Pugh score, and ascites. A higher Child-Pugh score, older age, refractory ascites to diuretics, and higher serum creatinine are all factors pointing towards a poor prognosis[125]. In recent years 5-year survival rates have improved in BCS. Improved survival is largely attributed to the improved management of hypercoagulable states, and endovascular intervention[95]. Although rarely performed now, surgical portosystemic shunting improved survival in BCS patients who were determined to have a poor prognosis[126]. In a retrospective analysis of 78 BCS patients both with and without MPNs similar outcomes were measured after liver transplantation[113]. Janssen *et al* studied 172 patients with EHPVO, 24 of which carried a diagnosis of MPN. The five-year survival rates were similar between both groups (92% *vs* 53%, $P = 0.18$) [127]. Significant consideration must also be given to the role of VKAs in the prognosis of BCS. De Stefano *et al*[128] performed a retrospective analysis of 94 patients with MPNs (PV or ET), significant reduction of re-thrombosis was independently achieved with VKAs (HR 0.32; 95%CI: 0.15–0.64) and antiplatelet agents (HR 0.42; 95%CI: 0.22–0.77). DOACs may improve outcomes in patients with BCS. Semmler *et al*[104] in 2022 performed a retrospective analysis of 46 patients across three Australian centers with BCS. Six patients were managed with DOACs and 16 were switched to DOACs from LMWHs ($n = 12$) or VKAs ($n = 4$). In total, 4 major and 7 minor bleeding events were reported. Larger prospective studies need to be conducted assessing the safety and prognosis of VKAs *vs* DOACs in patients with BCS. Based on these previous studies it is determined that identification of BCS in patients with MPNs should be promptly treated, thereby improving prognosis. Complications secondary to BCS can be determined based on the varying degree of ensuing hepatic injury and dysfunction. When untreated BCS can progress to fulminant liver failure, hepatorenal syndrome, hepatocellular carcinoma, and hepatic encephalopathy amongst other complications. In 2021, Asl *et al*[129] retrospectively reported on complications associated with liver transplantation (LT) in 4225 patients. 108 patients had BCS and were matched with a non-BCS group of 108 patients. One-, 3-, 5-, and 10- year survival rates were the same in both groups (82%, 78%, 76%, and 76% *vs* 83%, 83%, 83%, and 76%, $P = 0.556$). No differences were noted in the 6-month follow-up after LT. However, at a later period vascular thrombosis was more prevalent in the BCS group. In 2016, Ki *et al*[130] conducted a population-based study in South Korea identifying a total of 423 BCS patients from 2009-2013. Among them, 10.3% developed hepatic malignancy, and 3.3% underwent LT. The annual-case fatality rate was 2.8%. Hayek *et al*[131] performed a retrospective analysis on the long-term safety of patients with BCS who underwent TIPS. In total, 54 patients were identified, 34 (52%) of which suffered from MPNs. TIPS dysfunction was associated with MPNs (HR, 8.18; 95%CI: 1.45-46.18; $P = 0.017$).

CONCLUSION

Although BCS and MPNs are rare disorders, BCS can develop in the setting of MPNs. In this patient population, individualized, distinctive counseling and multidisciplinary surveillance and treatment strategies are crucial in achieving better possible outcomes. Individuals with MPNs should be managed in accordance with the most recent guidelines to avoid the occurrence of BCS, whereas a diagnosis of BCS should warrant an active search for the potential diagnosis of MPNs.

FOOTNOTES

Author contributions: All authors contributed to writing the original draft; Găman MA, Găman AM and Diaconu CC contributed to writing, review and editing, and methodology; Găman AM and Diaconu CC contributed equally as senior/last authors; all authors read and approved the final version of this paper.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Romania

ORCID number: Mihnea-Alexandru Găman 0000-0001-7133-8875; Matei-Alexandru Cozma 0000-0002-4998-0105; Arkadeep Dhali 0000-0002-1794-2569; L V Simhachalam Kutikuppala 0000-0002-5685-6049; Amelia Maria Găman 0000-0001-6974-2772; Camelia Cristina Diaconu 0000-0001-6837-260X.

S-Editor: Xing YX
L-Editor: A
P-Editor: Xing YX

REFERENCES

1 **Nangalia J**, Green AR. Myeloproliferative neoplasms: from origins to outcomes. *Blood* 2017; **130**: 2475-2483 [PMID: 29212804 DOI: 10.1182/blood-2017-06-782037]
2 **Barbui T**, Thiele J, Gisslinger H, Kvasnicka HM, Vannucchi AM, Guglielmelli P, Orazi A, Tefferi A. The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. *Blood Cancer J* 2018; **8**: 15 [PMID: 29426921 DOI: 10.1038/s41408-018-0054-y]
3 **Greenfield G**, McMullin MF, Mills K. Molecular pathogenesis of the myeloproliferative neoplasms. *J Hematol Oncol* 2021; **14**: 103 [PMID: 34193229 DOI: 10.1186/s13045-021-01116-z]
4 **Moliterno AR**, Ginzburg YZ, Hoffman R. Clinical insights into the origins of thrombosis in myeloproliferative neoplasms. *Blood* 2021; **137**: 1145-1153 [PMID: 33237986 DOI: 10.1182/blood.2020008043]
5 **Găman MA**, Cozma MA, Dobrică EC, Crețoiu SM, Găman AM, Diaconu CC. Liquid biopsy and potential liquid biopsy-based biomarkers in philadelphia-negative classical myeloproliferative neoplasms: A systematic review. *Life* 2021; 11 [PMID: 34357048 DOI: 10.3390/Life11070677]
6 **Darwish Murad S**, Plessier A, Hernandez-Guerra M, Fabris F, Eapen CE, Bahr MJ, Trebicka J, Morard I, Lasser L, Heller J, Hadengue A, Langlet P, Miranda H, Primignani M, Elias E, Leebeek FW, Rosendaal FR, Garcia-Pagan JC, Valla DC, Janssen HL; EN-Vie (European Network for Vascular Disorders of the Liver). Etiology, management, and outcome of the Budd-Chiari syndrome. *Ann Intern Med* 2009; **151**: 167-175 [PMID: 19652186 DOI: 10.7326/0003-4819-151-3-200908040-00004]
7 **Faraoun SA**, Boudjella Mel A, Debzi N, Afredj N, Guerrache Y, Benidir N, Bouzid C, Bentabak K, Soyer P, Bendib SE. Budd-Chiari syndrome: a prospective analysis of hepatic vein obstruction on ultrasonography, multidetector-row computed tomography and MR imaging. *Abdom Imaging* 2015; **40**: 1500-1509 [PMID: 25687630 DOI: 10.1007/s00261-015-0380-5]
8 **Ferral H**, Behrens G, Lopera J. Budd-Chiari syndrome. *AJR Am J Roentgenol* 2012; **199**: 737-745 [PMID: 22997363 DOI: 10.2214/AJR.12.9098]
9 **Menon KVN**, Shah V, Kamath PS. The Budd–Chiari Syndrome. *N Engl J Med* 2004; **350**: 578-585 [PMID: 14762185 DOI: 10.1056/NEJMra020282]
10 **Ponziani FR**, Zocco MA, Campanale C, Rinninella E, Tortora A, Di Maurizio L, Bombardieri G, De Cristofaro R, De Gaetano AM, Landolfi R, Gasbarrini A. Portal vein thrombosis: insight into physiopathology, diagnosis, and treatment. *World J Gastroenterol* 2010; **16**: 143-155 [PMID: 20066733 DOI: 10.3748/wjg.v16.i2.143]
11 **Plessier A**, Valla DC. Budd-Chiari syndrome. *Semin Liver Dis* 2008; **28**: 259-269 [PMID: 18814079 DOI: 10.1055/s-0028-1085094]
12 **Li Y**, De Stefano V, Li H, Zheng K, Bai Z, Guo X, Qi X. Epidemiology of Budd-Chiari syndrome: A systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2019; **43**: 468-474 [PMID: 30528513 DOI: 10.1016/j.clinre.2018.10.014]
13 **Wang ZG**, Zhang FJ, Yi MQ, Qiang LX. Evolution of management for Budd-Chiari syndrome: a team's view from 2564 patients. *ANZ J Surg* 2005; **75**: 55-63 [PMID: 15740519 DOI: 10.1111/j.1445-2197.2005.03135.x]
14 **Zhang W**, Qi X, Zhang X, Su H, Zhong H, Shi J, Xu K. Budd-Chiari Syndrome in China: A Systematic Analysis of Epidemiological Features Based on the Chinese Literature Survey. *Gastroenterol Res Pract* 2015; **2015**: 738548 [PMID: 26504461 DOI: 10.1155/2015/738548]
15 **Ollivier-Hourmand I**, Allaire M, Goutte N, Morello R, Chagneau-Derrode C, Goria O, Dumortier J, Cervoni JP, Dharancy S, Ganne-Carrié N, Bureau C, Carbonell N, Abergel A, Nousbaum JB, Anty R, Barraud H, Ripault MP, De Ledinghen V, Minello A, Oberti F, Radenne S, Bendersky N, Farges O, Archambeaud I, Guillygomarc'h A, Ecochard M, Ozenne V, Hilleret MN, Nguyen-Khac E, Dauvois B, Perarnau JM, Lefilliatre P, Raabe JJ, Doffoel M, Becquart JP, Saillard E, Valla D, Dao T, Plessier A; French Network for Vascular Disorders of the Liver. The epidemiology of Budd-Chiari syndrome in France. *Dig Liver Dis* 2018; **50**: 931-937 [PMID: 29803757 DOI: 10.1016/j.dld.2018.04.004]
16 **Shin N**, Kim YH, Xu H, Shi HB, Zhang QQ, Colon Pons JP, Kim D, Xu Y, Wu FY, Han S, Lee BB, Li LS. Redefining Budd-Chiari syndrome: A systematic review. *World J Hepatol* 2016; **8**: 691-702 [PMID: 27326316 DOI:

10.4254/wjh.v8.i16.691]
17 **Qi X**, Wu F, Ren W, He C, Yin Z, Niu J, Bai M, Yang Z, Wu K, Fan D, Han G. Thrombotic risk factors in Chinese Budd-Chiari syndrome patients. An observational study with a systematic review of the literature. *Thromb Haemost* 2013; **109**: 878-884 [PMID: 23447059 DOI: 10.1160/TH12-10-0784]
18 **Aydinli M**, Bayraktar Y. Budd-Chiari syndrome: etiology, pathogenesis and diagnosis. *World J Gastroenterol* 2007; **13**: 2693-2696 [PMID: 17569137 DOI: 10.3748/wjg.v13.i19.2693]
19 **Mahmoud AE**, Helmy AS, Billingham L, Elias E. Poor prognosis and limited therapeutic options in patients with Budd-Chiari syndrome and portal venous system thrombosis. *Eur J Gastroenterol Hepatol* 1997; **9**: 485-489 [PMID: 9187882 DOI: 10.1097/00042737-199705000-00014]
20 **Dacha S**, Devidi M, Osmundson E. Budd-Chiari syndrome in a patient with ulcerative colitis and no inherited coagulopathy. *World J Hepatol* 2011; **3**: 164-169 [PMID: 21860676 DOI: 10.4254/wjh.v3.i6.164]
21 **Meena DS**, Sonwal VS, Bohra GK, Balesa J, Rohila AK. Celiac disease with Budd-Chiari syndrome: A rare association. *SAGE Open Med Case Rep* 2019; **7**: 2050313X19842697 [PMID: 31001425 DOI: 10.1177/2050313X19842697]
22 **Bayraktar Y**, Balkanci F, Bayraktar M, Calguneri M. Budd-Chiari syndrome: A common complication of Behçet's disease. *Am J Gastroenterol* 1997; **92**: 858-862 [PMID: 9149201]
23 **Anagnostopoulos GK**, Margantinis G, Kostopoulos P, Papadopoulou G, Roulias A, Sakorafas G, Liassis N. Budd-Chiari syndrome and portal vein thrombosis due to right atrial myxoma. *Ann Thorac Surg* 2004; **78**: 333-334 [PMID: 15223461 DOI: 10.1016/S0003-4975(03)01265-7]
24 **Neelakantan S**, Babu AA, Anandarajan R, Philip B. Hepatic hydatid disease presenting as secondary Budd-Chiari syndrome. *BMJ Case Rep* 2016; **2016** [PMID: 27681355 DOI: 10.1136/bcr-2016-217118]
25 **de Menezes Neves PDM**, Balbo BEP, Watanabe EH, Rocha-Santos V, Andraus W, D'Albuquerque LAC, Onuchic LF. Functional Budd-Chiari Syndrome Associated With Severe Polycystic Liver Disease. *Clin Med Insights Gastroenterol* 2017; **10**: 1179552217713003 [PMID: 28611533 DOI: 10.1177/1179552217713003]
26 **Karadag O**, Akinci D, Aksoy DY, Bayraktar Y. Acute Budd-Chiari syndrome resulting from a pyogenic liver abscess. *Hepatogastroenterology* 2005; **52**: 1554-1556 [PMID: 16201117]
27 **Mahmoud AE**, Mendoza A, Meshikhes AN, Olliff S, West R, Neuberger J, Buckels J, Wilde J, Elias E. Clinical spectrum, investigations and treatment of Budd-Chiari syndrome. *QJM* 1996; **89**: 37-43 [PMID: 8730341 DOI: 10.1093/oxfordjournals.qjmed.a030135]
28 **Smalberg JH**, Arends LR, Valla DC, Kiladjian JJ, Janssen HL, Leebeek FW. Myeloproliferative neoplasms in Budd-Chiari syndrome and portal vein thrombosis: a meta-analysis. *Blood* 2012; **120**: 4921-4928 [PMID: 23043069 DOI: 10.1182/blood-2011-09-376517]
29 **Palandri F**, Latagliata R, Polverelli N, Tieghi A, Crugnola M, Martino B, Perricone M, Breccia M, Ottaviani E, Testoni N, Merli F, Aversa F, Alimena G, Cavo M, Martinelli G, Catani L, Baccarani M, Vianelli N. Mutations and long-term outcome of 217 young patients with essential thrombocythemia or early primary myelofibrosis. *Leukemia* 2015; **29**: 1344-1349 [PMID: 25801912 DOI: 10.1038/LEU.2015.87]
30 **Kralovics R**, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; **352**: 1779-1790 [PMID: 15858187 DOI: 10.1056/nejmoa051113]
31 **Smalberg JH**, Darwish Murad S, Braakman E, Valk PJ, Janssen HL, Leebeek FW. Myeloproliferative disease in the pathogenesis and survival of Budd-Chiari syndrome. *Haematologica* 2006; **91**: 1712-1713 [PMID: 17145613 DOI: 10.1182/blood.v108.11.1480.1480]
32 **Ibarrola C**, Castellano VM, Colina F. Focal hyperplastic hepatocellular nodules in hepatic venous outflow obstruction: a clinicopathological study of four patients and 24 nodules. *Histopathology* 2004; **44**: 172-179 [PMID: 14764061 DOI: 10.1111/j.1365-2559.2004.01795.x]
33 **Tanaka M**, Wanless IR. Pathology of the liver in Budd-Chiari syndrome: portal vein thrombosis and the histogenesis of veno-centric cirrhosis, veno-portal cirrhosis, and large regenerative nodules. *Hepatology* 1998; **27**: 488-496 [PMID: 9462648 DOI: 10.1002/hep.510270224]
34 **Lefkowitch JH**, Mendez L. Morphologic features of hepatic injury in cardiac disease and shock. *J Hepatol* 1986; **2**: 313-327 [PMID: 3722787 DOI: 10.1016/S0168-8278(86)80043-5]
35 **Cazals-Hatem D**, Vilgrain V, Genin P, Denninger MH, Durand F, Belghiti J, Valla D, Degott C. Arterial and portal circulation and parenchymal changes in Budd-Chiari syndrome: a study in 17 explanted livers. *Hepatology* 2003; **37**: 510-519 [PMID: 12601347 DOI: 10.1053/jhep.2003.50076]
36 **Gonzalez RS**, Gilger MA, Huh WJ, Washington MK. The Spectrum of Histologic Findings in Hepatic Outflow Obstruction. *Arch Pathol Lab Med* 2017; **141**: 98-103 [PMID: 27681331 DOI: 10.5858/arpa.2015-0388-OA]
37 **Brunt EM**, Gouw AS, Hubscher SG, Tiniakos DG, Bedossa P, Burt AD, Callea F, Clouston AD, Dienes HP, Goodman ZD, Roberts EA, Roskams T, Terracciano L, Torbenson MS, Wanless IR. Pathology of the liver sinusoids. *Histopathology* 2014; **64**: 907-920 [PMID: 24393125 DOI: 10.1111/his.12364]
38 **Arcidi JM**, Moore GW, Hutchins GM. Hepatic morphology in cardiac dysfunction. A clinicopathologic study of 1000 subjects at autopsy. *Am J Pathol* 1981; **104**: 159-166 [PMID: 6455066]
39 **Ghaferi AA**, Hutchins GM. Progression of liver pathology in patients undergoing the Fontan procedure: Chronic passive congestion, cardiac cirrhosis, hepatic adenoma, and hepatocellular carcinoma. *J Thorac Cardiovasc Surg* 2005; **129**: 1348-1352 [PMID: 15942576 DOI: 10.1016/j.jtcvs.2004.10.005]
40 **DeLeve LD**, Valla DC, Garcia-Tsao G; American Association for the Study Liver Diseases. Vascular disorders of the liver. *Hepatology* 2009; **49**: 1729-1764 [PMID: 19399912 DOI: 10.1002/hep.22772]
41 **Landolfi R**, Rocca B, Patrono C. Bleeding and thrombosis in myeloproliferative disorders: mechanisms and treatment. *Crit Rev Oncol Hematol* 1995; **20**: 203-222 [PMID: 8748010 DOI: 10.1016/1040-8428(94)00164-O]
42 **Landolfi R**, Di Gennaro L, Falanga A. Thrombosis in myeloproliferative disorders: pathogenetic facts and speculation. *Leukemia* 2008; **22**: 2020-2028 [PMID: 18800144 DOI: 10.1038/leu.2008.253]
43 **Pearson TC**, Wetherley-Mein G. Vascular occlusive episodes and venous haematocrit in primary proliferative

polycythaemia. *Lancet* 1978; **2**: 1219-1222 [PMID: 82733 DOI: 10.1016/S0140-6736(78)92098-6]
44 **Li M**, De Stefano V, Song T, Zhou X, Guo Z, Zhu J, Qi X. Prevalence of CALR mutations in splanchnic vein thrombosis: A systematic review and meta-analysis. *Thromb Res* 2018; **167**: 96-103 [PMID: 29803161 DOI: 10.1016/j.thromres.2018.05.007]
45 **De Stefano V**, Qi X, Betti S, Rossi E. Splanchnic vein thrombosis and myeloproliferative neoplasms: molecular-driven diagnosis and long-term treatment. *Thromb Haemost* 2016; **115**: 240-249 [PMID: 26333846 DOI: 10.1160/TH15-04-0326]
46 **Chaubal N**, Dighe M, Hanchate V, Thakkar H, Deshmukh H, Rathod K. Sonography in Budd-Chiari syndrome. *J Ultrasound Med* 2006; **25**: 373-379 [PMID: 16495498 DOI: 10.7863/jum.2006.25.3.373]
47 **Bansal V**, Gupta P, Sinha S, Dhaka N, Kalra N, Vijayvergiya R, Dutta U, Kochhar R. Budd-Chiari syndrome: imaging review. *Br J Radiol* 2018; **91**: 20180441 [PMID: 30004805 DOI: 10.1259/bjr.20180441]
48 **Patil P**, Deshmukh H, Popat B, Rathod K. Spectrum of imaging in Budd Chiari syndrome. *J Med Imaging Radiat Oncol* 2012; **56**: 75-83 [PMID: 22339749 DOI: 10.1111/j.1754-9485.2012.02341.x]
49 **Liu L**, Qi XS, Zhao Y, Chen H, Meng XC, Han GH. Budd-Chiari syndrome: Current perspectives and controversies. *Eur Rev Med Pharmacol Sci* 2016; **20**: 3273-3281 [PMID: 27467004]
50 **Falcão CK**, Fagundes GC, Lamos GC, Felipe-Silva A, Lovisolo SM, Martines JA, de Campos FP. Budd-Chiari Syndrome: an unnoticed diagnosis. *Autops Case Rep* 2015; **5**: 17-25 [PMID: 26484330 DOI: 10.4322/acr.2015.009]
51 **Okuda K**. Obliterative hepatocavopathy-inferior vena cava thrombosis at its hepatic portion. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 499-509 [PMID: 14607675]
52 **Sharma A**, Keshava SN, Eapen A, Elias E, Eapen CE. An Update on the Management of Budd-Chiari Syndrome. *Dig Dis Sci* 2021; **66**: 1780-1790 [PMID: 32691382 DOI: 10.1007/s10620-020-06485-y]
53 **Lupasco I**, Dumbrava VT. Diagnosis and therapy of Budd Chiari syndrome. *Med Pharm Rep* 2021; **94**: S68-S71 [PMID: 34527916 DOI: 10.15386/mpr-2235]
54 **Gavriilidis P**, Marangoni G, Ahmad J, Azoulay D. State of the Art, Current Perspectives, and Controversies of Budd-Chiari Syndrome: A Review. *J Clin Med Res* 2022; **14**: 147-157 [PMID: 35573933 DOI: 10.14740/jocmr4724]
55 **Parekh J**, Matei VM, Canas-Coto A, Friedman D, Lee WM; Acute Liver Failure Study Group. Budd-chiari syndrome causing acute liver failure: A multicenter case series. *Liver Transpl* 2017; **23**: 135-142 [PMID: 27656864 DOI: 10.1002/lt.24643]
56 **Shukla A**, Shreshtha A, Mukund A, Bihari C, Eapen CE, Han G, Deshmukh H, Cua IHY, Lesmana CRA, Al Meshtab M, Kage M, Chaiteeraki R, Treeprasertsuk S, Giri S, Punamiya S, Paradis V, Qi X, Sugawara Y, Abbas Z, Sarin SK. Budd-Chiari syndrome: consensus guidance of the Asian Pacific Association for the study of the liver (APASL). *Hepatol Int* 2021; **15**: 531-567 [PMID: 34240318 DOI: 10.1007/s12072-021-10189-4]
57 **Valla DC**. Hepatic vein thrombosis (Budd-Chiari syndrome). *Semin Liver Dis* 2002; **22**: 5-14 [PMID: 11928075 DOI: 10.1055/s-2002-23202]
58 **Abdel Hameed MR**, Elbeih EAS, Abd El-Aziz HM, Afifi OA, Khalaf LMR, Ali Abu Rahma MZ, Sabry A. Epidemiological Characteristics and Etiology of Budd-Chiari Syndrome in Upper Egypt. *J Blood Med* 2020; **11**: 515-524 [PMID: 33408545 DOI: 10.2147/JBM.S278678]
59 **Zhang W**, Wang QZ, Chen XW, Zhong HS, Zhang XT, Chen XD, Xu K. Budd-Chiari syndrome in China: A 30-year retrospective study on survival from a single center. *World J Gastroenterol* 2018; **24**: 1134-1143 [PMID: 29563757 DOI: 10.3748/wjg.v24.i10.1134]
60 **Okuda H**, Yamagata H, Obata H, Iwata H, Sasaki R, Imai F, Okudaira M, Ohbu M, Okuda K. Epidemiological and clinical features of Budd-Chiari syndrome in Japan. *J Hepatol* 1995; **22**: 1-9 [PMID: 7751574 DOI: 10.1016/0168-8278(95)80252-5]
61 **Alukal JJ**, Zhang T, Thuluvath PJ. A Nationwide Analysis of Budd-Chiari Syndrome in the United States. *J Clin Exp Hepatol* 2021; **11**: 181-187 [PMID: 33746442 DOI: 10.1016/j.jceh.2020.08.005]
62 **Afredj N**, Guessab N, Nani A, Faraoun SA, Ouled Cheikh I, Kerbouche R, Hannoun D, Amir ZC, Ait Kaci H, Bentabak K, Plessier A, Valla DC, Cazals-Hatem V, Denninger MH, Boucekkine T, Debzi N. Aetiological factors of Budd-Chiari syndrome in Algeria. *World J Hepatol* 2015; **7**: 903-909 [PMID: 25937867 DOI: 10.4254/wjh.v7.i6.903]
63 **Vannucchi AM**. Insights into the pathogenesis and management of thrombosis in polycythemia vera and essential thrombocythemia. *Intern Emerg Med* 2010; **5**: 177-184 [PMID: 19789961 DOI: 10.1007/s11739-009-0319-3]
64 **Denninger MH**, Chaït Y, Casadevall N, Hillaire S, Guillin MC, Bezeaud A, Erlinger S, Briere J, Valla D. Cause of portal or hepatic venous thrombosis in adults: the role of multiple concurrent factors. *Hepatology* 2000; **31**: 587-591 [PMID: 10706547 DOI: 10.1002/hep.510310307]
65 **Ponnatapura J**, Kielar A, Burke LMB, Lockhart ME, Abualruz AR, Tappouni R, Lalwani N. Hepatic complications of oral contraceptive pills and estrogen on MRI: Controversies and update - Adenoma and beyond. *Magn Reson Imaging* 2019; **60**: 110-121 [PMID: 31009688 DOI: 10.1016/j.mri.2019.04.010]
66 **Valla D**, Le MG, Poynard T, Zucman N, Rueff B, Benhamou JP. Risk of hepatic vein thrombosis in relation to recent use of oral contraceptives. A case-control study. *Gastroenterology* 1986; **90**: 807-811 [PMID: 3949113 DOI: 10.1016/0016-5085(86)90855-3]
67 **Valla D**, Dhumeaux D, Babany G, Hillon P, Rueff B, Rochant H, Benhamou JP. Hepatic vein thrombosis in paroxysmal nocturnal hemoglobinuria. A spectrum from asymptomatic occlusion of hepatic venules to fatal Budd-Chiari syndrome. *Gastroenterology* 1987; **93**: 569-575 [PMID: 3609666 DOI: 10.1016/0016-5085(87)90920-6]
68 **Altunayoglu V**, Turedi S, Gunduz A, Karaca Y, Akdogan RA. Cerebral venous thrombosis and hepatic venous thrombosis during pregnancy. *J Obstet Gynaecol Res* 2007; **33**: 78-82 [PMID: 17212671 DOI: 10.1111/j.1447-0756.2007.00479.x]
69 **Khuroo MS**, Datta DV. Budd-Chiari syndrome following pregnancy. Report of 16 cases, with roentgenologic, hemodynamic and histologic studies of the hepatic outflow tract. *Am J Med* 1980; **68**: 113-121 [PMID: 7350798 DOI: 10.1016/0002-9343(80)90180-1]
70 **Ren W**, Qi X, Yang Z, Han G, Fan D. Prevalence and risk factors of hepatocellular carcinoma in Budd-Chiari syndrome:

a systematic review. *Eur J Gastroenterol Hepatol* 2013; **25**: 830-841 [PMID: 23411869 DOI: 10.1097/MEG.0b013e32835eb8d4]

71 **Janssen HL**, Garcia-Pagan JC, Elias E, Mentha G, Hadengue A, Valla DC; European Group for the Study of Vascular Disorders of the Liver. Budd-Chiari syndrome: a review by an expert panel. *J Hepatol* 2003; **38**: 364-371 [PMID: 12586305 DOI: 10.1016/S0168-8278(02)00434-8]

72 **Hitawala AA**, Gupta V. Budd Chiari Syndrome. 2022 Feb 9. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan- [PMID: 32644367]

73 **Niknam R**, Hajizadegan N, Mohammadkarimi V, Mahmoudi L. A study of the different parameters in acute and chronic Budd–Chiari syndrome. *Egypt Liver J* 2020; 10: 1-8 [DOI: 10.1186/s43066-020-00058-4]

74 **Gupta P**, Bansal V, Kumar-M P, Sinha SK, Samanta J, Mandavdhare H, Sharma V, Dutta U, Kochhar R. Diagnostic accuracy of Doppler ultrasound, CT and MRI in Budd Chiari syndrome: systematic review and meta-analysis. *Br J Radiol* 2020; **93**: 20190847 [PMID: 32150462 DOI: 10.1259/bjr.20190847]

75 **Millener P**, Grant EG, Rose S, Duerinckx A, Schiller VL, Tessler FN, Perrella RR, Ragavendra N. Color Doppler imaging findings in patients with Budd-Chiari syndrome: correlation with venographic findings. *AJR Am J Roentgenol* 1993; **161**: 307-312 [PMID: 8333368 DOI: 10.2214/ajr.161.2.8333368]

76 **Mukund A**, Gamanagatti S. Imaging and interventions in Budd-Chiari syndrome. *World J Radiol* 2011; **3**: 169-177 [PMID: 21860712 DOI: 10.4329/wjr.v3.i7.169]

77 **Raju GS**, Felver M, Olin JW, Satti SD. Thrombolysis for acute Budd-Chiari syndrome: Case report and literature review. *Am J Gastroenterol* 1996; **91**: 1262-1263 [PMID: 8651186]

78 **Sharma S**, Texeira A, Texeira P, Elias E, Wilde J, Olliff SP. Pharmacological thrombolysis in Budd Chiari syndrome: a single centre experience and review of the literature. *J Hepatol* 2004; **40**: 172-180 [PMID: 14672630 DOI: 10.1016/j.jhep.2003.09.028]

79 **Fisher NC**, McCafferty I, Dolapci M, Wali M, Buckels JA, Olliff SP, Elias E. Managing Budd-Chiari syndrome: a retrospective review of percutaneous hepatic vein angioplasty and surgical shunting. *Gut* 1999; **44**: 568-574 [PMID: 10075967 DOI: 10.1136/gut.44.4.568]

80 **Eapen CE**, Velissaris D, Heydtmann M, Gunson B, Olliff S, Elias E. Favourable medium term outcome following hepatic vein recanalisation and/or transjugular intrahepatic portosystemic shunt for Budd Chiari syndrome. *Gut* 2006; **55**: 878-884 [PMID: 16174658 DOI: 10.1136/gut.2005.071423]

81 **Khan F**, Mehrzad H, Tripathi D. Timing of Transjugular Intrahepatic Portosystemic Stent-shunt in Budd-Chiari Syndrome: A UK Hepatologist's Perspective. *J Transl Int Med* 2018; **6**: 97-104 [PMID: 30425945 DOI: 10.2478/jtim-2018-0022]

82 **Seijo S**, Plessier A, Hoekstra J, Dell'era A, Mandair D, Rifai K, Trebicka J, Morard I, Lasser L, Abraldes JG, Darwish Murad S, Heller J, Hadengue A, Primignani M, Elias E, Janssen HL, Valla DC, Garcia-Pagan JC; European Network for Vascular Disorders of the Liver. Good long-term outcome of Budd-Chiari syndrome with a step-wise management. *Hepatology* 2013; **57**: 1962-1968 [PMID: 23389867 DOI: 10.1002/hep.26306]

83 **Tripathi D**, Macnicholas R, Kothari C, Sunderraj L, Al-Hilou H, Rangarajan B, Chen F, Mangat K, Elias E, Olliff S. Good clinical outcomes following transjugular intrahepatic portosystemic stent-shunts in Budd-Chiari syndrome. *Aliment Pharmacol Ther* 2014; **39**: 864-872 [PMID: 24611957 DOI: 10.1111/apt.12668]

84 **Fitsiori K**, Tsitskari M, Kelekis A, Filippiadis D, Triantafyllou K, Brountzos E. Transjugular intrahepatic portosystemic shunt for the treatment of Budd-Chiari syndrome patients: results from a single center. *Cardiovasc Intervent Radiol* 2014; **37**: 691-697 [PMID: 23860938 DOI: 10.1007/s00270-013-0697-9]

85 **Qi X**, Yang M, Fan D, Han G. Transjugular intrahepatic portosystemic shunt in the treatment of Budd-Chiari syndrome: a critical review of literatures. *Scand J Gastroenterol* 2013; **48**: 771-784 [PMID: 23506234 DOI: 10.3109/00365521.2013.777775]

86 **Sonavane AD**, Amarapurkar DN, Rathod KR, Punamiya SJ. Long Term Survival of Patients Undergoing TIPS in Budd-Chiari Syndrome. *J Clin Exp Hepatol* 2019; **9**: 56-61 [PMID: 30765940 DOI: 10.1016/j.jceh.2018.02.008]

87 **Aktas H**, Ozer A, Yilmaz TU, Keceoglu S, Can MG, Emiroglu R. Liver transplantation for Budd-Chiari syndrome: A challenging but handable procedure. *Asian J Surg* 2022; **45**: 1396-1402 [PMID: 34688524 DOI: 10.1016/j.asjsur.2021.09.007]

88 **Akamatsu N**, Sugawara Y, Kokudo N. Budd-Chiari syndrome and liver transplantation. *Intractable Rare Dis Res* 2015; **4**: 24-32 [PMID: 25674385 DOI: 10.5582/irdr.2014.01031]

89 **Ibach M**, Eurich D, Dobrindt E, Lurje G, Schöning W, Öllinger R, Pratschke J, Globke B. Orthotopic Liver Transplantation for Budd-Chiari Syndrome: Observations from a 30-Year Liver Transplant Program. *Medicina (Kaunas)* 2021; **57** [PMID: 34441027 DOI: 10.3390/medicina57080821]

90 **Verstovsek S**, Yu J, Scherber RM, Verma S, Dieyi C, Chen CC, Parasuraman S. Changes in the incidence and overall survival of patients with myeloproliferative neoplasms between 2002 and 2016 in the United States. *Leuk Lymphoma* 2022; **63**: 694-702 [PMID: 34689695 DOI: 10.1080/10428194.2021.1992756]

91 **Smalberg JH**, Spaander MV, Jie KS, Pattynama PM, van Buuren HR, van den Berg B, Janssen HL, Leebeek FW. Risks and benefits of transcatheter thrombolytic therapy in patients with splanchnic venous thrombosis. *Thromb Haemost* 2008; **100**: 1084-1088 [PMID: 19132234]

92 **Zhang Q**, Xu H, Zu M, Gu Y, Wei N, Wang W, Gao Z, Shen B. Catheter-directed thrombolytic therapy combined with angioplasty for hepatic vein obstruction in Budd-Chiari syndrome complicated by thrombosis. *Exp Ther Med* 2013; **6**: 1015-1021 [PMID: 24137308 DOI: 10.3892/etm.2013.1239]

93 **Mancuso A**. An update on the management of Budd-Chiari syndrome: the issues of timing and choice of treatment. *Eur J Gastroenterol Hepatol* 2015; **27**: 200-203 [PMID: 25590783 DOI: 10.1097/MEG.0000000000000282]

94 **Senzolo M**, Riggio O, Primignani M; Italian Association for the Study of the Liver (AISF) ad hoc. Vascular disorders of the liver: recommendations from the Italian Association for the Study of the Liver (AISF) ad hoc committee. *Dig Liver Dis* 2011; **43**: 503-514 [PMID: 21185794 DOI: 10.1016/j.dld.2010.11.006]

95 **Zeitoun G**, Escolano S, Hadengue A, Azar N, El Younsi M, Mallet A, Boudet MJ, Hay JM, Erlinger S, Benhamou JP,

Belghiti J, Valla D. Outcome of Budd-Chiari syndrome: a multivariate analysis of factors related to survival including surgical portosystemic shunting. *Hepatology* 1999; **30**: 84-89 [PMID: 10385643 DOI: 10.1002/hep.510300125]

96 **de Franchis R**, Baveno VI Faculty. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: 26047908 DOI: 10.1016/j.jhep.2015.05.022]

97 **Hadengue A**, Poliquin M, Vilgrain V, Belghiti J, Degott C, Erlinger S, Benhamou JP. The changing scene of hepatic vein thrombosis: recognition of asymptomatic cases. *Gastroenterology* 1994; **106**: 1042-1047 [PMID: 8143970 DOI: 10.1016/0016-5085(94)90765-X]

98 **Mentha G**, Giostra E, Majno PE, Bechstein WO, Neuhaus P, O'Grady J, Praseedom RK, Burroughs AK, Le Treut YP, Kirkegaard P, Rogiers X, Ericzon BG, Hockerstedt K, Adam R, Klempnauer J. Liver transplantation for Budd-Chiari syndrome: A European study on 248 patients from 51 centres. *J Hepatol* 2006; **44**: 520-528 [PMID: 16427719 DOI: 10.1016/j.jhep.2005.12.002]

99 **Ageno W**, Riva N, Schulman S, Beyer-Westendorf J, Bang SM, Senzolo M, Grandone E, Pasca S, Di Minno MN, Duce R, Malato A, Santoro R, Poli D, Verhamme P, Martinelli I, Kamphuisen P, Oh D, D'Amico E, Becattini C, De Stefano V, Vidili G, Vaccarino A, Nardo B, Di Nisio M, Dentali F. Long-term Clinical Outcomes of Splanchnic Vein Thrombosis: Results of an International Registry. *JAMA Intern Med* 2015; **175**: 1474-1480 [PMID: 26168152 DOI: 10.1001/jamainternmed.2015.3184]

100 **Westbrook RH**, Lea NC, Mohamedali AM, Smith AE, Orr DW, Roberts LN, Heaton ND, Wendon JA, O'Grady JG, Heneghan MA, Mufti GJ. Prevalence and clinical outcomes of the 46/1 haplotype, Janus kinase 2 mutations, and ten-eleven translocation 2 mutations in Budd-Chiari syndrome and their impact on thrombotic complications post liver transplantation. *Liver Transpl* 2012; **18**: 819-827 [PMID: 22467227 DOI: 10.1002/lt.23443]

101 **De Stefano V**, Vannucchi AM, Ruggeri M, Cervantes F, Alvarez-Larrán A, Iurlo A, Randi ML, Pieri L, Rossi E, Guglielmelli P, Betti S, Elli E, Finazzi MC, Finazzi G, Zetterberg E, Vianelli N, Gaidano G, Nichele I, Cattaneo D, Palova M, Ellis MH, Cacciola E, Tieghi A, Hernandez-Boluda JC, Pungolino E, Specchia G, Rapezzi D, Forcina A, Musolino C, Carobbio A, Griesshammer M, Barbui T. Splanchnic vein thrombosis in myeloproliferative neoplasms: risk factors for recurrences in a cohort of 181 patients. *Blood Cancer J* 2016; **6**: e493 [PMID: 27813534 DOI: 10.1038/bcj.2016.103]

102 **De Gottardi A**, Trebicka J, Klinger C, Plessier A, Seijo S, Terziroli B, Magenta L, Semela D, Buscarini E, Langlet P, Görtzen J, Puente A, Müllhaupt B, Navascuès C, Nery F, Deltenre P, Turon F, Engelmann C, Arya R, Caca K, Peck-Radosavljevic M, Leebeek FWG, Valla D, Garcia-Pagan JC; VALDIG Investigators. Antithrombotic treatment with direct-acting oral anticoagulants in patients with splanchnic vein thrombosis and cirrhosis. *Liver Int* 2017; **37**: 694-699 [PMID: 27778440 DOI: 10.1111/liv.13285]

103 **Jones C**, Levy Y, Tong AW. Elevated serum erythropoietin in a patient with polycythaemia vera presenting with Budd-Chiari syndrome. *BMJ Case Rep* 2014; **2014** [PMID: 25452296 DOI: 10.1136/bcr-2014-205663]

104 **Semmler G**, Lindorfer A, Schäfer B, Bartl S, Hametner-Schreil S, Gensluckner S, Balcar L, Pomej K, Lampichler K, Trauner M, Aigner E, Datz C, Zoller H, Hofer H, Schöfl R, Mandorfer M, Reiberger T, Scheiner B. Outcome of Budd-Chiari Syndrome Patients Treated With Direct Oral Anticoagulants: An Austrian Multicenter Study. *Clin Gastroenterol Hepatol* 2022; S1542-3565(22)00449 [PMID: 35533994 DOI: 10.1016/j.cgh.2022.04.024]

105 **Barbui T**, Tefferi A, Vannucchi AM, Passamonti F, Silver RT, Hoffman R, Verstovsek S, Mesa R, Kiladjian JJ, Hehlmann R, Reiter A, Cervantes F, Harrison C, Mc Mullin MF, Hasselbalch HC, Koschmieder S, Marchetti M, Bacigalupo A, Finazzi G, Kroeger N, Griesshammer M, Birgegard G, Barosi G. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. *Leukemia* 2018; **32**: 1057-1069 [PMID: 29515238 DOI: 10.1038/s41375-018-0077-1]

106 **Dentali F**, Squizzato A, Brivio L, Appio L, Campiotti L, Crowther M, Grandi AM, Ageno W. JAK2V617F mutation for the early diagnosis of Ph- myeloproliferative neoplasms in patients with venous thromboembolism: a meta-analysis. *Blood* 2009; **113**: 5617-5623 [PMID: 19273837 DOI: 10.1182/blood-2008-12-196014]

107 **De Stefano V**, Ruggeri M, Cervantes F, Alvarez-Larrán A, Iurlo A, Randi ML, Elli E, Finazzi MC, Finazzi G, Zetterberg E, Vianelli N, Gaidano G, Rossi E, Betti S, Nichele I, Cattaneo D, Palova M, Ellis MH, Cacciola R, Tieghi A, Hernandez-Boluda JC, Pungolino E, Specchia G, Rapezzi D, Forcina A, Musolino C, Carobbio A, Griesshammer M, Sant'Antonio E, Vannucchi AM, Barbui T. High rate of recurrent venous thromboembolism in patients with myeloproliferative neoplasms and effect of prophylaxis with vitamin K antagonists. *Leukemia* 2016; **30**: 2032-2038 [PMID: 27113812 DOI: 10.1038/leu.2016.85]

108 **Colaizzo D**, Amitrano L, Guardascione MA, Tiscia GL, D'Andrea G, Longo VA, Grandone E, Margaglione M. Outcome of patients with splanchnic venous thrombosis presenting without overt MPN: a role for the JAK2 V617F mutation re-evaluation. *Thromb Res* 2013; **132**: e99-e104 [PMID: 23916380 DOI: 10.1016/j.thromres.2013.07.014]

109 **Chinnakotla S**, Klintmalm GB, Kim P, Tomiyama K, Klintmalm E, Davis GL, Trotter JF, Saad R, Landaverde C, Levy MF, Goldstein RM, Stone MJ. Long-term follow-up of liver transplantation for Budd-Chiari syndrome with antithrombotic therapy based on the etiology. *Transplantation* 2011; **92**: 341-345 [PMID: 21659946 DOI: 10.1097/TP.0b013e3182247b05]

110 **Oldakowska-Jedynak U**, Ziarkiewicz M, Ziarkiewicz-Wróblewska B, Dwilewicz-Trojaczek J, Górnicka B, Nyckowski P, Paluszkiewicz R, Wróblewski T, Zieniewicz K, Patkowski W, Pączek L, Jedrzejczak WW, Krawczyk M. Myeloproliferative neoplasms and recurrent thrombotic events in patients undergoing liver transplantation for Budd-Chiari syndrome: a single-center experience. *Ann Transplant* 2014; **19**: 591-597 [PMID: 25394736 DOI: 10.12659/AOT.890834]

111 **De Stefano V**, Rossi E, Carobbio A, Ghirardi A, Betti S, Finazzi G, Vannucchi AM, Barbui T. Hydroxyurea prevents arterial and late venous thrombotic recurrences in patients with myeloproliferative neoplasms but fails in the splanchnic venous district. Pooled analysis of 1500 cases. *Blood Cancer J* 2018; **8**: 112 [PMID: 30420642 DOI: 10.1038/s41408-018-0151-y]

112 **Chait Y**, Condat B, Cazals-Hatem D, Rufat P, Atmani S, Chaoui D, Guilmin F, Kiladjian JJ, Plessier A, Denninger MH, Casadevall N, Valla D, Brière JB. Relevance of the criteria commonly used to diagnose myeloproliferative disorder in patients with splanchnic vein thrombosis. *Br J Haematol* 2005; **129**: 553-560 [PMID: 15877740 DOI:

10.1111/j.1365-2141.2005.05490.x]
113 **Potthoff A**, Attia D, Pischke S, Mederacke I, Beutel G, Rifai K, Deterding K, Heiringhoff K, Klempnauer J, Strassburg CP, Manns MP, Bahr MJ. Long-term outcome of liver transplant patients with Budd-Chiari syndrome secondary to myeloproliferative neoplasms. *Liver Int* 2015; **35**: 2042-2049 [PMID: 25736096 DOI: 10.1111/liv.12816]
114 **Payancé A**, Plessier A. Anticoagulation for budd-chiari syndrome. *Budd-Chiari Syndr* 2019; 131-145 [DOI: 10.1007/978-981-32-9232-1_10/COVER]
115 **Mancuso A**, Fung K, Mela M, Tibballs J, Watkinson A, Burroughs AK, Patch D. TIPS for acute and chronic Budd-Chiari syndrome: a single-centre experience. *J Hepatol* 2003; **38**: 751-754 [PMID: 12763367 DOI: 10.1016/s0168-8278(03)00118-1]
116 **Tavill AS**, Wood EJ, Kreel L, Jones EA, Gregory M, Sherlock S. The Budd-Chiari syndrome: correlation between hepatic scintigraphy and the clinical, radiological, and pathological findings in nineteen cases of hepatic venous outflow obstruction. *Gastroenterology* 1975; **68**: 509-518 [PMID: 1112452 DOI: 10.1016/S0016-5085(75)80090-4]
117 **Adams HP Jr**. Emergent use of anticoagulation for treatment of patients with ischemic stroke. *Stroke* 2002; **33**: 856-861 [PMID: 11872915 DOI: 10.1161/hs0302.104628]
118 **Sandercock PAG**, Counsell C, Gubitz GJ, Tseng MC. Antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev* 2018; **16**: CD000029 [PMID: 18646056 DOI: 10.1002/14651858.CD000029.pub2]
119 **Pullicino P**, Thompson JL, Barton B, Levin B, Graham S, Freudenberger RS; WARCEF Investigators. Warfarin *vs* aspirin in patients with reduced cardiac ejection fraction (WARCEF): rationale, objectives, and design. *J Card Fail* 2006; **12**: 39-46 [PMID: 16500579 DOI: 10.1016/j.cardfail.2005.07.007]
120 **Adams HP Jr**, Bendixen BH, Leira E, Chang KC, Davis PH, Woolson RF, Clarke WR, Hansen MD. Antithrombotic treatment of ischemic stroke among patients with occlusion or severe stenosis of the internal carotid artery: A report of the Trial of Org 10172 in Acute Stroke Treatment (TOAST). *Neurology* 1999; **53**: 122-125 [PMID: 10408547 DOI: 10.1212/wnl.53.1.122]
121 **Deltenre P**, Denninger MH, Hillaire S, Guillin MC, Casadevall N, Brière J, Erlinger S, Valla DC. Factor V Leiden related Budd-Chiari syndrome. *Gut* 2001; **48**: 264-268 [PMID: 11156651 DOI: 10.1136/gut.48.2.264]
122 **Hernández-Guerra M**, Turnes J, Rubinstein P, Olliff S, Elias E, Bosch J, García-Pagán JC. PTFE-covered stents improve TIPS patency in Budd-Chiari syndrome. *Hepatology* 2004; **40**: 1197-1202 [PMID: 15486923 DOI: 10.1002/hep.20436]
123 **Iliescu L**, Toma L, Mercan-Stanciu A, Grumeza M, Dodot M, Isac T, Ioanitescu S. Budd-Chiari syndrome - various etiologies and imagistic findings. A pictorial review. *Med Ultrason* 2019; **21**: 344-348 [PMID: 31476215 DOI: 10.11152/mu-1921]
124 **Kiladjian JJ**, Cervantes F, Leebeek FW, Marzac C, Cassinat B, Chevret S, Cazals-Hatem D, Plessier A, Garcia-Pagan JC, Darwish Murad S, Raffa S, Janssen HL, Gardin C, Cereja S, Tonetti C, Giraudier S, Condat B, Casadevall N, Fenaux P, Valla DC. The impact of JAK2 and MPL mutations on diagnosis and prognosis of splanchnic vein thrombosis: a report on 241 cases. *Blood* 2008; **111**: 4922-4929 [PMID: 18250227 DOI: 10.1182/blood-2007-11-125328]
125 **Langlet P**, Escolano S, Valla D, Coste-Zeitoun D, Denie C, Mallet A, Levy VG, Franco D, Vinel JP, Belghiti J, Lebrec D, Hay JM, Zeitoun G. Clinicopathological forms and prognostic index in Budd-Chiari syndrome. *J Hepatol* 2003; **39**: 496-501 [PMID: 12971957 DOI: 10.1016/S0168-8278(03)00323-4]
126 **Darwish Murad S**, Valla DC, de Groen PC, Zeitoun G, Hopmans JA, Haagsma EB, van Hoek B, Hansen BE, Rosendaal FR, Janssen HL. Determinants of survival and the effect of portosystemic shunting in patients with Budd-Chiari syndrome. *Hepatology* 2004; **39**: 500-508 [PMID: 14768004 DOI: 10.1002/hep.20064]
127 **Janssen HL**, Wijnhoud A, Haagsma EB, van Uum SH, van Nieuwkerk CM, Adang RP, Chamuleau RA, van Hattum J, Vleggaar FP, Hansen BE, Rosendaal FR, van Hoek B. Extrahepatic portal vein thrombosis: aetiology and determinants of survival. *Gut* 2001; **49**: 720-724 [PMID: 11600478 DOI: 10.1136/gut.49.5.720]
128 **De Stefano V**, Za T, Rossi E, Vannucchi AM, Ruggeri M, Elli E, Micò C, Tieghi A, Cacciola RR, Santoro C, Gerli G, Vianelli N, Guglielmelli P, Pieri L, Scognamiglio F, Rodeghiero F, Pogliani EM, Finazzi G, Gugliotta L, Marchioli R, Leone G, Barbui T; GIMEMA CMD-Working Party. Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. *Haematologica* 2008; **93**: 372-380 [PMID: 18268279 DOI: 10.3324/haematol.12053]
129 **Asl AA**, Lankarani KB, Nikeghbalian S, Kazemi K, Shamsaieefar A, Alizade-Naini M, Fattahi MR, Taghavi SA, Niknam R, Ejtehadi F, Dehghan M, Sivandzadeh G, Ghahramani S, Malek-Hosseini SA. Post liver transplant complications of Budd-Chiari syndrome. *Indian J Gastroenterol* 2021; **40**: 281-286 [PMID: 33743161 DOI: 10.1007/s12664-020-01139-3]
130 **Ki M**, Choi HY, Kim KA, Kim BH, Jang ES, Jeong SH. Incidence, prevalence and complications of Budd-Chiari syndrome in South Korea: a nationwide, population-based study. *Liver Int* 2016; **36**: 1067-1073 [PMID: 26558363 DOI: 10.1111/liv.13008]
131 **Hayek G**, Ronot M, Plessier A, Sibert A, Abdel-Rehim M, Zappa M, Rautou PE, Valla D, Vilgrain V. Long-term Outcome and Analysis of Dysfunction of Transjugular Intrahepatic Portosystemic Shunt Placement in Chronic Primary Budd-Chiari Syndrome. *Radiology* 2017; **283**: 280-292 [PMID: 27797679 DOI: 10.1148/radiol.2016152641]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 March 24; 14(3): 117-130

DOI: 10.5306/wjco.v14.i3.117

ISSN 2218-4333 (online)

MINIREVIEWS

Immune microenvironment of medulloblastoma: The association between its molecular subgroups and potential targeted immunotherapeutic receptors

Maher Kurdi, Nasser Mulla, Husam Malibary, Ahmed K Bamaga, Motaz M Fadul, Eyad Faizo, Sahar Hakamy, Saleh Baeesa

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Mduma E, Tanzania; Ogino S, United States

Received: December 14, 2022
Peer-review started: December 14, 2022
First decision: January 20, 2023
Revised: February 8, 2023
Accepted: February 22, 2023
Article in press: February 22, 2023
Published online: March 24, 2023

Maher Kurdi, Motaz M Fadul, Department of Pathology, Faculty of Medicine, King Abdulaziz University, Rabigh 213733, Saudi Arabia

Maher Kurdi, Neuromuscular Unit, King Fahad Medical Research Center, King Abdulaziz University, Jeddah 213733, Saudi Arabia

Nasser Mulla, Department of Internal Medicine, Faculty of Medicine, Taibah University, Medina 213733, Saudi Arabia

Husam Malibary, Department of Internal Medicine, Faculty of Medicine, King Abdulaziz University, Jeddah 213733, Saudi Arabia

Ahmed K Bamaga, Department of Paediatrics, Faculty of Medicine, King Abdulaziz University, Jeddah 213733, Saudi Arabia

Eyad Faizo, Division of Neurosurgery, Department of Surgery, Faculty of Medicine, Tabuk University, Tabuk 213733, Saudi Arabia

Sahar Hakamy, Neurmuscular Unit, Center of Excellence of Genomic Medicine, Jeddah 21423, Saudi Arabia

Saleh Baeesa, Department of Neuroscience, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia

Corresponding author: Maher Kurdi, FRCPC, MD, Associate Professor, Department of Pathology, Faculty of Medicine in Rabigh, King Abdulaziz University, Rabigh 213733, Saudi Arabia. ahkurdi@kau.edu.sa

Abstract

Medulloblastoma (MB) is considered the commonest malignant brain tumor in children. Multimodal treatments consisting of surgery, radiation, and chemotherapy have improved patients' survival. Nevertheless, the recurrence occurs in 30% of cases. The persistent mortality rates, the failure of current therapies to extend life expectancy, and the serious complications of non-targeted cytotoxic treatment indicate the need for more refined therapeutic approaches. Most MBs originating from the neurons of external granular layer line the outer surface of

neocerebellum and responsible for the afferent and efferent connections. Recently, MBs have been segregated into four molecular subgroups: Wingless-activated (WNT-MB) (Group 1); Sonic-hedgehog-activated (SHH-MB) (Group 2); Group 3 and 4 MBs. These molecular alterations follow specific gene mutations and disease-risk stratifications. The current treatment protocols and ongoing clinical trials against these molecular subgroups are still using common chemotherapeutic agents by which their efficacy have improved the progression-free survival but did not change the overall survival. However, the need to explore new therapies targeting specific receptors in MB microenvironment became essential. The immune microenvironment of MBs consists of distinctive cellular heterogeneities including immune cells and none-immune cells. Tumour associate macrophage and tumour infiltrating lymphocyte are considered the main principal cells in tumour microenvironment, and their role are still under investigation. In this review, we discuss the mechanism of interaction between MB cells and immune cells in the microenvironment, with an overview of the recent investigations and clinical trials

Key Words: Medulloblastoma; Tumour microenvironment; Tumour associated macrophages; Tumour infiltrating lymphocyte; Immunotherapies

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Medulloblastoma (MB) is the most common malignant childhood tumor of the brain. Multimodal treatments consisting of surgery, radiation, and chemotherapy have reduced the cumulative incidence of late mortality. Nevertheless, the recurrence rate remains high. In this review, we discuss the mechanism of interaction between tumour cells of MB and immune cells in the microenvironment, with an overview of the recent investigations and clinical trials.

Citation: Kurdi M, Mulla N, Malibary H, Bamaga AK, Fadul MM, Faizo E, Hakamy S, Baeesa S. Immune microenvironment of medulloblastoma: The association between its molecular subgroups and potential targeted immunotherapeutic receptors. *World J Clin Oncol* 2023; 14(3): 117-130
URL: https://www.wjgnet.com/2218-4333/full/v14/i3/117.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i3.117

INTRODUCTION

Brain tumors are the leading cause of oncological death during childhood, and medulloblastoma (MB) is the commonest malignant tumor of the brain, accounting for 20%-30% of all central nervous system (CNS) tumors[1]. Diverse treatment modalities consisting of surgery and chemoradiotherapy have improved the patient's survival. Nevertheless, more than 1/3 of children with MB die within 5-years after diagnosis[2]. Late mortality remains a significant problem in disease consequences, which is attributed to tumour recurrence[3]. The persistent mortality, the failure of current drug therapies to extend life expectancy, and the serious complications of cytotoxic therapies indicate the necessity to explore new targeted treatments. Over the past decades, several tumor-centric studies have identified mutant genes and signaling pathways dysfunction that encourage MB growth. Most of MBs originate from the granular layer of cerebellum, which reside in the external granular layer and line the neocerebellum of newborns[4]. The existence of irregular biological signaling pathways created signaling dysregulation and genetic mutations affecting cerebellar development. Hence, the anatomical and cellular complexity of developing human tissues within the rhombic lip germinal zone produces glutamatergic neuronal lineages before its centralization. Molecular signatures encoded within a human rhombic-lip-derived lineage trajectory aligned with photoreceptor and unipolar cell profiles that are maintained in some medulloblastomas, suggesting a convergent basis. The advanced genomic studies over decades led to the assemblage of large amount of genetic information which resulted in four distinguishing molecular subgroups of MB including (Group 1) Wingless-activated (WNT-MB); (Group 2) Sonic-hedgehog-activated (SHH-MB); and Group 3 and Group 4[5] (Figure 1). Each group is characterized by distinct genetic abnormalities, methylation profiles, and clinical outcome. WNT- and SHH-type MBs are clearly detached from the other groups with lack of signaling pathway dysregulation identified in Group 3 and 4[5].

Molecular subgroups of MB

WNT-MB is the least common type, accounting for about 10%-15% of all MB patients. They are classically absent in infants and are seen more among children above 10 years of age[6-8] (Figure 1). The

Molecular subtype	WNT	SHH	Group 3	Group 4
Prevalence	10- 15 %	25%	25%	35%
Age	10-12 years old	< 16 years old	< 3 years old	Children
Gender	1:1	1:1	2:1	3:1
Location	Midline 4th ventricle	Cerebellar vermis	Midline 4th ventricle	Midline 4th ventricle
Pathology	Classic, rare LCA	DN, classic, LCA	Classic, rarely LCA	Classic, rarely LCA
Metastasis	5 - 10%	15-20%	45%	30-40%
Recurrence	Rare	Local	Metastatic	Metastatic
Common driver genetic mutation	1.CTNNB1 (90%)-WNT 2.DDX3X (50 %) 3.SMARCA4 (25%) 4.TP53 (<20 %)	1.TERT (83%) 2.PTCH1 (45%) -SHH 3.TP53 (15%) 4.SUFU (10 %) 5.SMO (rare) 6.MYCN (rare) 7.GLI2 (very rare)	1.GFI1(30 %) 2.MYC (10-20 %) 3.PVT1 (10 %) 4.SMARCA4 (rare) 5.OTX2 (very rare)	1. KDM6A (15 %) 2.SNCAIP (10%) 3.MYCN (5%) 4.CDK6 (rare) 5.GFI1 (very rare)
Chromosome alteration	Monosomy 6	Loss of 9q (PTCH1)	Isochromosome 17q	Isochromosome 17q
MYC status	+	+	+++	-
5-year survival	>90%	70%	40%	70-80%

DOI: 10.5306/wjco.v14.i3.117 **Copyright** ©The Author(s) 2023.

Figure 1 Molecular subgroups of medulloblastoma based on 2021 World Health Organization classification of central nervous system tumours. SHH: Sonic-hedgehog; MYC: Myelocytomatosis oncogene; LCA: Life cycle assessment; WNT: Wingless.

clinical outcome of the disease under 16-years of age is usually good, with 90% 5-year survival[8]. The genetic mutation of the Catenin Beta-1 (*CTNNB1*) gene is the most common genetic alteration accounting for 85% of all WNT-MBs[9,10]. A gene expression with methylation profiling performed on several MB cases in 2016 has divided WNT- MBs into two variants: WNT-α, which consists of patients with chromosome 6 monosomy and WNT-β, that occurs in adults with chromosomal diploidy[11,12]. *CTNNB1* mutation usually occurs with other chromatin remodeling mutations such as Cyclic Adenosine Monophosphate Response Element Binding Protein (*CREBBP*), Mediator Complex Subunit 13 (*MED13*) and subunits of the nucleosome-remodeling complex such as SWI Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 4 (*SMARCA4*), At-rich interaction Domain 1A (*ARID1A*) [9,10,13]. Most of WNT-MBs carries DEAD-Box Helicase 3 X-Linked (*DDX3X*) mutations, which participates in mRNA translation[12,14]. The germline mutation of antigen presenting cells (APC) on chromosome 5 as inherited Turcot syndrome and Anaplastic Lymphoma Kinase (*ALK*) gene also contribute to the development of WNT-MBs[9,15].

SHH-MB accounts for about 25% of all MBs with a 70% 5-years overall survival (OS). It is frequently seen in infants and adult patients[16,17]. The majority shows histologically nodular or desmoplastic morphology, which predicts a favourable prognosis[18]. *TP53* mutation segregates SHH-MBs into tumors with *TP53*-wildtype, often seen in young children and associated with favorable prognosis, and *TP53* mutant SHH-MB classically seen among older children and associated with poorer prognosis. SHH-MB with Protein Patch Homolog-1 (*PTCH1*) and Suppressor of Fused Homolog (*SUFU*) mutation are associated with Gorlin syndrome[19,20]. In children, *TP53* mutations frequently occurs with *GLI2* and *MYCN*-amplifications[9] (Figure 1).

Group 3 MB, a classical histological variant, accounts for 25% of all MBs and considered the deadliest subtype[7,21]. Tumours in this group with *MYC*-amplification carries a 20% risk of 5-years survival[22]. However, the most common cytogenetic abnormalities seen in Group 3 is the 17 ploss followed 16q and 9q losses[19]. Rare genetic variants in Group 3 MBs include Orthodenticle Homebox-2 (*OTX2*) and Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit (*EZH2*) amplifications and *SMARCA4* mutations[23] (Figure 1).

Group 4 MB is the most frequent type among all MBs and often occurs in male more than females[6]. Isochromosome 17q is the most common cytogenetic aberration seen in this group. Other genetic variants include the loss of chromosome 8p, 10q, and the aberrations of 11p and 18q[2,17]. The clinical outcome is better in patients with chromosome 11 loss with an OS above 90%[19]. Zhou *et al*[24] reported that around 40% of Group 4 patients showed metastasis and treated as a high-risk disease. As we mentioned before, Group 3 and Group 4 MBs are genetically heterogeneous and not associated with germline mutations[25].

Current treatment options in MB

The magnitude of surgical resection in MB may not be as significant as earlier. After surgery, patients are treated with radiotherapy of the whole spinal axis with an additional boost targeting the tumor margins[26]. Radiotherapy usually starts 20-30 d after surgery however, delay of radiation may increase

risk of recurrence and is therefore not recommended for patients older than 3 years[27,28]. Post-operative radiotherapy for children less than 3 years of old may increase risk of cognitive dysfunction [18]. Postoperative chemotherapy in MB patients is essential strategy to reduce the radiation effects and improve the survival, particularly in young children. The treatment varies based on the risk of drug toxicity and recurrence rate. Both risks are correlated with MB molecular alterations and considered as prognostic factors prior treatment. The risk of toxicity should be taken carefully in infants and children younger than three years of age while the recurrence is usually high in metastatic cases or cases undergoing subtotal resection. Anaplastic and large cell variants may have poor response and worsening outcome[29] (Figure 2). The high-risk group consists of SHH-MBs with *MYCN*-amplification; SHH-MB with metastatic dissemination and wildtype *TP53*, and metastatic Group 4 MBs[7]. High-risk population includes mutant *TP53* SHH-MB patients and metastatic Group 3 MBs with *MYCN*-amplifications[7] (Figure 2).

Multi-modality treatments have been used in multiple clinical trials for ten years. The standard protocols included different chemotherapeutic agents with long-term or maintenance dose-related regime including ifosfamide, etoposide, methotrexate, cisplatin, and cytarabine, lomustine, and vincristine[30]. The maintenance regimen has improved the overall survival compared to the sandwich approach among patients with M0 or M1 disease[30,31]. Nonetheless, the most frequent and current treatment strategy includes risk-adapted radiotherapy followed by 4 cycles of cyclophosphamide, and a high dose of chemotherapy such as cisplatin, vincristine, followed by autologous stem cell transplantation. This protocol has improved the 5-year OS into 95%[16]. Additional clinical trials are ongoing to explore the efficacy of different treatment regimes in newly diagnosed MBs (Clinicaltrials.gov). The current treatment protocols and ongoing clinical trials are still using the same circulating chemotherapeutic agents but with different regimes. Multiple clinical trials have tested new therapies. Those trials were completed with positive and negative results (Clinicaltrials.gov). For example, a combined everolimus and ribociclib (cyclin D and CDK6 inhibitors) has been tested as a phase I trial (NCT03387020) in children with recurrent MBs. Some novel therapeutic strategies are currently recruiting, and their target are to reduce recurrence and to avoid the cytotoxic effects of chemoradiation (Table 1). For example, the usage of Entrectinib, a *TRK* inhibitor, and *ALK* inhibitor has been studied in a phase I/II trial (NCT02650401). There is a high tendency to discover the efficacy of molecularly targeted agents for MBs with dominant genetic alterations, regardless of the tumor subgroup. Patients with *FGFR*-gene mutation can be treated with erdafitinib (NCT03210714); MBs with *TSC*-gene mutations can be treated with samotolisib (NCT03213678); *SMARCA4*-gene mutations can be treated with tazemetostat, an *EZH2* inhibitor.

Immune microenvironment of MB

All the previously mentioned clinical trials are stratified based on disease risk, molecular subgroups, patients age, and all are targeting tumour cells. The necessity to explore MB microenvironment is encouraged to help discovering new targeted receptors. The immune microenvironment of any cancer represents all types of cells surrounding the tumour cells including immune and none-immune cells. The relationship between these cells is mechanical and heterogeneous, by which they can facilitate in promoting or inhibiting tumor growth[32]. Because some studies have indicated that MBs have fewer immune cells than glioblastoma[33,34], the role of immune microenvironment in promoting or suppressing MB progression was found to be difficult to understand. Some cellular factors in tumour microenvironment may act against immune reaction and can promote tumour growth progression and angiogenesis. The infiltration of immune cells in MB might be limited due to the blood–brain barrier (BBB), which acts as physical barrier for immune cells infiltration[35]. Despite of some immune cells bypass across BBB, there may be an increase in trafficking toward the brain under certain conditions due to destruction of the BBB[36]. Some experimental models showed that the reactive astrocytes surrounding the tumour microenvironment form perivascular barriers to restrict the immune cells infiltration to the brain through BBB[37].

The presence of inflammatory cells in the tumor microenvironment has been scientifically accepted as an essential element in tumour progression. A study done by Gururangan *et al*[38] found that treated MB patients exhibited more CD4+T-cell lymphopenia. We can also presume that pre-operative and post-operative steroid treatment may induce systemic immunosuppression which prevents antitumor immunity in MB patients. Tumours with a low mutational burden respond less efficiently to immune checkpoint inhibitor compared to tumors with a high mutational burden[39]. Moreover, the acidification of the tumour microenvironment causing glycolytic activity can encourage macrophages infiltration through G protein coupled receptor, which in turn enhances vascular endothelial growth factor, thus promoting M2-like features of tumor-associated macrophage (TAM)[40].

APC, the immune cells in microenvironment, were proven to infiltrate malignant brain tumours in children. APCs is expressed by Major Histocompatibility Complex (MHC) class-I on tumor cells to allow them to be identified and killed by CD8 cytotoxic T- cells. MBs and atypical teratoid/rhabdoid tumors showed the lowermost cellular infiltration of this type among all malignant brain tumors[34]. Microglia, resident macrophages in the brain, are the most dominant APCs in brain tumors[35]. It is not clear if microglia promote anti-MB immune response. Mundt *et al*[41] showed that microglia are dispensable for T-cell entry into the brain and for local reactivation of T-cells. The loss of MHC class-I expression on

Table 1 The most recent active and recruiting clinical trials of medulloblastoma that are targeting immune receptors or using different chemotherapeutic agents

Clinical Trial	Trial objective	Samples	Targeted subgroup	Completion date
NCT01878617	Clinical and molecular risk directed therapy of newly diagnosed MB	660	WNT, non-WNT, SHH	2028
NCT00089245	Intrathecal radioimmunotherapy using I-8H9	120	8H9 reactive MB confirmed by IHC	2024
NCT02905110	Simultaneous methotrexate/etoposide infusion	10	All MB subtypes	2023
NCT02962167	Modified measles virus (MV-NIS)	46	All MB subtypes	2024
NCT02271711	Expanded NK cells infusion with recurrent medulloblastoma	12	All MB subtypes	2023
NCT02359565	Pembrolizumab in patient with recurrent medulloblastoma	45	All MB subtypes	2023
NCT03389802	APX005M, a humanized IgG1κ monoclonal Ab that binds to CD40	45	MB with CD40 activity	2023
NCT03299309	PEP (CMV)-specific peptide vaccine in medulloblastoma	30	All MB subtypes	2024
NCT03598244	Volitinib, a small molecule inhibitor of c-Met in recurrent MB	50	All MB subtypes	2023
NCT03173950	Nivolumab, Immune check point inhibitor, in refractory MB	180	All MB subtypes	2024
NCT03500991	HER2-Specific CAR T-cell locoregional immunotherapy	48	Her-2 expressed medulloblastoma	2039
NCT01356290	Antiangiogenic therapy for recurrent medulloblastoma	100	All MB subtypes	2026
NCT03911388	G207, an oncolytic herpes simplex virus-1 (HSV)	15	All MB subtypes	2025
NCT03638167	EGFR806-specific CAR T-cell locoregional immunotherapy	36	EGFR positive tumours	2040
NCT03893487	Fimepinostat, a small molecule inhibitor in young MB	30	All MB subtypes	2027
NCT03709680	Palbociclib in combination with temozolomide and irinotecan	184	All MB subtypes	2028
NCT03904862	CX-4945 inhibitor of casein kinase II (CK2) tolerability	60	SHH-medulloblastoma	2028
NCT03936465	BMS-986158, a bromodomain inhibitor	66	MYCN amplification or BRD3 translocation MB	2024
NCT02650401	Entrectinib (RXDX-101), a TRKA/B/C, ROS1, and ALK inhibitor	68	MB harboring- NTRK1/2/3, ROS1, ALK fusions	2027
NCT03210714	Erdafitinib, an oral pan-FGFR inhibitor	49	Mutations in the FGFR1/2/3/4 pathway	2024
NCT03213678	Samotolisib, a PI3K/mTOR inhibitor	24	PI3K/MTOR activating mutations	2024
NCT03213704	Larotrectinib, NTRK fusion inhibitor for medulloblastoma	49	MB with NTRK fusions	2024
NCT03213665	Tazemetostat, a small molecule EZH2 inhibitor	20	EZH2, SMARCB1, or SMARCA4 mutations	2023
NCT03233204	Olaparib for refractory or aggressive medulloblastoma	29	Defects in DNA damage repair genes	2024
NCT04023669	LY2606368, a molecularly targeted CHK1/2 inhibitor	21	Group3/Group4; SHH; indeterminate types	2026
NCT03526250	Palbociclib (Pediatric MATCH treating trials	49	Rb positive solid tumours	2025
NCT02444546	Wild-Type Reovirus in Combination with Sargramostim	06	All MB subtypes	2026
NCT04185038	B7-H3-Specific CAR-T Cell Locoregional Immunotherapy	90	All MB subtypes	2041

NCT01601184	Vismodegib combined with Temozolomide	24	SHH-MB group	2023
NCT03155620	Targeted therapy directed by genetic testing	2316	All MB subtypes	2027
NCT00089245	Iodine I 131 monoclonal antibody 8H9	120	All MB subtypes	2025
NCT02271711	Natural killer cell therapy	12	All MB subtypes	
NCT04315064	Infusion of Panobinostat (MTX110)	5	All MB subtypes	2024
NCT04743661	131I-Omburtamab in recurrent medullo-blastoma	62	All MB subtypes	2030
NCT03257631	Pomalidomide onotherapy for recurrent or progressive MB	53	All MB subtypes	2023
NCT04320888	Selpercatinib for treatment of advanced medulloblastoma	49	Tumour with activating RET alteration	2027

ALK: Anaplastic Lymphoma Kinase; CMV: Cytomegalovirus; EGFR: Epidermal Growth Factor Receptor; MB: Medulloblastoma; PEP: Post-exposure prophylaxis; SHH: Sonic-hedgehog; IHC: Immunohistochemistry; RET: Rearranged in transfection; NK: Natural killer; WNT: Wingless.

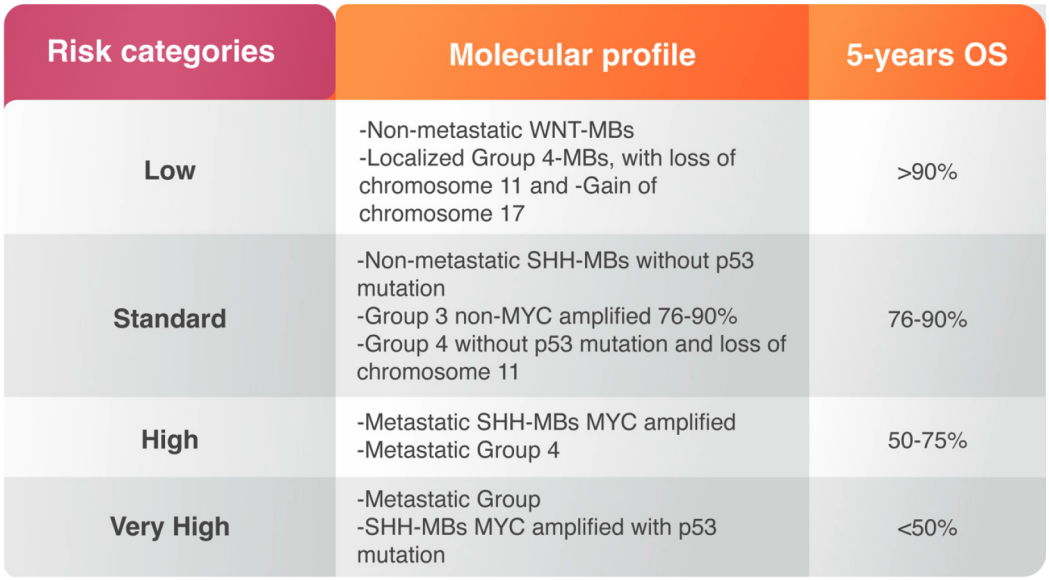
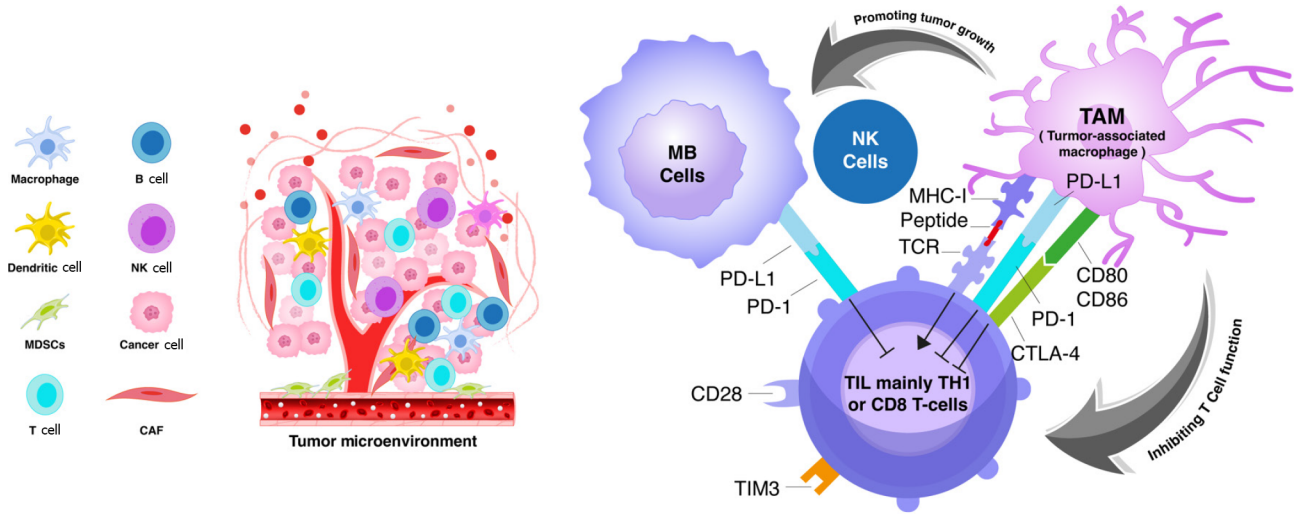


Figure 2 Risk groups and categories of medulloblastoma with their molecular profiles and the 5-years survival associated with each group. The information presented in this figure were taken with permission from the reference: Luzzi *et al*[91], 2020. SHH: Sonic-hedgehog; MB: Medulloblastoma; MYC: Myelocytomatosis oncogene; OS: Overall survival; WNT: Wingless.

tumor surface is also a common mechanism of immune escape in MB[42,43]. Because MHC class-I helps in the activation of CD8 cytotoxic T-cells, it acts as a passive regulator of natural killer (NK) cells. Thus, the loss of MHC-class I in tumor cells may increase tumour cell evasion[42,43].

Tumour associated macrophages in immune microenvironment

TAM is considered the major immune cell in the tumor microenvironment that can either support or inhibit tumor growth[44,45]. TAMs interact with tumour cells to promote tumour progression and invasion[46]. They are subclassified into two groups: (1) TAMs with M1 polarization, are induced by IFN-γ to release proinflammatory particles and are associated with some inflammatory response; and (2) TAMs with M2 polarization, are induced by interleukin-4 to release growth factors (*e.g.*, epidermal growth factor, fibroblast growth factor-1, vascular endothelial growth factor) and involved in tumour progression and immunosuppression[47-49]. Uncontrolled activation of M1-polarzed TAM can shift towards M2-polarization in long term. However, the M2-like macrophages, which mimic TAMs in the tumour microenvironment, can be stimulated by cytokines[50]. EGF released by TAMs stimulate carcinogenesis, while VEGF regulates angiogenesis. These processes emphasize the actual immune-suppressive function of TAMs[51]. TAMs infiltration in the tumour microenvironment was proven to be a poor prognostic factor[50]. Clinical data have indicated that a large number of M2-polarized TAMs expressing CD163 and CD204 were correlated with a poor outcome of several body cancers[47] (Figure 3). Moreover, the presence of TAMs, mainly M2- type, has been also noted in many adult malignancies including CNS tumors[52-54]. In response to hypoxia, TAMs overexpress the PD-1 ligands



DOI: 10.5306/wjco.v14.i3.117 **Copyright** ©The Author(s) 2023.

Figure 3 The signaling interaction between tumour cells, tumor-associated macrophages, and tumour infiltrating lymphocytes in medulloblastoma microenvironment. Tumour microenvironment represents diverse cellular heterogeneities including immune and none-immune cells. The targeted receptors linked between immune cells represent a potential targeted therapy. CAF: Cancer-associated fibroblasts; MB: Medulloblastoma; NK: Natural killer; TAM: Tumor-associated macrophage; TIL: Tumour infiltrating lymphocytes.

[55]. PD-L1 overexpression in TAM has been reported in glioblastoma[56] but it has never been explored well in other brain tumours such as medulloblastoma.

The current role of TAMs in the prognosis of MB is still controversial. Despite of the molecular insights provided by MB subgroups, less information were reported about the role of TAMs in MBs[33]. The genetic alterations and the disease risk would make diverse effects on immune microenvironment [57]. Because TAMs are composed of variable amounts of microglia and macrophages, the composition of TAMs are different in all MB subgroups. Margol *et al*[58] and Zhang *et al*[59] reported that TAMs were significantly higher in SHH-MB compared to other MB subgroups. This may be due to the high expression of monocyte chemotactic protein-1 (MCP-1], which helps in TAM recruitment and M2 polarization[60]. Another possibility, SHH-MB may exhibit molecular signatures predictive for fibroblast, T-cells, and macrophage infiltration[34]. Nevertheless, the role of TAMs in this era is not clear and the previous reported studies did not reveal the prognostic connotations of TAMs in SHH-MBs[58].

CD163 expression was observed in the small number of SHH-MBs, which suggested that TAMs may play a dynamic role in SHH-MB formation[58,61]. Another study done by Crotty *et al*[62], revealed that less TAMs in microenvironment was associated with a low recurrence and low risk of metastasis. Lee *et al*[63] suggested that a large number of M1-polarized TAMs was associated with worsening outcome in SHH-MB patients. Lee and his group has also investigated the correlation between TAM recruitment and outcome, and they revealed that expressed M1-polarized TAMs predicted better progression-free survival but, TAMs showed no significant effect on OS[59]. Few studies showed that the immunoreactivity in MB microenvironment, regardless the subtype, is age-related[64]. In a study done by Zhang *et al*, they divided the patients into three age groups. They found that the group between 0-3 years of age and the group between 11-18 year of age had more TAMs than the group aged between 4-10 years. It implies that TAMs in MBs are crucial in different age groups[59]. Zhang *et al*[59] also found that TAMs, mainly M1-polarized type, are prevalent in MBs with metastatic disease.

Tumour recurrence and metastases are the major obstacle for treatment success, and the disease recurrence is responsible for 90% of MB mortality[65]. Group 3 and 4 patients develop spinal metastases regardless of the type of chemotherapy given after resection[2]. The presence of *TP53-MYCN*-alteration in these groups is associated with rapid tumour progression[66]. The ability of Group 3 and 4 to metastasize indicates that these tumor cells participate in the epithelial-to-mesenchymal transition (EMT), thus warranting additional investigations into EMT[67]. It is not yet known why tumor cells enter the EMT phase. A study done by Bonde *et al*[68] showed that TGFβ triggers the EMT phase, shifting the cancer cells to gain a mesenchymal phenotype. The lack of local nutrients, loss of supportive cells in microenvironment, and repeated mutations can all be reasons for this aggressive behavior. Funakoshi *et al*[69] found that loss of CDH1 allows tumour cells to detach from each other and can invade and metastasize.

Tumour infiltrating lymphocyte in immune microenvironment

Generally, increased T-cells trafficking in the brain has been reported in some neurological diseases. The activated T-cells have the role to alter the BBB, allowing for immune cells recruitment and entry to the brain parenchyma[70]. Tumour infiltrating lymphocytes (TIL) are considered signaling interacted cells

between TAMs and tumour cells in the tumour microenvironment (Figure 3). The number of T-cells present in MB was found to be not significantly high compared to other control tissues[33]. Small amount of CD8 cytotoxic T-cells and NK cells suggest a less antitumor activity in MB[34]. However, a small percentage of helper T-cells (Th17) cells was also found at the site of the tumor but with uncertain significance[11]. Some experimental trials revealed that MB cells stimulate the release of the T-cells attractant (RANTES) from the endothelium, causing T-cell immigration[71]. Hence, increasing numbers of T-helper lymphocytes correlate with favourable prognosis in MB patients receiving chemotherapy [44].

T-regulatory cells (Tregs) control the activity of immune cells by releasing some anti-inflammatory cytokines such interleukin-10 (IL-10), and CTLA4-mediated trogocytosis[44]. Treg infiltration in MB microenvironment has been described by Gate *et al*[44]. Consequently, TGFβ drives the CD4 helper T-cells to Tregs, which in turn releases high levels of TGFβ. This process generates a feeding circuit to support immunosuppression. Elevated Treg in MBs can be therapy-induced, as Treg has been detected in the peripheral blood of some treated patients[38].

Interaction between TAMs and TILs in MB microenvironment

The interaction between TAMs and TILs were not scientifically explored in MB microenvironment (Figure 3). Kurdi *et al*[54] has explained the crosstalk between tumour cells, TAM sand TILs in glioblastoma. TAMs encircle cancer cells and supresses the killing action of T-cell thus, T-cells will not be able to help tumour cells against immune evasion. The TAMs accumulate in the microenvironment with less T-cells evolution[54]. Salsman *et al*[71] revealed that MB cell lines can interact with tumor endothelium to recruit T-cells to MB microenvironment, in particular macrophage migration inhibitory factor (MIF). MIF is the key molecule released by MB to stimulate the endothelial cells in the microenvironment to release more potent T-lymphocyte attractants[71].

Current immunotherapy in MB and possible targeted receptors

Immune checkpoints represent a family of proteins on T-cells surface that interact with some ligands on APCs or tumour cells while they inhibit TCR-mediated ligands. Certain cancers (colorectal, ovarian and brain cancers) are resistant to immune checkpoint inhibitor[72]. The number of studies utilizing immunotherapy in the treatment approach of MB is limited. The approach had few selected options. Most of studies were observational and contained a small sample size. There are two clinical trials currently investigating the blockade of inhibitory checkpoint pathways in MB including pembrolizumab and nivolumab (NCT02359565) (NCT03173950). CD276, another immune check point inhibitor on T-cell, is also under investigation[73]. CD40 [a TNF receptor] expressed by antigen presenting cells and B-cells expresses cytokines, activates T-cells, and in turn timulate programmed cell death[74]. CD40 has a significant cytotoxic effect on tumor cells. APX005M, a humanized IgG1κ monoclonal antibody agonist of CD40 is currently evaluated in a phase I trial (NCT03389802) in patients with recurrent MBs. The recent actively recruiting clinical trials are summarized in (Table 1).

Numerous studies revealed that TAMs may interfere with some anti-tumor treatments such as chemotherapies and other antibody-based immunotherapies targeting some molecules such as PD-1/PD-1[50,72]. These findings emphasize that TAMs might be a promising target of novel anti-tumor treatment particularly in patient not responding to the standard treatment. The ability of TAMs to limit the efficacy of immune check point blockade has been previously investigated in several cancers[75,76]. TAMs express multiple ligands for checkpoint receptors, such as PD-L1/2, CD80/86, and CD204/CD206, and the current checkpoint inhibitors are different from the targeted receptors as they maintain a state of effective immunosuppression[77] (Figure 3). These legends, representing M2-polarized TAMs, have not been investigated in MB microenvironment. Martin *et al*[78] showed that MBs expressing reduced levels of PD-L1 can help tumour cells to evade from the immunity, suggesting that an inflamed tumor microenvironment is necessary for PD-1 pathway stimulation. However, the efficacy of PD-PD-L1 inhibitor has not been yet proven to be formally used in MB treatment.

Trogocytosis is a process involved in immune microenvironment concerned with the transfer of membrane fragments and cell surface proteins between cells. It is not known if induced iTregs can undergo trogocytosis. The trogocytosis of CD80/CD86 occurring in CTLA-4 or PDL1-independent approach plays a significant role in the immune suppression[79]. CD80/86 expression and trogocytosis have never been explored in MB microenvironment. As a key mechanism, Treg-linked CTLA-4 inhibits the CD80/CD86 molecules expression on APCs. Tekguc *et al*[80] revealed that blockade of CTLA-4 and PD-1/PD-L1 pathways may impede Treg-mediated immunosuppression, which in turn enhances anti tumour activity response. This novel exploration has not been investigated in MB. Several investigations have demonstrated that activation of PI3Kγ signaling in macrophages suppresses NF-κB, thereby stimulating immunosuppression. TAMs in cancers treated with chemotherapies are often responsible for chemoresistance as they are more susceptible to the cytotoxic effect of macrophages[81]. This process occurs when there is excessive recruitment of anti-apoptotic process in tumour microenvironment[82].

Understanding the molecular events in the mechanism of TAMs activation allows for the development of anti-tumor treatment strategies. TAMs can be targeted to inhibit their infiltration in microenvironment through direct killing or through a TAM-polarization reprogramming. TAMs accumulate in tumour microenvironment because of the continuous recruitment of monocytes from the blood

circulation to TAMs through multiple tumour derived mediators. These mediators play a connection role between macrophages and tumour cells. CCL2 has been described as the main mediator involved in TAM recruitment. Indeed, the blockage of this pathway would cause less TAMs accumulation in tumour microenvironment[83]. Another pathway involved in monocytic recruitment into TAMs is the CXCL12/CXCR4 pathway[84]. It has been used in different trials of different cancers such as myeloid leukemia but never been tried in brain cancers.

CSF-1, a colony stimulating factor involved in the proliferation and the recruitment of monocytes-macrophages, is an essential target against TAM in tumour microenvironment. The expression of CSF-1 in tumour microenvirment was proven to be a poor prognosticator in multiple body cancers[85]. After treatment with CSF-1 inhibitor in one of clinical trials, the number of TAMs have depleted and there was an infiltration of CD8 cytotoxic T-cells in the tumor[86,87].

Reprogramming of TAM is another possible strategy to inhibit TAM activity. Several approaches attempted to switch M2-polarized TAMs into antitumor M1-like macrophages through monoclonal antibody inhibitors and Toll-like receptor (TLR) blockers. Alvarez-Arellano *et al*[88] revealed that TLR7 is a prognostic factor of survival in MB. Resiquimod, an agonist to TLR7/8, has shown an attention couple years ago for its efficacy to reprogram macrophages[89]. The CD47–SIRPα, involved in the regulation of phagocytosis, has never been used to reprogram TAMs. CD47 is expressed by tumor cells and interacts with the signal regulatory protein-α. Substantial evidence assumed that overexpression of CD47 in many cancers had a role in the phagocytic resistance[90]. However, this investigation has never been investigated in MB patients. Promising results were obtained in lymphoma patients in a combination of anti-CD47 with anti-CD20. Despite these results, the *in vivo* application of CD47 for the treatment of cancer is still limited.

CONCLUSION

Medulloblastoma is the most common malignant pediatric tumour in CNS that are subclassified into four distinguishing molecular subgroups. The current treatments failed to improve the patient's survival significantly while the serious complications associated with these cytotoxic therapies warrant for exploring new therapeutic approaches targeting different immune receptors. The identification of tumour microenvironment has facilitated the scientists understanding how tumor growth and progression are regulated. TAMs and TILs, the main dominant immune cells in microenvironment, seem to have a major role in immune mechanism and tumor progression. Their infiltration in microenvironment has prompted researchers to evaluate the interaction of new targeted immune receptors with the current signaling pathways. Their infiltration in microenvironment may also be targeted through different reprogramming mechanisms. However, the ability of TAMs to limit the efficacy of immune check point blockade in MB requires further investigations. These strategic thoughts emphasize that TAMs might be a promising targeted treatment particularly in patients with recurrent or progressive MB. Further studies to explore new targeted receptors in tumour microenvironment and understanding the conventional relationship between TAMs, TILs and tumour cells are essential to develop new therapeutic approaches.

FOOTNOTES

Author contributions: All authors shared in writing the review.

Conflict-of-interest statement: All the author declare no conflict of interests for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial [CC BY-NC 4.0] license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Saudi Arabia

ORCID number: Maher Kurdi 0000-0002-8979-3849.

S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

REFERENCES

1 **Pollack IF**, Boyett JM, Yates AJ, Burger PC, Gilles FH, Davis RL, Finlay JL; Children's Cancer Group. The influence of central review on outcome associations in childhood malignant gliomas: results from the CCG-945 experience. *Neuro Oncol* 2003; **5**: 197-207 [PMID: 12816726 DOI: 10.1215/S1152851703000097]

2 **Ramaswamy V**, Remke M, Bouffet E, Bailey S, Clifford SC, Doz F, Kool M, Dufour C, Vassal G, Milde T, Witt O, von Hoff K, Pietsch T, Northcott PA, Gajjar A, Robinson GW, Padovani L, André N, Massimino M, Pizer B, Packer R, Rutkowski S, Pfister SM, Taylor MD, Pomeroy SL. Risk stratification of childhood medulloblastoma in the molecular era: the current consensus. *Acta Neuropathol* 2016; **131**: 821-831 [PMID: 27040285 DOI: 10.1007/s00401-016-1569-6]

3 **Ning MS**, Perkins SM, Dewees T, Shinohara ET. Evidence of high mortality in long term survivors of childhood medulloblastoma. *J Neurooncol* 2015; **122**: 321-327 [PMID: 25557108 DOI: 10.1007/s11060-014-1712-y]

4 **Altman J**, Bayer SA. The generation, movements, and settling of cerebellar granule cells and the formation of parallel fibers in development of the cerebellar system in relation to its evolution structure and function. CRC Press 1997; New York pp. 334-361

5 **Louis DN**, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol* 2016; **131**: 803-820 [PMID: 27157931 DOI: 10.1007/s00401-016-1545-1]

6 **Taylor MD**, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, Ellison DW, Lichter P, Gilbertson RJ, Pomeroy SL, Kool M, Pfister SM. Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol* 2012; **123**: 465-472 [PMID: 22134537 DOI: 10.1007/s00401-011-0922-z]

7 **Thompson MC**, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, Chintagumpala M, Adesina A, Ashley DM, Kellie SJ, Taylor MD, Curran T, Gajjar A, Gilbertson RJ. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol* 2006; **24**: 1924-1931 [PMID: 16567768 DOI: 10.1200/JCO.2005.04.4974]

8 **Ellison DW**, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, Kenney AM, Brat DJ, Perry A, Yong WH, Taylor RE, Bailey S, Clifford SC, Gilbertson RJ. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta Neuropathol* 2011; **121**: 381-396 [PMID: 21267586 DOI: 10.1007/s00401-011-0800-8]

9 **Northcott PA**, Buchhalter I, Morrissy AS, Hovestadt V, Weischenfeldt J, Ehrenberger T, Gröbner S, Segura-Wang M, Zichner T, Rudneva VA, Warnatz HJ, Sidiropoulos N, Phillips AH, Schumacher S, Kleinheinz K, Waszak SM, Erkek S, Jones DTW, Worst BC, Kool M, Zapatka M, Jäger N, Chavez L, Hutter B, Bieg M, Paramasivam N, Heinold M, Gu Z, Ishaque N, Jäger-Schmidt C, Imbusch CD, Jugold A, Hübschmann D, Risch T, Amstislavskiy V, Gonzalez FGR, Weber UD, Wolf S, Robinson GW, Zhou X, Wu G, Finkelstein D, Liu Y, Cavalli FMG, Luu B, Ramaswamy V, Wu X, Koster J, Ryzhova M, Cho YJ, Pomeroy SL, Herold-Mende C, Schuhmann M, Ebinger M, Liau LM, Mora J, McLendon RE, Jabado N, Kumabe T, Chuah E, Ma Y, Moore RA, Mungall AJ, Mungall KL, Thiessen N, Tse K, Wong T, Jones SJM, Witt O, Milde T, Von Deimling A, Capper D, Korshunov A, Yaspo ML, Kriwacki R, Gajjar A, Zhang J, Beroukhim R, Fraenkel E, Korbel JO, Brors B, Schlesner M, Eils R, Marra MA, Pfister SM, Taylor MD, Lichter P. The whole-genome landscape of medulloblastoma subtypes. *Nature* 2017; **547**: 311-317 [PMID: 28726821 DOI: 10.1038/nature22973]

10 **Robinson G**, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, Phoenix TN, Hedlund E, Wei L, Zhu X, Chalhoub N, Baker SJ, Huether R, Kriwacki R, Curley N, Thiruvenkatam R, Wang J, Wu G, Rusch M, Hong X, Becksfort J, Gupta P, Ma J, Easton J, Vadodaria B, Onar-Thomas A, Lin T, Li S, Pounds S, Paugh S, Zhao D, Kawauchi D, Roussel MF, Finkelstein D, Ellison DW, Lau CC, Bouffet E, Hassall T, Gururangan S, Cohn R, Fulton RS, Fulton LL, Dooling DJ, Ochoa K, Gajjar A, Mardis ER, Wilson RK, Downing JR, Zhang J, Gilbertson RJ. Novel mutations target distinct subgroups of medulloblastoma. *Nature* 2012; **488**: 43-48 [PMID: 22722829 DOI: 10.1038/nature11213]

11 **Zhao F**, Ohgaki H, Xu L, Giangaspero F, Li C, Li P, Yang Z, Wang B, Wang X, Wang Z, Ai L, Zhang J, Luo L, Liu P. Molecular subgroups of adult medulloblastoma: a long-term single-institution study. *Neuro Oncol* 2016; **18**: 982-990 [PMID: 27106407 DOI: 10.1093/neuonc/now050]

12 **Cavalli FMG**, Remke M, Rampasek L, Peacock J, Shih DJH, Luu B, Garzia L, Torchia J, Nor C, Morrissy AS, Agnihotri S, Thompson YY, Kuzan-Fischer CM, Farooq H, Isaev K, Daniels C, Cho BK, Kim SK, Wang KC, Lee JY, Grajkowska WA, Perek-Polnik M, Vasiljevic A, Faure-Conter C, Jouvet A, Giannini C, Nageswara Rao AA, Li KKW, Ng HK, Eberhart CG, Pollack IF, Hamilton RL, Gillespie GY, Olson JM, Leary S, Weiss WA, Lach B, Chambless LB, Thompson RC, Cooper MK, Vibhakar R, Hauser P, van Veelen MC, Kros JM, French PJ, Ra YS, Kumabe T, López-Aguilar E, Zitterbart K, Sterba J, Finocchiaro G, Massimino M, Van Meir EG, Osuka S, Shofuda T, Klekner A, Zollo M, Leonard JR, Rubin JB, Jabado N, Albrecht S, Mora J, Van Meter TE, Jung S, Moore AS, Hallahan AR, Chan JA, Tirapelli DPC, Carlotti CG, Fouladi M, Pimentel J, Faria CC, Saad AG, Massimi L, Liau LM, Wheeler H, Nakamura H, Elbabaa SK, Perezpeña-Diazconti M, Chico Ponce de León F, Robinson S, Zapotocky M, Lassaletta A, Huang A, Hawkins CE, Tabori U, Bouffet E, Bartels U, Dirks PB, Rutka JT, Bader GD, Reimand J, Goldenberg A, Ramaswamy V, Taylor MD. Intertumoral Heterogeneity within Medulloblastoma Subgroups. *Cancer Cell* 2017; **31**: 737-754.e6 [PMID: 28609654 DOI: 10.1016/j.ccell.2017.05.005]

13 **Pugh TJ**, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, Carneiro MO, Carter SL, Cibulskis K, Erlich RL, Greulich H, Lawrence MS, Lennon NJ, McKenna A, Meldrim J, Ramos AH, Ross MG, Russ C, Shefler E, Sivachenko A, Sogoloff B, Stojanov P, Tamayo P, Mesirov JP, Amani V, Teider N, Sengupta S, Francois JP, Northcott PA, Taylor MD, Yu F, Crabtree GR, Kautzman AG, Gabriel SB, Getz G, Jäger N, Jones DT, Lichter P, Pfister SM, Roberts TM, Meyerson M, Pomeroy SL, Cho YJ. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* 2012; **488**: 106-110 [PMID: 22820256 DOI: 10.1038/nature11329]

14 **Schröder M**. Human DEAD-box protein 3 has multiple functions in gene regulation and cell cycle control and is a prime target for viral manipulation. *Biochem Pharmacol* 2010; **79**: 297-306 [PMID: 19782656 DOI: 10.1016/j.bcp.2009.08.032]

15 **Trubicka J**, Szperl M, Grajkowska W, Karkucińska-Więckowska A, Tarasińska M, Falana K, Dembowska-Bagińska B, Łastowska M. Identification of a novel inherited ALK variant M1199L in the WNT type of medulloblastoma. *Folia Neuropathol* 2016; **54**: 23-30 [PMID: 27179218 DOI: 10.5114/fn.2016.58912]

16 **Gajjar AJ**, Robinson GW. Medulloblastoma-translating discoveries from the bench to the bedside. *Nat Rev Clin Oncol*

2014; **11**: 714-722 [PMID: 25348790 DOI: 10.1038/nrclinonc.2014.181]

17 **Cho YJ**, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, Berhoukim R, Amani V, Goumnerova L, Eberhart CG, Lau CC, Olson JM, Gilbertson RJ, Gajjar A, Delattre O, Kool M, Ligon K, Meyerson M, Mesirov JP, Pomeroy SL. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol* 2011; **29**: 1424-1430 [PMID: 21098324 DOI: 10.1200/JCO.2010.28.5148]

18 **Rutkowski S**, Gerber NU, von Hoff K, Gnekow A, Bode U, Graf N, Berthold F, Henze G, Wolff JE, Warmuth-Metz M, Soerensen N, Emser A, Ottensmeier H, Deinlein F, Schlegel PG, Kortmann RD, Pietsch T, Kuehl J; German Pediatric Brain Tumor Study Group. Treatment of early childhood medulloblastoma by postoperative chemotherapy and deferred radiotherapy. *Neuro Oncol* 2009; **11**: 201-210 [PMID: 18818397 DOI: 10.1215/15228517-2008-084]

19 **Kool M**, Jones DT, Jäger N, Northcott PA, Pugh TJ, Hovestadt V, Piro RM, Esparza LA, Markant SL, Remke M, Milde T, Bourdeaut F, Ryzhova M, Sturm D, Pfaff E, Stark S, Hutter S, Seker-Cin H, Johann P, Bender S, Schmidt C, Rausch T, Shih D, Reimand J, Sieber L, Wittmann A, Linke L, Witt H, Weber UD, Zapatka M, König R, Beroukhim R, Bergthold G, van Sluis P, Volckmann R, Koster J, Versteeg R, Schmidt S, Wolf S, Lawerenz C, Bartholomae CC, von Kalle C, Unterberg A, Herold-Mende C, Hofer S, Kulozik AE, von Deimling A, Scheurlen W, Felsberg J, Reifenberger G, Hasselblatt M, Crawford JR, Grant GA, Jabado N, Perry A, Cowdrey C, Croul S, Zadeh G, Korbel JO, Doz F, Delattre O, Bader GD, McCabe MG, Collins VP, Kieran MW, Cho YJ, Pomeroy SL, Witt O, Brors B, Taylor MD, Schüller U, Korshunov A, Eils R, Wechsler-Reya RJ, Lichter P, Pfister SM; ICGC PedBrain Tumor Project. Genome sequencing of SHH medulloblastoma predicts genotype-related response to smoothened inhibition. *Cancer Cell* 2014; **25**: 393-405 [PMID: 24651015 DOI: 10.1016/j.ccr.2014.02.004]

20 **Smith MJ**, Beetz C, Williams SG, Bhaskar SS, O'Sullivan J, Anderson B, Daly SB, Urquhart JE, Bholah Z, Oudit D, Cheesman E, Kelsey A, McCabe MG, Newman WG, Evans DG. Germline mutations in SUFU cause Gorlin syndrome-associated childhood medulloblastoma and redefine the risk associated with PTCH1 mutations. *J Clin Oncol* 2014; **32**: 4155-4161 [PMID: 25403219 DOI: 10.1200/JCO.2014.58.2569]

21 **Northcott PA**, Jones DT, Kool M, Robinson GW, Gilbertson RJ, Cho YJ, Pomeroy SL, Korshunov A, Lichter P, Taylor MD, Pfister SM. Medulloblastomics: the end of the beginning. *Nat Rev Cancer* 2012; **12**: 818-834 [PMID: 23175120 DOI: 10.1038/nrc3410]

22 **Sursal T**, Ronecker JS, Dicpinigaitis AJ, Mohan AL, Tobias ME, Gandhi CD, Jhanwar-Uniyal M. Molecular Stratification of Medulloblastoma: Clinical Outcomes and Therapeutic Interventions. *Anticancer Res* 2022; **42**: 2225-2239 [PMID: 35489737 DOI: 10.21873/anticanres.15703]

23 **Menyhárt O**, Giangaspero F, Győrffy B. Molecular markers and potential therapeutic targets in non-WNT/non-SHH (group 3 and group 4) medulloblastomas. *J Hematol Oncol* 2019; **12**: 29 [PMID: 30876441 DOI: 10.1186/s13045-019-0712-y]

24 **Zhou P**, Sha H, Zhu J. The role of T-helper 17 (Th17) cells in patients with medulloblastoma. *J Int Med Res* 2010; **38**: 611-619 [PMID: 20515574 DOI: 10.1177/147323001003800223]

25 **Waszak SM**, Northcott PA, Buchhalter I, Robinson GW, Sutter C, Groebner S, Grund KB, Brugières L, Jones DTW, Pajtler KW, Morrissy AS, Kool M, Sturm D, Chavez L, Ernst A, Brabetz S, Hain M, Zichner T, Segura-Wang M, Weischenfeldt J, Rausch T, Mardin BR, Zhou X, Baciu C, Lawerenz C, Chan JA, Varlet P, Guerrini-Rousseau L, Fults DW, Grajkowska W, Hauser P, Jabado N, Ra YS, Zitterbart K, Shringarpure SS, De La Vega FM, Bustamante CD, Ng HK, Perry A, MacDonald TJ, Hernáiz Driever P, Bendel AE, Bowers DC, McCowage G, Chintagumpala MM, Cohn R, Hassall T, Fleischhack G, Eggen T, Wesenberg F, Feychting M, Lannering B, Schüz J, Johansen C, Andersen TV, Röösli M, Kuehni CE, Grotzer M, Kjaerheim K, Monoranu CM, Archer TC, Duke E, Pomeroy SL, Shelagh R, Frank S, Sumerauer D, Scheurlen W, Ryzhova MV, Milde T, Kratz CP, Samuel D, Zhang J, Solomon DA, Marra M, Eils R, Bartram CR, von Hoff K, Rutkowski S, Ramaswamy V, Gilbertson RJ, Korshunov A, Taylor MD, Lichter P, Malkin D, Gajjar A, Korbel JO, Pfister SM. Spectrum and prevalence of genetic predisposition in medulloblastoma: a retrospective genetic study and prospective validation in a clinical trial cohort. *Lancet Oncol* 2018; **19**: 785-798 [PMID: 29753700 DOI: 10.1016/S1470-2045(18)30242-0]

26 **Wolden SL**, Dunkel IJ, Souweidane MM, Happersett L, Khakoo Y, Schupak K, Lyden D, Leibel SA. Patterns of failure using a conformal radiation therapy tumor bed boost for medulloblastoma. *J Clin Oncol* 2003; **21**: 3079-3083 [PMID: 12915597 DOI: 10.1200/JCO.2003.11.140]

27 **Schwalbe EC**, Lindsey JC, Nakjang S, Crosier S, Smith AJ, Hicks D, Rafiee G, Hill RM, Iliasova A, Stone T, Pizer B, Michalski A, Joshi A, Wharton SB, Jacques TS, Bailey S, Williamson D, Clifford SC. Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study. *Lancet Oncol* 2017; **18**: 958-971 [PMID: 28545823 DOI: 10.1016/S1470-2045(17)30243-7]

28 **Kann BH**, Park HS, Lester-Coll NH, Yeboa DN, Benitez V, Khan AJ, Bindra RS, Marks AM, Roberts KB. Postoperative Radiotherapy Patterns of Care and Survival Implications for Medulloblastoma in Young Children. *JAMA Oncol* 2016; **2**: 1574-1581 [PMID: 27491009 DOI: 10.1001/jamaoncol.2016.2547]

29 **Northcott PA**, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, Rutka JT, Pfister S, Taylor MD. Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 2011; **29**: 1408-1414 [PMID: 20823417 DOI: 10.1200/JCO.2009.27.4324]

30 **Hoff KV**, Hinkes B, Gerber NU, Deinlein F, Mittler U, Urban C, Benesch M, Warmuth-Metz M, Soerensen N, Zwiener I, Goette H, Schlegel PG, Pietsch T, Kortmann RD, Kuehl J, Rutkowski S. Long-term outcome and clinical prognostic factors in children with medulloblastoma treated in the prospective randomised multicentre trial HIT'91. *Eur J Cancer* 2009; **45**: 1209-1217 [PMID: 19250820 DOI: 10.1016/j.ejca.2009.01.015]

31 **Franceschi E**, Giannini C, Furtner J, Pajtler KW, Asioli S, Guzman R, Seidel C, Gatto L, Hau P. Adult Medulloblastoma: Updates on Current Management and Future Perspectives. *Cancers (Basel)* 2022; **14** [PMID: 35954372 DOI: 10.3390/cancers14153708]

32 **Quail DF**, Joyce JA. The Microenvironmental Landscape of Brain Tumors. *Cancer Cell* 2017; **31**: 326-341 [PMID: 28292436 DOI: 10.1016/j.ccell.2017.02.009]

33 **Griesinger AM**, Birks DK, Donson AM, Amani V, Hoffman LM, Waziri A, Wang M, Handler MH, Foreman NK.

Characterization of distinct immunophenotypes across pediatric brain tumor types. *J Immunol* 2013; **191**: 4880-4888 [PMID: 24078694 DOI: 10.4049/jimmunol.1301966]

34 **Bockmayr M**, Mohme M, Klauschen F, Winkler B, Budczies J, Rutkowski S, Schüller U. Subgroup-specific immune and stromal microenvironment in medulloblastoma. *Oncoimmunology* 2018; **7**: e1462430 [PMID: 30228931 DOI: 10.1080/2162402X.2018.1462430]

35 **Eisemann T**, Wechsler-Reya RJ. Coming in from the cold: overcoming the hostile immune microenvironment of medulloblastoma. *Genes Dev* 2022; **36**: 514-532 [PMID: 35680424 DOI: 10.1101/gad.349538.122]

36 **Weiss N**, Miller F, Cazaubon S, Couraud PO. The blood-brain barrier in brain homeostasis and neurological diseases. *Biochim Biophys Acta* 2009; **1788**: 842-857 [PMID: 19061857 DOI: 10.1016/j.bbamem.2008.10.022]

37 **Voskuhl RR**, Peterson RS, Song B, Ao Y, Morales LB, Tiwari-Woodruff S, Sofroniew MV. Reactive astrocytes form scar-like perivascular barriers to leukocytes during adaptive immune inflammation of the CNS. *J Neurosci* 2009; **29**: 11511-11522 [PMID: 19759299 DOI: 10.1523/JNEUROSCI.1514-09.2009]

38 **Gururangan S**, Reap E, Schmittling R, Kocak M, Reynolds R, Grant G, Onar-Thomas A, Baxter P, Pollack IF, Phillips P, Boyett J, Fouladi M, Mitchell D. Regulatory T cell subsets in patients with medulloblastoma at diagnosis and during standard irradiation and chemotherapy (PBTC N-11). *Cancer Immunol Immunother* 2017; **66**: 1589-1595 [PMID: 28825123 DOI: 10.1007/s00262-017-2051-6]

39 **Rizvi NA**, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; **348**: 124-128 [PMID: 25765070 DOI: 10.1126/science.aaa1348]

40 **Colegio OR**, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, Cline GW, Phillips AJ, Medzhitov R. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 2014; **513**: 559-563 [PMID: 25043024 DOI: 10.1038/nature13490]

41 **Mundt S**, Mrdjen D, Utz SG, Greter M, Schreiner B, Becher B. Conventional DCs sample and present myelin antigens in the healthy CNS and allow parenchymal T cell entry to initiate neuroinflammation. *Sci Immunol* 2019; **4** [PMID: 30679199 DOI: 10.1126/sciimmunol.aau8380]

42 **Raffaghello L**, Nozza P, Morandi F, Camoriano M, Wang X, Garrè ML, Cama A, Basso G, Ferrone S, Gambini C, Pistoia V. Expression and functional analysis of human leukocyte antigen class I antigen-processing machinery in medulloblastoma. *Cancer Res* 2007; **67**: 5471-5478 [PMID: 17545629 DOI: 10.1158/0008-5472.CAN-06-4735]

43 **Smith C**, Santi M, Rajan B, Rushing EJ, Choi MR, Rood BR, Cornelison R, MacDonald TJ, Vukmanovic S. A novel role of HLA class I in the pathology of medulloblastoma. *J Transl Med* 2009; **7**: 59 [PMID: 19594892 DOI: 10.1186/1479-5876-7-59]

44 **Haberthur K**, Brennan K, Hoglund V, Balcaitis S, Chinn H, Davis A, Kreuser S, Winter C, Leary SE, Deutsch GH, Ellenbogen RG, Crane CA. NKG2D ligand expression in pediatric brain tumors. *Cancer Biol Ther* 2016; **17**: 1253-1265 [PMID: 27834580 DOI: 10.1080/15384047.2016.1250047]

45 **Dehne N**, Mora J, Namgaladze D, Weigert A, Brüne B. Cancer cell and macrophage cross-talk in the tumor microenvironment. *Curr Opin Pharmacol* 2017; **35**: 12-19 [PMID: 28538141 DOI: 10.1016/j.coph.2017.04.007]

46 **Feng X**, Szulzewsky F, Yerevanian A, Chen Z, Heinzmann D, Rasmussen RD, Alvarez-Garcia V, Kim Y, Wang B, Tamagno I, Zhou H, Li X, Kettenmann H, Ransohoff RM, Hambardzumyan D. Loss of CX3CR1 increases accumulation of inflammatory monocytes and promotes gliomagenesis. *Oncotarget* 2015; **6**: 15077-15094 [PMID: 25987130 DOI: 10.18632/oncotarget.3730]

47 **Fridman WH**, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol* 2017; **14**: 717-734 [PMID: 28741618 DOI: 10.1038/nrclinonc.2017.101]

48 **Gieryng A**, Pszczolkowska D, Walentynowicz KA, Rajan WD, Kaminska B. Immune microenvironment of gliomas. *Lab Invest* 2017; **97**: 498-518 [PMID: 28287634 DOI: 10.1038/labinvest.2017.19]

49 **Ye XZ**, Xu SL, Xin YH, Yu SC, Ping YF, Chen L, Xiao HL, Wang B, Yi L, Wang QL, Jiang XF, Yang L, Zhang P, Qian C, Cui YH, Zhang X, Bian XW. Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells *via* TGF-β1 signaling pathway. *J Immunol* 2012; **189**: 444-453 [PMID: 22664874 DOI: 10.4049/jimmunol.1103248]

50 **Mantovani A**, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol* 2017; **14**: 399-416 [PMID: 28117416 DOI: 10.1038/nrclinonc.2016.217]

51 **Wenes M**, Shang M, Di Matteo M, Goveia J, Martín-Pérez R, Serneels J, Prenen H, Ghesquière B, Carmeliet P, Mazzone M. Macrophage Metabolism Controls Tumor Blood Vessel Morphogenesis and Metastasis. *Cell Metab* 2016; **24**: 701-715 [PMID: 27773694 DOI: 10.1016/j.cmet.2016.09.008]

52 **Hussain SF**, Yang D, Suki D, Aldape K, Grimm E, Heimberger AB. The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. *Neuro Oncol* 2006; **8**: 261-279 [PMID: 16775224 DOI: 10.1215/15228517-2006-008]

53 **Engler JR**, Robinson AE, Smirnov I, Hodgson JG, Berger MS, Gupta N, James CD, Molinaro A, Phillips JJ. Increased microglia/macrophage gene expression in a subset of adult and pediatric astrocytomas. *PLoS One* 2012; **7**: e43339 [PMID: 22937035 DOI: 10.1371/journal.pone.0043339]

54 **Kurdi M**, Alghamdi B, Butt NS, Baeesa S. The relationship between CD204 (M2)-polarized tumour-associated macrophages (TAMs), tumour-infiltrating lymphocytes (TILs), and microglial activation in glioblastoma microenvironment: a novel immune checkpoint receptor target. *Discov Oncol* 2021; **12**: 28 [PMID: 35201470 DOI: 10.1007/s12672-021-00423-8]

55 **Noman MZ**, Desantis G, Janji B, Hasmim M, Karray S, Dessen P, Bronte V, Chouaib S. PD-L1 is a novel direct target of HIF-1α, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *J Exp Med* 2014; **211**: 781-790 [PMID: 24778419 DOI: 10.1084/jem.20131916]

56 **Bloch O**, Crane CA, Kaur R, Safaee M, Rutkowski MJ, Parsa AT. Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. *Clin Cancer Res* 2013; **19**: 3165-3175 [PMID: 23613317 DOI: 10.1158/1078-0432.CCR-12-3314]

57 **Gordon SR**, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, Gupta R, Tsai JM, Sinha R, Corey D, Ring AM, Connolly AJ, Weissman IL. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* 2017; **545**: 495-499 [PMID: 28514441 DOI: 10.1038/nature22396]

58 **Margol AS**, Robison NJ, Gnanachandran J, Hung LT, Kennedy RJ, Vali M, Dhall G, Finlay JL, Erdreich-Epstein A, Krieger MD, Drissi R, Fouladi M, Gilles FH, Judkins AR, Sposto R, Asgharzadeh S. Tumor-associated macrophages in SHH subgroup of medulloblastomas. *Clin Cancer Res* 2015; **21**: 1457-1465 [PMID: 25344580 DOI: 10.1158/1078-0432.CCR-14-1144]

59 **Zhang J**, Yuan X, Wang Y, Liu J, Li Z, Li S, Liu Y, Gong X, Sun Y, Wu W, Sun L, Du S, Wang T. Tumor-Associated Macrophages Correlate With Prognosis in Medulloblastoma. *Front Oncol* 2022; **12**: 893132 [PMID: 35860588 DOI: 10.3389/fonc.2022.893132]

60 **O'Connor T**, Heikenwalder M. CCL2 in the Tumor Microenvironment. *Adv Exp Med Biol* 2021; **1302**: 1-14 [PMID: 34286437 DOI: 10.1007/978-3-030-62658-7_1]

61 **Maximov V**, Chen Z, Wei Y, Robinson MH, Herting CJ, Shanmugam NS, Rudneva VA, Goldsmith KC, MacDonald TJ, Northcott PA, Hambardzumyan D, Kenney AM. Tumour-associated macrophages exhibit anti-tumoural properties in Sonic Hedgehog medulloblastoma. *Nat Commun* 2019; **10**: 2410 [PMID: 31160587 DOI: 10.1038/s41467-019-10458-9]

62 **Crotty EE**, Smith SMC, Brasel K, Pakiam F, Girard EJ, Connor YD, Zindy F, Mhyre AJ, Roussel MF, Olson JM. Medulloblastoma recurrence and metastatic spread are independent of colony-stimulating factor 1 receptor signaling and macrophage survival. *J Neurooncol* 2021; **153**: 225-237 [PMID: 33963961 DOI: 10.1007/s11060-021-03767-x]

63 **Lee C**, Lee J, Choi SA, Kim SK, Wang KC, Park SH, Kim SH, Lee JY, Phi JH. M1 macrophage recruitment correlates with worse outcome in SHH Medulloblastomas. *BMC Cancer* 2018; **18**: 535 [PMID: 29739450 DOI: 10.1186/s12885-018-4457-8]

64 **Patt S**, Zimmer C. Age-related immunoreactivity pattern in medulloblastoma. *Childs Nerv Syst* 1992; **8**: 326-331 [PMID: 1382852 DOI: 10.1007/BF00296563]

65 **Pizer BL**, Clifford SC. The potential impact of tumour biology on improved clinical practice for medulloblastoma: progress towards biologically driven clinical trials. *Br J Neurosurg* 2009; **23**: 364-375 [PMID: 19637007 DOI: 10.1080/02688690903121807]

66 **Hill RM**, Kuijper S, Lindsey JC, Petrie K, Schwalbe EC, Barker K, Boult JK, Williamson D, Ahmad Z, Hallsworth A, Ryan SL, Poon E, Robinson SP, Ruddle R, Raynaud FI, Howell L, Kwok C, Joshi A, Nicholson SL, Crosier S, Ellison DW, Wharton SB, Robson K, Michalski A, Hargrave D, Jacques TS, Pizer B, Bailey S, Swartling FJ, Weiss WA, Chesler L, Clifford SC. Combined MYC and P53 defects emerge at medulloblastoma relapse and define rapidly progressive, therapeutically targetable disease. *Cancer Cell* 2015; **27**: 72-84 [PMID: 25533335 DOI: 10.1016/j.ccell.2014.11.002]

67 **Yang J**, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell* 2008; **14**: 818-829 [PMID: 18539112 DOI: 10.1016/j.devcel.2008.05.009]

68 **Bonde AK**, Tischler V, Kumar S, Soltermann A, Schwendener RA. Intratumoral macrophages contribute to epithelial-mesenchymal transition in solid tumors. *BMC Cancer* 2012; **12**: 35 [PMID: 22273460 DOI: 10.1186/1471-2407-12-35]

69 **Funakoshi Y**, Sugihara Y, Uneda A, Nakashima T, Suzuki H. Recent advances in the molecular understanding of medulloblastoma. *Cancer Sci* 2022 [PMID: 36520034 DOI: 10.1111/cas.15691]

70 **Engelhardt B**, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. *Trends Immunol* 2012; **33**: 579-589 [PMID: 22926201 DOI: 10.1016/j.it.2012.07.004]

71 **Salsman VS**, Chow KK, Shaffer DR, Kadikoy H, Li XN, Gerken C, Perlaky L, Metelitsa LS, Gao X, Bhattacharjee M, Hirschi K, Heslop HE, Gottschalk S, Ahmed N. Crosstalk between medulloblastoma cells and endothelium triggers a strong chemotactic signal recruiting T lymphocytes to the tumor microenvironment. *PLoS One* 2011; **6**: e20267 [PMID: 21647415 DOI: 10.1371/journal.pone.0020267]

72 **Quaranta V**, Schmid MC. Macrophage-Mediated Subversion of Anti-Tumour Immunity. *Cells* 2019; **8** [PMID: 31331034 DOI: 10.3390/cells8070747]

73 **Picarda E**, Ohaegbulam KC, Zang X. Molecular Pathways: Targeting B7-H3 (CD276) for Human Cancer Immunotherapy. *Clin Cancer Res* 2016; **22**: 3425-3431 [PMID: 27208063 DOI: 10.1158/1078-0432.CCR-15-2428]

74 **Vonderheide RH**. Prospect of targeting the CD40 pathway for cancer therapy. *Clin Cancer Res* 2007; **13**: 1083-1088 [PMID: 17317815 DOI: 10.1158/1078-0432.CCR-06-1893]

75 **Arlauckas SP**, Garris CS, Kohler RH, Kitaoka M, Cuccarese MF, Yang KS, Miller MA, Carlson JC, Freeman GJ, Anthony RM, Weissleder R, Pittet MJ. In vivo imaging reveals a tumor-associated macrophage-mediated resistance pathway in anti-PD-1 therapy. *Sci Transl Med* 2017; **9** [PMID: 28490665 DOI: 10.1126/scitranslmed.aal3604]

76 **Gordon S**, Plüddemann A. Tissue macrophages: heterogeneity and functions. *BMC Biol* 2017; **15**: 53 [PMID: 28662662 DOI: 10.1186/s12915-017-0392-4]

77 **Winograd R**, Byrne KT, Evans RA, Odorizzi PM, Meyer AR, Bajor DL, Clendenin C, Stanger BZ, Furth EE, Wherry EJ, Vonderheide RH. Induction of T-cell Immunity Overcomes Complete Resistance to PD-1 and CTLA-4 Blockade and Improves Survival in Pancreatic Carcinoma. *Cancer Immunol Res* 2015; **3**: 399-411 [PMID: 25678581 DOI: 10.1158/2326-6066.CIR-14-0215]

78 **Martin AM**, Nirschl CJ, Polanczyk MJ, Bell WR, Nirschl TR, Harris-Bookman S, Phallen J, Hicks J, Martinez D, Ogurtsova A, Xu H, Sullivan LM, Meeker AK, Raabe EH, Cohen KJ, Eberhart CG, Burger PC, Santi M, Taube JM, Pardoll DM, Drake CG, Lim M. PD-L1 expression in medulloblastoma: an evaluation by subgroup. *Oncotarget* 2018; **9**: 19177-19191 [PMID: 29721192 DOI: 10.18632/oncotarget.24951]

79 **Gu P**, Gao JF, D'Souza CA, Kowalczyk A, Chou KY, Zhang L. Trogocytosis of CD80 and CD86 by induced regulatory T cells. *Cell Mol Immunol* 2012; **9**: 136-146 [PMID: 22307040 DOI: 10.1038/cmi.2011.62]

80 **Tekguc M**, Wing JB, Osaki M, Long J, Sakaguchi S. Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells. *Proc Natl Acad Sci U S A* 2021; **118** [PMID: 34301886 DOI: 10.1073/pnas.2023739118]

81 **Colotta F**, Peri G, Villa A, Mantovani A. Rapid killing of actinomycin D-treated tumor cells by human mononuclear cells. I. Effectors belong to the monocyte-macrophage lineage. *J Immunol* 1984; **132**: 936-944 [PMID: 6690624]

82 **Anfray C**, Ummarino A, Andón FT, Allavena P. Current Strategies to Target Tumor-Associated-Macrophages to Improve Anti-Tumor Immune Responses. *Cells* 2019; **9** [PMID: 31878087 DOI: 10.3390/cells9010046]

83 **Canè S**, Ugel S, Trovato R, Marigo I, De Sanctis F, Sartoris S, Bronte V. The Endless Saga of Monocyte Diversity. *Front Immunol* 2019; **10**: 1786 [PMID: 31447834 DOI: 10.3389/fimmu.2019.01786]

84 **Sánchez-Martín L**, Estecha A, Samaniego R, Sánchez-Ramón S, Vega MÁ, Sánchez-Mateos P. The chemokine CXCL12 regulates monocyte-macrophage differentiation and RUNX3 expression. *Blood* 2011; **117**: 88-97 [PMID: 20930067 DOI: 10.1182/blood-2009-12-258186]

85 **Koh YW**, Park C, Yoon DH, Suh C, Huh J. CSF-1R expression in tumor-associated macrophages is associated with worse prognosis in classical Hodgkin lymphoma. *Am J Clin Pathol* 2014; **141**: 573-583 [PMID: 24619759 DOI: 10.1309/AJCPR92TDDFARISU]

86 **DeNardo DG**, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, Gallagher WM, Wadhwani N, Keil SD, Junaid SA, Rugo HS, Hwang ES, Jirström K, West BL, Coussens LM. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov* 2011; **1**: 54-67 [PMID: 22039576 DOI: 10.1158/2159-8274.CD-10-0028]

87 **Stafford JH**, Hirai T, Deng L, Chernikova SB, Urata K, West BL, Brown JM. Colony stimulating factor 1 receptor inhibition delays recurrence of glioblastoma after radiation by altering myeloid cell recruitment and polarization. *Neuro Oncol* 2016; **18**: 797-806 [PMID: 26538619 DOI: 10.1093/neuonc/nov272]

88 **Alvarez-Arellano L**, Eguía-Aguilar P, Piña-Sánchez P, González-García N, Palma-Guzman A, Perezpeña-Diazconti M, Maldonado-Bernal C. High expression of Toll-like receptor 7 is a survival factor in pediatric medulloblastoma. *Childs Nerv Syst* 2021; **37**: 3743-3752 [PMID: 34480601 DOI: 10.1007/s00381-021-05347-w]

89 **Thauvin C**, Widmer J, Mottas I, Hocevar S, Allémann E, Bourquin C, Delie F. Development of resiquimod-loaded modified PLA-based nanoparticles for cancer immunotherapy: A kinetic study. *Eur J Pharm Biopharm* 2019; **139**: 253-261 [PMID: 30981947 DOI: 10.1016/j.ejpb.2019.04.007]

90 **Murata Y**, Kotani T, Ohnishi H, Matozaki T. The CD47-SIRPα signalling system: its physiological roles and therapeutic application. *J Biochem* 2014; **155**: 335-344 [PMID: 24627525 DOI: 10.1093/jb/mvu017]

91 **Luzzi S**, Giotta Lucifero A, Brambilla I, Semeria Mantelli S, Mosconi M, Foiadelli T, Savasta S. Targeting the medulloblastoma: a molecular-based approach. *Acta Biomed* 2020; **91**: 79-100 [PMID: 32608377 DOI: 10.23750/abm.v91i7-S.9958]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 March 24; 14(3): 131-137

DOI: 10.5306/wjco.v14.i3.131

ISSN 2218-4333 (online)

CASE REPORT

Unusual breast metastasis of gastrointestinal stromal tumor: A case report and literature review

Daria Filonenko, Nikolay Karnaukhov, Gurami Kvetenadze, Lyudmila Zhukova

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Guo F, China; Machaku D, Tanzania; Wang Z, China; Zhao G, China

Received: September 21, 2022
Peer-review started: September 21, 2022
First decision: November 14, 2022
Revised: November 27, 2022
Accepted: February 21, 2023
Article in press: February 21, 2023
Published online: March 24, 2023

Daria Filonenko, Department of Oncology Chemotherapy, SBIH Moscow Clinical Scientific and Practical Center Named After A.S.Loginov of DHM Moscow, Moscow 111123, Russia

Nikolay Karnaukhov, Department of Pathomorphology, SBIH "Moscow Clinical Scientific and Practical Center Named After A.S. Loginov" DHM, Moscow 111123, Russia

Gurami Kvetenadze, Department of Surgery, SBIH "Moscow Clinical Scientific and Practical Center Named After A.S. Loginov" DHM, Moscow 111123, Russia

Lyudmila Zhukova, Department of Oncology, SBIH "Moscow Clinical Scientific and Practical Center Named After A.S. Loginov" DHM, Moscow 111123, Russia

Corresponding author: Daria Filonenko, PhD, Chief Physician, Department of Oncology Chemotherapy, SBIH Moscow Clinical Scientific and Practical Center Named After A.S.Loginov of DHM Moscow, Shosse Enthuziastov 86, Moscow 111123, Russia.
shubina_d@mail.ru

Abstract

BACKGROUND

Gastrointestinal stromal tumors (GISTs) are the most frequent mesenchymal tumors of gastrointestinal tract. The most common sites of metastases are the liver and the peritoneum, whereas breast metastases from GIST are extremely rare. We present a second case of GIST breast metastasis.

CASE SUMMARY

We found a case of breast metastasis from rectum GIST. A 55-year-old female patient presented with rectum tumor with multiply liver lesions and metastasis in the right breast. Abdominal-perineal extirpation of rectum was performed, histology and immunohistochemistry study showed GIST, mixed type with CD117 and DOG-1 positive staining. The patient was taking imatinib 400 mg for 22 mo with stable disease. Because of growth of the breast metastasis the treatment was changed twice: The dose of imatinib was doubled with further progression in the breast lesion and then the patient was receiving sunitinib for 26 mo with partial response in the right breast and stable disease in the liver lesions. The breast lesion increased and right breast resection was done – surgery on local progression, the liver metastases were stable. Histology and immunohisto-chemistry studies revealed GIST metastasis, CD 117 and DOG 1 positive with KIT exon 11 mutation. After surgery the patient resumed imatinib. Until now the patient has been taking imatinib 400 mg for 19 mo without progression, last

follow up was in November 2022.

CONCLUSION
GISTs breast metastases are extremely rare, we described the second case. At the same time second primary tumors have been reported frequently in patients diagnosed with GISTs and breast cancer is one of the most common second primary tumors in patients with GISTs. That is why it is very important to distinguish primary from metastatic breast lesions. Surgery on local progression made it possible to resume less toxic treatment.

Key Words: Gastrointestinal stromal tumors; Metastases; Breast; Limited progression; Case report

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: We presented the second case of gastrointestinal stromal tumor (GIST) metastasis to the breast, which is a very extraordinary condition. The most common metastatic sites of GIST are the liver and the peritoneum and at the same time metastasis to the breast from extramammary carcinomas is extremely rare and in this clinical situation it is obligatory to exclude breast cancer. Our patient received two lines of treatment due to metastatic disease and had a local progression on imatinib and sunitinib therapy, growth only lesion in the breast, we removed increased metastasis (surgery on local progression), that allowed to return to less toxic treatment, the patient resumed imatinib until now.

Citation: Filonenko D, Karnaukhov N, Kvetenadze G, Zhukova L. Unusual breast metastasis of gastrointestinal stromal tumor: A case report and literature review. *World J Clin Oncol* 2023; 14(3): 131-137
URL: https://www.wjgnet.com/2218-4333/full/v14/i3/131.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i3.131

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are rare tumors, with an incidence 1%-2% and at the same time are the most frequent mesenchymal tumors of gastrointestinal tract[1]. Approximately 10% to 20% of patients present with metastatic disease[2]. Many evidences showed that gastrointestinal tumors can metastasize to other parts of the body[3]. The most common metastatic sites of GISTs are the liver (60%-70%) and the peritoneum (20%-30%)[4]. Lung metastases (2%-9%), bone and soft tissue (1%-6%) and skin (1%) can occur but very rare. Casuistic bizzare cases of GISTs metastasis to brain[5], core[6], ovary [7,8], and breast[9] are described in the literature. We made a literature review, breast metastases from GIST have been previously described only in one case.

Breast tumors are usually primary. The incidence of metastatic spread from extramammary sites to the breast varies between 0.4% and 1.5% of all breast malignancies. The breast is considered to be resistant to metastasis because it contains large areas of fibrous tissue with a relatively low supply of blood. Most common malignancies that metastasize into the breast are lymphoma, leukemia, melanoma and carcinomas of stomach, ovary, lung, kidney and others[10-12].

In the article we report a second case of GIST patient presenting breast metastasis, highlighting the pathological/molecular features of this unusual site of metastatic presentation and the clinical implications.

CASE PRESENTATION

Chief complaints

The 55-year-old female complained of the tumor in her right breast.

History of present illness

In April 2016 55-year-old female patient presented with recurrent rectum tumor. Abdominal-perineal extirpation of rectum was performed, histology and immunohistochemistry study showed GIST, mixed type with CD117 and DOG-1 positive staining. After the surgery computer tomography (CT) revealed multiply cystic liver lesions that were estimated as metastases, biopsy was not done. At the same time, the patient found lesion in her right breast 30 mm, biopsy was not performed. The patient was taking imatinib 400 mg from June 2016 until August 2018 for 22 mo with stable disease.

In September 2018 Lesion in the right breast increased, liver lesions were stable and the dose of imatinib was doubled, the patient received imatinib 800mg from September until December 2018 with further growth of breast lesion. From December 2018 until March 2020 for 15 mo the patient was receiving sunitinib 50 mg 4/2 regiment. In March 2020 the dose of sunitinib was reduced to 25 mg every day without a break because of hand–food skin reaction grade 2 and then until February 2021 for 11 mo the patient continued the treatment with partial response lesion in the right breast and stable disease in the liver lesions. The toxicity of modified regiment was acceptable. The patient became hypothyroid and received levothyroxine 25 mcg.

In August 2020 breast ultrasound and magnetic resonance imaging were done at the first time and revealed heterogeneous lesion 47 mm on the border of the upper quadrants of the right breast with central zone of necrosis and peripheral vascularization, BIRADS 5 (Figure 1). The biopsy of the right breast was done to exclude primary breast cancer, histology and immunohistochemistry showed metastasis of GIST, mixed type, 10 mitoses with CD117 and DOG -1 positive staining.

In February 2021 control positron emission tomography-computed tomography (PET-CT) in comparison with September 2020 was obtained and demonstrated progression in the right breast lesion, size increased from 39 mm to 48 mm and FDG uptake increased from 7 to 12 and invasion to the large pectoral muscle was detected (Figure 2). The multiply liver metastases were stable.

History of past illness

The patient had a rectum leiomyoma resection twice in 2012 and 2013 then the patient was on follow up until April 2016.

Personal and family history

The patient's other medical history was not noteworthy.

Physical examination

In the right breast on the border of the upper quadrants the solid lesion was revealed, 50 mm in size.

Laboratory examinations

Laboratory testing showed any clinically significant abnormalities.

Imaging examinations

In April 2021, histology and immunohistochemistry studies showed tumor macroscopical size 50 mm with thick fibrous capsule, with histologically negative margins (Figure 3); microscopic examination showed predominantly epithelioid type with focuses of spindle cell that occupied 15% of the square, with prominent hyperchromatic nuclei, high mitotic index (72 mitoses per 50 HPF), small foci of necrosis and large hemorrhagic areas; immunohistochemistry study showed immunophenotype typical for GIST: Strong cytoplasmic expression CD34, membrane-cytoplasmic expression CD117 and DOG-1 (Figure 4).

MULTIDISCIPLINARY EXPERT CONSULTATION

The clinical situation was estimated as local progression: increase of breast lesion and stable of the liver lesions. Because of growth of the breast metastasis the treatment was changed twice: The patient received double dose of imatinib and sunitinib. Taking into account that the patient had local progression we decided to remove increasing lesion.

FINAL DIAGNOSIS

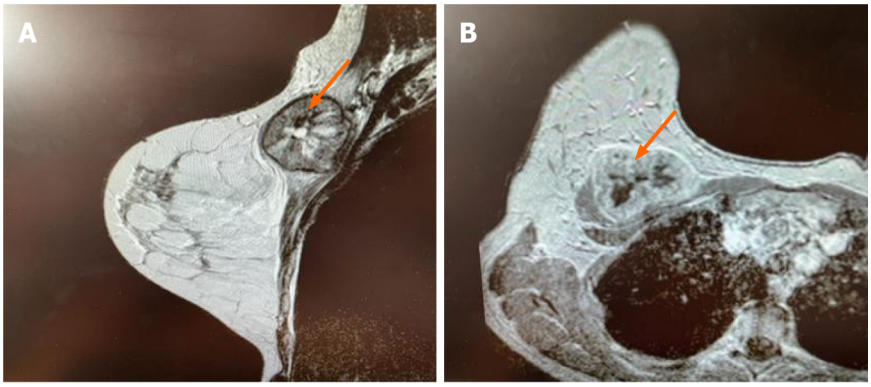
The final diagnosis was local progression: Increase of breast lesion and stable of the liver lesions.

TREATMENT

In April 2021 right breast resection with partial resection of large pectoral muscle was done. The patient had undergone surgery in 2012, 2013 and 2016 in different clinics, unfortunately histology materials were lost and we had no opportunity to compare histological specimens.

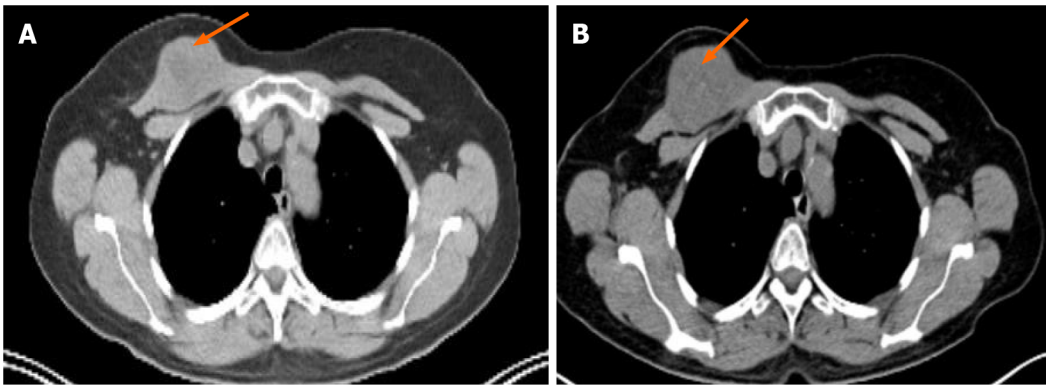
Molecular analysis was performed on the breast metastasis by direct Sanger sequencing and revealed a KIT exon 11 mutation, with a consequent 557-559 deletion.

After surgery we decided to resume imatinib. Previously we changed treatment because of the growth of breast lesion (increased imatinib dose, prescribed sunitinib) and then we removed increased



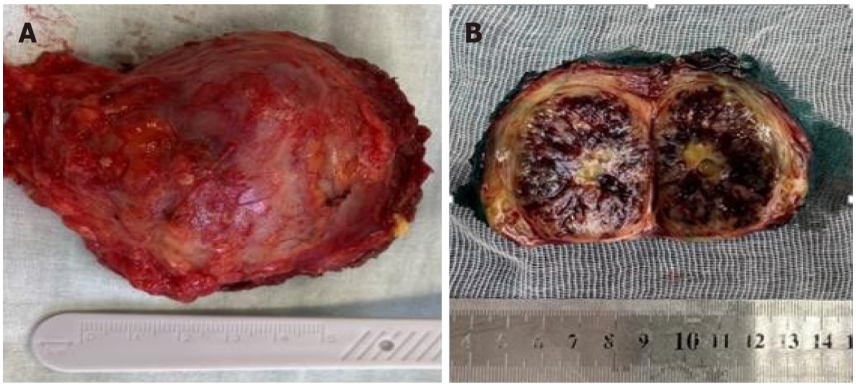
DOI: 10.5306/wjco.v14.i3.131 **Copyright** ©The Author(s) 2023.

Figure 1 Breast magnetic resonance imaging. Heterogeneous lesion 47 mm (orange arrows) on the border of the upper quadrants of the right breast with central zone of necrosis and peripheral vascularization. A: Axial; B: Frontal.



DOI: 10.5306/wjco.v14.i3.131 **Copyright** ©The Author(s) 2023.

Figure 2 Positron emission tomography-computed tomography. A: Computed tomography (CT) on September 2020; B: CT on February 2021 demonstrated progression in the right breast lesion (orange arrows), size increased from 39 mm to 48 mm.



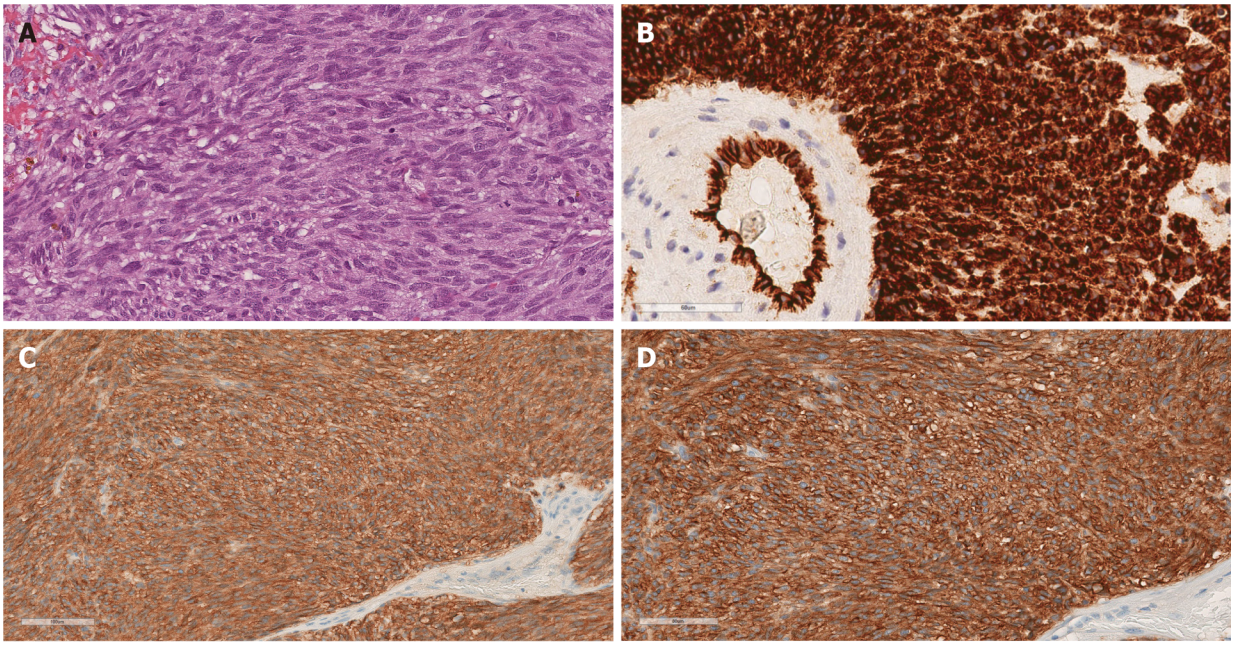
DOI: 10.5306/wjco.v14.i3.131 **Copyright** ©The Author(s) 2023.

Figure 3 Macroscopic examination. A, B: Macroscopical size 50 mm, thick fibrous capsule.

metastasis that is why we decided to return to less toxic treatment.

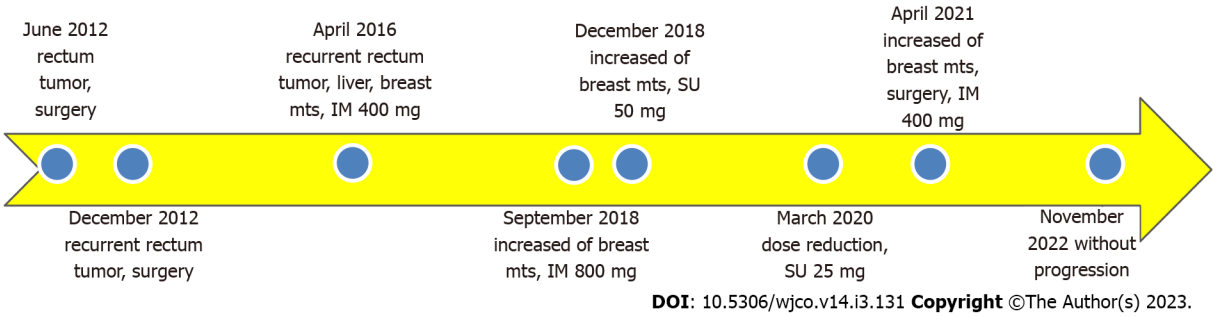
OUTCOME AND FOLLOW-UP

From April 2021 until now patient has been taking imatinib for 19 mo without progression, PET-CT was done in November 2022. By November 2022 the live duration with metastatic GIST is 77 mo (Figure 5).



DOI: 10.5306/wjco.v14.i3.131 **Copyright** ©The Author(s) 2023.

Figure 4 Microscopic examination. A: Histology, original magnification ×200, hematoxylin-eosin stain; B: Immunohistochemistry original magnification ×200, CD34 positive stain; C: Immunohistochemistry ×200, CD117 positive stain, D: Immunohistochemistry original magnification×200, DOG-1 positive stain.



DOI: 10.5306/wjco.v14.i3.131 **Copyright** ©The Author(s) 2023.

Figure 5 Treatment timeline.

DISCUSSION

We made a literature review, breast metastases from GIST have been previously described only in one case[9]. Hasbay *et al*[9] reported the clinical case of 46-year-old women with metastases of GIST to liver, bone, abdominal lymph nodes and left breast. We reported the second case of GIST metastases to the breast.

In this case we came across with diagnostic challenges because the most common metastatic sites of GIST are the liver and the peritoneum and at the same time metastasis to the breast from extra-mammary carcinomas is extremely rare and varies between 0.4% and 1.5% of all breast malignancies [10]. We usually deal with primary breast cancer because that is the most common female malignancies [13].

Recently, there is an increasing evidence regarding the association of sporadic GISTs with second neoplasia. In a systematic review and meta-analysis conducted the rate of secondary tumors with GISTs was reported to be 20%[14]. Breast cancers are the most common malignancies together with GISTs.

Taking into account that breast metastasis from GISTs are extremely rare and that second tumors including breast cancer are common, at first in this clinical situation it is obligatory to exclude breast cancer that we have done.

Our patient had a local progression, growth only lesion in the breast, we removed increased metastasis that allowed us to return to less toxic treatment, the patient resumed imatinib. The critical question of whether surgery provides additional benefit over remaining on tyrosine kinase inhibitors (TKIs) therapy alone without surgical resection is unanswered. Randomized trials failed to recruit quickly enough to meet target accrual. In the absence of randomized trials, single-institution and multi-institutional retrospective studies document long-term disease control and longer overall survival for

selected patients who undergo metastasectomy of increased lesions while other lesions under control (local progression) on imatinib therapy. Removal of increased metastases let to continue imatinib therapy and not to change the TKIs that have less efficacy and not so favorable profile of toxicity. The median time to progression on sunitinib therapy is 6 mo, on regorafenib only 4 mo. Fairweather *et al*[15] published the largest series of patients with metastatic GIST treated with TKI undergoing surgical resection (n = 323). The median time to progression during imatinib therapy on local progression was 47 mo from the start of imatinib and 11 mo from cytoreductive surgery, removal increasing lesions[15]. These data are consistent with the result of treatment of our patient; the duration of imatinib therapy after surgery is 19 mo that is more than four times higher than the median PFS on regorafenib therapy.

CONCLUSION

In conclusion, breast metastases from GISTs are very rare, but it is clinically very important to distinguish primary from metastatic breast lesions.

FOOTNOTES

Author contributions: Filonenko D has been treating the patient, developing the treatment strategy of the patient; made the literature review, analyzed the data and wrote the text of the article and revised the article according to editor's revisions; Karnaukhov N is pathologist who made the morphology, histology and immunohistochemistry investigations, take a photo of these investigations; Kvetenadze G is a surgeon who performed resection of the right breast; Zhukov L is developing the treatment strategy of the patient, made the literature review, analyzed the data and correct the text of the article and revised the article according to editor's revisions.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to report.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Russia

ORCID number: Daria Filonenko 0000-0002-7224-3111; Nikolay Karnaukhov 0000-0003-0889-2720; Lyudmila Zhukova 0000-0003-4848-6938.

S-Editor: Wang JL
L-Editor: A
P-Editor: Wang JL

REFERENCES

1 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors--definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch* 2001; **438**: 1-12 [PMID: 11213830 DOI: 10.1007/s004280000338]
2 **Miettinen M**, Sarlomo-Rikala M, Lasota J. Gastrointestinal stromal tumors: recent advances in understanding of their biology. *Hum Pathol* 1999; **30**: 1213-1220 [PMID: 10534170 DOI: 10.1016/s0046-8177(99)90040-0]
3 **Tulsi R**, Ul Haque MM, Hanif FM, Devi A, Mubarak M, Hassan Luck N. Metastasis of Duodenal Adenocarcinoma to the Urinary Bladder Presenting as Hematuria. *J Transl Int Med* 2021; **9**: 143-145 [PMID: 34497753 DOI: 10.2478/jtim-2021-0010]
4 **Liu P**, Tan F, Liu H, Ge J, Liu S, Lei T, Zhao X. Skin Metastasis of Gastrointestinal Stromal Tumors: A Case Series and Literature Review. *Cancer Manag Res* 2020; **12**: 7681-7690 [PMID: 32904396 DOI: 10.2147/CMAR.S261823]
5 **Wong CS**, Chu YC. Intra-cranial metastasis of gastrointestinal stromal tumor. *Chin Med J (Engl)* 2011; **124**: 3595-3597 [PMID: 22340184 DOI: 10.3760/cma.j.issn.0366-6999.2011.21.029]
6 **Bashir U**, Qureshi A, Khan HA, Uddin N. Gastrointestinal stromal tumor with skeletal muscle, adrenal and cardiac metastases: an unusual occurrence. *Indian J Pathol Microbiol* 2011; **54**: 362-364 [PMID: 21623091 DOI:

10.4103/0377-4929.81638]
7 **De Leo A**, Nannini M, Dondi G, Santini D, Urbini M, Gruppioni E, De Iaco P, Perrone AM, Pantaleo MA. Unusual bilateral ovarian metastases from ileal gastrointestinal stromal tumor (GIST): a case report. *BMC Cancer* 2018; **18**: 301 [PMID: 29548310 DOI: 10.1186/s12885-018-4204-1]
8 **Irving JA**, Lerwill MF, Young RH. Gastrointestinal stromal tumors metastatic to the ovary: a report of five cases. *Am J Surg Pathol* 2005; **29**: 920-926 [PMID: 15958857 DOI: 10.1097/01.pas.0000155161.55915.c3]
9 **Hasbay B**, Aytaç HÖ, Kayaselçuk F, Torun N. An Unusual Gastrointestinal Stromal Tumor Presentain: Breast, Liver and Lymph Node Metastasis. *Eur J Breast Health* 2017; **13**: 216-218 [PMID: 29082381 DOI: 10.5152/ejbh.2017.3492]
10 **McFarlane ME**. Metastasis to the breast: a rare site for secondary malignancy. *Int J Surg* 2006; **4**: 204-205 [PMID: 17462351 DOI: 10.1016/j.ijsu.2005.10.008]
11 **Klingen TA**, Klaasen H, Aas H, Chen Y, Akslen LA. Secondary breast cancer: a 5-year population-based study with review of the literature. *APMIS* 2009; **117**: 762-767 [PMID: 19775345 DOI: 10.1111/j.1600-0463.2009.02529.x]
12 **Wang Y**, Hou K, Jin Y, Bao B, Tang S, Qi J, Yang Y, Che X, Liu Y, Hu X, Zheng C. Lung adenocarcinoma-specific three-integrin signature contributes to poor outcomes by metastasis and immune escape pathways. *J Transl Int Med* 2021; **9**: 249-263 [PMID: 35136724 DOI: 10.2478/jtim-2021-0046]
13 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin* 2021; **71**: 7-33 [PMID: 33433946 DOI: 10.3322/caac.21654]
14 **Petrelli F**, Tomasello G, Barni S, Varricchio A, Costanzo A, Rampulla V, Cabiddu M, Turati L, Russo A, Seghezzi S, Passalacqua R, Ghidini M. Risk of second primary tumors in GIST survivors: A systematic review and meta-analysis. *Surg Oncol* 2019; **29**: 64-70 [PMID: 31196495 DOI: 10.1016/j.suronc.2019.03.001]
15 **Fairweather M**, Balachandran VP, Li GZ, Bertagnolli MM, Antonescu C, Tap W, Singer S, DeMatteo RP, Raut CP. Cytoreductive Surgery for Metastatic Gastrointestinal Stromal Tumors Treated With Tyrosine Kinase Inhibitors: A 2-institutional Analysis. *Ann Surg* 2018; **268**: 296-302 [PMID: 28448384 DOI: 10.1097/SLA.0000000000002281]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 April 24; 14(4): 138-189

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 4 April 24, 2023

REVIEW

138 Immunotherapy in glioblastoma treatment: Current state and future prospects

Rocha Pinheiro SL, Lemos FFB, Marques HS, Silva Luz M, de Oliveira Silva LG, Faria Souza Mendes dos Santos C, da Costa Evangelista K, Calmon MS, Sande Loureiro M, Freire de Melo F

MINIREVIEWS

160 Integration of molecular testing for the personalized management of patients with diffuse large B-cell lymphoma and follicular lymphoma

Stuckey R, Luzardo Henríquez H, de la Nuez Melian H, Rivero Vera JC, Bilbao-Sieyro C, Gómez-Casares MT

171 Current progress on the endoscopic features of colorectal sessile serrated lesions

Wang RG, Wei L, Jiang B

ORIGINAL ARTICLE

Retrospective Cohort Study

179 Interaction between age and gender on survival outcomes in extramedullary multiple myeloma over the past two decades

Bangolo AI, Fwelo P, Trivedi C, Sagireddy S, Aljanaahi H, Auda A, Mohamed M, Onyeka S, Fisher M, Thapa J, Tabucanon EJ, Georgiev L, Wishart A, Kumari S, Erikson C, Bangura M, Paddy O, Madhukar R, Gomez EL, Rathod J, Naria M, Hajal B, Awadhalla M, Siegel D, Parmar H, Biran N, Vesole DH, Phull P, Weissman S

World Journal of Clinical Oncology

Monthly Volume 14 Number 4 April 24, 2023

Contents

ABOUT COVER

Peer Reviewer of of *World Journal of Clinical Oncology*, Xiang Wang, MD, PhD, Associate Professor, Associate Chief Physician, Department of Oral Medicine, Nanjing Stomatological Hospital, Medical School of Nanjing University, No. 30 Zhongyang Road, Nanjing 210008, Jiangsu Province, China. wangxiang@nju.edu.cn

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 edition of Journal Citation Reports® cites the 2021 Journal Citation Indicator (JCI) for *WJCO* as 0.35.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Yu-Jie Ma*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
April 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 April 24; 14(4): 138-159

DOI: 10.5306/wjco.v14.i4.138

ISSN 2218-4333 (online)

REVIEW

Immunotherapy in glioblastoma treatment: Current state and future prospects

Samuel Luca Rocha Pinheiro, Fabian Fellipe Bueno Lemos, Hanna Santos Marques, Marcel Silva Luz, Luís Guilherme de Oliveira Silva, Clara Faria Souza Mendes dos Santos, Karolaine da Costa Evangelista, Mariana Santos Calmon, Matheus Sande Loureiro, Fabrício Freire de Melo

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Chen YH, China; Rizzo A, Italy

Received: December 28, 2022
Peer-review started: December 28, 2022
First decision: January 17, 2023
Revised: February 6, 2023
Accepted: April 12, 2023
Article in press: April 12, 2023
Published online: April 24, 2023

Samuel Luca Rocha Pinheiro, Fabian Fellipe Bueno Lemos, Marcel Silva Luz, Luís Guilherme de Oliveira Silva, Clara Faria Souza Mendes dos Santos, Karolaine da Costa Evangelista, Mariana Santos Calmon, Matheus Sande Loureiro, Fabrício Freire de Melo, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Vitória da Conquista 45029-094, Bahia, Brazil

Hanna Santos Marques, Campus Vitória da Conquista, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista 45029-094, Bahia, Brazil

Corresponding author: Fabrício Freire de Melo, PhD, Professor, Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Rua Hormindo Barros, 58, Quadra 17, Lote 58, Vitória da Conquista 45029-094, Bahia, Brazil. freiremeloufba@gmail.com

Abstract

Glioblastoma remains as the most common and aggressive malignant brain tumor, standing with a poor prognosis and treatment prospective. Despite the aggressive standard care, such as surgical resection and chemoradiation, median survival rates are low. In this regard, immunotherapeutic strategies aim to become more attractive for glioblastoma, considering its recent advances and approaches. In this review, we provide an overview of the current status and progress in immunotherapy for glioblastoma, going through the fundamental knowledge on immune targeting to promising strategies, such as Chimeric antigen receptor T-Cell therapy, immune checkpoint inhibitors, cytokine-based treatment, oncolytic virus and vaccine-based techniques. At last, it is discussed innovative methods to overcome diverse challenges, and future perspectives in this area.

Key Words: Glioblastoma; Immunotherapy; Tumor microenvironment; Chimeric antigen receptor T cell; Oncolytic viruses; Immune-checkpoint inhibitors; Brain cancer

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: This study aims to review the ongoing status and improvement made in immunotherapy for glioblastoma, a malignant brain tumor. Thus, this review goes through the general concepts of the tumor microenvironment, standard treatment and its limitations and immune targeting promising methods, such as Chimeric antigen receptor T-Cell therapy, immune checkpoint inhibitors, cytokine-based treatment, oncolytic virus and vaccine-based techniques. Finally, it is explained some methods to surpass the various challenges, and future prospects in this field.

Citation: Rocha Pinheiro SL, Lemos FFB, Marques HS, Silva Luz M, de Oliveira Silva LG, Faria Souza Mendes dos Santos C, da Costa Evangelista K, Calmon MS, Sande Loureiro M, Freire de Melo F. Immunotherapy in glioblastoma treatment: Current state and future prospects. *World J Clin Oncol* 2023; 14(4): 138-159
URL: https://www.wjgnet.com/2218-4333/full/v14/i4/138.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i4.138

INTRODUCTION

Glioblastomas (GBM) are the most common type of malignant tumor affecting the central nervous system. It is more common among men and its incidence is significantly related to age, being rare among young people and more common among the elderly, especially those aged between 74 and 85 years. It has a very poor prognosis, with survival of 12 to 15 mo after diagnosis, and, when untreated, of only 3 mo[1].

Regarding clinical manifestations, the symptoms are quite diverse and common to other types of brain tumors and include manifestations associated with intracranial hypertension such as intense headache, which can be accompanied by nausea and vomiting, focal neurological deficits, memory and personality changes, and seizures[2].

GBMs are tumors that originate from glial cells and are classified according to their histological characteristics as high-grade gliomas by the WHO, and the characteristics that define this denomination include hypercellularity, nuclear atypia and dysregulation of mitotic activity, besides microvascular proliferation and tumor necrosis[3]. So, they are classified as primary if there is no pre-existing involvement or secondary if they have progressed from low-grade astrocytomas; primary GBMs represent the majority of cases and secondary GBMs correspond to only 5 to 10% and usually affect young people[4].

In addition to histopathological analysis, molecular markers are essential for the understanding of the disease, since different genetic alterations can originate this type of tumor and determine subtypes that behave differently in terms of evolution and response to treatments used, which makes the identification of these factors essential for the establishment of therapeutic strategies. In this sense, GBMs can be grouped into 4 subtypes according to their molecular characteristics: classic, neural, pro-neural and mesenchymal[3,4].

Among the mutations related to the pathogenesis of GBM, we can cite 3 main pathways: receptor tyrosine kinase signaling, inhibition of the p53 pathway, and RB, and in most cases all three types of alterations are present. These mutations are associated with activation of oncogenes that act mainly in neoplastic proliferation, apoptosis disturbances, and cell cycle checkpoint failures that promote tumor cell survival[5]. Moreover, when compared to a normal brain, GBMs present a higher expression of genes related to immune cell infiltration, especially macrophages, and angiogenesis, noticing that hypoxia, which is characteristic of necrotic tumor regions, induces a higher expression of vascular endothelial growth factor (VEGF) and, consequently, a higher vascular proliferation[6].

Due to the characteristics of its pathogenesis, there is a diversity of cells that are found in the analysis of these tumors, including non-neoplastic components of the immune system. This is related to the tumor microenvironment of glioblastoma, since it has an inflammatory and pro-angiogenic characteristic that affects the permeability of the blood-brain barrier and allows the infiltration of defense cells, especially tumor-associated macrophages (TAM). The immune system in the early stages of the disease is responsible for controlling the development of the cancer, however, as proliferation progresses, the tumor cells become able to escape this surveillance and the defense cells not only become unable to perform this control, but start contributing to the growth of the tumor[7].

The available treatment is complex and usually requires a combination of different approaches and is dependent on a number of factors. Although there are other options and studies for the development of new treatments, the therapeutic strategies are still controversial and the prognosis is unsatisfactory with a high recurrence rate[8].

In this review, we provide an analysis of the ongoing status and progress in immunotherapy for glioblastoma, going through the general information about the tumor microenvironment, fundamental knowledge on immune targeting to promising strategies like Chimeric antigen receptor (CAR) T-Cell therapy, cytokine-based treatment, oncolytic virus and vaccine-based approaches. Finally, we discuss

contemporary methods to prevail distinct challenges, and future perspectives in this field.

CURRENT STANDARD CARE LIMITATIONS

The treatment of primary brain tumors such as GBM is still quite limited and, therefore, a major challenge in oncology. Although the treatment is difficult, expensive and subject to therapeutic failure, management protocols for patients with GBM consider multimodal therapeutic strategies that act in synergy in order to destroy the tumor. For this, such strategies must be individualized based on each patient according to their functional status, imaging exam, speed of disease progression, quality of life and clinical diagnosis. However, for new methods to be developed and current ones to be improved, it is necessary to think about the limitations of existing treatments. The Figure 1 synthesizes the current GBM treatment strategies and its advantages and limitations.

Surgical method

The surgical method is based on the maximum safe resection of the tumor and currently comprises the backbone of therapy for GBM[9], as in addition to reducing the volume of the neoplastic mass and the symptoms associated with parenchymal compression, the histological diagnosis and genetic study of the tumor are also possible by surgical intervention[10]. The aim of surgical treatment is to achieve a gross total resection as completely and safely as possible without risking the patient's functional status. Complete resection has been associated with a greater chance of survival and no progression than partial resection or biopsy. In this sense, some tools were developed to maximize the surgical procedure and alleviate as much as possible the neurological deficits that may be associated with the method. Among these tools, monitoring using fluorescence of tumor tissue with 5-aminolevulinic acid in conjunction with functional magnetic resonance imaging shows beneficial results[10,11].

However, GBMs are not cured with surgery alone, as almost all are recurrent and the biological pleomorphism of each tumor influences the degree of resectability of the cancer, with less malignant brain tumors being the most resectable[12]. Furthermore, the surgical method is extremely complex, delicate and expensive, because it demands a qualified neurosurgeon and sophisticated imaging equipment, in addition to the fact that the patient has the possibility of developing a neurological deficit as a result of the intervention, which may even prevent the following steps of the standard treatment, such as radiotherapy and chemotherapy[13]. Thus, it is necessary to accurately weigh the risks and benefits of the surgical technique.

Radiotherapy

Radiotherapy (RT) became popular in the 1970s and 1980s and is currently a therapeutic strategy based on the use of radiation volumes focused on specific regions. This method has become standard for GBMs since 2005, as it was in that year that a phase III clinical trial solidified the role of radiotherapy and adjuvant chemotherapy in the postoperative period of GBM[14]. After the surgical diagnosis, the patient is submitted to doses of 2 Gy for 6 wk until reaching a dose of 60 Gy[13]. It is an effective method that increases patient survival in different types of doses provided, especially hypofractionated doses, which make this method viable in elderly people (over 65 years old) with glioblastoma[9].

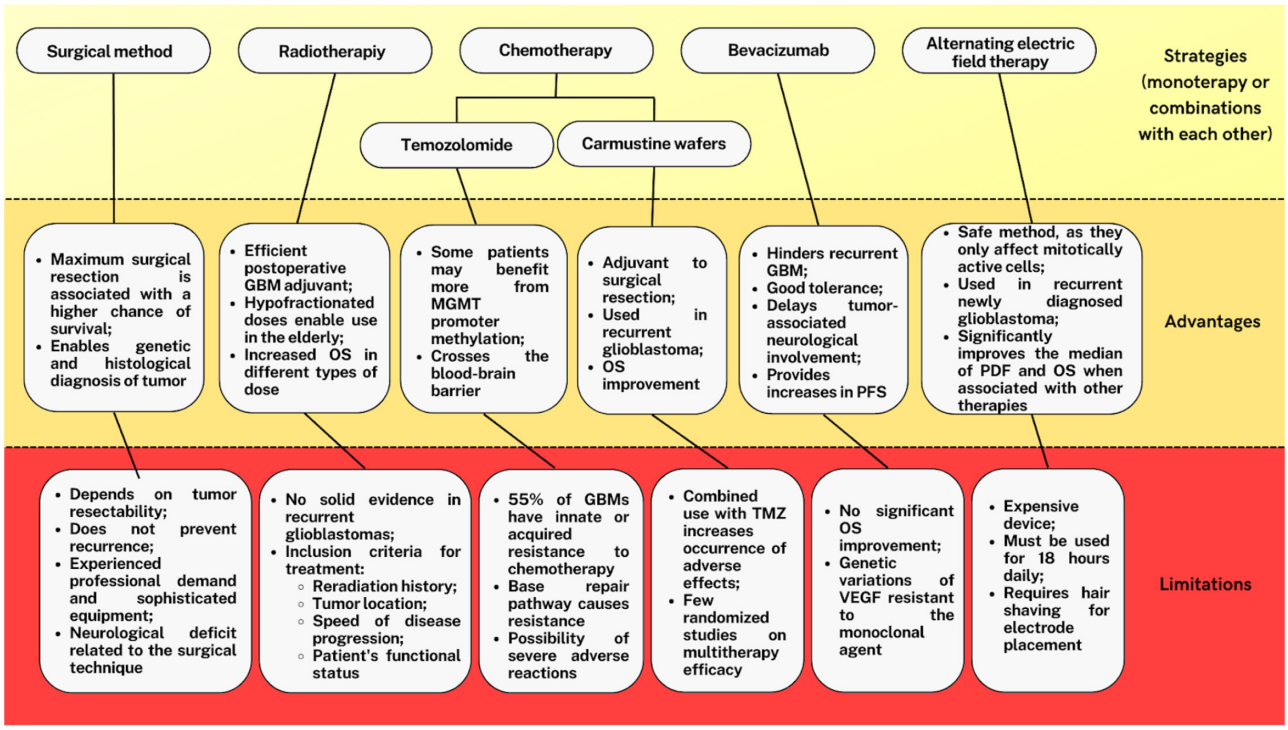
The combination of radiotherapy for 6 wk and chemotherapy with adjuvant Temozolomide 75 mg/m² for 6 wk and 150-200 mg/m² every 28 d for 6 mo is the gold standard treatment for young patients with glioblastoma. This combination of strategies significantly improved the survival of younger patients between 2 and 5 years[14].

RT has an important limitation in the sense that its use does not have much favorable evidence in recurrent gliomas, although it is extremely useful as a palliative therapy for small recurrent tumors[15]. In addition, it is necessary to be wise in the use of radiation, since the treatment protocol requires the patient's history of previous radiation, as well as the location of the tumor and the maximum dose for the structure in which it is allocated[16]. Finally, the therapeutic algorithm assesses the speed of disease progression and the patient's functional status. Thus, the use of chemoradiotherapy is not indicated for individuals over 70 years of age who do not have a good functional status, which is measured by the Functional Status Score for the Intensive Care Unit scale[15].

Chemotherapy

Temozolomide: Temozolomide (TMZ) is an alkylating agent that is cell cycle independent and is the most effective chemotherapy for GBM to current date. This efficiency is due to the ability to cross the blood-brain barrier and transportable cytosolic transformation to the cell nucleus[17]. The current standard of care in newly diagnosed GBM includes administration of 75 mg/m² of TMZ daily during the 6 wk of radiotherapy. Then, 150-200 mg/m² are maintained for 5 d at each 28-d cycle with 6 cycles of the drug[13].

However, this therapeutic strategy is variable based on the age of the patient, performance status according to the Karnofsky performance score, the promoter methylation status of the repair enzyme O(6)-Methylguanine-DNA-methyltransferase (MGMT) and the tumor recurrence[14], since TMZ does



DOI: 10.5306/wjco.v14.i4.138 **Copyright** ©The Author(s) 2023.

Figure 1 Scheme about current glioblastomas treatment strategies and its advantages and limitations. GBM: Glioblastomas; OS: Overall survival; PFS: Progression-free survival; TMZ: Temozolomide; VEGF: Vascular endothelial growth factor;

not prevent this event. This enzyme can cause patient resistance to TMZ, and some patients who have MGMT gene promoter methylation in the tumor may benefit from reduced drug resistance.

About 55% GBMs[12] have innate or acquired resistance to chemotherapy due to non-methylation of the MGMT promoter. In this way, the alkyl groups are removed from the O6 position of the guanine, reducing the pharmacological efficacy of the alkylating agents[18]. Another important mechanism of resistance to chemotherapy is the reduction of TMZ cytotoxicity by the base excision repair pathway. This pathway, mainly composed of poly (ADP-ribose) polymerase-1, is capable of repairing the bases methylated by the alkylating agent in the DNA and, therefore, reducing the occurrence of apoptotic events in tumor cells[19,20]. Thus, the use of iniparib and velparib is promising, either alone or in combination with TMZ, to reduce drug resistance[20,21].

It is noteworthy that the MGMT promoter methylation status is not routinely evaluated for all patients with the discussed disease and, if evaluated, the result may not be taken into account for TMZ treatment decision making in some clinics, as there may be lower availability of treatment agents, presence of severe adverse reactions to chemotherapy, associated comorbidities and preference for treatment by the patient.

Carmustine wafers: Carmustine wafers are biodegradable chemotherapy intratumoral implants[22] used as an adjunct to surgical resection since 1995 in patients with recurrent GBM, since there is an improvement in overall survival (OS) of 7.2 mo in the carmustine group *vs* 5.4 mo in the placebo group [23]. However, its combined use with TMZ still divides authors, since some scientists believe that concomitant use is associated with an increase in the occurrence of adverse effects[24]. Therefore, it is necessary to have a randomized controlled clinical trial to support or refute the safety and efficacy of simultaneous use of carmustine wafer with TMZ.

Biological agent: Bevacizumab, a drug containing antiangiogenic monoclonal antibodies that has been in use since 2009 against the progressive form of the disease, binds to the VEGF making it difficult for recurrent GBM and rapid neurological involvement associated with the tumor, being a well-tolerated drug and capable of reducing cerebral edema, which allows a reduction in the use of corticosteroids and associated adverse effects[25].

The aforementioned drug is recommended as monotherapy or in association with other chemotherapy drugs, such as irinotecan, carmustine, lomustine, carboplatin or temozolomide[26,27], in newly diagnosed or recurrent glioblastoma. Several clinical trials over the past decade in patients with newly diagnosed GBM have shown improvements in progression-free survival (PFS), although they have not shown significant improvement in overall survival (OS). A recent study evaluated the combination of lomustine and bevacizumab in recurrent GBM and concluded with a survival of 5.1 mo [28].

However, there are genetic variations of VEGF that can determine the success or failure of bevacizumab therapy, requiring great care in the administration of this biological agent. Moreover, as the anti-VEGF method did not convincingly show improvement in OS as a monotherapy, it is necessary to evaluate the combination of this type of drug with other known therapeutic options used in neuro oncology.

Alternating electric field therapy

Tumor treatment fields (TTFs) are a therapeutic method that uses alternating currents of low intensity (1-2 V/cm) and intermediate frequency through electrodes placed on the skin around the region of a malignant tumor to stop growth and to induce apoptosis of mitotically active cells[29,30], which is considered a safe method, as it does not affect non-dividing cells.

A 2015 study revealed that the combination of TTFs and TMZ significantly improves median PFS and OS compared to TMZ monotherapy during maintenance therapy with less occurrence of electrical device-related adverse effects[31]. Current treatment guidelines incorporate TFT into the therapeutic regimen of patients with newly diagnosed and recurrent GBM[13].

However, the device is expensive, must be used at least 18 h a day and requires hair shaving of users for proper application of electrodes[32]. This can affect the patient's self-esteem and quality of life, in addition to causing a possible low adherence to treatment.

PIVOTAL ROLE OF THE TUMOR MICROENVIRONMENT

The central nervous system as an immune-distinct site

The role of the tumor microenvironment in the modulation of antitumor immune responses is becoming clearer[33]. The central nervous system (CNS) is usually described as an immune-privileged site, which means that it shows attenuated responses to alloantigen challenges[34]. Classically, the property of CNS immune privilege has been attributed to two mechanisms: (1) The blood-brain barrier (BBB); and (2) the absence of classical lymphatic drainage of CNS antigens[35]. The BBB is a semi-permeable cellular barrier composed of specialized endo-thelial cells (non-fenestrated, firmly attached by tight junctions), astrocyte end-feet, and pericytes. Its main function is to tightly regulate the movement of ions, molecules, and cells (*e.g.*, immune cells) between the blood and the brain[36,37]. The ability to block the entry of possibly neurotoxic molecules, primarily through ATP-binding cassette transporter-mediated efflux, is one of the main challenges posed to immunotherapy[38]. On the other hand, the lack of professional antigen-presenting cells in the CNS parenchyma, low expression of MHC class I and II, and the first apparent absence of classic CNS lym-phatic drainage also limit the ability of an immune response to CNS-derived antigens[39,40]. Given that efficient anti-tumor responses require not only that cancer-specific T cells be generated, but also that these T cells come into direct contact with the tumor cells, it becomes evident that the CNS provides an immune-privileged microenvironment for tumor growth and proliferation.

Fortunately, increasing evidence has pointed to the CNS, not as an immune-privileged site, but rather as an immune-distinct site that remains accessible to the onset of antitumor immune responses and immunotherapy[35]. Recent studies suggest the existence of a functional meningeal lymphatic system that drains cerebrospinal fluid (CSF), macromolecules, and immune cells from the CNS into the deep cervical lymph nodes[41]. Investigating these antigenic presentation routes will be an important step in understanding the immune-distinct properties of the GBM microenvironment.

Immunosuppressive mechanisms in GBM

Although revolutionary in the treatment of cancer patients, immunotherapy is critically dependent on the availability of preexisting anti-tumor immunity[42,43]. GBM is widely recognized to induce local and systemic immunosuppression, which is a hindrance to the use of immune-modulating therapies [44].

GBM cells can evade immune surveillance through the release of various soluble mediators that exert a variety of immunosuppressive effects[45]. The best-characterized GBM-derived immunomodulatory factors are the transforming growth factor β (TGF- β), interleukin 10 (IL-10), and prostaglandin E2 (PGE-2)[45-48]. In the presence of TGF-β, CD4+ T cells upregulate FoxP3 and differentiate into Treg cells with potent immunosuppressive potential. These converted suppressor cells not only do not respond to TCR stimulation and produce neither Th-1 nor Th-2 cytokines, but also express TGF-β and inhibit normal T cell proliferation *in vitro*[49,50]. It has also been shown that this cytokine inhibits the expression of five cytolytic gene products - specifically, perforin, granzyme A, granzyme B, Fas ligand, and interferon (IFN)-γ - which are co-responsible for CD8+ T cell-mediated tumor cytotoxicity[51]. Additionally, there is a TGF-β1-mediated downregulation of activating receptor NKG2D on the surface of CD8+ T cells and natural killer (NK) cells, thereby precluding cytotoxicity against GBM cells[52]. On the other hand, TGF-β2 can prevent neoantigen presentation and facilitate immune escape from T lymphocytes through the down-regulation of HLA-DR antigen expression on tumor cells[53]. Altogether, these immunosuppressive stimuli of T or NK cell activity prevent the effective immune-mediated clearance of tumor cells

[54,55].

IL-10 also plays a pivotal role in modulating the activity of resident and infiltrating immune cells and tumor cells in GBM, predominantly inducing an immunosuppressive phenotype[47]. Upon activation by GBM cell-derived IL-10, tumor-microglia and macrophages are then elicited to produce most of the IL-10 in the tumor microenvironment[56]. Increased secretion of IL-10 was associated with enhanced expression of other anti-inflammatory cytokines, such as IL-4, CCL2, and TGF-β[57]. In the presence of IL-10, TAMs downregulate the expression of antigen-presenting molecules, thereby impairing CD4+ T cell activation[58]. Along with TGF-β, IL-10 is also able to exert FOXP3-expressing naive T cells differentiation into Treg cells, hence leading to Treg-driven immunosuppression[59-61]. Conversely, recent data have shown that a subset of IL-10-releasing HMOX1+ myeloid cells, spatially localizing to mesenchymal-like tumor regions, also in-duce T-cell exhaustion and thus contribute to the tumor microenvironment[62].

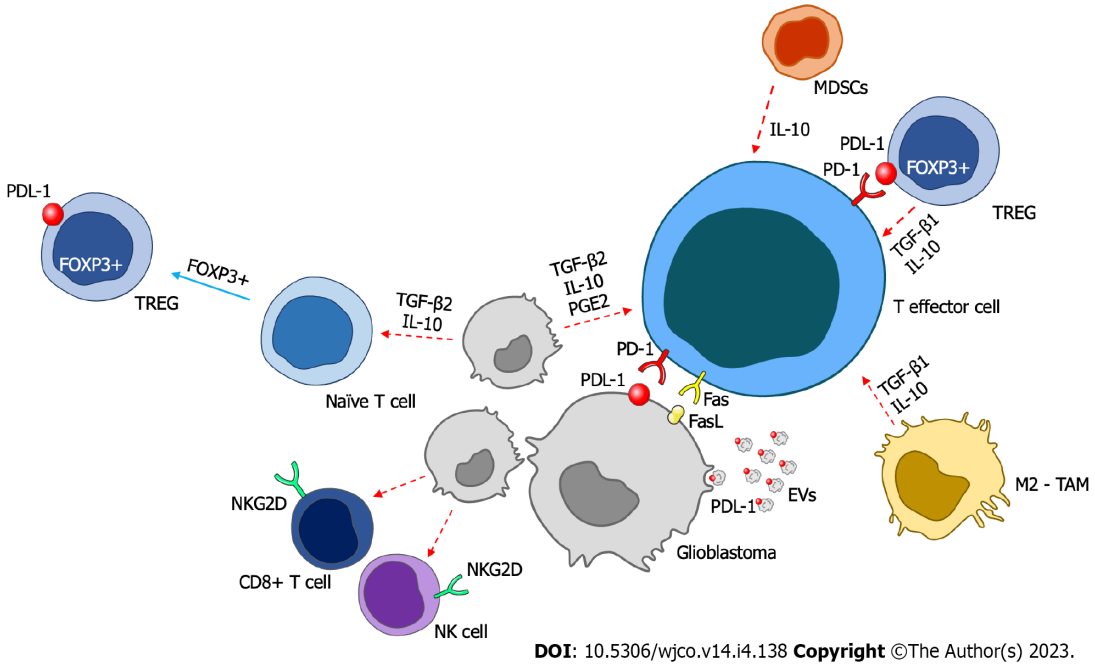
In turn, PGE-2 has been shown as a key mediator of immunosuppressive activity through the expansion of myeloid-derived suppressor cells (MDSCs)[48,63]. VEGF, on the other hand, is the most important mediator of angiogenesis in glioblastoma, which has made it one of the main therapeutic targets in GBM treatment[64]. Finally, through the activation of hypoxia-inducible factor 1- α, hypoxia regulates the expression levels of the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death-ligand 1 (PDL-1), and other immunomodulatory surface ligands, which hinder effective anti-tumor immune responses[65].

GBM cells can attenuate anti-tumor responses through the expression of a plethora of cell surface immunosuppressive factors, including the so-called immune Checkpoint molecules (ICs). Coupled with programmed cell death-1 (PD-1) located on the surface of activated T-cells, GBM and immunosuppressive (*e.g.*, Treg) cells membrane-bound PDL-1 can exert T-cell exhaustion and anergy[66,67]. Hence, PDL-1 upregulation in the tumor microenvironment propitiates resistance against T cell-mediated killing, in a protective process termed a "molecular shield"[68]. Conversely, the expression of the CD95 (Fas) ligand by GBM cells can also attenuate immune attack through the induction of CD95-Dependent apoptosis in infiltrating lymphocytes[69]. In turn, CTLA-4 is also an important ICs due to its capacity to compete with CD28 for binding to costimulatory molecules (CD80 and CD86) on antigen-presenting cells, thereby precluding the activation of T cells[67,68,70,71]. Lastly, indoleamine 2,3-dioxygenase 1 (IDO) and Lectin-like transcript-1 (LLT-1), are known to increase intratumoral Treg and myeloid-derived suppressor cells, and to repress NK cell activity, respectively[72,73].

Increasing evidence has reaffirmed the pivotal role of immunosuppressive monocytes, including MDSCs, and tumor-derived extracellular vesicles (EVs) in GBM-induced local and systemic immunosuppression[74]. EVs are defined as biologically active particles that carry both GBM-derived soluble factors and membrane-bound receptors that can be functionally delivered to target cells[74]. In combination with the tumor milieu, these particles can induce the conversion of monocytes to an immunosuppressive phenotype[75]. The role of EVs in direct T-cell inhibition has also been demonstrated. Ricklefs *et al*[76] recently showed that glioblastoma EVs block T cell activation and proliferation in response to T cell receptor stimulation. This mechanism of immunosuppression and its local and systemic effects have great potential for exploration in the context of immunotherapy. The Figure 2 synthesizes the GBM-induced immunosuppressive microenvironment.

CYTOKINE THERAPY

Cytokine therapy in the treatment of GBM is based on the use of pro-inflammatory cytokines, in order to promote reversal of the immunosuppressive microenvironment triggered by this tumor and subsequent activation of the immune response[76,77]. Mainly, IFN-α, TNF-α and IL-12 have been assessed as possible therapeutic options for glioblastoma[78,79]. In this sense, IFN-α is related to increased activity and reduced exhaustion of T cells and macrophages, besides inhibiting tumor angiogenesis and immune suppression-related gene expression[79]. On the other hand, TNF-α promotes dendritic cells maturation and, consequently, T cell stimulation, while IL-12 is related to enhanced CAR-T cell efficacy, increased infiltration of CD4+ T cells and decreased frequency of T-regulatory cells in the tumor microenvironment[80,81]. Nevertheless, the therapy with IFN-α presents high toxic systemic potential and low efficiency in maximum tolerated doses[82]. The possibility of collateral effects implies a damage to the user, clinical trials reveal hyperthermia, shivering, headaches, gastrointestinal symptoms, decline in systolic and diastolic blood pressure and associated orthostatic hypotension[83]. This means that the therapy is a resource with limited use at least at this moment. It is expected that, in the future, this route will be used in conjunction with other therapeutic forms, such as inhibitors of anti-apoptotic proteins, to increase efficacy and tolerability[84]. In another perspective, glioma cells infected by a vector capable of transducing TNF-α decreased tumor growth rate in a mouse animal model, which constitutes a different therapeutic strategy for the treatment[82]. Additionally, the administration of TNF-α is also a problem to solve because the intravenous administration is known for the capacity to induce toxicities for the patients[76]. Recently, the discover of a interleukin-7 agonist had shown the ability to repair the lymphopenia caused by the standard treatment for GBM and also improved the



DOI: 10.5306/wjco.v14.i4.138 **Copyright** ©The Author(s) 2023.

Figure 2 Simplified scheme of glioblastomas-induced immunosuppressive microenvironment. MDSCs: myeloid-derived suppressor cells; NK: Natural killer. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

immune system by elevating the CD8 serial lymphocytes in murine models, but this discover needs more studies to be apply for patients with this primary glioma[85].

IMMUNE CHECKPOINT INHIBITORS

Immune checkpoints are molecular receptors that perform an inhibitory function in order to control exacerbated immune activity and prevent uncontrolled activity of this system[86]. These receptors are found on T cells (CD4 and CD8), dendritic cells (DC), NK cells and B cells[87].

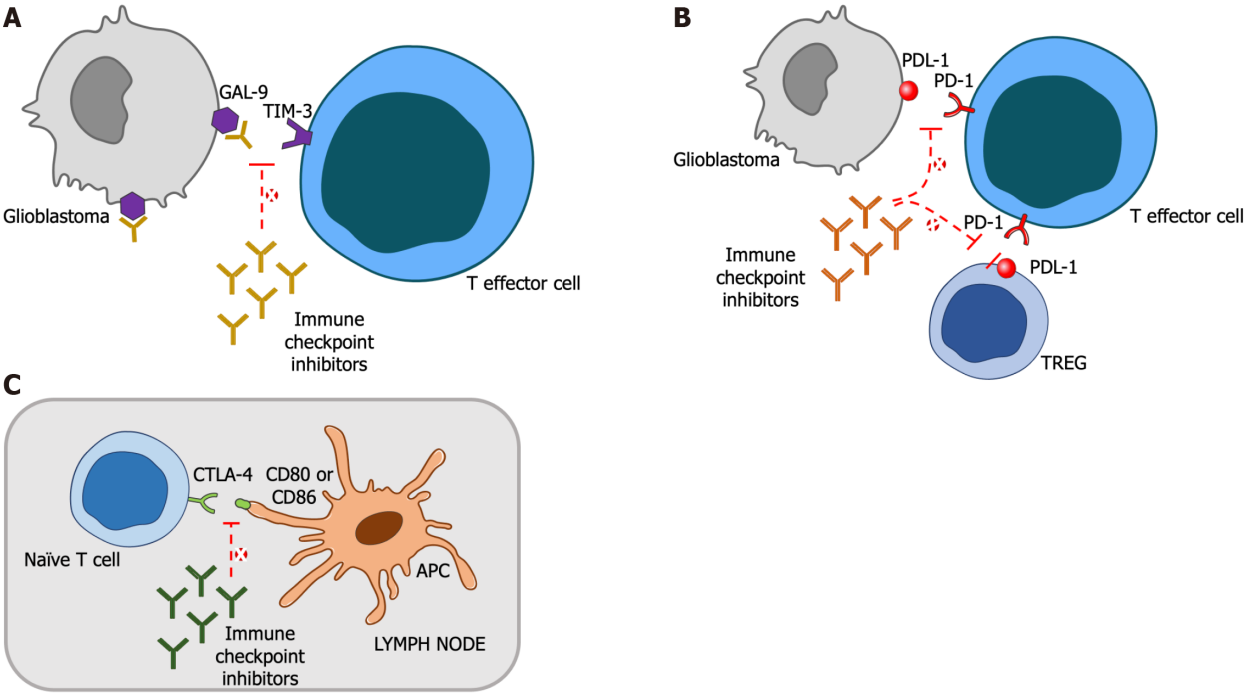
Cancer cells have some mechanisms that allow them to reduce the effectiveness of the immune system during the attack on mutated cells[88]. One of these mechanisms is the expression of molecules that interact directly with the immune checkpoint receptors resulting in reduced immune activity from the inhibition of essential cells of the protection system. Thus, immune checkpoint inhibitors have emerged as a therapeutic alternative, in order to prevent the occurrence of inhibition of immune cells from the interaction of receptors of these cells and molecules produced by glioblastoma cancer cells[87].

In this regard, studies have identified the main receptors of immune checkpoints and that have physiological importance in glioblastoma. PD-1, T cell immunoglobulin and mucin domain 3 (TIM3), CTLA4, lymphocyte activation gene 3 (LAG3), T-cell immunoglobulin and ITIM domain (TIGIT) and CD96 are inhibitory receptors expressed on immune system cells, such as lymphocytes (T and B) and NK, and have corresponding ligands produced by cancer cells[87].

Thus, studies aimed at blocking the immune checkpoint in glioblastoma have been initiated[89,90]. A study conducted in murines, associated anti-PD-1 and temozolomide (chemotherapeutic agent used in the treatment of GBM) in the treatment of glioblastoma and obtained a good antitumor efficacy[89]. However, the response in humans did not show the same efficacy, as evidenced by the randomized phase III clinical trial of 369 patients diagnosed with GBM who were treated with nivolumab (anti-PD-1) and did not show improved survival compared to the control group[90]. However, the preclinical trials are promising and the therapeutic model is still recent. This means that therapy based on blocking ICIs may yet yield an important efficiency in the lives of patients diagnosed with GBM. In Figure 3, there is a representation of immune checkpoint inhibition targets: TIM-3/Galactin 9 (GAL-9), PD-1/PDL-1, and CTL-4/CD80 or CD86.

PD-1/PD-L1

The PD-1 receptor is expressed on T cells, B cells, TAMs, MDSCs and NK cells[91]. For inhibition of these cells to occur the PD-1 receptor interacts with PD-L1, which is expressed on GBM tumor cells. This interaction results in T-cell apoptosis, inhibition of T-cell cytotoxicity, and blockage of inflammatory mediator production. Thus, immunotherapy aims to target the PD-1/PD-L1 pathway and generate an antitumor response[87].



DOI: 10.5306/wjco.v14.i4.138 **Copyright** ©The Author(s) 2023.

Figure 3 Immune checkpoint inhibition targets: T cell immunoglobulin and mucin domain 3/ Galactin 9, programmed cell death-1/programmed death-ligand 1, and cytotoxic T-lymphocyte-associated protein 4 /CD80 or CD86. A: T cell immunoglobulin and mucin domain 3/ Galactin 9; B: programmed cell death-1/programmed death-ligand 1; C: cytotoxic T-lymphocyte-associated protein 4/CD80 or CD86. TIM-3: T cell immunoglobulin and mucin domain 3; GAL-9: Galactin 9; PD-1: Programmed cell death-1; PDL-1: Programmed death-ligand 1; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

The anti-PD-1/PD-L1 class is a category that includes pembrolizumab, nivolumab, durvalumab and atezo-lizumab[92]. These ICIs have shown good results in some types of cancer, such as melanoma and non-small cell lung cancer[93,94], but for GBM, the overall efficacy is not yet optimal, especially in monotherapy, since GBM is a disease with unique peculiarities. However, studies using combination therapy with other ICIs are ongoing and have brought positive preliminary results, despite difficulties that still need to be overcome[92]. One of these challenges is the need for these ICIs to cross the blood brain barrier, which is very peculiar to brain tumors and makes chemical therapy of this type of cancer difficult[95].

TIM3/GAL9

TIM3 is a membrane protein, normally found on CD4+ and CD8+ T lymphocytes, and is also an inhibitory receptor for antitumor T cell activity[11]. GAL9 is a binding protein to TIM3. This binding results in the activation of the TIM3/GAL9 pathway, which induces T cell apoptosis, a fact that directly impacts antitumor immune activity[96,97].

The expression of GAL9 is higher in tissues from glioma patients and the TIM3/GAL9 interaction is involved with a higher malignancy of this type of CNS tumor. Thus, TIM3 has also become a potential target of immune checkpoint inhibitors in an attempt to boost immune activity against tumor invasion and result in a better prognosis for the patient[97].

CTLA4

CTLA4 is an inhibitory receptor expressed on T cells and has relevance when dealing with GBM and a worse prognosis of this disease from the activation of this receptor[70]. The process is based on the interaction of T cells with antigen-presenting cells in the peripheral lymphatic tissue through co-stimulatory and coinhibitory receptors, such as CTLA4[98]. CTLA4 binds to CD80/CD86 receptors on antigen-presenting cells. Thus, this receptor is involved with the initial process (antigen presentation) of immune activity and its activation reduces the activation and proliferation of antigen-specific T cells that will act directly on the CNS and tumor cells[87].

CTLA4 has a higher expression in more serious gliomas and is related to a worse disease prognosis, as it is related to reduced antitumor immune activity[71].

Based on this, in 2011, the Food and Drug Administration approved the use of ipilimumab in the therapy of some tumors. Ipilimumab is a monoclonal antibody that binds to CTLA4 receptors and blocks the inhibition of T cells that occurs through this molecule[87].

LAG3

LAG3 is a regulatory protein expressed on the membrane of T cells and when activated by specific ligands, it generates an inhibitory effect on immunity. It is believed that one of these ligands is FGL1 and that it is expressed by cancer cells and induces a decrease in antitumor activity, but this mechanism is still not well known, especially in relation to gliomas[99].

In addition, it is possible that LAG3 generates immunosuppression by acting in conjunction with other immune checkpoints, such as PD-1[99]. A process that has already been reported in breast cancer studies, which identified a co-expression of LAG3 and PD-1 in the tumor process, generating T-cell inhibition[100].

TIGIT/CD96

TIGIT and CD96 are co-inhibitory receptors[87]. TIGIT is expressed on various immune cells such as T cells, regulatory T cells (Tregs) and natural killer (NK) cells[101]. CD96, on the other hand, has been found mainly on conventional T cells, NK cells and NKT cells[87].

High expression of PD-1 and TIGIT was found in CNS infiltrating lymphocytes, acting at the site of GBM[101]. Thus, a combined blockade therapy for PD-1 and TIGIT has shown improved efficacy and survival for patients with GBM[101].

CD96 is directly linked to the inflammatory response in GBM and additionally, a direct and synergistic correlation of this receptor with other immune checkpoints such as PD-1, CTLA-4, TIGIT and TIM-3 has been described[102]. With this, it was found that a simultaneous blockade of CD96 and other ICIs results in enhanced antitumor immunity and better prognosis[102].

CAR T-CELL THERAPY

Chimeric antigen receptors are synthetic receptors capable of redirecting the immune functions of T lymphocytes to a specific target antigen and thus, T cells exert short and long-term effects by triggering complex antitumor responses[103]. CAR-Ts have an extracellular domain with a tumor binding site as the single-chain variable fragment (scFv), a flexible hinge, a transmembrane region, and an intracellular signaling domain of T cells. In addition, CARs can be subdivided, according to the amount of CD3ζ stimulatory domains, into first, second and third generation, and the most modern CARs have two costimulatory domains linked to CD3ζ in order to potentiate its ability of signaling activation[104]. Since CAR-Ts has been used effectively against hematological tumors, the objective is to adapt the method for solid tumors such as GBM so that the activation of T cells in the tumor microenvironment promotes targeted immunological mechanisms of cell death to specific targets in the tumor, achieving the same success as the treatment in non-solid tumors, regardless of the presentation of the peptide by histocompatibility complexes[105]. The most promising studies addressing T cell therapy against GBM have explored CAR-T cells targeting human epidermal growth factor receptor 2 (HER2), variant epidermal growth factor receptor III (EGFRvIII) and alpha receptor 2 of IL-13 (IL-13 Rα2) mainly, as well as evaluating the different forms of therapy administration (local or systemic)[106-108].

EGFRvIII consists of an oncogenic mutation pattern existing in human tumors that allows the identification of specific tumor antigens by the immune system. EGFRvIII is relatively common, especially when it comes to GBM, in which the mutation is present in approximately 30% of scenarios[109]. EGFRvIII expression in patients with GBM is considered a marker of poor prognosis probably because the receptor enhances tumor oncogenic signaling[110]. In this sense, the first clinical study that investigated CAR-Ts therapy directed at EGFRvIII was conducted by O'Rourke *et al*[107] and evaluated 10 patients with recurrent EGFRvIII + GBM. The results demonstrated that the administration of CAR-Ts Cells by infusion is a safe route to be used, as there was no evidence of toxicity outside the tumor microenvironment or cytokine release syndrome. Although the study did not have the objective of evaluating the effectiveness of the therapy, it was observed that no patient had GBM regression and one patient remained in stable disease for more than 18 mo. Therefore, the assay also revealed a consistent response with immunological checkpoints and immunosuppressive molecules such as IDO 1, PD-L1, TGF-β and IL-10 and this indicated that EGFRvIII+ led to an antitumor response[107]. Complementarily, a recent study evaluated apheresis and infusion products from the previous study to explore EGFRvIII as a therapeutic target for GBM and concluded that PD1 is a predictive marker of peripheral graft and progression-free survival in transduction products of patients with targeted CAR-Ts to EGFRvIII. Furthermore, it was also observed that PD1 was expressed concomitantly with ICIs (CTLA4, TIM3, LAG3) and activation markers (GRZB, HLA-DR) suggesting that PD1 is the protagonist of these correlations with the clinical response surrogates in the study. However, the aforementioned correlations were not present before the generation of CAR-Ts. Therefore, it has been proposed that the PD1 marker may predict better response to therapy against recurrent GBM and that the preparation of the infusion product is responsible for the differences in therapeutic results found in the study[111].

HER2 is also a tumor-associated antigen that is expressed by about 80% of GBM, however, the receptor is also expressed in physiological host cells and this gives HER2 the potential to generate autoimmunity when used as a specific target antigen[112]. An early trial involving HER2 CAR T Cells in

cancer patients did not produce positive effects. The study was associated with acute toxicity with fatal outcome in one patient[113]. However, a subsequent preclinical study yielded a more favorable outcome as CD28-costimulated HER2-CER T cells were tolerated by 17 patients with GBM without dose-associated toxic effects. Trial findings showed that one patient had a partial response to therapy for 9 mo, 7 remained with stable disease for 8 wk to 29 mo, and 8 had tumor progression. Additionally, patients had an overall survival of about 11 mo from T cell infusion (95%CI: 4.1–27.2 mo) and HER2 CAR T cells were present in blood at up to one year of follow-up[106]. IL-13Rα2 is another tumor-associated antigen that is expressed in up to 50% of GBM and despite being expressed in normal tissue, it is not expressed at significant levels in normal brain tissue[114,115]. Interestingly, the first trial that evaluated the safety and feasibility of CAR-T-s targeting IL-13Rα2 for the treatment of recurrent GBM was done by Brown *et al*[116] and included three patients with the malignancy. Among the three patients included, one had reduced global expression of IL-13Rα2 in the tumor after treatment and another patient showed an increase in the necrotic portion of the tumor where IL-13-zetacin + T cells had been administered. Despite the small sample, the findings of the work were favorable and were fundamental for the advancement in knowledge about the therapeutic method[116]. In this regard, new initial studies, albeit promising, have emerged with the aim of improving the CAR-Ts. Some works, for example, such as that of Muhammad *et al*[117], validated a new TanCAR [IL-13 (4MS) and EphA2 scFv] that proved effective in destroying GBM cancer cells recognizing IL-13Rα2 or EphA2 receptors and did not damage normal IL-13Rα1/ IL-4Rα. Therefore, it proved to be an option with the potential to remedy difficulties in current therapy by preventing antigen escape and reducing extra tumor toxicity[117]. In addition, another initial work constructed an IL-13Rα2 directed to humanized third-generation CAR and evaluated its efficacy against GBM *in vitro* and reported that the receptor achieved satisfactory results that support its use in clinical trials[118].

Therefore, CAR-T-s therapy targeting specific antigens is very promising and has the potential to become a therapeutic option for solid malignancies with poor prognosis such as GBM. However, the evidence is still limited, which creates a series of challenges to be overcome by the therapeutic method. The main obstacles to a safe and effective CAR-Ts therapy are the access of immune cells to the CNS and the heterogeneity of the tumor microenvironment. The first is mainly due to the existence of the endothelial blood-brain barrier and the epithelial blood-brain barrier[119]. The second occurs because GBM is characterized by a complex and active tumor microenvironment capable of evading the functionality of CAR-T-s, as well as hindering the recognition of a single specific target antigen[120]. In this regard, one way to improve access to the CNS would be to add property to CAR-T cells through gene editing. The development of innovative CAR-Ts that can target different tumor-associated antigens or program different CAR-Ts to recognize a single tumor-associated antigen is a possible solution to immune escape or target antigen escape. A recent study targeted 3 antigens using a single universal tricistronic (U) transgene product of CAR-T-s specific for HER2, IL-13Rα2 and EphA2 showing an effective alternative to the interpatient variability that is one of the obstacles to therapy. The *in vitro* test of the study showed an improvement in the survival of the animals, corroborating the initial hypothesis [121]. The work by Muhammad *et al*[117], cited above, starts from the same premise that the new TanCAR destroyed tumor cells by recognizing both IL-13Rα2 and EphA2 alone or together, also corroborating for a more effective therapy by avoiding immune escape and recognition of non-target antigens. Another possibility to deal with difficulties in therapy with CAR-Ts cells is the remodeling of immune cells in the tumor microenvironment. This technique is based on the use of CAR-T cells with the objective of recruiting pro-inflammatory cytokines, mainly OL-7, IL-8 and IL-12, enhancing the death of GBM cells[122-124]. In addition, the blocking of immune suppression signals through chimeric decoy and switch receptors has also been explored. For example, Liu *et al*[125] added genetically modified switch receptors including the extracellular domain of PD1 and the transmembrane and cytoplasmic signaling domains of CD28 in order to stimulate the performance of CAR-T cells in solid tumors and the study data revealed a strategy potentially efficient therapy. Finally, the expansion of the use of bispecific T cell couplers (BiTE) in combination with CAR-T cells as a new artifice for the recognition of multiple antigens has also been discussed[126]. Bearing in mind that EGFRvIII-specific CAR-T cells may not be satisfactorily efficient in view of the heterogeneity of the GBM tumor microenvironment, Choi *et al*[127] proposed the use of CARBiTE cells capable of secreting wild-type EGFR-specific BiTEs. The results of the initial study were positive and showed that BiTE cells annihilated heterogeneous GBM tumors in mice and did not promote toxicity against human skin grafts *in vivo*.

ONCOLYTIC VIRUSES

Over the last few years, oncolytic viruses (OVs) have gained prominence in tumor treatment, including GBM. OVs are particularly suitable for GBM therapy due to its privileges, such as lack of distant metastasis and tumor's limitations, allowing the use of viruses at this site as a promising form of immunotherapy[128]. They are administered intravenously or intratumorally to achieve its neutralizing effects.

OVs can be defined as weakly pathogenic viruses that can selectively infect, replicate in, and kill cancer cells without damaging normal cells and leading to tumor cells apoptosis[129]. This occurs through antitumor reactions of tumor-specific cell killing and the induction of the host's systemic antitumor and/or antiviral immunity. Thus, OVs activate the innate immune system *via* pattern recognition receptors and pathogen-associated molecular patterns, leading to a physiological response of immune cells recruitment, such as neutrophils, natural killer cells, macrophages, Th1 cells and its associated cytokines that promotes cell lysis[128,130]. Moreover, this response induces an adaptive immune reaction to new cancer antigens and may possibly develop a long-term immunotherapy repercussion[131]. Besides this, OVs can also be used as non-replicating viral vectors to deliver therapeutic genes, serving as vehicles to efficiently achieve tumor cells[104]. In Figure 4, there is a graphical representation of how OV therapy for GBM works.

Currently, OVs are being tested for their effectiveness against GBM in leading clinical trials using over 20 distinct viral strains like herpes simplex virus[132], adenovirus[133], measles virus[134], parvovirus[135], Newcastle disease virus[136], reovirus[137], poliovirus[138] and zika virus[139]. In Table 1, the clinical trials using virotherapy for GBM are summarized.

As aforementioned, the cooperation of the innate and adaptive immune systems is crucial in oncolytic virotherapy response, and matching it with other immunotherapy strategies such as checkpoint inhibitors increases the immunological response and tumor regression[140-142].

VACCINE-BASED THERAPY

In recent years, it has been discussed the great possibility of combating and stabilizing oncological conditions through immunotherapy, and the proposal of vaccine therapies is a remarkable point. In this sense, when thinking about GBM, the proposal of an alternative therapy that generates a more positive prognosis for patients, through vaccination, is a matter of much research and debate.

Many vaccines with a variety of immunological bases have been developed and tested in the treatment of GBM. There are four commonly used approaches to base GBM vaccines on: Peptide and DNA vaccines, which use genetic information from the tumor itself, and are more specific in their use. Cellular vaccines, based on dendritic cells prepared also with tumor antigens, and mRNA-based ones, with viral vectors[143]. In general, the principle behind this bet is on the immune response, thinking about the ability of the tumor to evade the individual immune response.

Thus, one of the ways found to "combat" this disease is to use the immune system itself, more specifically, a response coordinated by T lymphocytes capable of recognizing tumor antigens and reacting against them. In this sense, the initial proposal aims to use specific tumor antigens (TSAs) to obtain an immune response, having as a basis for this process peptides based on the tumor characteristics that trigger an anti-tumor immune response by mimicking neoantigens in glioblastoma cells[144,145].

Personalized neoantigen vaccines are a different approach to anti-tumor vaccine development, with trials already showing increased survival in patients with a recent diagnosis of GBM, demonstrating a potential to alter the immune environment in GBM[85].

However, there are some points of conflict within this vaccine therapy, since the tumor heterogeneity, with factors expressed differently among individuals, which would generate a high specificity in the manufacture of the vaccine, a need for customization, not being extremely effective on a large scale, hindering the inclusion of patients[146]. This treatment also has a limitation, generated by antigenic escape in the face of tumors that do not express this antigen. In addition, the collection of peptides for the vaccine base, meets a barrier, since the association of a disparate tumor profile, with possible formations of nonspecific epitopes - a tumor formation not from mutations, but from exacerbated expressions of factors that are expressed in normal tissues - raises a predisposition to responses beyond the tumor affection, such as autoimmune responses and inflammatory processes in other regions[146].

Another point of study that has been gaining prominence are DC vaccines, being considered one of the most promising at the moment. This is due to the role they play in immune regulation and in the GBM picture. Thus, they are extremely important for the induction of acquired immunity, also influencing the lymphocytic response, its differentiation, and antigen presentation. With this in mind, within GBM pictures, DCs are found with reduced function, being in an inhibited or immature state, which can be related to the severe tumor microenvironment, DCs are kept with low function due to the inhibitory effect of the immune microenvironment, and this status is problematic for body function, but reversed by DC vaccines[147]. This is due to the fact that the advantages of DCs vaccines are based on *in vitro* matured dendritic cells, usually from the affected individual himself, which can activate previously inhibited Ts lymphocytes, increasing the patient's adaptive response, increasing the expression of MHCs, cytokines and chemokines, and promoting an intense migration of immune cells to the immunosuppressive microenvironment found in GBM[147].

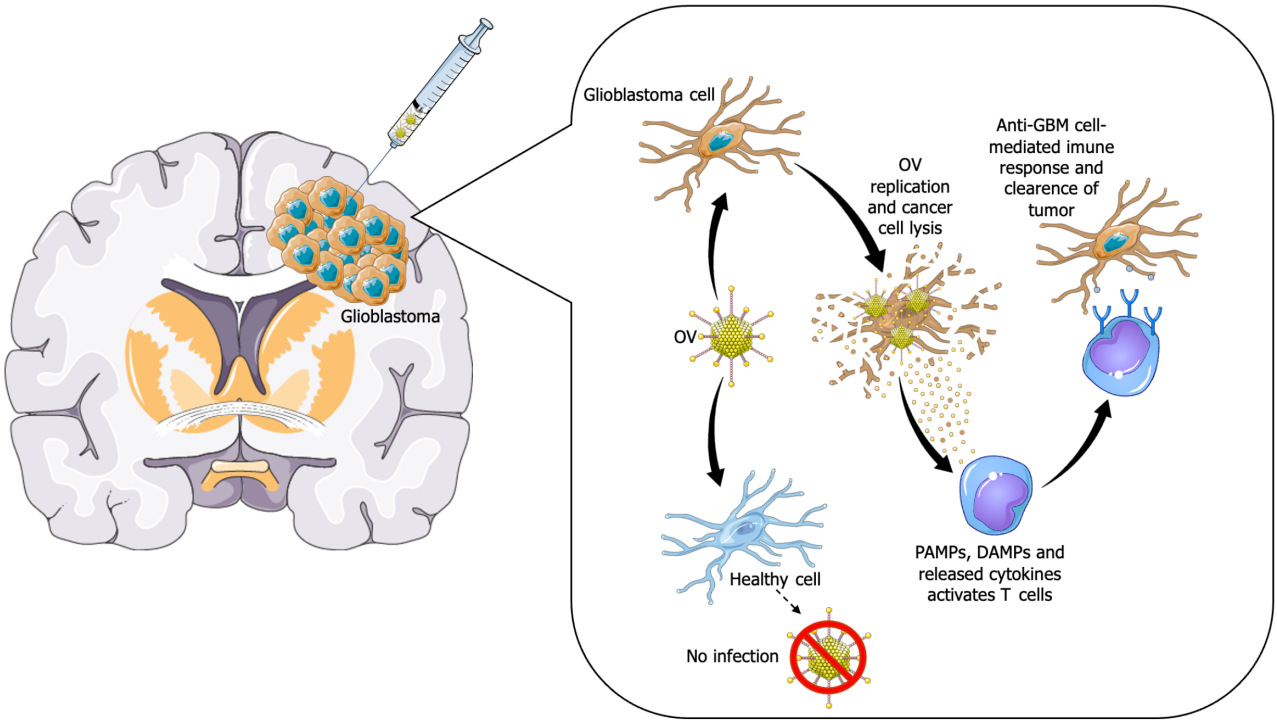
Currently, some studies have shown that DC vaccines can improve the picture of GBM, with some age-related factors seeing a better prognosis in younger patients. Another study, in phase II clinical trial, showed that the use of the vaccine after tumor resection, obtained a median overall survival of 23.4 mo,

Table 1 Ongoing and completed clinical trials of oncolytic virus therapy in glioblastoma

NCT Number	Title	Status	Enrolled patients	Interventions	Country	Phase
NCT03714334	DNX-2440 Oncolytic Adenovirus for Recurrent Glioblastoma	Unknown status	24	Drug: DNX-2440 injection	Spain	Phase 1
NCT03294486	Safety and Efficacy of the oncolytic virus Armed for Local Chemotherapy, TG6002/5- FC, in Recurrent Glioblastoma Patients	Unknown status	78	Drug: Combination of TG6002 and 5-flucytosine (5-FC, Ancotil®)	France	Phase 1 and 2
NCT02197169	DNX-2401 With Interferon Gamma (IFN-#) for Recurrent Glioblastoma or Gliosarcoma Brain Tumors	Completed	37	Drug: Single intratumoral injection of DNX-2401; Drug: Interferon-gamma	United States	Phase 1
NCT01956734	Virus DNX2401 and Temozolomide in Recurrent Glioblastoma	Completed	31	Procedure: DNX2401 and Temozo-lomide	Spain	Phase 1
NCT05095441	A Clinical Study of Intratumoral MVR-C5252 (C5252) in Patients With Recurrent or Progressive Glioblastoma	Not yet recruiting	51	Biological: C5252	United States	Phase 1
NCT01174537	New Castle Disease Virus (NDV) in Glioblastoma Multiforme (GBM), Sarcoma and Neuroblastoma	Withdrawn	0	Biological: New castle disease virus	Israel	Phase 1 and 2
NCT01491893	PVSRIPO for Recurrent Glioblastoma (GBM)	Completed	61	Biological: Recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO)	United States	Phase 1
NCT00028158	Safety and Effectiveness Study of G207, a Tumor-Killing Virus, in Patients With Recurrent Brain Cancer	Completed	65	Drug: G207, an oncolytic virus	Not provided	Phase 1 and 2
NCT03896568	MSC-DNX-2401 in Treating Patients With Recurrent High Grade Glioma	Recruiting	36	Biological: Oncolytic Adenovirus Ad5- DNX-2401; Procedure: Therapeutic conventional surgery	United States	Phase 1
NCT01582516	Safety Study of Replication competent Adenovirus (Delta-24-rgd) in Patients With Recurrent Glioblastoma	Completed	20	Biological: Delta-24- RGD adenovirus	Netherlands	Phase 1 and 2
NCT03072134	Neural Stem Cell Based Virotherapy of Newly Diagnosed Malignant Glioma	Completed	13	Biological: Neural stem cells loaded with an oncolytic adenovirus	United States	Phase 1
NCT01301430	0 Parvovirus H-1 (ParvOryx) in Patients With Progressive Primary or Recurrent Glioblastoma Multiforme.	Completed	18	Drug: H-1PV	Germany	Phase 1 and 2
NCT05084430	Study of Pembrolizumab and M032 (NSC 733972)	Active, not recruiting	28	Drug: M032; Drug: Pembrolizumab	United States	Phase 1 and 2
NCT02031965	Oncolytic HSV-1716 in Treating Younger Patients With Refractory or Recurrent High Grade Glioma That Can Be Removed By Surgery	Terminated	2	Biological: Oncolytic HSV-1716; Drug: Dexamethasone; Procedure: Therapeutic conventional surgery	United States	Phase 1
NCT02798406	Combination Adenovirus + Pembrol-izumab to Trigger Immune Virus Effects	Completed	49	Biological: DNX-2401; Biological: Pembrolizumab	United States	Phase 2
NCT03657576	Trial of C134 in Patients With Recurrent GBM	Active, not recruiting	24	Biological: C134	United States	Phase 1
NCT03152318	A Study of the Treatment of Recurrent Malignant Glioma With rQNestin34.5v.2	Recruiting	62	Drug: rQNestin; Drug: Cyclophos-phamide Procedure: Stereotactic biopsy	United States	Phase 1
NCT03043391	Phase 1b Study PVSRIPO for Recurrent Malignant Glioma in Children	Active, not recruiting	12	Biological: Polio/ Rhinovirus Recombinant (PVSRIPO)	United States	Phase 1
NCT05139056	Multiple Doses of Neural Stem Cell Virotherapy (NSC-CRAdS-pk7) for the Treatment of Recurrent High-Grade Gliomas	Withdrawn	0	Biological: Neural Stem Cells expressing CRAdS-pk7; Procedure: Resection	Not provided	Phase 1
NCT02062827	Genetically Engineered HSV-1 Phase 1 Study for the Treatment of Recurrent Malignant Glioma	Active, not recruiting	24	Biological: M032 (NSC 733972)	United States	Phase 1
NCT04482933	HSV G207 With a Single Radiation Dose in Children With Recurrent High-Grade	Not yet recruiting	40	Drug: Biological G207	United States	Phase 2

	Glioma					
NCT02986178	PVSRIPO in Recurrent Malignant Glioma	Active, not recruiting	122	Biological: PVSRIPO	United States	Phase 2
NCT03911388	HSV G207 in Children With Recurrent or Refractory Cerebellar Brain Tumors	Recruiting	15	Biological: G207	United States	Phase 1
NCT02457845	HSV G207 Alone or With a Single Radiation Dose in Children With Progressive or Recurrent Supratentorial Brain Tumors	Active, not recruiting	13	Biological: G207	United States	Phase 1
NCT00528684	Safety and Efficacy Study of REOLYSIN® in the Treatment of Recurrent Malignant Gliomas	Completed	18	Biological: REOLYSIN®	United States	Phase 1
NCT03973879	Combination of PVSRIPO and Atezolizumab for Adults With Recurrent Malignant Glioma	Withdrawn	0	Biological: PVSRIPO; Drug: Atezolizumab	Not provided	Phase 1 and 2
NCT00314925	Safety Study of Seneca Valley Virus in Patients With Solid Tumors With Neuroendocrine Features	Unknown status	60	Drug: Seneca Valley virus (biological agent)	United States	Phase 1

Most data were obtained from findings from www.clinicaltrials.gov using the search terms "glioblastoma" and "oncolytic" filter.



DOI: 10.5306/wjco.v14.i4.138 **Copyright** ©The Author(s) 2023.

Figure 4 Simplified scheme of oncolytic virotherapy for glioblastomas. GBM: Glioblastoma; OV: Oncolytic virus; PAMPs: Pathogen-associated molecular patterns; DAMPs: Damage-associated molecular patterns. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

among some patients[85]. However, a meta-analysis of randomized controlled trials on the efficacy of DC vaccines demonstrated that the use of the vaccine in newly diagnosed glioblastoma patients did not show a substantial effect on overall patient survival[148]. Thus, it is still an area that needs more studies and trials with more advanced phases, and the ability to inhibit glioma is still a point to be better tested in future studies.

Some other vaccine ideas have already been proposed, such as using isocitrate dehydrogenase as the basis for the vaccine, since mutation in this enzyme occurs purely in tumor cells, making it an interesting tumor-specific antigen to use[146]. In addition, vaccines that inactivate tumors are also an attraction for research, given their success in other pathologies, not only in treatment but also in prevention, but there is still a low efficiency for the treatment of neoplasms, requiring more research for the development and application in GBM. More advanced research is needed for the use of these other

vaccine approaches.

Another alternative attempt for the treatment of GBM, are oncolytic virotherapies, using previously known viruses, which would be injected intratumorally, enabling an inflammatory reaction and an immune response against the tumor-virus unit. Many researches and vaccines have already been approved with this type of technology, and it is a promising therapy that acts both by selectively infecting tumor cells, replicating and leading to tumor death, and by being used to transport factors for gene therapy, through viruses with alterations in their replication[104]. Regarding GBM, some vaccines, such as DNX-2401, have already gone through initial testing phases and showed positive results. However, updates of the studies are needed to better understand the spectrum and efficiency of the action of this vaccine. In addition, other vaccines are under study such as ParvOryx, Toca 511, Reovirus, and HSV type 1, being tested in patients with GBM, but still in early stages of testing[86].

Furthermore, vaccination focused on eliminating EGFRvIII is also an important resource against GBM, as it is an important TSA in this pathology[146]. Thus, the EGFRvIII anti-tumor vaccine is another interesting therapy. Some late stage studies were able to observe a good humoral induction and cytotoxic T response with the use of this TSA, after good conduct in animal studies. However, the results were not as significant as expected in survival and remission rate, in human trials[146]. Besides that many adverse effects have been found such as seizures, edema, thrombocytopenia and pulmonary embolism, and these complications when coupled with the fact that not all GBM patients express EGFRvIII, become a limitation for this therapy, since not all patients could use this vaccine[140].

The benefits of vaccination are already found in some studies, demonstrating an increase in patient survival when compared to other measures used, including the surgical approach, demonstrating the advances in this research[148,149]. However, only 3 vaccination agents have reached phase III clinical trial: Rindopepimut, DCvax and PPV[143].

Thus, the key point for vaccine therapy is the choice of the appropriate immune target with a reduction of vaccine toxicity. The search for TSA and possible alternatives must take into account the immune alterations caused by the tumor microenvironment, the immune status of the affected individual and possible adverse effects, which need to be reduced to their maximum. Moreover, there is a very important factor, even with the momentary trend towards personalized vaccines, the questioning of how to make this new reality feasible, generates a need to search for a combination of antigens of greater spectrum, having in mind also, how the vaccine process will reverberate in the organism, thinking about a long-term immune response, and what are the predictions for the future, which makes the development of studies with more solid results indispensable[143]. In addition, the possibility of combining vaccines with other immunotherapies has shown considerable benefit when compared to the use of some vaccines alone, and needs to be further investigated as an approach to be considered in patient management[86,104].

IMMUNOTHERAPY LIMITATIONS AND CHALLENGES

Immunotherapy options currently available for the treatment of GBM are vast. These include vaccines, oncolytic viruses, immune checkpoint inhibitors, and genetically modified T cells[85]. In this sense, the various ongoing studies and clinical trials may provide favorable outcomes in expanding the use of these therapies in the near future, and, given the potential to manipulate or enhance the immune system apparatus to attack and kill tumor cells, immunotherapy has enlightened and generated a lot of excitement in the treatment of GBM. However, so far, there are some limiting factors that hinder the applicability of immunotherapy in the treatment of glioblastoma, whether related to individual anatomical and immunological factors or to routes of administration and adverse effects[140-142].

The blood-brain barrier is one of the major limitations to GBM immunotherapy. These specialized endothelial cells attached to astrocytes and pericytes hinder drug delivery, leading to inefficient therapeutic action[104,150]. Also, GBM is able to induce alterations in the BBB, forming a structurally different barrier (i.e., brain tumor barrier) that also contributes to poor penetration of therapeutic agents [77]. Furthermore, intratumoral heterogeneity plays a pivotal role in immunotherapy resistance, given the rapid growth of resistant clones after the selective destruction of susceptible ones[151]. The immunosuppressive microenvironment of this tumor also poses a challenge in the immunotherapeutic approach[152]. Treg cell upregulation leads to inhibition of effector T cells, thus impairing the use of CAR-T cells[145]. Regarding cytokine therapy, despite its ability to modulate the microenvironment of GBM, leading to increased DC cells maturation, T cell infiltration and reduced exhaustion[81], its systemic use presents severe toxicity and poor absorption, which greatly hampers the use of this therapy[78]. In this regard, future studies on the topic might provide further options for these limitations to be overcome in the near future.

In order to increase the therapeutic effectiveness of the current immunotherapy approaches, various strategies have been developed to increase drug penetration and decrease the occurrence of adverse effects. Of note, we highlight (1) the use of combined therapies, for synergistic action[153]; (2) targeted drug delivery, which increases pharmacokinetic properties and reduces toxicity[79]; and (3) intrathecal administration, to overcome the blood-brain barrier[140-142].

Furthermore, given the intrinsic heterogeneous nature of GBM and its ability to evade and resist single treatments, it is crucial that future interventions should explore the combination of biological (immunomodulators and cell based delivery systems), physical (ultrasound, 3D printed implants, heat) and chemical (delivery technologies, radiation, chemotherapy) approaches to not only treat GBM more adequately but also improve the patient's prognosis, selecting ideal combination strategies to overcome the limiting barriers. In this regard, techniques using anti-PD-1/PD-L1 antibodies combined with antibodies targeting CTLA-4, TIM-3, LAG-3, 4-1BB, or OX-40 are under study[154]. Furthermore, anti-PD-1/PD-L1 therapy combined with tumor-specific peptide vaccination or CAR-T cell therapy is also worth exploring, and can provide a harmonious combination approach to surpass the obstacles[155, 156].

Finally, exploring effective predictive biomarkers of clinical efficacy, combined with other therapeutic strategies, is a critical issue to avoid treatment delay and early mortality[157,158]. In this sense, there is a demanding need to incorporate the status of known biomarkers into daily clinical practice, which may assist not only in patient selection, but also in the adjustment of treatment schedule based on the patient-specific diagnosis.

With various ongoing clinical trials for new molecular targeted therapies, cancer vaccines and immune-modulators, it can be expected that in the near future more compelling interventions against GBM will become available.

CONCLUSION

In this way, it is possible to see that the treatment for GBM is advancing and discoveries are being made. However, the immunosuppressive nature of this primary glioma and the pleomorphism presented by the constitutional cells represents important challenges to implant a successful therapy with less harm for the patient. The need for resolutions to prevent the collateral damage caused by the current standard treatment and for the alternative immunotherapies, which are being developed, demonstrates potential to be the next stage in this field alongside the increase of searching for other approaches. The main objective is to better manage this aggressive malignant brain tumor to modify the current prognostic perspective. This review shows an overview of this reality and it is stated that, based on particular pathogenesis of GBM, it is necessary an individualized treatment according to the tumor progress follow-up.

The potential of the immunotherapy presented by previous and current clinical trials reveals a hopeful perspective for patients with GBM. It is expected that a combination of therapies would be used to avoid collateral damages and improve the recovery. Risks and costs of the surgical method, radiotherapy and chemotherapy suggest several issues that alternative approaches do not have and it is more favorable as a palliative therapy than as a healing mechanism, and still usage problems must be solved for them to be applied. Biological agents and Tumor treatment fields also have benefits, even though they are, respectively, susceptible to genetic variabilities and need expensive devices to put into practice as the Figure 1 illustrates. The intervention with cytokine therapy and agonists are a recently explored field and demonstrates the ability to use different inflammatory cytokines to remodel the immune response, nevertheless there are also problems with the form of administration and the doses due to systemic toxicity. Immune checkpoints inhibitors reveal the ability to curb the immunosuppressive strategies of GBM, but the response in humans has not shown yet the same efficacy demonstrated in animal models. Chimeric antigen receptor T cell therapy is also a hopeful route of treatment due to its potential to redirect the immune response for specific targets, however the difficult to transpass the BBB and the microenvironment possessed by the active tumor, which enables evasion and difficult to recognize, are also challenges to be solved for highly functional deployment. Vaccine-based therapy is also being developed and four approaches are more currently discussed. In summary, the immunotherapy options display advantages and limitations. Thus, more advancements in ways to prevent toxic activity or/and ineffectiveness of the hopeful new recently discovered immunotherapies are fundamental to increase life expectancy and reduce suffering for the patients.

FOOTNOTES

Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version; all authors agree to be accountable for all aspects of the work in ensuring that questions that are related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Supported by the Scientific Initiation Scholarship Programme (PIBIC) of National Council for Scientific and Technological Development, CNPq, Brazil; the Scientific Initiation Scholarship Programme (PIBIC) of Bahia State Research Support Foundation, FAPESB, Brazil.

Conflict-of-interest statement: All authors declare that they have no conflict of interest.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Brazil

ORCID number: Samuel Luca Rocha Pinheiro 0000-0002-8877-892X; Fabian Fellipe Bueno Lemos 0000-0002-4686-7086; Hanna Santos Marques 0000-0001-5741-1570; Marcel Silva Luz 0000-0003-1650-5807; Luís Guilherme de Oliveira Silva 0000-0001-7275-7182; Clara Faria Souza Mendes dos Santos 0000-0003-2424-7780; Karolaine da Costa Evangelista 0000-0003-4048-7370; Mariana Santos Calmon 0000-0002-3871-7408; Matheus Sande Loureiro 0000-0002-5140-2996; Fabrício Freire de Melo 0000-0002-5680-2753.

S-Editor: Wang JL
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Thakkar JP**, Dolecek TA, Horbinski C, Ostrom QT, Lightner DD, Barnholtz-Sloan JS, Villano JL. Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 1985-1996 [PMID: 25053711 DOI: 10.1158/1055-9965.EPI-14-0275]

2 **Wen PY**, Kesari S. Malignant gliomas in adults. *N Engl J Med* 2008; **359**: 492-507 [PMID: 18669428 DOI: 10.1056/NEJMra0708126]

3 **Aldape K**, Zadeh G, Mansouri S, Reifenberger G, von Deimling A. Glioblastoma: pathology, molecular mechanisms and markers. *Acta Neuropathol* 2015; **129**: 829-848 [PMID: 25943888 DOI: 10.1007/s00401-015-1432-1]

4 **Soomro SH**, Ting LR, Qing YY, Ren M. Molecular biology of glioblastoma: Classification and mutational locations. *J Pak Med Assoc* 2017; **67**: 1410-1414 [PMID: 28924284]

5 **Cancer Genome Atlas Research Network**. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008; **455**: 1061-1068 [PMID: 18772890 DOI: 10.1038/nature07385]

6 **Liang Y**, Diehn M, Watson N, Bollen AW, Aldape KD, Nicholas MK, Lamborn KR, Berger MS, Botstein D, Brown PO, Israel MA. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *Proc Natl Acad Sci U S A* 2005; **102**: 5814-5819 [PMID: 15827123 DOI: 10.1073/pnas.0402870102]

7 **Chen Z**, Hambardzumyan D. Immune Microenvironment in Glioblastoma Subtypes. *Front Immunol* 2018; **9**: 1004 [PMID: 29867979 DOI: 10.3389/fimmu.2018.01004]

8 **Lowe S**, Bhat KP, Olar A. Current clinical management of patients with glioblastoma. *Cancer Rep (Hoboken)* 2019; **2**: e1216 [PMID: 32721125 DOI: 10.1002/cnr2.1216]

9 **Kelly PJ**, Daumas-Duport C, Scheithauer BW, Kall BA, Kispert DB. Stereotactic histologic correlations of computed tomography- and magnetic resonance imaging-defined abnormalities in patients with glial neoplasms. *Mayo Clin Proc* 1987; **62**: 450-459 [PMID: 3553757 DOI: 10.1016/s0025-6196(12)65470-6]

10 **Bush NA**, Chang SM, Berger MS. Current and future strategies for treatment of glioma. *Neurosurg Rev* 2017; **40**: 1-14 [PMID: 27085859 DOI: 10.1007/s10143-016-0709-8]

11 **Stummer W**, Suero Molina E. Fluorescence Imaging/Agents in Tumor Resection. *Neurosurg Clin N Am* 2017; **28**: 569-583 [PMID: 28917285 DOI: 10.1016/j.nec.2017.05.009]

12 **Rodríguez-Camacho A**, Flores-Vázquez JG, Moscardini-Martelli J, Torres-Ríos JA, Olmos-Guzmán A, Ortiz-Arce CS, Cid-Sánchez DR, Pérez SR, Macías-González MDS, Hernández-Sánchez LC, Heredia-Gutiérrez JC, Contreras-Palafox GA, Suárez-Campos JJE, Celis-López MÁ, Gutiérrez-Aceves GA, Moreno-Jiménez S. Glioblastoma Treatment: State-of-the-Art and Future Perspectives. *Int J Mol Sci* 2022; **23** [PMID: 35806212 DOI: 10.3390/ijms23137207]

13 **Nabors LB**, Portnow J, Ahluwalia M, Baehring J, Brem H, Brem S, Butowski N, Campian JL, Clark SW, Fabiano AJ, Forsyth P, Hattangadi-Gluth J, Holdhoff M, Horbinski C, Junck L, Kaley T, Kumthekar P, Loeffler JS, Mrugala MM, Nagpal S, Pandey M, Parney I, Peters K, Puduvalli VK, Robins I, Rockhill J, Rusthoven C, Shonka N, Shrieve DC, Swinnen LJ, Weiss S, Wen PY, Willmarth NE, Bergman MA, Darlow SD. Central Nervous System Cancers, Version 3.2020, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2020; **18**: 1537-1570 [PMID: 33152694 DOI: 10.6004/jnccn.2020.0052]

14 **Stupp R**, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; **352**: 987-996 [PMID: 15758009 DOI: 10.1056/NEJMoa043330]

15 **Batash R**, Asna N, Schaffer P, Francis N, Schaffer M. Glioblastoma Multiforme, Diagnosis and Treatment; Recent Literature Review. *Curr Med Chem* 2017; **24**: 3002-3009 [PMID: 28521700 DOI: 10.2174/0929867324666170516123206]

16 **Patel M**, Siddiqui F, Jin JY, Mikkelsen T, Rosenblum M, Movsas B, Ryu S. Salvage reirradiation for recurrent glioblastoma with radiosurgery: radiographic response and improved survival. *J Neurooncol* 2009; **92**: 185-191 [PMID: 19066727 DOI: 10.1007/s11060-008-9752-9]

17 **Wesolowski JR**, Rajdev P, Mukherji SK. Temozolomide (Temodar). *AJNR Am J Neuroradiol* 2010; **31**: 1383-1384 [PMID: 20538821 DOI: 10.3174/ajnr.A2170]

18 **Hegi ME**, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; **352**: 997-1003 [PMID: 15758010 DOI: 10.1056/NEJMoa043331]

19 **Denny BJ**, Wheelhouse RT, Stevens MF, Tsang LL, Slack JA. NMR and molecular modeling investigation of the mechanism of activation of the antitumor drug temozolomide and its interaction with DNA. *Biochemistry* 1994; **33**: 9045-9051 [PMID: 8049205 DOI: 10.1021/bi00197a003]

20 **Zhang J**, Stevens MF, Bradshaw TD. Temozolomide: mechanisms of action, repair and resistance. *Curr Mol Pharmacol* 2012; **5**: 102-114 [PMID: 22122467 DOI: 10.2174/1874467211205010102]

21 **Xu J**, Keenan TE, Overmoyer B, Tung NM, Gelman RS, Habin K, Garber JE, Ellisen LW, Winer EP, Goss PE, Yeap BY, Chabner BA, Isakoff SJ. Phase II trial of veliparib and temozolomide in metastatic breast cancer patients with and without BRCA1/2 mutations. *Breast Cancer Res Treat* 2021; **189**: 641-651 [PMID: 34417675 DOI: 10.1007/s10549-021-06292-7]

22 **Fukai J**, Nishibayashi H, Uematsu Y, Kanemura Y, Fujita K, Nakao N. Rapid regression of glioblastoma following carmustine wafer implantation: A case report. *Mol Clin Oncol* 2016; **5**: 153-157 [PMID: 27330789 DOI: 10.3892/mco.2016.894]

23 **Brem H**, Piantadosi S, Burger PC, Walker M, Selker R, Vick NA, Black K, Sisti M, Brem S, Mohr G. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. *Lancet* 1995; **345**: 1008-1012 [PMID: 7723496 DOI: 10.1016/s0140-6736(95)90755-6]

24 **Gutenberg A**, Lumenta CB, Braunsdorf WE, Sabel M, Mehdorn HM, Westphal M, Giese A. The combination of carmustine wafers and temozolomide for the treatment of malignant gliomas. A comprehensive review of the rationale and clinical experience. *J Neurooncol* 2013; **113**: 163-174 [PMID: 23535992 DOI: 10.1007/s11060-013-1110-x]

25 **Diaz RJ**, Ali S, Qadir MG, De La Fuente MI, Ivan ME, Komotar RJ. The role of bevacizumab in the treatment of glioblastoma. *J Neurooncol* 2017; **133**: 455-467 [PMID: 28527008 DOI: 10.1007/s11060-017-2477-x]

26 **Norden AD**, Young GS, Setayesh K, Muzikansky A, Klufas R, Ross GL, Ciampa AS, Ebbeling LG, Levy B, Drappatz J, Kesari S, Wen PY. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. *Neurology* 2008; **70**: 779-787 [PMID: 18316689 DOI: 10.1212/01.wnl.0000304121.57857.38]

27 **Mrugala MM**, Crew LK, Fink JR, Spence AM. Carboplatin and bevacizumab for recurrent malignant glioma. *Oncol Lett* 2012; **4**: 1082-1086 [PMID: 23162656 DOI: 10.3892/ol.2012.839]

28 **Tonder M**, Weller M, Eisele G, Roth P. Carboplatin and Etoposide in Heavily Pretreated Patients with Progressive High-Grade Glioma. *Chemotherapy* 2014; **60**: 375-378 [PMID: 26496463 DOI: 10.1159/000440678]

29 **Pless M**, Weinberg U. Tumor treating fields: concept, evidence and future. *Expert Opin Investig Drugs* 2011; **20**: 1099-1106 [PMID: 21548832 DOI: 10.1517/13543784.2011.583236]

30 **Burri SH**, Gondi V, Brown PD, Mehta MP. The Evolving Role of Tumor Treating Fields in Managing Glioblastoma: Guide for Oncologists. *Am J Clin Oncol* 2018; **41**: 191-196 [PMID: 28832384 DOI: 10.1097/COC.0000000000000395]

31 **Hottinger AF**, Pacheco P, Stupp R. Tumor treating fields: a novel treatment modality and its use in brain tumors. *Neuro Oncol* 2016; **18**: 1338-1349 [PMID: 27664860 DOI: 10.1093/neuonc/now182]

32 **Stupp R**, Taillibert S, Kanner AA, Kesari S, Steinberg DM, Toms SA, Taylor LP, Lieberman F, Silvani A, Fink KL, Barnett GH, Zhu JJ, Henson JW, Engelhard HH, Chen TC, Tran DD, Sroubek J, Tran ND, Hottinger AF, Landolfi J, Desai R, Caroli M, Kew Y, Honnorat J, Idbaih A, Kirson ED, Weinberg U, Palti Y, Hegi ME, Ram Z. Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. *JAMA* 2015; **314**: 2535-2543 [PMID: 26670971 DOI: 10.1001/jama.2015.16669]

33 **Labani-Motlagh A**, Ashja-Mahdavi M, Loskog A. The Tumor Microenvironment: A Milieu Hindering and Obstructing Antitumor Immune Responses. *Front Immunol* 2020; **11**: 940 [PMID: 32499786 DOI: 10.3389/fimmu.2020.00940]

34 **Forrester JV**, McMenamin PG, Dando SJ. CNS infection and immune privilege. *Nat Rev Neurosci* 2018; **19**: 655-671 [PMID: 30310148 DOI: 10.1038/s41583-018-0070-8]

35 **Louveau A**, Harris TH, Kipnis J. Revisiting the Mechanisms of CNS Immune Privilege. *Trends Immunol* 2015; **36**: 569-577 [PMID: 26431936 DOI: 10.1016/j.it.2015.08.006]

36 **Ballabh P**, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 2004; **16**: 1-13 [PMID: 15207256 DOI: 10.1016/j.nbd.2003.12.016]

37 **Daneman R**, Prat A. The blood-brain barrier. *Cold Spring Harb Perspect Biol* 2015; **7**: a020412 [PMID: 25561720 DOI: 10.1101/cshperspect.a020412]

38 **Pardridge WM**. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx* 2005; **2**: 3-14 [PMID: 15717053 DOI: 10.1602/neurorx.2.1.3]

39 **Weller RO**, Djuanda E, Yow HY, Carare RO. Lymphatic drainage of the brain and the pathophysiology of neurological disease. *Acta Neuropathol* 2009; **117**: 1-14 [PMID: 19002474 DOI: 10.1007/s00401-008-0457-0]

40 **Carson MJ**, Doose JM, Melchior B, Schmid CD, Ploix CC. CNS immune privilege: hiding in plain sight. *Immunol Rev* 2006; **213**: 48-65 [PMID: 16972896 DOI: 10.1111/j.1600-065X.2006.00441.x]

41 **Louveau A**, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J. Structural and functional features of central nervous system lymphatic vessels. *Nature* 2015; **523**: 337-341 [PMID: 26030524 DOI: 10.1038/nature14432]

42 **Waldman AD**, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* 2020; **20**: 651-668 [PMID: 32433532 DOI: 10.1038/s41577-020-0306-5]

43 **Crittenden MR**, Zebertavage L, Kramer G, Bambina S, Friedman D, Troesch V, Blair T, Baird JR, Alice A, Gough MJ.

Tumor cure by radiation therapy and checkpoint inhibitors depends on pre-existing immunity. *Sci Rep* 2018; **8**: 7012 [PMID: 29725089 DOI: 10.1038/s41598-018-25482-w]

44 **Pombo Antunes AR**, Scheyltjens I, Duerinck J, Neyns B, Movahedi K, Van Ginderachter JA. Understanding the glioblastoma immune microenvironment as basis for the development of new immunotherapeutic strategies. *Elife* 2020; **9** [PMID: 32014107 DOI: 10.7554/eLife.52176]

45 **Mangani D**, Weller M, Roth P. The network of immunosuppressive pathways in glioblastoma. *Biochem Pharmacol* 2017; **130**: 1-9 [PMID: 28017775 DOI: 10.1016/j.bcp.2016.12.011]

46 **Han J**, Alvarez-Breckenridge CA, Wang QE, Yu J. TGF-β signaling and its targeting for glioma treatment. *Am J Cancer Res* 2015; **5**: 945-955 [PMID: 26045979]

47 **Widodo SS**, Dinevska M, Furst LM, Stylli SS, Mantamadiotis T. IL-10 in glioma. *Br J Cancer* 2021; **125**: 1466-1476 [PMID: 34349251 DOI: 10.1038/s41416-021-01515-6]

48 **Jiang J**, Qiu J, Li Q, Shi Z. Prostaglandin E2 Signaling: Alternative Target for Glioblastoma? *Trends Cancer* 2017; **3**: 75-78 [PMID: 28718447 DOI: 10.1016/j.trecan.2016.12.002]

49 **Chen W**, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003; **198**: 1875-1886 [PMID: 14676299 DOI: 10.1084/jem.20030152]

50 **Guo XY**, Zhang GH, Wang ZN, Duan H, Xie T, Liang L, Cui R, Hu HR, Wu Y, Dong JJ, He ZQ, Mou YG. A novel Foxp3-related immune prognostic signature for glioblastoma multiforme based on immunogenomic profiling. *Aging (Albany NY)* 2021; **13**: 3501-3517 [PMID: 33429364 DOI: 10.18632/aging.202282]

51 **Thomas DA**, Massagué J. TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell* 2005; **8**: 369-380 [PMID: 16286245 DOI: 10.1016/j.ccr.2005.10.012]

52 **Crane CA**, Han SJ, Barry JJ, Ahn BJ, Lanier LL, Parsa AT. TGF-beta downregulates the activating receptor NKG2D on NK cells and CD8+ T cells in glioma patients. *Neuro Oncol* 2010; **12**: 7-13 [PMID: 20150362 DOI: 10.1093/neuonc/nop009]

53 **Zuber P**, Kuppner MC, De Tribolet N. Transforming growth factor-beta 2 down-regulates HLA-DR antigen expression on human malignant glioma cells. *Eur J Immunol* 1988; **18**: 1623-1626 [PMID: 3142781 DOI: 10.1002/eji.1830181023]

54 **Barcia C Jr**, Gómez A, Gallego-Sanchez JM, Perez-Vallés A, Castro MG, Lowenstein PR, Barcia C Sr, Herrero MT. Infiltrating CTLs in human glioblastoma establish immunological synapses with tumorigenic cells. *Am J Pathol* 2009; **175**: 786-798 [PMID: 19628762 DOI: 10.2353/ajpath.2009.081034]

55 **Castriconi R**, Daga A, Dondero A, Zona G, Poliani PL, Melotti A, Griffero F, Marubbi D, Spaziante R, Bellora F, Moretta L, Moretta A, Corte G, Bottino C. NK cells recognize and kill human glioblastoma cells with stem cell-like properties. *J Immunol* 2009; **182**: 3530-3539 [PMID: 19265131 DOI: 10.4049/jimmunol.0802845]

56 **Wu A**, Wei J, Kong LY, Wang Y, Priebe W, Qiao W, Sawaya R, Heimberger AB. Glioma cancer stem cells induce immunosuppressive macrophages/microglia. *Neuro Oncol* 2010; **12**: 1113-1125 [PMID: 20667896 DOI: 10.1093/neuonc/noq082]

57 **Li Z**, Zhang J, Zheng H, Li C, Xiong J, Wang W, Bao H, Jin H, Liang P. Modulating lncRNA SNHG15/CDK6/miR-627 circuit by palbociclib, overcomes temozolomide resistance and reduces M2-polarization of glioma associated microglia in glioblastoma multiforme. *J Exp Clin Cancer Res* 2019; **38**: 380 [PMID: 31462285 DOI: 10.1186/s13046-019-1371-0]

58 **Mittal SK**, Cho KJ, Ishido S, Roche PA. Interleukin 10 (IL-10)-mediated Immunosuppression: March-I Induction Regulates Antigen Presentation by Macrophages but Not Dendritic Cells. *J Biol Chem* 2015; **290**: 27158-27167 [PMID: 26408197 DOI: 10.1074/jbc.M115.682708]

59 **Li Z**, Liu X, Guo R, Wang P. CD4(+)Foxp3(-) type 1 regulatory T cells in glioblastoma multiforme suppress T cell responses through multiple pathways and are regulated by tumor-associated macrophages. *Int J Biochem Cell Biol* 2016; **81**: 1-9 [PMID: 27644153 DOI: 10.1016/j.biocel.2016.09.013]

60 **Sawant DV**, Yano H, Chikina M, Zhang Q, Liao M, Liu C, Callahan DJ, Sun Z, Sun T, Tabib T, Pennathur A, Corry DB, Luketich JD, Lafyatis R, Chen W, Poholek AC, Bruno TC, Workman CJ, Vignali DAA. Adaptive plasticity of IL-10(+) and IL-35(+) T(reg) cells cooperatively promotes tumor T cell exhaustion. *Nat Immunol* 2019; **20**: 724-735 [PMID: 30936494 DOI: 10.1038/s41590-019-0346-9]

61 **Fecci PE**, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, Herndon JE 2nd, Bigner DD, Dranoff G, Sampson JH. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. *Cancer Res* 2006; **66**: 3294-3302 [PMID: 16540683 DOI: 10.1158/0008-5472.CAN-05-3773]

62 **Ravi VM**, Neidert N, Will P, Joseph K, Maier JP, Kückelhaus J, Vollmer L, Goeldner JM, Behringer SP, Scherer F, Boerries M, Follo M, Weiss T, Delev D, Kernbach J, Franco P, Schallner N, Dierks C, Carro MS, Hofmann UG, Fung C, Sankowski R, Prinz M, Beck J, Salié H, Bengsch B, Schnell O, Heiland DH. T-cell dysfunction in the glioblastoma microenvironment is mediated by myeloid cells releasing interleukin-10. *Nat Commun* 2022; **13**: 925 [PMID: 35177622 DOI: 10.1038/s41467-022-28523-1]

63 **Fujita M**, Kohanbash G, Fellows-Mayle W, Hamilton RL, Komohara Y, Decker SA, Ohlfest JR, Okada H. COX-2 blockade suppresses gliomagenesis by inhibiting myeloid-derived suppressor cells. *Cancer Res* 2011; **71**: 2664-2674 [PMID: 21324923 DOI: 10.1158/0008-5472.CAN-10-3055]

64 **Weathers SP**, de Groot J. VEGF Manipulation in Glioblastoma. *Oncology (Williston Park)* 2015; **29**: 720-727 [PMID: 26470893]

65 **Hu M**, Li Y, Lu Y, Wang M, Wang C, Li Q, Zhao H. The regulation of immune checkpoints by the hypoxic tumor microenvironment. *PeerJ* 2021; **9**: e11306 [PMID: 34012727 DOI: 10.7717/peerj.11306]

66 **Nduom EK**, Wei J, Yaghi NK, Huang N, Kong LY, Gabrusiewicz K, Ling X, Zhou S, Ivan C, Chen JQ, Burks JK, Fuller GN, Calin GA, Conrad CA, Creasy C, Ritthipichai K, Radvanyi L, Heimberger AB. PD-L1 expression and prognostic impact in glioblastoma. *Neuro Oncol* 2016; **18**: 195-205 [PMID: 26323609 DOI: 10.1093/neuonc/nov172]

67 **Davidson TB**, Lee A, Hsu M, Sedighim S, Orpilla J, Treger J, Mastall M, Roesch S, Rapp C, Galvez M, Mochizuki A, Antonios J, Garcia A, Kotecha N, Bayless N, Nathanson D, Wang A, Everson R, Yong WH, Cloughesy TF, Liau LM,

Herold-Mende C, Prins RM. Expression of PD-1 by T Cells in Malignant Glioma Patients Reflects Exhaustion and Activation. *Clin Cancer Res* 2019; **25**: 1913-1922 [PMID: 30498094 DOI: 10.1158/1078-0432.CCR-18-1176]

68 **Azuma T**, Yao S, Zhu G, Flies AS, Flies SJ, Chen L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. *Blood* 2008; **111**: 3635-3643 [PMID: 18223165 DOI: 10.1182/blood-2007-11-123141]

69 **Gratas C**, Tohma Y, Van Meir EG, Klein M, Tenan M, Ishii N, Tachibana O, Kleihues P, Ohgaki H. Fas ligand expression in glioblastoma cell lines and primary astrocytic brain tumors. *Brain Pathol* 1997; **7**: 863-869 [PMID: 9217971 DOI: 10.1111/j.1750-3639.1997.tb00889.x]

70 **Guan X**, Wang Y, Sun Y, Zhang C, Ma S, Zhang D, Li D, Jia W. CTLA4-Mediated Immunosuppression in Glioblastoma is Associated with the Infiltration of Macrophages in the Tumor Microenvironment. *J Inflamm Res* 2021; **14**: 7315-7329 [PMID: 34992419 DOI: 10.2147/JIR.S341981]

71 **Liu F**, Huang J, Liu X, Cheng Q, Luo C, Liu Z. CTLA-4 correlates with immune and clinical characteristics of glioma. *Cancer Cell Int* 2020; **20**: 7 [PMID: 31911758 DOI: 10.1186/s12935-019-1085-6]

72 **Zhai L**, Bell A, Ladomersky E, Lauing KL, Bollu L, Nguyen B, Genet M, Kim M, Chen P, Mi X, Wu JD, Schipma MJ, Wray B, Griffiths J, Unwin RD, Clark SJ, Acharya R, Bao R, Horbinski C, Lukas RV, Schiltz GE, Wainwright DA. Tumor Cell IDO Enhances Immune Suppression and Decreases Survival Independent of Tryptophan Metabolism in Glioblastoma. *Clin Cancer Res* 2021; **27**: 6514-6528 [PMID: 34479957 DOI: 10.1158/1078-0432.CCR-21-1392]

73 **Roth P**, Mittelbronn M, Wick W, Meyermann R, Tatagiba M, Weller M. Malignant glioma cells counteract antitumor immune responses through expression of lectin-like transcript-1. *Cancer Res* 2007; **67**: 3540-3544 [PMID: 17440061 DOI: 10.1158/0008-5472.CAN-06-4783]

74 **Himes BT**, Geiger PA, Ayasoufi K, Bhargav AG, Brown DA, Parney IF. Immunosuppression in Glioblastoma: Current Understanding and Therapeutic Implications. *Front Oncol* 2021; **11**: 770561 [PMID: 34778089 DOI: 10.3389/fonc.2021.770561]

75 **Himes BT**, Peterson TE, de Mooij T, Garcia LMC, Jung MY, Uhm S, Yan D, Tyson J, Jin-Lee HJ, Parney D, Abukhadra Y, Gustafson MP, Dietz AB, Johnson AJ, Dong H, Maus RL, Markovic S, Lucien F, Parney IF. The role of extracellular vesicles and PD-L1 in glioblastoma-mediated immunosuppressive monocyte induction. *Neuro Oncol* 2020; **22**: 967-978 [PMID: 32080744 DOI: 10.1093/neuonc/noaa029]

76 **Ricklefs FL**, Alayo Q, Krenzlin H, Mahmoud AB, Speranza MC, Nakashima H, Hayes JL, Lee K, Balaj L, Passaro C, Rooj AK, Krasemann S, Carter BS, Chen CC, Steed T, Treiber J, Rodig S, Yang K, Nakano I, Lee H, Weissleder R, Breakefield XO, Godlewski J, Westphal M, Lamszus K, Freeman GJ, Bronisz A, Lawler SE, Chiocca EA. Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles. *Sci Adv* 2018; **4**: eaar2766 [PMID: 29532035 DOI: 10.1126/sciadv.aar2766]

77 **Janjua TI**, Rewatkar P, Ahmed-Cox A, Saeed I, Mansfeld FM, Kulshreshtha R, Kumeria T, Ziegler DS, Kavallaris M, Mazzieri R, Popat A. Frontiers in the treatment of glioblastoma: Past, present and emerging. *Adv Drug Deliv Rev* 2021; **171**: 108-138 [PMID: 33486006 DOI: 10.1016/j.addr.2021.01.012]

78 **Han SJ**, Zygourakis C, Lim M, Parsa AT. Immunotherapy for glioma: promises and challenges. *Neurosurg Clin N Am* 2012; **23**: 357-370 [PMID: 22748649 DOI: 10.1016/j.nec.2012.05.001]

79 **Weiss T**, Puca E, Silginer M, Hemmerle T, Pazahr S, Bink A, Weller M, Neri D, Roth P. Immunocytokines are a promising immunotherapeutic approach against glioblastoma. *Sci Transl Med* 2020; **12** [PMID: 33028706 DOI: 10.1126/scitranslmed.abb2311]

80 **Enderlin M**, Kleinmann EV, Struyf S, Buracchi C, Vecchi A, Kinscherf R, Kiessling F, Paschek S, Sozzani S, Rommelaere J, Cornelis JJ, Van Damme J, Dinsart C. TNF-alpha and the IFN-gamma-inducible protein 10 (IP-10/CXCL-10) delivered by parvoviral vectors act in synergy to induce antitumor effects in mouse glioblastoma. *Cancer Gene Ther* 2009; **16**: 149-160 [PMID: 18670452 DOI: 10.1038/cgt.2008.62]

81 **Agliardi G**, Liuzzi AR, Hotblack A, De Feo D, Núñez N, Stowe CL, Friebel E, Nannini F, Rindlisbacher L, Roberts TA, Ramasawmy R, Williams IP, Siow BM, Lythgoe MF, Kalber TL, Quezada SA, Pule MA, Tugues S, Straathof K, Becher B. Intratumoral IL-12 delivery empowers CAR-T cell immunotherapy in a pre-clinical model of glioblastoma. *Nat Commun* 2021; **12**: 444 [PMID: 33469002 DOI: 10.1038/s41467-020-20599-x]

82 **Birocchi F**, Cusimano M, Rossari F, Beretta S, Rancoita PMV, Ranghetti A, Colombo S, Costa B, Angel P, Sanvito F, Callea M, Norata R, Chaabane L, Canu T, Spinelli A, Genua M, Ostuni R, Merelli I, Coltella N, Naldini L. Targeted inducible delivery of immunoactivating cytokines reprograms glioblastoma microenvironment and inhibits growth in mouse models. *Sci Transl Med* 2022; **14**: eabl4106 [PMID: 35857642 DOI: 10.1126/scitranslmed.abl4106]

83 **Feinberg B**, Kurzrock R, Talpaz M, Blick M, Saks S, Gutterman JU. A phase I trial of intravenously-administered recombinant tumor necrosis factor-alpha in cancer patients. *J Clin Oncol* 1988; **6**: 1328-1334 [PMID: 3411344]

84 **Kiladjian JJ**, Mesa RA, Hoffman R. The renaissance of interferon therapy for the treatment of myeloid malignancies. *Blood* 2011; **117**: 4706-4715 [PMID: 21389325]

85 **Sener U**, Ruff MW, Campian JL. Immunotherapy in Glioblastoma: Current Approaches and Future Perspectives. *Int J Mol Sci* 2022; **23** [PMID: 35806051 DOI: 10.3390/ijms23137046]

86 **Huang B**, Li X, Li Y, Zhang J, Zong Z, Zhang H. Current Immunotherapies for Glioblastoma Multiforme. *Front Immunol* 2020; **11**: 603911 [PMID: 33767690 DOI: 10.3389/fimmu.2020.603911]

87 **Ghouzlani A**, Kandoussi S, Tall M, Reddy KP, Rafii S, Badou A. Immune Checkpoint Inhibitors in Human Glioma Microenvironment. *Front Immunol* 2021; **12**: 679425 [PMID: 34305910 DOI: 10.3389/fimmu.2021.679425]

88 **Drake CG**, Jaffee E, Pardoll DM. Mechanisms of immune evasion by tumors. *Adv Immunol* 2006; **90**: 51-81 [PMID: 16730261 DOI: 10.1016/S0065-2776(06)90002-9]

89 **Park J**, Kim CG, Shim JK, Kim JH, Lee H, Lee JE, Kim MH, Haam K, Jung I, Park SH, Chang JH, Shin EC, Kang SG. Effect of combined anti-PD-1 and temozolomide therapy in glioblastoma. *Oncoimmunology* 2019; **8**: e1525243 [PMID: 30546966 DOI: 10.1080/2162402X.2018.1525243]

90 **Reardon DA**, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, Baehring J, Ahluwalia MS, Roth P, Bähr O, Phuphanich S, Sepulveda JM, De Souza P, Sahebjam S, Carleton M, Tatsuoka K, Taitt C, Zwirtes R, Sampson J, Weller M. Effect of Nivolumab vs Bevacizumab in Patients With Recurrent Glioblastoma: The CheckMate 143 Phase 3

Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: 1003-1010 [PMID: 32437507 DOI: 10.1001/jamaoncol.2020.1024]

91 **Shu C**, Li Q. Current advances in PD-1/PD-L1 axis-related tumour-infiltrating immune cells and therapeutic regimens in glioblastoma. *Crit Rev Oncol Hematol* 2020; **151**: 102965 [PMID: 32442903 DOI: 10.1016/j.critrevonc.2020.102965]

92 **Yang T**, Kong Z, Ma W. PD-1/PD-L1 immune checkpoint inhibitors in glioblastoma: clinical studies, challenges and potential. *Hum Vaccin Immunother* 2021; **17**: 546-553 [PMID: 32643507 DOI: 10.1080/21645515.2020.1782692]

93 **Robert C**, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A; KEYNOTE-006 investigators. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med* 2015; **372**: 2521-2532 [PMID: 25891173 DOI: 10.1056/NEJMoa1503093]

94 **Herbst RS**, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, Majem M, Fidler MJ, de Castro G Jr, Garrido M, Lubiniecki GM, Shentu Y, Im E, Dolled-Filhart M, Garon EB. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387**: 1540-1550 [PMID: 26712084 DOI: 10.1016/S0140-6736(15)01281-7]

95 **van Tellingen O**, Yetkin-Arik B, de Gooijer MC, Wesseling P, Wurdinger T, de Vries HE. Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. *Drug Resist Updat* 2015; **19**: 1-12 [PMID: 25791797 DOI: 10.1016/j.drup.2015.02.002]

96 **Kandel S**, Adhikary P, Li G, Cheng K. The TIM3/Gal9 signaling pathway: An emerging target for cancer immunotherapy. *Cancer Lett* 2021; **510**: 67-78 [PMID: 33895262 DOI: 10.1016/j.canlet.2021.04.011]

97 **Liu Z**, Han H, He X, Li S, Wu C, Yu C, Wang S. Expression of the galectin-9-Tim-3 pathway in glioma tissues is associated with the clinical manifestations of glioma. *Oncol Lett* 2016; **11**: 1829-1834 [PMID: 26998085 DOI: 10.3892/ol.2016.4142]

98 **Huang J**, Liu F, Liu Z, Tang H, Wu H, Gong Q, Chen J. Immune Checkpoint in Glioblastoma: Promising and Challenging. *Front Pharmacol* 2017; **8**: 242 [PMID: 28536525 DOI: 10.3389/fphar.2017.00242]

99 **Shi AP**, Tang XY, Xiong YL, Zheng KF, Liu YJ, Shi XG, Lv Y, Jiang T, Ma N, Zhao JB. Immune Checkpoint LAG3 and Its Ligand FGL1 in Cancer. *Front Immunol* 2021; **12**: 785091 [PMID: 35111155 DOI: 10.3389/fimmu.2021.785091]

100 **Du H**, Yi Z, Wang L, Li Z, Niu B, Ren G. The co-expression characteristics of LAG3 and PD-1 on the T cells of patients with breast cancer reveal a new therapeutic strategy. *Int Immunopharmacol* 2020; **78**: 106113 [PMID: 31841754 DOI: 10.1016/j.intimp.2019.106113]

101 **Hung AL**, Maxwell R, Theodros D, Belcaid Z, Mathios D, Luksik AS, Kim E, Wu A, Xia Y, Garzon-Muvdi T, Jackson C, Ye X, Tyler B, Selby M, Korman A, Barnhart B, Park SM, Youn JI, Chowdhury T, Park CK, Brem H, Pardoll DM, Lim M. TIGIT and PD-1 dual checkpoint blockade enhances antitumor immunity and survival in GBM. *Oncoimmunology* 2018; **7**: e1466769 [PMID: 30221069 DOI: 10.1080/2162402X.2018.1466769]

102 **Zhang Q**, Zhong H, Fan Y, Liu Q, Song J, Yao S, Cao F. Immune and Clinical Features of CD96 Expression in Glioma by in silico Analysis. *Front Bioeng Biotechnol* 2020; **8**: 592 [PMID: 32695752 DOI: 10.3389/fbioe.2020.00592]

103 **Sadelain M**, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discov* 2013; **3**: 388-398 [PMID: 23550147 DOI: 10.1158/2159-8290.CD-12-0548]

104 **Rong L**, Li N, Zhang Z. Emerging therapies for glioblastoma: current state and future directions. *J Exp Clin Cancer Res* 2022; **41**: 142 [PMID: 35428347 DOI: 10.1186/s13046-022-02349-7]

105 **Akhavan D**, Alizadeh D, Wang D, Weist MR, Shepphird JK, Brown CE. CAR T cells for brain tumors: Lessons learned and road ahead. *Immunol Rev* 2019; **290**: 60-84 [PMID: 31355493 DOI: 10.1111/imr.12773]

106 **Ahmed N**, Brawley V, Hegde M, Bielamowicz K, Kalra M, Landi D, Robertson C, Gray TL, Diouf O, Wakefield A, Ghazi A, Gerken C, Yi Z, Ashoori A, Wu MF, Liu H, Rooney C, Dotti G, Gee A, Su J, Kew Y, Baskin D, Zhang YJ, New P, Grilley B, Stojakovic M, Hicks J, Powell SZ, Brenner MK, Heslop HE, Grossman R, Wels WS, Gottschalk S. HER2-Specific Chimeric Antigen Receptor-Modified Virus-Specific T Cells for Progressive Glioblastoma: A Phase 1 Dose-Escalation Trial. *JAMA Oncol* 2017; **3**: 1094-1101 [PMID: 28426845 DOI: 10.1001/jamaoncol.2017.0184]

107 **O'Rourke DM**, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrissette JJD, Martinez-Lage M, Brem S, Maloney E, Shen A, Isaacs R, Mohan S, Plesa G, Lacey SF, Navenot JM, Zheng Z, Levine BL, Okada H, June CH, Brogdon JL, Maus MV. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med* 2017; **9** [PMID: 28724573 DOI: 10.1126/scitranslmed.aaa0984]

108 **Brown CE**, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, Ostberg JR, Blanchard MS, Kilpatrick J, Simpson J, Kurien A, Priceman SJ, Wang X, Harshbarger TL, D'Apuzzo M, Ressler JA, Jensen MC, Barish ME, Chen M, Portnow J, Forman SJ, Badie B. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. *N Engl J Med* 2016; **375**: 2561-2569 [PMID: 28029927 DOI: 10.1056/NEJMoa1610497]

109 **Li G**, Wong AJ. EGF receptor variant III as a target antigen for tumor immunotherapy. *Expert Rev Vaccines* 2008; **7**: 977-985 [PMID: 18767947 DOI: 10.1586/14760584.7.7.977]

110 **Heimberger AB**, Suki D, Yang D, Shi W, Aldape K. The natural history of EGFR and EGFRvIII in glioblastoma patients. *J Transl Med* 2005; **3**: 38 [PMID: 16236164 DOI: 10.1186/1479-5876-3-38]

111 **Tang OY**, Tian L, Yoder T, Xu R, Kulikovskaya I, Gupta M, Melenhorst JJ, Lacey SF, O'Rourke DM, Binder ZA. PD1 Expression in EGFRvIII-Directed CAR T Cell Infusion Product for Glioblastoma Is Associated with Clinical Response. *Front Immunol* 2022; **13**: 872756 [PMID: 35603165 DOI: 10.3389/fimmu.2022.872756]

112 **Sampson JH**, Choi BD, Sanchez-Perez L, Suryadevara CM, Snyder DJ, Flores CT, Schmittling RJ, Nair SK, Reap EA, Norberg PK, Herndon JE 2nd, Kuan CT, Morgan RA, Rosenberg SA, Johnson LA. EGFRvIII mCAR-modified T-cell therapy cures mice with established intracerebral glioma and generates host immunity against tumor-antigen loss. *Clin Cancer Res* 2014; **20**: 972-984 [PMID: 24352643 DOI: 10.1158/1078-0432.CCR-13-0709]

113 **Morgan RA**, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther* 2010; **18**: 843-851 [PMID: 20179677 DOI: 10.1038/mt.2010.24]

114 **Jarboe JS**, Johnson KR, Choi Y, Lonser RR, Park JK. Expression of interleukin-13 receptor alpha2 in glioblastoma

multiforme: implications for targeted therapies. *Cancer Res* 2007; **67**: 7983-7986 [PMID: 17804706 DOI: 10.1158/0008-5472.CAN-07-1493]

115 **Mintz A**, Gibo DM, Slagle-Webb B, Christensen ND, Debinski W. IL-13Ralpha2 is a glioma-restricted receptor for interleukin-13. *Neoplasia* 2002; **4**: 388-399 [PMID: 12192597 DOI: 10.1038/sj.neo.7900234]

116 **Brown CE**, Badie B, Barish ME, Weng L, Ostberg JR, Chang WC, Naranjo A, Starr R, Wagner J, Wright C, Zhai Y, Bading JR, Ressler JA, Portnow J, D'Apuzzo M, Forman SJ, Jensen MC. Bioactivity and Safety of IL13Rα2-Redirected Chimeric Antigen Receptor CD8+ T Cells in Patients with Recurrent Glioblastoma. *Clin Cancer Res* 2015; **21**: 4062-4072 [PMID: 26059190 DOI: 10.1158/1078-0432.CCR-15-0428]

117 **Muhammad N**, Wang R, Li W, Zhang Z, Chang Y, Hu Y, Zhao J, Zheng X, Mao Q, Xia H. A novel TanCAR targeting IL13Rα2 and EphA2 for enhanced glioblastoma therapy. *Mol Ther Oncolytics* 2022; **24**: 729-741 [PMID: 35317513 DOI: 10.1016/j.omto.2022.02.012]

118 **Xu C**, Bai Y, An Z, Hu Y, Zhang C, Zhong X. IL-13Rα2 humanized scFv-based CAR-T cells exhibit therapeutic activity against glioblastoma. *Mol Ther Oncolytics* 2022; **24**: 443-451 [PMID: 35141400 DOI: 10.1016/j.omto.2022.01.002]

119 **Engelhardt B**, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. *Trends Immunol* 2012; **33**: 579-589 [PMID: 22926201 DOI: 10.1016/j.it.2012.07.004]

120 **Bagley SJ**, O'Rourke DM. Clinical investigation of CAR T cells for solid tumors: Lessons learned and future directions. *Pharmacol Ther* 2020; **205**: 107419 [PMID: 31629009 DOI: 10.1016/j.pharmthera.2019.107419]

121 **Bielamowicz K**, Fousek K, Byrd TT, Samaha H, Mukherjee M, Aware N, Wu MF, Orange JS, Sumazin P, Man TK, Joseph SK, Hegde M, Ahmed N. Trivalent CAR T cells overcome interpatient antigenic variability in glioblastoma. *Neuro Oncol* 2018; **20**: 506-518 [PMID: 29016929 DOI: 10.1093/neuonc/nox182]

122 **Luo H**, Su J, Sun R, Sun Y, Wang Y, Dong Y, Shi B, Jiang H, Li Z. Coexpression of IL7 and CCL21 Increases Efficacy of CAR-T Cells in Solid Tumors without Requiring Preconditioned Lymphodepletion. *Clin Cancer Res* 2020; **26**: 5494-5505 [PMID: 32816947 DOI: 10.1158/1078-0432.CCR-20-0777]

123 **Chmielewski M**, Abken H. CAR T Cells Releasing IL-18 Convert to T-Bet(high) FoxO1(low) Effectors that Exhibit Augmented Activity against Advanced Solid Tumors. *Cell Rep* 2017; **21**: 3205-3219 [PMID: 29241547 DOI: 10.1016/j.celrep.2017.11.063]

124 **Liu Y**, Di S, Shi B, Zhang H, Wang Y, Wu X, Luo H, Wang H, Li Z, Jiang H. Armored Inducible Expression of IL-12 Enhances Antitumor Activity of Glypican-3-Targeted Chimeric Antigen Receptor-Engineered T Cells in Hepatocellular Carcinoma. *J Immunol* 2019; **203**: 198-207 [PMID: 31142602 DOI: 10.4049/jimmunol.1800033]

125 **Liu X**, Ranganathan R, Jiang S, Fang C, Sun J, Kim S, Newick K, Lo A, June CH, Zhao Y, Moon EK. A Chimeric Switch-Receptor Targeting PD1 Augments the Efficacy of Second-Generation CAR T Cells in Advanced Solid Tumors. *Cancer Res* 2016; **76**: 1578-1590 [PMID: 26979791 DOI: 10.1158/0008-5472.CAN-15-2524]

126 **Goebeler ME**, Bargou RC. T cell-engaging therapies - BiTEs and beyond. *Nat Rev Clin Oncol* 2020; **17**: 418-434 [PMID: 32242094 DOI: 10.1038/s41571-020-0347-5]

127 **Choi BD**, Yu X, Castano AP, Bouffard AA, Schmidts A, Larson RC, Bailey SR, Boroughs AC, Frigault MJ, Leick MB, Scarfò I, Cetrulo CL, Demehri S, Nahed BV, Cahill DP, Wakimoto H, Curry WT, Carter BS, Maus MV. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. *Nat Biotechnol* 2019; **37**: 1049-1058 [PMID: 31332324 DOI: 10.1038/s41587-019-0192-1]

128 **Wollmann G**, Ozduman K, van den Pol AN. Oncolytic virus therapy for glioblastoma multiforme: concepts and candidates. *Cancer J* 2012; **18**: 69-81 [PMID: 22290260 DOI: 10.1097/PPO.0b013e31824671c9]

129 **Mahmoud AB**, Ajina R, Aref S, Darwish M, Alsayb M, Taher M, AlSharif SA, Hashem AM, Alkayyal AA. Advances in immunotherapy for glioblastoma multiforme. *Front Immunol* 2022; **13**: 944452 [PMID: 36311781 DOI: 10.3389/fimmu.2022.944452]

130 **Foreman PM**, Friedman GK, Cassady KA, Markert JM. Oncolytic Virotherapy for the Treatment of Malignant Glioma. *Neurotherapeutics* 2017; **14**: 333-344 [PMID: 28265902 DOI: 10.1007/s13311-017-0516-0]

131 **Peruzzi P**, Chiocca EA. Viruses in cancer therapy - from benchwarmers to quarterbacks. *Nat Rev Clin Oncol* 2018; **15**: 657-658 [PMID: 30057404 DOI: 10.1038/s41571-018-0077-0]

132 **Alessandrini F**, Menotti L, Avitabile E, Appolloni I, Ceresa D, Marubbi D, Campadelli-Fiume G, Malatesta P. Eradication of glioblastoma by immuno-virotherapy with a retargeted oncolytic HSV in a preclinical model. *Oncogene* 2019; **38**: 4467-4479 [PMID: 30755732 DOI: 10.1038/s41388-019-0737-2]

133 **Kiyokawa J**, Wakimoto H. Preclinical And Clinical Development Of Oncolytic Adenovirus For The Treatment Of Malignant Glioma. *Oncolytic Virother* 2019; **8**: 27-37 [PMID: 31750274 DOI: 10.2147/OV.S196403]

134 **Rajaraman S**, Canjuga D, Ghosh M, Codrea MC, Sieger R, Wedekink F, Tatagiba M, Koch M, Lauer UM, Nahnsen S, Rammensee HG, Mühlebach MD, Stevanovic S, Tabatabai G. Measles Virus-Based Treatments Trigger a Pro-inflammatory Cascade and a Distinctive Immunopeptidome in Glioblastoma. *Mol Ther Oncolytics* 2019; **12**: 147-161 [PMID: 30775418 DOI: 10.1016/j.omto.2018.12.010]

135 **Geletneky K**, Hajda J, Angelova AL, Leuchs B, Capper D, Bartsch AJ, Neumann JO, Schöning T, Hüsing J, Beelte B, Kiprianova I, Roscher M, Bhat R, von Deimling A, Brück W, Just A, Frehtman V, Löbhard S, Terletskaia-Ladwig E, Fry J, Jochims K, Daniel V, Krebs O, Dahm M, Huber B, Unterberg A, Rommelaere J. Oncolytic H-1 Parvovirus Shows Safety and Signs of Immunogenic Activity in a First Phase I/IIa Glioblastoma Trial. *Mol Ther* 2017; **25**: 2620-2634 [PMID: 28967558 DOI: 10.1016/j.ymthe.2017.08.016]

136 **Freeman AI**, Zakay-Rones Z, Gomori JM, Linetsky E, Rasooly L, Greenbaum E, Rozenman-Yair S, Panet A, Libson E, Irving CS, Galun E, Siegal T. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. *Mol Ther* 2006; **13**: 221-228 [PMID: 16257582 DOI: 10.1016/j.ymthe.2005.08.016]

137 **Kicielinski KP**, Chiocca EA, Yu JS, Gill GM, Coffey M, Markert JM. Phase 1 clinical trial of intratumoral reovirus infusion for the treatment of recurrent malignant gliomas in adults. *Mol Ther* 2014; **22**: 1056-1062 [PMID: 24553100 DOI: 10.1038/mt.2014.21]

138 **Gromeier M**, Lachmann S, Rosenfeld MR, Gutin PH, Wimmer E. Intergeneric poliovirus recombinants for the treatment of malignant glioma. *Proc Natl Acad Sci U S A* 2000; **97**: 6803-6808 [PMID: 10841575 DOI: 10.1073/pnas.97.12.6803]

139 **Zhu Z**, Mesci P, Bernatchez JA, Gimple RC, Wang X, Schafer ST, Wettersten HI, Beck S, Clark AE, Wu Q, Prager BC, Kim LJY, Dhanwani R, Sharma S, Garancher A, Weis SM, Mack SC, Negraes PD, Trujillo CA, Penalva LO, Feng J, Lan Z, Zhang R, Wessel AW, Dhawan S, Diamond MS, Chen CC, Wechsler-Reya RJ, Gage FH, Hu H, Siqueira-Neto JL, Muotri AR, Cheresh DA, Rich JN. Zika Virus Targets Glioblastoma Stem Cells through a SOX2-Integrin α(v)β(5) Axis. *Cell Stem Cell* 2020; **26**: 187-204.e10 [PMID: 31956038 DOI: 10.1016/j.stem.2019.11.016]

140 **Chowdhury S**, Bappy MH, Clocchiatti-Tuozzo S, Cheeti S, Chowdhury S, Patel V. Current Advances in Immunotherapy for Glioblastoma Multiforme and Future Prospects. *Cureus* 2021; **13**: e20604 [PMID: 35103180 DOI: 10.7759/cureus.20604]

141 **Lu D**, Gao Y. Immune Checkpoint Inhibitor-related Endocrinopathies. *J Transl Int Med* 2022; **10**: 9-14 [PMID: 35702188 DOI: 10.2478/jtim-2022-0009]

142 **Alok A**, Seok K, Wesolow J. A Case of Abdominal Pain and Diarrhea Post Immunotherapy: Hypophysitis Associated with Immune Checkpoint Inhibitors. *J Transl Int Med* 2022; **10**: 178-180 [PMID: 35959448 DOI: 10.2478/jtim-2022-0030]

143 **Yuan B**, Wang G, Tang X, Tong A, Zhou L. Immunotherapy of glioblastoma: Recent advances and future prospects. *Hum Vaccin Immunother* 2022; **18**: 2055417 [PMID: 35344682 DOI: 10.1080/21645515.2022.2055417]

144 **Apavaloaei A**, Hardy MP, Thibault P, Perreault C. The Origin and Immune Recognition of Tumor-Specific Antigens. *Cancers (Basel)* 2020; **12** [PMID: 32932620 DOI: 10.3390/cancers12092607]

145 **Majc B**, Novak M, Kopitar-Jerala N, Jewett A, Breznik B. Immunotherapy of Glioblastoma: Current Strategies and Challenges in Tumor Model Development. *Cells* 2021; **10** [PMID: 33572835 DOI: 10.3390/cells10020265]

146 **Zhao T**, Li C, Ge H, Lin Y, Kang D. Glioblastoma vaccine tumor therapy research progress. *Chin Neurosurg J* 2022; **8**: 2 [PMID: 35045874 DOI: 10.1186/s41016-021-00269-7]

147 **Zhou J**, Li L, Jia M, Liao Q, Peng G, Luo G, Zhou Y. Dendritic cell vaccines improve the glioma microenvironment: Influence, challenges, and future directions. *Cancer Med* 2023; **12**: 7207-7221 [PMID: 36464889 DOI: 10.1002/cam4.5511]

148 **Tan L**, Peng J, Liu P, Wu Q. The Efficacy of Dendritic Cell Vaccine for Newly Diagnosed Glioblastoma: A Meta-analysis of Randomized Controlled Studies. *Clin Neuropharmacol* 2021; **44**: 216-221 [PMID: 34767325 DOI: 10.1097/WNF.0000000000000452]

149 **Liau LM**, Ashkan K, Brem S, Campian JL, Trusheim JE, Iwamoto FM, Tran DD, Ansstas G, Cobbs CS, Heth JA, Salacz ME, D'Andre S, Aiken RD, Moshel YA, Nam JY, Pillainayagam CP, Wagner SA, Walter KA, Chaudhary R, Goldlust SA, Lee IY, Bota DA, Elinzano H, Grewal J, Lillehei K, Mikkelsen T, Walbert T, Abram S, Brenner AJ, Ewend MG, Khagi S, Lovick DS, Portnow J, Kim L, Loudon WG, Martinez NL, Thompson RC, Avigan DE, Fink KL, Geoffroy FJ, Giglio P, Gligich O, Krex D, Lindhorst SM, Lutzky J, Meisel HJ, Nadji-Ohl M, Sanchin L, Sloan A, Taylor LP, Wu JK, Dunbar EM, Etame AB, Kesari S, Mathieu D, Piccioni DE, Baskin DS, Lacroix M, May SA, New PZ, Pluard TJ, Toms SA, Tse V, Peak S, Villano JL, Battiste JD, Mulholland PJ, Pearlman ML, Petrecca K, Schulder M, Prins RM, Boynton AL, Bosch ML. Association of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination With Extension of Survival Among Patients With Newly Diagnosed and Recurrent Glioblastoma: A Phase 3 Prospective Externally Controlled Cohort Trial. *JAMA Oncol* 2023; **9**: 112-121 [PMID: 36394838 DOI: 10.1001/jamaoncol.2022.5370]

150 **Alahmari A**. Blood-Brain Barrier Overview: Structural and Functional Correlation. *Neural Plast* 2021; **2021**: 6564585 [PMID: 34912450 DOI: 10.1155/2021/6564585]

151 **Jackson CM**, Choi J, Lim M. Mechanisms of immunotherapy resistance: lessons from glioblastoma. *Nat Immunol* 2019; **20**: 1100-1109 [PMID: 31358997 DOI: 10.1038/s41590-019-0433-y]

152 **Medikonda R**, Dunn G, Rahman M, Fecci P, Lim M. A review of glioblastoma immunotherapy. *J Neurooncol* 2021; **151**: 41-53 [PMID: 32253714 DOI: 10.1007/s11060-020-03448-1]

153 **Reits EA**, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, Camphausen K, Luiten RM, de Ru AH, Neijssen J, Griekspoor A, Mesman E, Verreck FA, Spits H, Schlom J, van Veelen P, Neefjes JJ. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med* 2006; **203**: 1259-1271 [PMID: 16636135 DOI: 10.1084/jem.20052494]

154 **Omuro A**, Vlahovic G, Lim M, Sahebjam S, Baehring J, Cloughesy T, Voloschin A, Ramkissoon SH, Ligon KL, Latek R, Zwirtes R, Strauss L, Paliwal P, Harbison CT, Reardon DA, Sampson JH. Nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. *Neuro Oncol* 2018; **20**: 674-686 [PMID: 29106665 DOI: 10.1093/neuonc/nox208]

155 **Schuessler A**, Smith C, Beagley L, Boyle GM, Rehan S, Matthews K, Jones L, Crough T, Dasari V, Klein K, Smalley A, Alexander H, Walker DG, Khanna R. Autologous T-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma. *Cancer Res* 2014; **74**: 3466-3476 [PMID: 24795429 DOI: 10.1158/0008-5472.CAN-14-0296]

156 **Nehama D**, Di Ianni N, Musio S, Du H, Patané M, Pollo B, Finocchiaro G, Park JJH, Dunn DE, Edwards DS, Damrauer JS, Hudson H, Floyd SR, Ferrone S, Savoldo B, Pellegatta S, Dotti G. B7-H3-redirected chimeric antigen receptor T cells target glioblastoma and neurospheres. *EBioMedicine* 2019; **47**: 33-43 [PMID: 31466914 DOI: 10.1016/j.ebiom.2019.08.030]

157 **Viscardi G**, Tralongo AC, Massari F, Lambertini M, Mollica V, Rizzo A, Comito F, Di Liello R, Alfieri S, Imbimbo M, Della Corte CM, Morgillo F, Simeon V, Lo Russo G, Proto C, Prelaj A, De Toma A, Galli G, Signorelli D, Ciardiello F, Remon J, Chaput N, Besse B, de Braud F, Garassino MC, Torri V, Cinquini M, Ferrara R. Comparative assessment of early mortality risk upon immune checkpoint inhibitors alone or in combination with other agents across solid malignancies: a systematic review and meta-analysis. *Eur J Cancer* 2022; **177**: 175-185 [PMID: 36368251 DOI: 10.1016/j.ejca.2022.09.031]

158 **Pan S**, Sun S, Liu B, Hou Y. Pan-cancer Landscape of the RUNX Protein Family Reveals their Potential as Carcinogenic Biomarkers and the Mechanisms Underlying their Action. *J Transl Int Med* 2022; **10**: 156-174 [PMID: 35959452 DOI: 10.2478/jtim-2022-0013]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 April 24; 14(4): 160-170

DOI: 10.5306/wjco.v14.i4.160

ISSN 2218-4333 (online)

MINIREVIEWS

Integration of molecular testing for the personalized management of patients with diffuse large B-cell lymphoma and follicular lymphoma

Ruth Stuckey, Hugo Luzardo Henríquez, Haridian de la Nuez Melian, José Carlos Rivero Vera, Cristina Bilbao-Sieyro, María Teresa Gómez-Casares

Specialty type: Hematology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Luo Y, China; Shahriari M, Iran

Received: December 21, 2022
Peer-review started: December 21, 2022
First decision: March 24, 2023
Revised: March 24, 2023
Accepted: April 4, 2023
Article in press: April 4, 2023
Published online: April 24, 2023

Ruth Stuckey, Hugo Luzardo Henríquez, Haridian de la Nuez Melian, Department of Hematology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas 35019, Spain

José Carlos Rivero Vera, Department of Anatomical Pathology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas 35019, Spain

Cristina Bilbao-Sieyro, María Teresa Gómez-Casares, Department of Hematology, Hospital Universitario de Gran Canaria Dr. Negrin, Las Palmas de Gran Canaria 35019, Las Palmas de Gran Canaria, Spain

Cristina Bilbao-Sieyro, Department of Morphology, Universitario de Las Palmas de Gran Canaria, Las Palmas 35001, Spain

María Teresa Gómez-Casares, Medical Science, Universitario de Las Palmas de Gran Canaria, Las Palmas 35001, Spain

Corresponding author: Cristina Bilbao-Sieyro, PhD, Lecturer, Technician, Department of Hematology, Hospital Universitario de Gran Canaria Dr. Negrin, Barranco de la Ballena, Las Palmas de Gran Canaria 35019, Las Palmas de Gran Canaria, Spain. bilbaocristina@gmail.com

Abstract

Diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) are the most common forms of aggressive and indolent lymphoma, respectively. The majority of patients are cured by standard R-CHOP immunochemotherapy, but 30%–40% of DLBCL and 20% of FL patients relapse or are refractory (R/R). DLBCL and FL are phenotypically and genetically hereterogenous B-cell neoplasms. To date, the diagnosis of DLBCL and FL has been based on morphology, immunophenotyping and cytogenetics. However, next-generation sequencing (NGS) is widening our understanding of the genetic basis of the B-cell lymphomas. In this review we will discuss how integrating the NGS-based characterization of somatic gene mutations with diagnostic or prognostic value in DLBCL and FL could help refine B-cell lymphoma classification as part of a multidisciplinary pathology work-up. We will also discuss how molecular testing can identify candidates for clinical trials with targeted therapies and help predict therapeutic outcome to currently available treatments, including chimeric antigen receptor T-cell, as well as explore the application of circulating cell-free DNA, a non-invasive method for patient monitoring. We conclude that molecular analyses can drive improvements in patient outcomes due to an increased understanding

of the different pathogenic pathways affected by each DLBCL subtype and indolent FL *vs* R/R FL.

Key Words: Next-generation sequencing; Prognosis; Molecular analysis; Targeted therapy; Chimeric antigen receptor T-cell therapy; Personalized medicine

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Molecular studies in the past decade have improved our understanding of the biological heterogeneity of B-cell lymphomas such as diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. Next-generation sequencing studies are helping to reveal the different pathogenic pathways affected by each DLBCL subtype and identify new targets for directed therapy. Molecular analysis can also help predict therapeutic outcome to currently available treatments, including chimeric antigen receptor T-cell therapy, and identify candidates for clinical trials with targeted therapies, ultimately leading to improvements in patient outcomes. As such, the incorporation of precision medicine *via* the integration of molecular analyses in clinical practice can improve clinical outcomes in patients and thus contribute to a new standard of care for patients with B-cell lymphomas.

Citation: Stuckey R, Luzardo Henríquez H, de la Nuez Melian H, Rivero Vera JC, Bilbao-Sieyro C, Gómez-Casares MT. Integration of molecular testing for the personalized management of patients with diffuse large B-cell lymphoma and follicular lymphoma. *World J Clin Oncol* 2023; 14(4): 160-170
URL: https://www.wjgnet.com/2218-4333/full/v14/i4/160.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i4.160

INTRODUCTION

B-cell lymphomas are classified into over 19 distinct entities, as defined by the 2022 World Health Organization (WHO) classification[1]. Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma (NHL), representing approximately 30% of lymphomas of mature B-cells[2], while follicular lymphoma (FL) is the second most common NHL. However, both DLBCL and FL are phenotypically and genetically hereterogenous B-cell neoplasms. For example, the majority of DLBCL patients are cured by standard rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) immunochemotherapy, but 30%–40% relapse or are refractory (R/R), while for FL, approximately 20% of patients treated with chemoimmunotherapy will progress within the first two years of diagnosis (POD24)[3]. Thus, improvements in patient outcomes will rely on an increased understanding of the different pathogenic DLBCL and FL pathways that lead to treatment failure and/or progression.

Next-generation sequencing (NGS) studies together with copy-number analysis are determining genes with recurrent alterations in DLBCL and FL, some of which can refine diagnosis and prognostic stratification. In this minireview, we will describe how molecular analyses are revealing differences in somatic mutations according to disease subtypes, helping with differential diagnosis, as well as determining new targets for the development of directed therapies. We will also explore the application of circulating cell-free DNA, a non-invasive method for patient monitoring. Finally, we will discuss how the incorporation of precision medicine can identify candidates for clinical trials with targeted therapies and help predict therapeutic outcome to currently available treatments in a drive towards a more personalized treatment approach.

We aim to convince the reader that the incorporation of molecular testing for somatic gene mutations can improve the diagnosis and prognosis of patients with DLBCL and FL as part of a multidisciplinary pathology work-up.

CONVENTIONAL CLASSIFICATION OF DLBCL

Currently, using immunohistochemistry (IHC), fluorescence in situ hybridization (FISH) and flow cytometry techniques, patients with DLBCL are divided into three subtypes depending on the stage of differentiation and maturation of the B cells of origin: germinal center B-cell like (GCB) or activated B-cell like (ABC), with the remaining 10% to 20% "unclassified" or not otherwise specified (NOS). For example, IHC analysis of *CD10*, *BCL6* and *MUM1* markers helps determine the GCB and ABC subtypes according to the Hans algorithm[4]. Nevertheless, the Hans algorithm doesn't distinguish the NOS DLBCL subtype and gives an incorrect classification in approximately 20% of cases[4].

DLBCL patients show distinct clinical outcomes according to the subtype: patients with the GCB phenotype have a more favorable outcome than ABC in terms of survival when treated with standard chemotherapy, 60% at 5 years *vs* 35% in ABC[5]. In addition, IHC of *MYC* and *BCL2/BCL6* can identify tumors as double- or triple-expressor, associated with worse prognosis[6]. Studies involving FISH analysis of *MYC* rearrangements have shown that *MYC* rearranged with an immunoglobulin (IG) gene has worse prognosis compared to *MYC* with a non-IG partner, with *MYC*/IG double hits associated with an even poorer prognosis[7]. Thus, genomic tests used in routine clinical practice are already adding prognostic value. Even so, the diagnostic work-up and treatment are practically identical for all DLBCL patients despite the high genetic heterogeneity.

In terms of treatment, R-CHOP has been the standard of care for over two decades, and is still administered to the majority of DLBCL patients[8]. To improve treatment response, and predict which patients are likely to be R/R, elucidation of the molecular determinants related to treatment response will be fundamental. One advancement in this area is the observation that high *EZH2* expression (> 70%, detected by IHC) is associated with superior survival of DLBCL patients following R-CHOP[9].

CONVENTIONAL CLASSIFICATION OF FL

FL is characterized by the t(14;18)(q32;q21) translocation, present in 90% of FL patients, resulting in overexpression of *BCL2* under the *IGH* promoter. In cases lacking t(14;18), BCL6 and CD10 expression patterns confirm FL diagnosis[10]. Rearrangement of *BCL6* (3q27) may also be found in grade 3 FL, with or without t(14;18)[11].

Prognostic biological factors include age > 60 years and hemoglobin < 12 g/dL, as well as other biomarkers, such as LDH or β2-microglobin above normal, according to the FLIPI and FLIPI-2 scores, respectively[12,13].

Several first-lines of immunochemotherapy exist, including bendamustine + rituximab, rituximab alone, or R-CHOP, with choice largely down to the clinician's preference. Treatment improvements are a necessity, given that POD24 is a predictor of overall survival (OS), with rates of just 50% for patients with POD24 *vs* 90% in those with no POD24 following R-CHOP treatment[3]. As highlighted in the recent editorial by Leonard[14], there is currently "no reliable way" to determine at diagnosis whether a patient with FL is likely to respond optimally to immunochemotherapy. The hope is that molecular analyses could help identify a subgroup of at-risk patients who would benefit from upfront treatment with a specific targeted therapy.

NGS APPLICATION IN LYMPHOMAS

According to the 2022 WHO and ICC classifications and the European Society for Medical Oncology's 2021 clinical guidelines, no molecular analyses are currently recommended at diagnosis for DLBCL or FL[1,15,16]. To date, only a few entities of lymphoid neoplasms are defined by genomic criteria. This is in stark contrast to other hematological malignancies, in particular myeloid neoplasms, where the use of NGS is well-established in diagnosis and risk-stratification[1,16,17]. For example, for acute myeloid leukemia a complete genomic evaluation, including NGS panel, is obligatory at diagnosis to define disease subtypes and to direct therapies[1,16,17]. Nevertheless, both international consortiums acknowledge that molecular analyses in B-cell lymphomas have identified genomic alterations "with diagnostic, prognostic, and predictive impact in different entities"[18] and explicitly state that it is highly probable that more entities will be defined by genomic criteria in the near future[1,16,18].

MOLECULAR ANALYSES IN DLBCL

In recent years, advances in next-generation sequencing (NGS) techniques are redefining our understanding of the genetic basis of lymphomas. Molecular studies are revealing recurrent genetic events and thus are helping to identify the key pathways that are important in DLBCL pathogenicity and evolution, and may even have prognostic impact[19].

Mutations in the genes *MYD88, CARD11, EZH2* and *CD79A/CD79B* have been identified in approximately 40% of DLBCL and are considered drivers of lymphomagenesis[20]. Moreover, NGS studies have revealed that GCB and ABC have a distinct profile of somatic mutations. For instance, mutations in *GNA13* are found in GCB but are rare in other B-cell lymphoma subtypes[21], whereas the MYD88 L265P mutation is found in ABC but is rarely identified in GCB DLBCL[21]. Thus, mutational information can assist in providing an accurate diagnosis, for example for the differential diagnosis of DLBCL from primary mediastinal large B-cell lymphoma (PMBCL, a relatively rare NHL with large B-cell morphology)[22], mantle cell lymphoma, or grade 3 FL. In addition, relapse has been associated with mutations in certain genes, such as the *B2M* and *CD58* immune surveillance genes[23].

Recent findings suggest that tumor genotype also influences treatment response. For example, whole genome sequencing analysis of 20 patients with high-risk GCB DLBCL revealed that those with cryptic rearrangements of *MYC* or *BCL2* (not detectable by FISH) had worse outcomes to R-CHOP[24]. Moreover, ABC tumors that harboured both a mutation in *CD79B* and the *MYD88* L265P mutation were more sensitive to the BTK inhibitor ibrutinib, whereas NOS subtype tumors with the *MYD88* L265P mutation and *CD79B* wild-type showed a poor response to ibrutinib[25].

Although NGS of DLBCL is not currently recommended in routine clinical practice[1,16], huge efforts are underway to characterize the prognostic value and thus functional impact of driver mutations, for instance *via* the whole-exome sequencing of 1001 DLBCL samples[26].

Such large-scale studies using NGS techniques together with copy-number analysis, to identify genes with recurrent alterations with prognostic value, have led to the proposition of new DLBCL classifications. After studying 574 DLBCL biopsy samples Schmitz *et al*[27] proposed four genetic subtypes termed MCD (based on the co-occurrence of *MYD88* and *CD79B* mutations), BN2 (based on *BCL6* fusions and *NOTCH2* mutations), N1 (based on *NOTCH1* mutations), and EZB (based on *EZH2* mutations and *BCL2* translocations). These subtypes differed in their responses to immunochemotherapy, with favorable survival in the BN2 and EZB subtypes and inferior outcomes in the MCD and N1 subtypes[27], whereas MCD and N1 subtypes responded well to R-CHOP with ibrutinib[28]. Importantly, data on the BN2 subtype, with overlap with the NOS subgroup, revealed that patients are likely to be responsive to antagonists of B-cell receptor signaling such as the BTK inhibitors. Similarly, Chapuy *et al*[29] studied 304 DLBCL biopsies and identified six genetic subgroups. Of note, mutations in *CD79B* were associated with relapse independently of the subtype or International Prognostic Index (IPI) risk group[30].

MOLECULAR ANALYSES IN FL

Mutations in genes encoding epigenetic modifiers (and the resultant pattern of aberrant DNA methylation) are a molecular hallmark of FL (Table 1)[31,32]. Moreover, such mutations are likely to be early driver events[33].

NGS studies have revealed that the acquisition of additional mutations contributes to disease progression and the risk of transformation of FL to DLBCL. For example, *TP53* mutations have been associated with shorter progression-free survival and OS[34,35], while the gain of mutations in genes such as *EBF1*, *MYD88* and *TNFAIP3* are associated with progression to a more aggressive disease[32]. Additionally, expression studies have revealed chromosome regions, such as 1p36 and 6q21 deletion associated with transformation[36]. Thus, genetic analyses can improve the prognostication of patients with FL[37].

In the case of FL, the mutational status of seven genes (*EZH2*, *ARID1A*, *MEF2B*, *EP300*, *FOXO1*, *CREBBP* and *CARD11*) was added to the preexisting Eastern Cooperative Oncology Group (ECOG) performance status, FL International Prognostic Index (ECOG PS and FLIPI) risk stratification algorithms to develop the m7-FLIPI risk score[38]. Application of the m7-FLIPI risk score defined a high-risk group with a significantly shorter failure-free survival after receiving first-line R-CHOP.

Specifically, mutations in *EZH2* were associated with the low-risk m7-FLIPI group and with higher OS[38]. As such, the presence or absence of the *EZH2* Y646 point mutation can help decide the chemotherapy regime in a patient-specific manner, since patients with such a mutation were shown to respond well to R-CHOP[38] (higher OS and lower relapse rate) while patients without this mutation responded better to bendamustine[39].

Although the m7-FLIPI was not prognostic for FL patients who received rituximab, patients with *EZH2* mutations had longer time to treatment failure while *EP300* mutations were associated with shorter time to treatment failure[40]. Therefore, it remains to be determined if the m7-FLIPI risk score is prognostic for FL patients treated with other chemotherapy regimes other than R-CHOP. Furthermore, the use of such risk scores in the routine clinical practice is not common, partly due to lack of availability of mutational studies in some centers[41].

TARGETED THERAPIES IN DLBCL

It is clear that improvements in DLBCL outcomes will rely on an increased understanding of the different pathogenic pathways affected by each DLBCL subtype. Indeed, an *in silico* drug discovery analysis showed that 46% of cases harbored at least one genomic alteration considered to be a potential drug response target (according to early clinical trials or preclinical assays in DLBCL or other B-cell lymphomas)[30].

But, to date, only one targeted therapy against a molecular driver has been approved for DLBCL – selinexor – although many others are in development. Selinexor is a specific inhibitor of the XPO1 nuclear export transporter protein that was approved by the FDA in June 2020 for the treatment of adults with R/R DLBCL NOS, including DLBCL progressed from FL, after at least two previous lines of

Table 1 Frequent mutations detected in follicular lymphoma. Adapted from[32,37]

Gene	Frequency in FL, %
KMT2D	82
CREBBP	65-70
HIST1H1C and/or HIST1H1E	28
EZH2	20-25
EP300	14
STAT6	12
CARD11	11
TNFAIP3	11
SOCS1	8
TP53	6

FL: Follicular lymphoma.

therapy[42].

Besides targeted agents, many immunotherapy strategies are in development, with the aim to promote the immune recognition and cytotoxic attack of T cells or macrophages. Some have achieved approval, such as rituximab, a monoclonal antibody against the common surface antigen CD20, and polatuzumab vedotin (Pola; Polivy™), an antibody-drug conjugate. Pola includes an anti-CD79B monoclonal antibody for cell targeting, which upon binding allows the antineoplastic agent monomethyl auristatin E to enter the cell and inhibit microtubule assembly, preventing cell mitosis and ultimately causing apoptosis[43]. Pola was approved by the FDA in June 2019 in combination with rituximab and bendamustine for the treatment of adults with R/R DLBCL after at least two previous lines of therapy. Such strategies have the advantage that the surface antigens they target are universally expressed on all DLBCL subtypes.

TARGETED THERAPIES IN FL

Genetic studies have identified epigenetic mechanisms in the pathogenesis of both DLBCL and FL, such as acetylation/deacetylation affected by *CREBBP* and *EP300* mutations, or histone methylation changes affected by *EZH2* mutations. Indeed, Tazemetostat (Tazverik™), an EZH2 inhibitor, was the first directed therapy to be approved by the FDA (in June 2020) for the treatment of R/R FL after two lines of previous therapy[44]. The *EZH2* mutation is predictive of Tazemetostat response but, interestingly, this targeted agent was also shown to improve the outcome of patients without an *EZH2* mutation[44].

Other FDA-approved agents for R/R FL include four PI3K signaling inhibitors: Idelalisib (July 2014), copanlisib (September 2017), duvelisib (September 2018), and umbralisib (February 2021)[45-48]. Further information on FL therapies in development can be found in this recent review[37].

CAR-T

Besides targeted therapies, an improved understanding of the genetic and immune biology of DLBCL and FL has led to the development of chimeric antigen receptor T-cell (CAR-T) therapies, considered a major scientific breakthrough and offering an alternative treatment option for patients with R/R B-cell lymphomas[49,50].

In 2020, our center obtained the license to provide the European Medicine Agency-approved anti-CD19 CAR-T axicabtagene ciloleucel (Yescarta™) and Tisagenlecleucel (Kymriah™) for the treatment of adult patients with R/R DLBCL or PMBCL after two or more previous lines of treatment. As of February 2021, the third CAR-T lisocabtagene maraleucel (Breyanzi™) also obtained FDA approval for the treatment of R/R DLBCL[51].

CAR-T is also an option for the treatment of adult R/R FL patients after two or more previous lines of treatment[52,53], following the FDA approval of axicabtagene ciloleucel in March 2021.

The proliferation and persistence of CAR-T cells in the body is an important factor influencing therapy durability, with the loss of a CAR-T signal associated with progression of the disease[54]. A quantitative TaqMan PCR (qPCR) assay can be used to monitor the number of CAR-T cells circulating in

peripheral blood *via* detection of the quimeric CD19 recognition domain (FMC63)[55]. Flow cytometry is an alternative method for CAR-T cell monitoring, but has the disadvantage that it needs to be carried out on fresh samples and has lower sensitivity. Future studies are required to explore the correlation between the expansion/persistence of CAR-T cells and clinical outcomes including treatment efficacy and clinical symptoms.

CIRCULATING CELL-FREE TUMOR DNA

Surgical excision biopsies are the gold-standard technique used in the diagnosis and follow-up of patients with lymphomas, although core needle biopsies are a useful and viable alternative under certain conditions[56]. However, both surgical excision and core needle biopsies are resource intensive, can be painful, and impact negatively on patients, and surgical excision biopsies, in particular, have an associated risk of morbidity due to bleeding and infection. Additionally, some lymphomas may not be easily accessible which can limit the availability of tissue for genomic studies. Moreover, the extraction of genomic DNA from formalin-fixed, paraffin-embedded biopsies for downstream NGS applications is not ideal since chemicals used in the fixation can degrade nucleic acids, thus decreasing NGS sensitivity.

Liquid biopsy techniques are currently being explored as non-invasive methods for tumor diagnosis and disease monitoring[57]. Circulating cell-free DNA (ctDNA), consisting of highly fragmented DNA in plasma that is released by normal or tumor cells that undergo apoptosis or necrosis[58], may better reflect intratumoral heterogeneity than can be obtained from a single tissue biopsy. Indeed, in comparison with the sequencing of genomic DNA extracted from the diagnostic tissue biopsy, the sequencing of ctDNA can identify somatic mutations with a similar accuracy and identified additional clinically relevant mutations that were not detected in the diagnostic tissue biopsy[59]. Moreover, the analysis of ctDNA could overcome some other limitations of biopsies. For example, in the case of a biopsy at an extranodal site, it is not uncommon for the paraffin block to also contain other non-tumoral tissue.

Due to their easy accessibility through non-invasive procedures (such as a simple peripheral blood draw), ctDNA analyses can be repeated regularly to track lymphomas over time, such as to monitor treatment response. Indeed, studies have shown that changes in ctDNA quantification correlated with positive responses to chemotherapy and could even detect relapse, months earlier than conventional CT scan monitoring[60]. Thus they may also be useful as "surveillance" methods in patients who have completed treatment but may be at risk of relapse, *e.g.* those with mutations in *CD79B* or those with a high pretreatment ctDNA quantitative burden for early relapse detection[32,59].

Future studies are required to optimize the application of ctDNA analyses in the management of patients with B-cell lymphomas. Nevertheless, ctDNA is currently used in the clinic in some fields of oncology, such as in the molecular profiling of patients with non-squamous non-small cell lung cancer at diagnosis, as recommended by the National Comprehensive Cancer Network[61].

IMPLEMENTATION OF MOLECULAR TESTING IN CLINICAL PRACTICE

The application of NGS, together with other molecular techniques, is key to the integration of personalized medicine approaches into healthcare services. The use of NGS targeted panels, which focus on a limited and relevant set of genes or gene regions that have known associations with a particular pathology, produce large quantities of genetic information with diagnostic, prognostic and theranostic value with a high sensitivity. The simultaneous analysis of an elevated number of genes (15-200) is more resource efficient as it drastically reduces the cost and time required to obtain such genetic information enabling a more precise diagnosis and prognosis. Furthermore, the use of NGS permits the detection of emerging clones which can help inform disease follow-up and may be associated with treatment resistance, thus providing data that can help guide individualized patient therapeutic plans.

In 2016 our team implemented NGS into the routine diagnosis and prognosis of patients with acute myeloid leukemia[62]. Since then, the use of NGS has expanded to include a targeted myeloid panel for the diagnosis of patients with myeloproliferative neoplasms and myelodysplastic syndromes, a chronic lymphocytic leukemia-specific panel, and a panel for the detection of germline hematologic malignancies. However, the molecular analysis of B-cell lymphoma samples in our center is currently limited to the qPCR-based analysis of several individual genes with prognostic value (including *MYD88, TP53,* and *EZH2*) to complement the conventional cytometry, IHC and FISH tests used in routine clinical practice.

Several commercial gene panels are currently available on the market for the detection of mutations with diagnostic, prognostic or theranostic value in DLBCL and FL, given the considerable overlap of genetic alterations between GCB DLBCL and FL[32], including Oncomine™ Lymphoma (ThermoFisher), FusionPlex® Lymphoma (Archer) and Lymphoma Solution® (SOPHiA).

Incorporating a comprehensive NGS-based characterization of somatic gene mutations as a precision medicine strategy for B-cell lymphomas would assist in the daily practice by refining DLBCL and FL classification and prognosis. Importantly, it would also facilitate individualized therapeutic decision-making for patients and increase treatment opportunities by identifying candidates for clinical trials with targeted therapies. However, feasibility studies would be required to determine the clinical utility and added value of incorporating an NGS panel in the multidisciplinary diagnostic work-up, since "while many stakeholders believe that personalized medicine can provide benefits to patients and the healthcare system, payer and providers are often reluctant to change policies and practices without convincing evidence of clinical and economic value"[63].

It is also important to consider the limitations of introducing such molecular analyses for B-cell lymphomas into routine hematology laboratories. Difficulties arise in interpretation of the results generated by extensive NGS panels due to the data's complexity and uncertainty about the biological relevance as not all molecular variants are clinically actionable. For this reason, it is essential to have highly trained staff with experience in the interpretation of the clinical impact of tumor variants. Other limitations include the economic cost of molecular analyses and the turnaround time, which has a large impact on the applicability of genomic tests to clinical decision-making. The potential to multiplex lymphoma samples with other targeted panels in the same sequencing run would help optimize the resources dedicated to library preparation and sequencing, and minimize the time required to analyze patient samples and report results to guide clinical decision-making. This is essential for aggressive B-cell lymphomas where immediate treatment is frequently required.

CONCLUSION

The incorporation of molecular testing into the routine clinical management of patients with B-cell lymphomas *via* the implementation of a targeted NGS panel would help improve disease subtype classification, allow the prediction of therapeutic outcome to currently available treatments, and identify patients for personalized treatment. Moreover, the optimization of non-invasive ctDNA analysis could allow for closer patient monitoring and earlier relapse detection.

FOOTNOTES

Author contributions: Stuckey R performed the majority of the writing, and prepared the tables; Luzardo Henríquez H, de la Nuez Melian H and Rivero Vera JC provided input in writing the paper; Bilbao-Sieyro C and Gómez-Casares MT coordinated the writing of the paper; all authors approved the final version of the manuscript.

Conflict-of-interest statement: All the authors have no conflicts of interest to declare.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Spain

ORCID number: Ruth Stuckey 0000-0001-6955-2290; Cristina Bilbao-Sieyro 0000-0002-4796-1445; María Teresa Gómez-Casares 0000-0003-0505-5126.

S-Editor: Liu JH
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Alaggio R**, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, Bhagat G, Borges AM, Boyer D, Calaminici M, Chadburn A, Chan JKC, Cheuk W, Chng WJ, Choi JK, Chuang SS, Coupland SE, Czader M, Dave SS, de Jong D, Du MQ, Elenitoba-Johnson KS, Ferry J, Geyer J, Gratzinger D, Guitart J, Gujral S, Harris M, Harrison CJ, Hartmann S, Hochhaus A, Jansen PM, Karube K, Kempf W, Khoury J, Kimura H, Klapper W, Kovach AE, Kumar S, Lazar AJ, Lazzi S, Leoncini L, Leung N, Leventaki V, Li XQ, Lim MS, Liu WP, Louissaint A Jr, Marcogliese A, Medeiros LJ, Michal M, Miranda RN, Mitteldorf C, Montes-Moreno S, Morice W, Nardi V, Naresh KN, Natkunam Y, Ng SB, Oschlies I, Ott G, Parrens M, Pulitzer M, Rajkumar SV, Rawstron AC, Rech K, Rosenwald A, Said J, Sarkozy C, Sayed S, Saygin C, Schuh A, Sewell W, Siebert R, Sohani AR, Tooze R, Traverse-Glehen A, Vega F, Vergier B,

Wechalekar AD, Wood B, Xerri L, Xiao W. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leukemia* 2022; **36**: 1720-1748 [PMID: 35732829 DOI: 10.1038/s41375-022-01620-2]

2 **Bastos-Oreiro M**, Muntañola A, Panizo C, Gonzalez-Barca E, de Villambrosia SG, Córdoba R, López JLB, González-Sierra P, Terol MJ, Gutierrez A, Grande C, Ramirez MJ, Iserte L, Perez E, Navarro B, Gomez P, Salar A, Luzardo H, López A, Del Campo R, García-Belmonte D, Vida MJ, Infante M, Queizan-Hernandez JA, Novelli S, Moreno M, Penarrubia M, Gómez J, Domingo A, Donato E, Viguria MC, López F, Rodriguez MJ, Pardal E, Noriega V, Andreu R, Peñalver J, Martín A, Caballero D, López-Guillermo A. RELINF: prospective epidemiological registry of lymphoid neoplasms in Spain. A project from the GELTAMO group. *Ann Hematol* 2020; **99**: 799-808 [PMID: 32076827 DOI: 10.1007/s00277-020-03918-6]

3 **Casulo C**, Byrtek M, Dawson KL, Zhou X, Farber CM, Flowers CR, Hainsworth JD, Maurer MJ, Cerhan JR, Link BK, Zelenetz AD, Friedberg JW. Early Relapse of Follicular Lymphoma After Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone Defines Patients at High Risk for Death: An Analysis From the National LymphoCare Study. *J Clin Oncol* 2015; **33**: 2516-2522 [PMID: 26124482 DOI: 10.1200/JCO.2014.59.7534]

4 **Hans CP**, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, Müller-Hermelink HK, Campo E, Braziel RM, Jaffe ES, Pan Z, Farinha P, Smith LM, Falini B, Banham AH, Rosenwald A, Staudt LM, Connors JM, Armitage JO, Chan WC. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004; **103**: 275-282 [PMID: 14504078 DOI: 10.1182/blood-2003-05-1545]

5 **Rosenwald A**, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM, Hurt EM, Zhao H, Averett L, Yang L, Wilson WH, Jaffe ES, Simon R, Klausner RD, Powell J, Duffey PL, Longo DL, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Montserrat E, López-Guillermo A, Grogan TM, Miller TP, LeBlanc M, Ott G, Kvaloy S, Delabie J, Holte H, Krajci P, Stokke T, Staudt LM; Lymphoma/Leukemia Molecular Profiling Project. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346**: 1937-1947 [PMID: 12075054 DOI: 10.1056/NEJMoa012914]

6 **Friedberg JW**. How I treat double-hit lymphoma. *Blood* 2017; **130**: 590-596 [PMID: 28600333 DOI: 10.1182/blood-2017-04-737320]

7 **Pedersen MØ**, Gang AO, Poulsen TS, Knudsen H, Lauritzen AF, Nielsen SL, Klausen TW, Nørgaard P. MYC translocation partner gene determines survival of patients with large B-cell lymphoma with MYC- or double-hit MYC/BCL2 translocations. *Eur J Haematol* 2014; **92**: 42-48 [PMID: 24118498 DOI: 10.1111/ejh.12212]

8 **Roschewski M**, Staudt LM, Wilson WH. Diffuse large B-cell lymphoma-treatment approaches in the molecular era. *Nat Rev Clin Oncol* 2014; **11**: 12-23 [PMID: 24217204 DOI: 10.1038/nrclinonc.2013.197]

9 **Lee HJ**, Shin DH, Kim KB, Shin N, Park WY, Lee JH, Choi KU, Kim JY, Lee CH, Sol MY. Polycomb protein EZH2 expression in diffuse large B-cell lymphoma is associated with better prognosis in patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone. *Leuk Lymphoma* 2014; **55**: 2056-2063 [PMID: 24304372 DOI: 10.3109/10428194.2013.858816]

10 **Leich E**, Salaverria I, Bea S, Zettl A, Wright G, Moreno V, Gascoyne RD, Chan WC, Braziel RM, Rimsza LM, Weisenburger DD, Delabie J, Jaffe ES, Lister A, Fitzgibbon J, Staudt LM, Hartmann EM, Mueller-Hermelink HK, Campo E, Ott G, Rosenwald A. Follicular lymphomas with and without translocation t(14;18) differ in gene expression profiles and genetic alterations. *Blood* 2009; **114**: 826-834 [PMID: 19471018 DOI: 10.1182/blood-2009-01-198580]

11 **Nann D**, Ramis-Zaldivar JE, Müller I, Gonzalez-Farre B, Schmidt J, Egan C, Salmeron-Villalobos J, Clot G, Mattern S, Otto F, Mankel B, Colomer D, Balagué O, Szablewski V, Lome-Maldonado C, Leoncini L, Dojcinov S, Chott A, Copie-Bergman C, Bonzheim I, Fend F, Jaffe ES, Campo E, Salaverria I, Quintanilla-Martinez L. Follicular lymphoma t(14;18)-negative is genetically a heterogeneous disease. *Blood Adv* 2020; **4**: 5652-5665 [PMID: 33211828 DOI: 10.1182/bloodadvances.2020002944]

12 **Solal-Céligny P**, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, Au WY, Bellei M, Brice P, Caballero D, Coiffier B, Conde-Garcia E, Doyen C, Federico M, Fisher RI, Garcia-Conde JF, Guglielmi C, Hagenbeek A, Haïoun C, LeBlanc M, Lister AT, Lopez-Guillermo A, McLaughlin P, Milpied N, Morel P, Mounier N, Proctor SJ, Rohatiner A, Smith P, Soubeyran P, Tilly H, Vitolo U, Zinzani PL, Zucca E, Montserrat E. Follicular lymphoma international prognostic index. *Blood* 2004; **104**: 1258-1265 [PMID: 15126323 DOI: 10.1182/blood-2003-12-4434]

13 **Federico M**, Bellei M, Marcheselli L, Luminari S, Lopez-Guillermo A, Vitolo U, Pro B, Pileri S, Pulsoni A, Soubeyran P, Cortelazzo S, Martinelli G, Martelli M, Rigacci L, Arcaini L, Di Raimondo F, Merli F, Sabattini E, McLaughlin P, Solal-Céligny P. Follicular lymphoma international prognostic index 2: a new prognostic index for follicular lymphoma developed by the international follicular lymphoma prognostic factor project. *J Clin Oncol* 2009; **27**: 4555-4562 [PMID: 19652063 DOI: 10.1200/JCO.2008.21.3991]

14 **Leonard JP**. POD24 in follicular lymphoma: time to be "wise". *Blood* 2022; **139**: 1609-1610 [PMID: 35298602 DOI: 10.1182/blood.2021013437]

15 **Dreyling M**, Ghielmini M, Rule S, Salles G, Vitolo U, Ladetto M; ESMO Guidelines Committee. Newly diagnosed and relapsed follicular lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016; **27**: v83-v90 [PMID: 27664263 DOI: 10.1093/annonc/mdw400]

16 **Campo E**, Jaffe ES, Cook JR, Quintanilla-Martinez L, Swerdlow SH, Anderson KC, Brousset P, Cerroni L, de Leval L, Dirnhofer S, Dogan A, Feldman AL, Fend F, Friedberg JW, Gaulard P, Ghia P, Horwitz SM, King RL, Salles G, San-Miguel J, Seymour JF, Treon SP, Vose JM, Zucca E, Advani R, Ansell S, Au WY, Barrionuevo C, Bergsagel L, Chan WC, Cohen JI, d'Amore F, Davies A, Falini B, Ghobrial IM, Goodlad JR, Gribben JG, Hsi ED, Kahl BS, Kim WS, Kumar S, LaCasce AS, Laurent C, Lenz G, Leonard JP, Link MP, Lopez-Guillermo A, Mateos MV, Macintyre E, Melnick AM, Morschhauser F, Nakamura S, Narbaitz M, Pavlovsky A, Pileri SA, Piris M, Pro B, Rajkumar V, Rosen ST, Sander B, Sehn L, Shipp MA, Smith SM, Staudt LM, Thieblemont C, Tousseyn T, Wilson WH, Yoshino T, Zinzani PL, Dreyling M, Scott DW, Winter JN, Zelenetz AD. The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. *Blood* 2022; **140**: 1229-1253 [PMID: 35653592 DOI: 10.1182/blood.2022015851]

17 **Duncavage EJ**, Bagg A, Hasserjian RP, DiNardo CD, Godley LA, Iacobucci I, Jaiswal S, Malcovati L, Vannucchi AM, Patel KP, Arber DA, Arcila ME, Bejar R, Berliner N, Borowitz MJ, Branford S, Brown AL, Cargo CA, Döhner H, Falini B, Garcia-Manero G, Haferlach T, Hellström-Lindberg E, Kim AS, Klco JM, Komrokji R, Lee-Cheun Loh M, Loghavi S, Mullighan CG, Ogawa S, Orazi A, Papaemmanuil E, Reiter A, Ross DM, Savona M, Shimamura A, Skoda RC, Solé F, Stone RM, Tefferi A, Walter MJ, Wu D, Ebert BL, Cazzola M. Genomic profiling for clinical decision making in myeloid neoplasms and acute leukemia. *Blood* 2022; **140**: 2228-2247 [PMID: 36130297 DOI: 10.1182/blood.2022015853]

18 **de Leval L**, Alizadeh AA, Bergsagel PL, Campo E, Davies A, Dogan A, Fitzgibbon J, Horwitz SM, Melnick AM, Morice WG, Morin RD, Nadel B, Pileri SA, Rosenquist R, Rossi D, Salaverria I, Steidl C, Treon SP, Zelenetz AD, Advani RH, Allen CE, Ansell SM, Chan WC, Cook JR, Cook LB, d'Amore F, Dirnhofer S, Dreyling M, Dunleavy K, Feldman AL, Fend F, Gaulard P, Ghia P, Gribben JG, Hermine O, Hodson DJ, Hsi ED, Inghirami G, Jaffe ES, Karube K, Kataoka K, Klapper W, Kim WS, King RL, Ko YH, LaCasce AS, Lenz G, Martin-Subero JI, Piris MA, Pittaluga S, Pasqualucci L, Quintanilla-Martinez L, Rodig SJ, Rosenwald A, Salles GA, San-Miguel J, Savage KJ, Sehn LH, Semenzato G, Staudt LM, Swerdlow SH, Tam CS, Trotman J, Vose JM, Weigert O, Wilson WH, Winter JN, Wu CJ, Zinzani PL, Zucca E, Bagg A, Scott DW. Genomic profiling for clinical decision making in lymphoid neoplasms. *Blood* 2022; **140**: 2193-2227 [PMID: 36001803 DOI: 10.1182/blood.2022015854]

19 **Rosenquist R**, Beà S, Du MQ, Nadel B, Pan-Hammarström Q. Genetic landscape and deregulated pathways in B-cell lymphoid malignancies. *J Intern Med* 2017; **282**: 371-394 [PMID: 28631441 DOI: 10.1111/joim.12633]

20 **Bohers E**, Mareschal S, Bouzelfen A, Marchand V, Ruminy P, Maingonnat C, Ménard AL, Etancelin P, Bertrand P, Dubois S, Alcantara M, Bastard C, Tilly H, Jardin F. Targetable activating mutations are very frequent in GCB and ABC diffuse large B-cell lymphoma. *Genes Chromosomes Cancer* 2014; **53**: 144-153 [PMID: 24327543 DOI: 10.1002/gcc.22126]

21 **Ngo VN**, Young RM, Schmitz R, Jhavar S, Xiao W, Lim KH, Kohlhammer H, Xu W, Yang Y, Zhao H, Shaffer AL, Romesser P, Wright G, Powell J, Rosenwald A, Muller-Hermelink HK, Ott G, Gascoyne RD, Connors JM, Rimsza LM, Campo E, Jaffe ES, Delabie J, Smeland EB, Fisher RI, Braziel RM, Tubbs RR, Cook JR, Weisenburger DD, Chan WC, Staudt LM. Oncogenically active MYD88 mutations in human lymphoma. *Nature* 2011; **470**: 115-119 [PMID: 21179087 DOI: 10.1038/nature09671]

22 **Mottok A**, Wright G, Rosenwald A, Ott G, Ramsower C, Campo E, Braziel RM, Delabie J, Weisenburger DD, Song JY, Chan WC, Cook JR, Fu K, Greiner T, Smeland E, Holte H, Savage KJ, Glinsmann-Gibson BJ, Gascoyne RD, Staudt LM, Jaffe ES, Connors JM, Scott DW, Steidl C, Rimsza LM. Molecular classification of primary mediastinal large B-cell lymphoma using routinely available tissue specimens. *Blood* 2018; **132**: 2401-2405 [PMID: 30257882 DOI: 10.1182/blood-2018-05-851154]

23 **Jiang Y**, Redmond D, Nie K, Eng KW, Clozel T, Martin P, Tan LH, Melnick AM, Tam W, Elemento O. Deep sequencing reveals clonal evolution patterns and mutation events associated with relapse in B-cell lymphomas. *Genome Biol* 2014; **15**: 432 [PMID: 25123191 DOI: 10.1186/s13059-014-0432-0]

24 **Hilton LK**, Tang J, Ben-Neriah S, Alcaide M, Jiang A, Grande BM, Rushton CK, Boyle M, Meissner B, Scott DW, Morin RD. The double-hit signature identifies double-hit diffuse large B-cell lymphoma with genetic events cryptic to FISH. *Blood* 2019; **134**: 1528-1532 [PMID: 31527075 DOI: 10.1182/blood.2019002600]

25 **Wilson WH**, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, Lih CJ, Williams PM, Shaffer AL, Gerecitano J, de Vos S, Goy A, Kenkre VP, Barr PM, Blum KA, Shustov A, Advani R, Fowler NH, Vose JM, Elstrom RL, Habermann TM, Barrientos JC, McGreivy J, Fardis M, Chang BY, Clow F, Munneke B, Moussa D, Beaupre DM, Staudt LM. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med* 2015; **21**: 922-926 [PMID: 26193343 DOI: 10.1038/nm.3884]

26 **Reddy A**, Zhang J, Davis NS, Moffitt AB, Love CL, Waldrop A, Leppa S, Pasanen A, Meriranta L, Karjalainen-Lindsberg ML, Nørgaard P, Pedersen M, Gang AO, Høgdall E, Heavican TB, Lone W, Iqbal J, Qin Q, Li G, Kim SY, Healy J, Richards KL, Fedoriw Y, Bernal-Mizrachi L, Koff JL, Staton AD, Flowers CR, Paltiel O, Goldschmidt N, Calaminici M, Clear A, Gribben J, Nguyen E, Czader MB, Ondrejka SL, Collie A, Hsi ED, Tse E, Au-Yeung RKH, Kwong YL, Srivastava G, Choi WWL, Evens AM, Pilichowska M, Sengar M, Reddy N, Li S, Chadburn A, Gordon LI, Jaffe ES, Levy S, Rempel R, Tzeng T, Happ LE, Dave T, Rajagopalan D, Datta J, Dunson DB, Dave SS. Genetic and Functional Drivers of Diffuse Large B Cell Lymphoma. *Cell* 2017; **171**: 481-494.e15 [PMID: 28985567 DOI: 10.1016/j.cell.2017.09.027]

27 **Schmitz R**, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ, Roulland S, Kasbekar M, Young RM, Shaffer AL, Hodson DJ, Xiao W, Yu X, Yang Y, Zhao H, Xu W, Liu X, Zhou B, Du W, Chan WC, Jaffe ES, Gascoyne RD, Connors JM, Campo E, Lopez-Guillermo A, Rosenwald A, Ott G, Delabie J, Rimsza LM, Tay Kuang Wei K, Zelenetz AD, Leonard JP, Bartlett NL, Tran B, Shetty J, Zhao Y, Soppet DR, Pittaluga S, Wilson WH, Staudt LM. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med* 2018; **378**: 1396-1407 [PMID: 29641966 DOI: 10.1056/NEJMoa1801445]

28 **Wilson WH**, Wright GW, Huang DW, Hodkinson B, Balasubramanian S, Fan Y, Vermeulen J, Shreeve M, Staudt LM. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. *Cancer Cell* 2021; **39**: 1643-1653.e3 [PMID: 34739844 DOI: 10.1016/j.ccell.2021.10.006]

29 **Chapuy B**, Stewart C, Dunford AJ, Kim J, Kamburov A, Redd RA, Lawrence MS, Roemer MGM, Li AJ, Ziepert M, Staiger AM, Wala JA, Ducar MD, Leshchiner I, Rheinbay E, Taylor-Weiner A, Coughlin CA, Hess JM, Pedamallu CS, Livitz D, Rosebrock D, Rosenberg M, Tracy AA, Horn H, van Hummelen P, Feldman AL, Link BK, Novak AJ, Cerhan JR, Habermann TM, Siebert R, Rosenwald A, Thorner AR, Meyerson ML, Golub TR, Beroukhim R, Wulf GG, Ott G, Rodig SJ, Monti S, Neuberg DS, Loeffler M, Pfreundschuh M, Trümper L, Getz G, Shipp MA. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med* 2018; **24**: 679-690 [PMID: 29713087 DOI: 10.1038/s41591-018-0016-8]

30 **Karube K**, Enjuanes A, Dlouhy I, Jares P, Martin-Garcia D, Nadeu F, Ordóñez GR, Rovira J, Clot G, Royo C, Navarro A, Gonzalez-Farre B, Vaghefi A, Castellano G, Rubio-Perez C, Tamborero D, Briones J, Salar A, Sancho JM, Mercadal S, Gonzalez-Barca E, Escoda L, Miyoshi H, Ohshima K, Miyawaki K, Kato K, Akashi K, Mozos A, Colomo L, Alcoceba M, Valera A, Carrió A, Costa D, Lopez-Bigas N, Schmitz R, Staudt LM, Salaverria I, López-Guillermo A, Campo E.

Integrating genomic alterations in diffuse large B-cell lymphoma identifies new relevant pathways and potential therapeutic targets. *Leukemia* 2018; **32**: 675-684 [PMID: 28804123 DOI: 10.1038/leu.2017.251]

31 **Pasqualucci L**, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, Kasper LH, Lerach S, Tang H, Ma J, Rossi D, Chadburn A, Murty VV, Mullighan CG, Gaidano G, Rabadan R, Brindle PK, Dalla-Favera R. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. *Nature* 2011; **471**: 189-195 [PMID: 21390126 DOI: 10.1038/nature09730]

32 **Okosun J**, Bödör C, Wang J, Araf S, Yang CY, Pan C, Boller S, Cittaro D, Bozek M, Iqbal S, Matthews J, Wrench D, Marzec J, Tawana K, Popov N, O'Riain C, O'Shea D, Carlotti E, Davies A, Lawrie CH, Matolcsy A, Calaminici M, Norton A, Byers RJ, Mein C, Stupka E, Lister TA, Lenz G, Montoto S, Gribben JG, Fan Y, Grosschedl R, Chelala C, Fitzgibbon J. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. *Nat Genet* 2014; **46**: 176-181 [PMID: 24362818 DOI: 10.1038/ng.2856]

33 **Pasqualucci L**, Khiabanian H, Fangazio M, Vasishtha M, Messina M, Holmes AB, Ouillette P, Trifonov V, Rossi D, Tabbò F, Ponzoni M, Chadburn A, Murty VV, Bhagat G, Gaidano G, Inghirami G, Malek SN, Rabadan R, Dalla-Favera R. Genetics of follicular lymphoma transformation. *Cell Rep* 2014; **6**: 130-140 [PMID: 24388756 DOI: 10.1016/j.celrep.2013.12.027]

34 **Sander CA**, Yano T, Clark HM, Harris C, Longo DL, Jaffe ES, Raffeld M. p53 mutation is associated with progression in follicular lymphomas. *Blood* 1993; **82**: 1994-2004 [PMID: 8400252 DOI: 10.1182/blood.V82.7.1994.1994]

35 **O'Shea D**, O'Riain C, Taylor C, Waters R, Carlotti E, Macdougall F, Gribben J, Rosenwald A, Ott G, Rimsza LM, Smeland EB, Johnson N, Campo E, Greiner TC, Chan WC, Gascoyne RD, Wright G, Staudt LM, Lister TA, Fitzgibbon J. The presence of TP53 mutation at diagnosis of follicular lymphoma identifies a high-risk group of patients with shortened time to disease progression and poorer overall survival. *Blood* 2008; **112**: 3126-3129 [PMID: 18628487 DOI: 10.1182/blood-2008-05-154013]

36 **Cheung KJ**, Shah SP, Steidl C, Johnson N, Relander T, Telenius A, Lai B, Murphy KP, Lam W, Al-Tourah AJ, Connors JM, Ng RT, Gascoyne RD, Horsman DE. Genome-wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. *Blood* 2009; **113**: 137-148 [PMID: 18703704 DOI: 10.1182/blood-2008-02-140616]

37 **Cahill KE**, Smith SM. Follicular Lymphoma: a Focus on Current and Emerging Therapies. *Oncology (Williston Park)* 2022; **36**: 97-106 [PMID: 35180337 DOI: 10.46883/2022.25920946]

38 **Pastore A**, Jurinovic V, Kridel R, Hoster E, Staiger AM, Szczepanowski M, Pott C, Kopp N, Murakami M, Horn H, Leich E, Moccia AA, Mottok A, Sunkavalli A, Van Hummelen P, Ducar M, Ennishi D, Shulha HP, Hother C, Connors JM, Sehn LH, Dreyling M, Neuberg D, Möller P, Feller AC, Hansmann ML, Stein H, Rosenwald A, Ott G, Klapper W, Unterhalt M, Hiddemann W, Gascoyne RD, Weinstock DM, Weigert O. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol* 2015; **16**: 1111-1122 [PMID: 26256760 DOI: 10.1016/S1470-2045(15)00169-2]

39 **Martínez-Laperche C**, Sanz-Villanueva L, Díaz Crespo FJ, Muñiz P, Martín Rojas R, Carbonell D, Chicano M, Suárez-González J, Menárguez J, Kwon M, Diez Martín JL, Buño I, Bastos Oreiro M. EZH2 mutations at diagnosis in follicular lymphoma: a promising biomarker to guide frontline treatment. *BMC Cancer* 2022; **22**: 982 [PMID: 36104682 DOI: 10.1186/s12885-022-10070-z]

40 **Lockmer S**, Ren W, Brodtkorb M, Østenstad B, Wahlin BE, Pan-Hammarström Q, Kimby E. M7-FLIPI is not prognostic in follicular lymphoma patients with first-line rituximab chemo-free therapy. *Br J Haematol* 2020; **188**: 259-267 [PMID: 31423576 DOI: 10.1111/bjh.16159]

41 **Cartron G**, Trotman J. Time for an individualized approach to first-line management of follicular lymphoma. *Haematologica* 2022; **107**: 7-18 [PMID: 34985230 DOI: 10.3324/haematol.2021.278766]

42 **Kalakonda N**, Maerevoet M, Cavallo F, Follows G, Goy A, Vermaat JSP, Casasnovas O, Hamad N, Zijlstra JM, Bakhshi S, Bouabdallah R, Choquet S, Gurion R, Hill B, Jaeger U, Sancho JM, Schuster M, Thieblemont C, De la Cruz F, Egyed M, Mishra S, Offner F, Vassilakopoulos TP, Warzocha K, McCarthy D, Ma X, Corona K, Saint-Martin JR, Chang H, Landesman Y, Joshi A, Wang H, Shah J, Shacham S, Kauffman M, Van Den Neste E, Canales MA. Selinexor in patients with relapsed or refractory diffuse large B-cell lymphoma (SADAL): a single-arm, multinational, multicentre, open-label, phase 2 trial. *Lancet Haematol* 2020; **7**: e511-e522 [PMID: 32589977 DOI: 10.1016/S2352-3026(20)30120-4]

43 **Sehn LH**, Herrera AF, Flowers CR, Kamdar MK, McMillan A, Hertzberg M, Assouline S, Kim TM, Kim WS, Ozcan M, Hirata J, Penuel E, Paulson JN, Cheng J, Ku G, Matasar MJ. Polatuzumab Vedotin in Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *J Clin Oncol* 2020; **38**: 155-165 [PMID: 31693429 DOI: 10.1200/JCO.19.00172]

44 **Morschhauser F**, Tilly H, Chaidos A, McKay P, Phillips T, Assouline S, Batlevi CL, Campbell P, Ribrag V, Damaj GL, Dickinson M, Jurczak W, Kazmierczak M, Opat S, Radford J, Schmitt A, Yang J, Whalen J, Agarwal S, Adib D, Salles G. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. *Lancet Oncol* 2020; **21**: 1433-1442 [PMID: 33035457 DOI: 10.1016/S1470-2045(20)30441-1]

45 **Gopal AK**, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ, Flinn IW, Flowers CR, Martin P, Viardot A, Blum KA, Goy AH, Davies AJ, Zinzani PL, Dreyling M, Johnson D, Miller LL, Holes L, Li D, Dansey RD, Godfrey WR, Salles GA. PI3Kδ inhibition by idelalisib in patients with relapsed indolent lymphoma. *N Engl J Med* 2014; **370**: 1008-1018 [PMID: 24450858 DOI: 10.1056/NEJMoa1314583]

46 **Dreyling M**, Santoro A, Mollica L, Leppä S, Follows GA, Lenz G, Kim WS, Nagler A, Panayiotidis P, Demeter J, Özcan M, Kosinova M, Bouabdallah K, Morschhauser F, Stevens DA, Trevarthen D, Giurescu M, Cupit L, Liu L, Köchert K, Seidel H, Peña C, Yin S, Hiemeyer F, Garcia-Vargas J, Childs BH, Zinzani PL. Phosphatidylinositol 3-Kinase Inhibition by Copanlisib in Relapsed or Refractory Indolent Lymphoma. *J Clin Oncol* 2017; **35**: 3898-3905 [PMID: 28976790 DOI: 10.1200/JCO.2017.75.4648]

47 **Flinn IW**, Miller CB, Ardeshna KM, Tetreault S, Assouline SE, Mayer J, Merli M, Lunin SD, Pettitt AR, Nagy Z, Tournilhac O, Abou-Nassar KE, Crump M, Jacobsen ED, de Vos S, Kelly VM, Shi W, Steelman L, Le N, Weaver DT, Lustgarten S, Wagner-Johnston ND, Zinzani PL. DYNAMO: A Phase II Study of Duvelisib (IPI-145) in Patients With

Refractory Indolent Non-Hodgkin Lymphoma. *J Clin Oncol* 2019; **37**: 912-922 [PMID: 30742566 DOI: 10.1200/JCO.18.00915]

48 **Fowler NH**, Samaniego F, Jurczak W, Ghosh N, Derenzini E, Reeves JA, Knopińska-Posłuszny W, Cheah CY, Phillips T, Lech-Maranda E, Cheson BD, Caimi PF, Grosicki S, Leslie LA, Chavez JC, Fonseca G, Babu S, Hodson DJ, Shao SH, Burke JM, Sharman JP, Law JY, Pagel JM, Miskin HP, Sportelli P, O'Connor OA, Weiss MS, Zinzani PL. Umbralisib, a Dual PI3Kδ/CK1ε Inhibitor in Patients With Relapsed or Refractory Indolent Lymphoma. *J Clin Oncol* 2021; **39**: 1609-1618 [PMID: 33683917 DOI: 10.1200/JCO.20.03433]

49 **Ramos CA**, Heslop HE, Brenner MK. CAR-T Cell Therapy for Lymphoma. *Annu Rev Med* 2016; **67**: 165-183 [PMID: 26332003 DOI: 10.1146/annurev-med-051914-021702]

50 **Sadelain M**. CAR therapy: the CD19 paradigm. *J Clin Invest* 2015; **125**: 3392-3400 [PMID: 26325036 DOI: 10.1172/JCI80010]

51 **Abramson JS**, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason J, Mehta A, Purev E, Maloney DG, Andreadis C, Sehgal A, Solomon SR, Ghosh N, Albertson TM, Garcia J, Kostic A, Mallaney M, Ogasawara K, Newhall K, Kim Y, Li D, Siddiqi T. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet* 2020; **396**: 839-852 [PMID: 32888407 DOI: 10.1016/S0140-6736(20)31366-0]

52 **Fowler NH**, Dickinson M, Dreyling M, Martinez-Lopez J, Kolstad A, Butler J, Ghosh M, Popplewell L, Chavez JC, Bachy E, Kato K, Harigae H, Kersten MJ, Andreadis C, Riedell PA, Ho PJ, Pérez-Simón JA, Chen AI, Nastoupil LJ, von Tresckow B, Ferreri AJM, Teshima T, Patten PEM, McGuirk JP, Petzer AL, Offner F, Viardot A, Zinzani PL, Malladi R, Zia A, Awasthi R, Masood A, Anak O, Schuster SJ, Thieblemont C. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. *Nat Med* 2022; **28**: 325-332 [PMID: 34921238 DOI: 10.1038/s41591-021-01622-0]

53 **Locke FL**, Miklos DB, Jacobson CA, Perales MA, Kersten MJ, Oluwole OO, Ghobadi A, Rapoport AP, McGuirk J, Pagel JM, Muñoz J, Farooq U, van Meerten T, Reagan PM, Sureda A, Flinn IW, Vandenberghe P, Song KW, Dickinson M, Minnema MC, Riedell PA, Leslie LA, Chaganti S, Yang Y, Filosto S, Shah J, Schupp M, To C, Cheng P, Gordon LI, Westin JR; All ZUMA-7 Investigators and Contributing Kite Members. Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma. *N Engl J Med* 2022; **386**: 640-654 [PMID: 34891224 DOI: 10.1056/NEJMoa2116133]

54 **Roschewski M**, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, Kong K, Shovlin M, Jaffe ES, Staudt LM, Lai C, Steinberg SM, Chen CC, Zheng J, Willis TD, Faham M, Wilson WH. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol* 2015; **16**: 541-549 [PMID: 25842160 DOI: 10.1016/S1470-2045(15)70106-3]

55 **Wang H**, Du X, Chen WH, Lou J, Xiao HL, Pan YM, Chen H, An N, Zhang QX. Establishment of a Quantitative Polymerase Chain Reaction Assay for Monitoring Chimeric Antigen Receptor T Cells in Peripheral Blood. *Transplant Proc* 2018; **50**: 104-109 [PMID: 29407291 DOI: 10.1016/j.transproceed.2017.11.028]

56 **Gonçalves MC**, de Oliveira CRGCM, Sandes AF, Rodrigues CA, Novis Y, Viana PCC, Serra MMP, Zerbini MCN. Core Needle Biopsy in Lymphoma Diagnosis: The Diagnostic Performance and the Role of the Multidisciplinary Approach in the Optimization of Results. *Am J Surg Pathol* 2023; **47**: 111-123 [PMID: 36395467 DOI: 10.1097/PAS.0000000000001991]

57 **Kurtz DM**, Scherer F, Jin MC, Soo J, Craig AFM, Esfahani MS, Chabon JJ, Stehr H, Liu CL, Tibshirani R, Maeda LS, Gupta NK, Khodadoust MS, Advani RH, Levy R, Newman AM, Dührsen U, Hüttmann A, Meignan M, Casasnovas RO, Westin JR, Roschewski M, Wilson WH, Gaidano G, Rossi D, Diehn M, Alizadeh AA. Circulating Tumor DNA Measurements As Early Outcome Predictors in Diffuse Large B-Cell Lymphoma. *J Clin Oncol* 2018; **36**: 2845-2853 [PMID: 30125215 DOI: 10.1200/JCO.2018.78.5246]

58 **Gormally E**, Caboux E, Vineis P, Hainaut P. Circulating free DNA in plasma or serum as biomarker of carcinogenesis: practical aspects and biological significance. *Mutat Res* 2007; **635**: 105-117 [PMID: 17257890 DOI: 10.1016/j.mrrev.2006.11.002]

59 **Scherer F**, Kurtz DM, Newman AM, Stehr H, Craig AF, Esfahani MS, Lovejoy AF, Chabon JJ, Klass DM, Liu CL, Zhou L, Glover C, Visser BC, Poultsides GA, Advani RH, Maeda LS, Gupta NK, Levy R, Ohgami RS, Kunder CA, Diehn M, Alizadeh AA. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. *Sci Transl Med* 2016; **8**: 364ra155 [PMID: 27831904 DOI: 10.1126/scitranslmed.aai8545]

60 **Heitzer E**, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. *Nat Rev Genet* 2019; **20**: 71-88 [PMID: 30410101 DOI: 10.1038/s41576-018-0071-5]

61 **NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines)**. Non-small cell lung cancer. Version 6.2020. Published 2017. Accessed July 20, 2020. Available from: http://www.nccn.org

62 **Stuckey R**, Bilbao-Sieyro C, Gómez-Casares MT. A summary of the molecular testing recommended in acute myeloid leukemia. *Ann Mol Genet Med* 2020; **4**: 012-017 [DOI: 10.17352/amgm.000007]

63 **Novartis Oncology**. The precision oncology annual trend report: perspectives from payers, oncologists, and pathologists. Published 2016. Accessed July 21, 2020. Available from: http://www.hcp.novartis.com/care-management

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 April 24; 14(4): 171-178

DOI: 10.5306/wjco.v14.i4.171

ISSN 2218-4333 (online)

MINIREVIEWS

Current progress on the endoscopic features of colorectal sessile serrated lesions

Rui-Gang Wang, Lai Wei, Bo Jiang

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Choi YS, South Korea; Sano W, Japan

Received: February 13, 2023
Peer-review started: February 13, 2023
First decision: March 28, 2023
Revised: April 6, 2023
Accepted: April 13, 2023
Article in press: April 13, 2023
Published online: April 24, 2023

Rui-Gang Wang, Bo Jiang, Department of Gastroenterology, Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing 102218, China

Lai Wei, Department of Digestive Medicine, Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, Beijing 102218, China

Corresponding author: Bo Jiang, MD, Director, Department of Gastroenterology, Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University, No. 168 Litang Road, Changping District, Beijing 102218, China. jba00920@btch.edu.cn

Abstract

Along with the discovery and refinement of serrated pathways, the World Health Organization amended the classification of digestive system tumors in 2019, recommending the renaming of sessile serrated adenomas/polyps to sessile serrated lesions (SSLs). Given the particularity of the endoscopic appearance of SSLs, it could easily be overlooked and missed in colonoscopy screening, which is crucial for the occurrence of interval colorectal cancer. Existing literature has found that adequate bowel preparation, reasonable withdrawal time, and awareness of colorectal SSLs have improved the quality and accuracy of detection. More particularly, with the continuous advancement and development of endoscopy technology, equipment, and accessories, a potent auxiliary tool is provided for accurate observation and immediate diagnosis of SSLs. High-definition white light endoscopy, chromoendoscopy, and magnifying endoscopy have distinct roles in the detection of colorectal SSLs and are valuable in identifying the size, shape, character, risk degree, and potential malignant tendency. This article delves into the relevant factors influencing the detection rate of colorectal SSLs, reviews its characteristics under various endoscopic techniques, and expects to attract the attention of colonoscopists.

Key Words: Colorectal cancer; Sessile serrated lesions; Endoscopic features

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Because of its unique endoscopic patterns and behavior, sessile serrated lesions (SSLs) are easily missing during colonoscopy. SSL is a critical cause of interval colorectal cancer, so it is necessary to summarize the endoscopic features of the sessile serrated lesion to help endoscopists make a better identification and diagnosis.

Citation: Wang RG, Wei L, Jiang B. Current progress on the endoscopic features of colorectal sessile serrated lesions. *World J Clin Oncol* 2023; 14(4): 171-178
URL: https://www.wjgnet.com/2218-4333/full/v14/i4/171.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i4.171

INTRODUCTION

Colorectal cancer (CRC) is a common gastrointestinal malignancy with the third-highest incidence and the second-highest mortality rate. In China, more than 550000 new cases were diagnosed and 280000 deaths took place in 2020[1], severely threatening people's lives and health. With the improvements in awareness concerning colonoscopic screening, the occurrence of interval CRC has garnered significant attention. Interval CRC refers to the CRC that is not detected during colorectal screening but discovered prior to the next recommended screening date[2]. The incidence of interval CRC is a vital indicator in assessing the quality of colonoscopic screening. Interval CRC detection in the proximal colon goes beyond being merely a test of the patient's bowel preparation since the colonoscopist's experience is also highly relevant.

Colorectal sessile serrated lesions (SSLs) are poorly defined and pale, covered with mucus, and hardly distinguishable from the surrounding mucosa. For endoscopists who lack awareness of the features of colorectal SSLs, missing or overlooking them becomes inevitable, resulting in the incidence of interval CRCs[3]. Related research observed that the occurrence of interval CRCs three years after colonoscopy was 3.4%-9.0%[2,4].

Colorectal SSLs may proceed to CRCs through the pathways of BRAF mutation, microsatellite instability, CpG island methylation, and the deletion gene of DNA damage repair[5]. It has been exhibited that a 15%-30% incidence of CRCs occurs *via* the serrated pathway, which is known as the vital cancer pathway[5,6].

UPDATE ON THE PATHOLOGICAL CLASSIFICATION OF SERRATED LESIONS

During the previous decade, related studies have generated a more accurate description of the pathogenesis of intestinal adenocarcinoma. Population-based screening for CRCs has led to a comprehensive understanding of precancerous lesions and established a foundation for investigating the molecular pathways and biological behaviors of cancerous lesions. Accordingly, WHO renamed the sessile serrated adenoma/polyp as SSLs, as these may be flat rather than polypoid, and the association with BRAF or KRAS mutation delineates two separate neoplastic pathways.

Currently, the categorization of gastrointestinal tumors classifies serrated lesions as Hyperplastic Polyps (HP), SSL, SSL with dysplasia (SSL-D), Traditional serrated adenomas (TSA), and serrated tubular villous adenoma (STVA)[7-9].

HP is a benign lesion, and the pathological features are primarily epithelial hyperplasia in the upper 2/3 of the saphenous fossa, forming small papillae protruding into the lumen of the saphenous fossa, which then gives the luminal surface a serrated shape. Based on the cell composition and molecular genetic alterations, the two types of HP are the Microvesicular type of Hyperplastic Polyp and the Goblet Cell-rich type of Hyperplastic Polyp. Generally, the TSA is pedicled and has villous structures but is potentially malignant[10-12]. In contrast to the conventional tubulovillous adenoma, STVA usually presents histological changes in advanced adenomas, and the glands are frequently serrated when high-grade dysplasia and invasive carcinoma appear.

The histological diagnosis of SSLs necessitates the detection of at least one abnormal crypt. By way of illustration, the entire saphenous fossa is serrated and grows horizontally along the mucosal muscular layer, the basal expansion, abnormal maturation, and asymmetric proliferation, in which asymmetric proliferation causes structural changes in the entire saphenous fossa. This is the fundamental difference from the HP[8]. Moreover, SSL-D is histologically heterogeneous. Its abnormal crypt structures—being its core feature—differ from the surrounding glands like the appearance of villous structures, which are longer and more crowded, complex branching, sieve-shaped crypt, and increased or decreased serration compared with the background SSL. The morphology of SSL-Ds is often tanglesome and mixed with different subtypes, making it challenging to distinguish the degree of heterogeneous hyperplasia.

COLORECTAL SSL-RELATED RISKS

It has been widely recognized that colorectal SSLs essentially differ from HPs, both with regard to morphological and pathological characteristics analysis, and instead behave comparably to neoplastic lesions with malignant potential.

Meta-analyses have demonstrated that SSLs are associated with an increased risk of concurrent progressive tumors. Patients with larger proximal colorectal serrated lesions are at significant risk and may require closer monitoring and further completion of a colon examination[13,14]. In light of this, a population-based, case-control study from Danish revealed that having an SSL was associated with 3-fold increased odds for CRC, while having SSL-D was associated with a nearly 5-fold increased odds for CRC[15,16].

Reports have confirmed that the risk of developing CRCs in cases with SSL-D is 4.4% within a decade, which is higher than that of conventional adenoma (2.3%). This highlights a significantly increased long-term risk of CRC in patients with SSL[16]. Similarly, correlative studies have found that SSLs have a mean duration of 7-15 years before developing SSL-Ds. Then, 3.03%-12.5% of SSLs develop into CRCs 5-7 years after follow-up[17,18]. However, SSL-Ds progress to CRCs at a much faster rate, and there are reports of SSL-Ds rapidly aggravating submucosal invasive carcinomas within one or two years[19-21].

FACTORS AFFECTING THE DETECTION OF COLORECTAL SSLS

As a critical influencing factor in the occurrence of interval CRCs, the detection rate of SSLs can effectively evaluate the quality of colonoscopy and assess the level of colonoscopists. A retrospective study that included more than 10000 colonoscopies found that bowel preparation, exit time, polyp diameter, and adenoma detection rate were linked to the SSL detection rate. Equally important, a multivariate analysis underlined that adenoma detection rate was an independent predictor of SSL detection rate, implying that patients who developed colorectal adenomas were at higher risk of complicating SSL[22].

Additionally, a colonoscopist's professional experience is vital to the timely and accurate detection of colorectal SSLs. Li *et al*[23] noted that different colonoscopists are independent risk factors for the detection rate of proximal colonic serrated lesions. It underscores that inexperienced colonoscopists detected serrated lesions at only 16%-83% compared with their experienced counterparts. They also found that proximal serrated polyps are more common in men over 50 years old.

ENDOSCOPIC FEATURES OF COLORECTAL SSLS

The development of an endoscopic technique delivers a reliable tool for detecting colorectal SSLs, which are not easily distinguishable from the background mucosa. To effectively prevent the incidence of interval CRCs, an early diagnosis and treatment of SSLs are crucial, thereby improving the quality of life and disease prognosis of patients.

Colorectal SSL characteristics under white light endoscopy

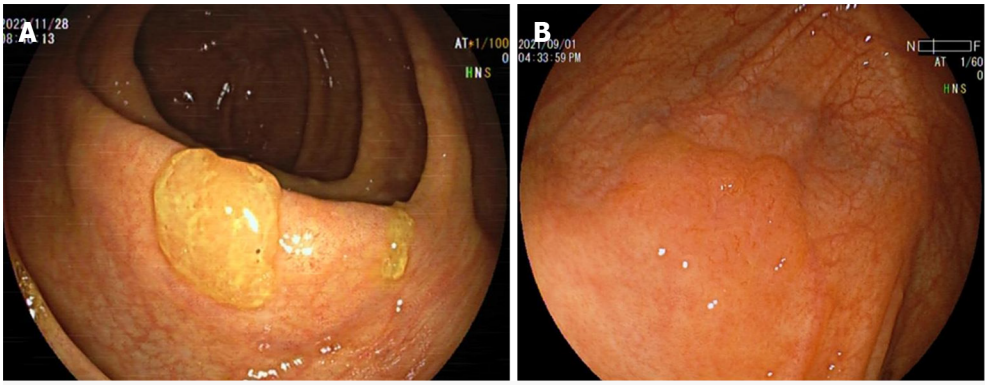
Colorectal SSL and SSL-D are prevalent in the proximal colon, usually > 5 mm in size, accounting for approximately 20%-25% of all serrated lesions. Additionally, colorectal SSLs often present with faint borders and a pale surface under white light endoscopy. Consequently, distinguishing them from the surrounding mucosa is difficult, making them prone to adverse events like missed or delayed diagnoses and incomplete resections. Most colorectal SSLs are accompanied by a mucus cap (Figure 1A), which, when flushed is not easily differentiated from HP. A further study also uncovered that inconspicuous borders and cloud-like surfaces are two independent diagnostic features of colorectal SSL in white light endoscopy[24-26]. Meanwhile, colorectal SSL-Ds are often associated with pedicled, bimodal appearance, central depression, and reddish color (Figure 1B), which can differentiate SSLs from SSL-Ds, with one of such features having a sensitivity of 97.7% and a specificity of 85.3% for the diagnosis of SSL-Ds[27].

Colorectal SSL characteristics under chromoendoscopy

Both HPs and SSLs are generally challenging and complex to identify when small (< 5 mm). To address this issue, the chromoendoscopy technique is adopted.

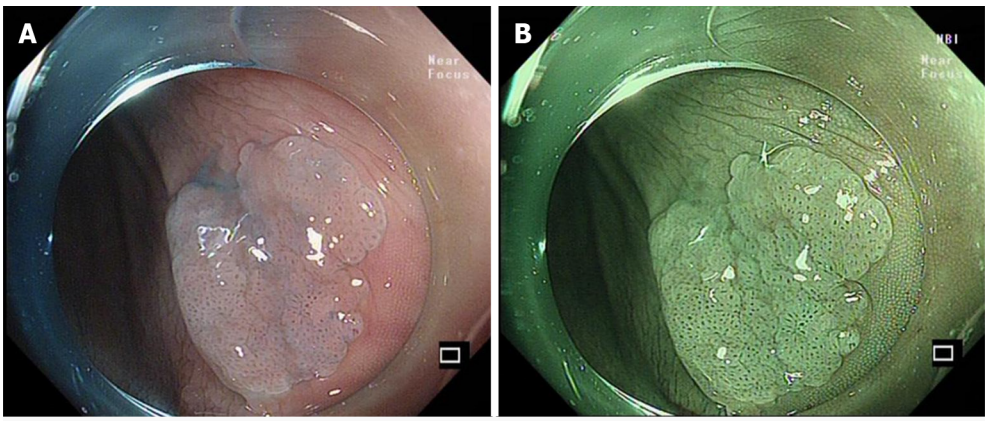
During endoscopy, chemical dyes (indigo carmine, crystalline violet, acetic acid, among several others) spray on the surface of the lesions, so the particles of the stains are deposited within the folds of the colorectal SSL lesion and surrounding mucosa. Then, the outlining of the lesion border and surface microstructure facilitates the assessment of SSL size and character.

It is important to note that acetic acid spray plays an important role in showing the borders and diameter of colorectal SSLs (Figure 2A). The surface morphology of the SSL is clearer and more easily



DOI: 10.5306/wjco.v14.i4.171 **Copyright** ©The Author(s) 2023.

Figure 1 White light endoscopic features of colorectal sessile serrated lesion cases. A: The sessile serrated lesions (SSLs) case with mucus cap under white light endoscopy; B: The borders of SSLs are not clearly distinguishable from the surrounding mucosa, and the morphology are cloud-like surface under white light. The above figure shows a case of SSL-D which has a reddish surface and a central depression.



DOI: 10.5306/wjco.v14.i4.171 **Copyright** ©The Author(s) 2023.

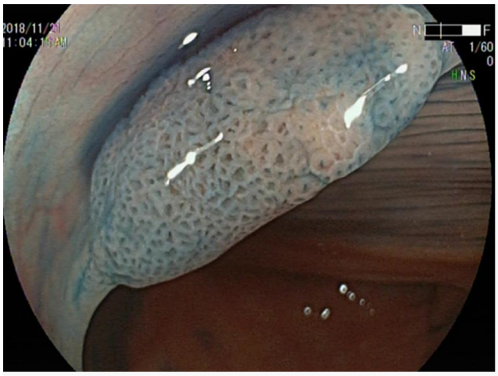
Figure 2 Endoscopic features of the colorectal sessile serrated lesion case after acetic acid spray. A: The border of the sessile serrated lesions (SSLs) is clearly revealed under acetic acid spray; B: The combined application of acetic acid spray and narrow band imaging can clearly show the borders and surface microstructure of SSLs, which is more conducive to endoscopic treatment.

described after acetic acid spray, and its useful in better delineation of the recurrent colorectal SSL[28-30]. In addition, it has been demonstrated that acetic acid spray can help endoscopists perform cold resection of colorectal SSLs more accurately[31] (Figure 2B).

One study classified the chromoendoscopy images of the surface glands of more than 300 SSLs and indicated that open Type II (Pit II-O) structures, compared with the conventional Pit II type glands opening pattern, were endoscopic characteristics in colorectal SSLs[24]. Moreover, the opening pattern of the Pit II-O gland is similar to that of Pit II, and the former is typically surrounded by the latter, but the former features an expanded and more rounded shape, reflecting the expansion of the SSL crypt (Figure 3).

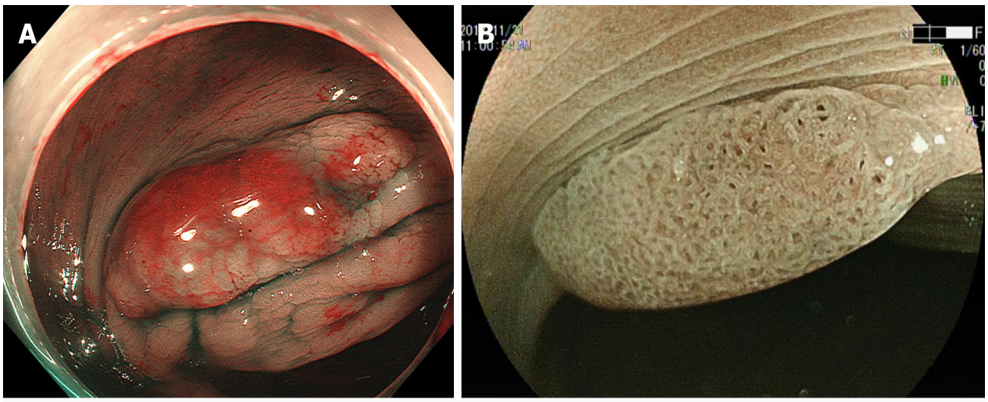
The image enhanced endoscopy (IEE) is the most common mode of electronic staining used in colonoscopy. Narrow band imaging (NBI) is a widely used IEE, which utilizes a filter to screen the broadband spectrum of the red, blue, and green light emitted by the light source, leaving only the narrowband spectrum for the diagnosis of various digestive disorders. Linked color imaging and blue laser imaging (BLI) are the next-generation IEEs, considering that their imaging principle is founded on light absorption and reflection by the mucosa of the digestive tract. Then, the lesions appear in a different color from the surrounding tissues, yielding a clear distinction between the superficial mucosal microvasculature and microstructure. It is also worth noting that the IEE has a brighter and higher resolution and is known as the "electron chromatography" technique given that the image observed by the IEE resembles a dye-stained image.

Furthermore, the NBI pattern enhances the visibility of colorectal SSLs with a mucus cap and gives it a concentrated red color that contrasts more prominently with the background mucosa[32] (Figure 4A). Also, both the NBI and BLI generally feature small black spots within the glandular opening of SSLs (Figure 4B), which is a critical histological feature within dependent diagnostic value that aids the endoscopist to differentiate SSLs from HPs during colonoscopy[25,33]. It has been confirmed that



DOI: 10.5306/wjco.v14.i4.171 **Copyright** ©The Author(s) 2023.

Figure 3 Endoscopic features of the colorectal sessile serrated lesion case after indigo carmine spray. The opening pattern of the Pit II-O gland, features an expanded and more rounded shape, dilation of the colorectal sessile serrated lesion surface crypt.



DOI: 10.5306/wjco.v14.i4.171 **Copyright** ©The Author(s) 2023.

Figure 4 Endoscopic features of colorectal sessile serrated lesion cases under narrow band imaging mode. A: The mucus cap of sessile serrated lesions (SSLs) shows a brick-red appearance under narrow band imaging (NBI); B: The expansion of the surface crypt in the SSLs shows a black spot under NBI.

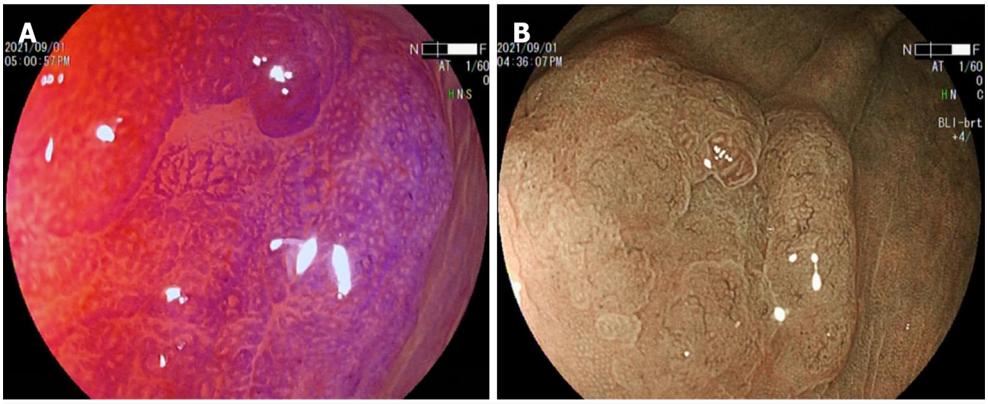
dilated and branching vessels in NBI endoscopy differs from the vascular surrounding superficial mucosal glands, and irregular capillaries may be observed at sites of colorectal SSL that show dysplasia [3,34,35].

Additional research showed that the multivariate analysis of the location (proximal colon), size (≥ 10 mm), glandular opening, and microvascular morphology of the serrated lesions exhibited more than 90% positive diagnosis of the SSL, which was 2.3 times more advantageous than its single factor diagnosis[36,37].

Colorectal SSL characteristics under magnified endoscopy

In identifying neoplastic and non-neoplastic lesions, the value of magnified endoscopy combined with chromoscopy has been extensively evident. Close observation of the surface pattern of lesions with a specific combination can effectively predict its pathological characteristics and even the depth of invasion. Relevant literature has demonstrated that Type II-O glands can be used as an indicator to differentiate between SSLs and HPs. The Pit II-O glands also suggest histological variation in the morphology of colorectal SSL glands, significantly boosting the accuracy of diagnosing SSLs[38]. For large colorectal SSLs, magnified endoscopic findings of not only Type II-O glands but also those possibly mixed with Types IIIL, IV, Vi, and Vn glands at the same time often prompt SSL-Ds or cancers [24,27] (Figure 5A).

Magnified endoscopy combined with IEE can further develop the visualization of microvessels (Figure 5B). The varicose microvessels, running through the deep layer of mucosa, on the lesion surface of colorectal SSL, differ from those around the mucosal glands[37]. A similar study in China pointed out a statistical difference between magnified endoscopy and chromic endoscopy for varicose microvessels in predicting colorectal SSLs and HPs[35].



DOI: 10.5306/wjco.v14.i4.171 **Copyright** ©The Author(s) 2023.

Figure 5 Endoscopic features of the colorectal sessile serrated lesion case under chromoscopy combined with magnified endoscopy. A: Crystalline violet spray makes the surface glandular structure of colorectal sessile serrated lesions (SSLs) more visible, and combined with magnified endoscopic observation is useful for inferring the pathological characteristics of the lesion; B: Blue light imaging combined with magnified endoscopic observation of the microstructure of the SSL surface revealed that the SSL-D case have a Pit III and/or Pit IV type of glandular duct opening pattern based on Pit II-O, and varicose microvessels on the surface of the lesion are found.

CONCLUSION

Colorectal SSL is potentially malignant and has a higher risk of malignancy than conventional tubular adenomas, thereby making an immediate diagnosis or early detection in colonoscopic screening especially important. However, the current diagnosis of SSLs in screening colonoscopy is undeniably insufficiently high and often depends on the histopathological diagnosis post-biopsy or resection. With advances in endoscopy equipment and imaging techniques, we have witnessed the role of cytoendoscopy in diagnosing gastrointestinal tract tumors[39]. In the future, we hope to discover a more objective and accurate factor in order to characterize the endoscopic presentation of colorectal SSLs, which can swiftly and efficiently identify lesions, reduce missed or delayed diagnoses, and effectively decrease the incidence of interval CRCs.

For colorectal SSLs, good bowel preparation is the foundation, and the endoscopist's knowledge and experience play an essential role. Ultimately, combining all the predictive factors in colonoscopy screening to generate an immediate diagnosis can improve the detection rate.

FOOTNOTES

Author contributions: Wang RG reviewed the literature, wrote and revised the review; Wei L guided the literature search and review writing; Jiang B guided the topic selection, reviewed and suggested revisions.

Conflict-of-interest statement: All authors declare that there is no conflict-of-interest.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Rui-Gang Wang 0000-0002-9053-5329; Lai Wei 0000-0003-2326-1257; Bo Jiang 0000-0002-7768-7600.

S-Editor: Ma YJ
L-Editor: A
P-Editor: Li X

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021;

71: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]
2 **Sanduleanu S**, le Clercq CM, Dekker E, Meijer GA, Rabeneck L, Rutter MD, Valori R, Young GP, Schoen RE; Expert Working Group on 'Right-sided lesions and interval cancers', Colorectal Cancer Screening Committee, World Endoscopy Organization. Definition and taxonomy of interval colorectal cancers: a proposal for standardising nomenclature. *Gut* 2015; **64**: 1257-1267 [PMID: 25193802 DOI: 10.1136/gutjnl-2014-307992]
3 **Murakami T**, Sakamoto N, Fukushima H, Shibuya T, Yao T, Nagahara A. Usefulness of the Japan narrow-band imaging expert team classification system for the diagnosis of sessile serrated lesion with dysplasia/carcinoma. *Surg Endosc* 2021; **35**: 4528-4538 [PMID: 32909209 DOI: 10.1007/s00464-020-07967-w]
4 **Burr NE**, Derbyshire E, Taylor J, Whalley S, Subramanian V, Finan PJ, Rutter MD, Valori R, Morris EJA. Variation in post-colonoscopy colorectal cancer across colonoscopy providers in English National Health Service: population based cohort study. *BMJ* 2019; **367**: l6090 [PMID: 31722875 DOI: 10.1136/bmj.l6090]
5 **Dekker E**, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet* 2019; **394**: 1467-1480 [PMID: 31631858 DOI: 10.1016/S0140-6736(19)32319-0]
6 **East JE**, Atkin WS, Bateman AC, Clark SK, Dolwani S, Ket SN, Leedham SJ, Phull PS, Rutter MD, Shepherd NA, Tomlinson I, Rees CJ. British Society of Gastroenterology position statement on serrated polyps in the colon and rectum. *Gut* 2017; **66**: 1181-1196 [PMID: 28450390 DOI: 10.1136/gutjnl-2017-314005]
7 **Ahadi M**, Sokolova A, Brown I, Chou A, Gill AJ. The 2019 World Health Organization Classification of appendiceal, colorectal and anal canal tumours: an update and critical assessment. *Pathology* 2021; **53**: 454-461 [PMID: 33461799 DOI: 10.1016/j.pathol.2020.10.010]
8 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020; **76**: 182-188 [PMID: 31433515 DOI: 10.1111/his.13975]
9 **Li J**, Teng XD, Lai MD. [Colorectal non-invasive epithelial lesions: an update on the pathology of colorectal serrated lesions and polyps]. *Zhonghua Bing Li Xue Za Zhi* 2020; **49**: 1339-1344 [PMID: 33287530 DOI: 10.3760/cma.j.cn112151-20200330-00272]
10 **Ng SC**, Ching JY, Chan VC, Wong MC, Tang R, Wong S, Luk AK, Lam TY, Gao Q, Chan AW, Wu JC, Chan FK, Lau JY, Sung JJ. Association between serrated polyps and the risk of synchronous advanced colorectal neoplasia in average-risk individuals. *Aliment Pharmacol Ther* 2015; **41**: 108-115 [PMID: 25339583 DOI: 10.1111/apt.13003]
11 **Crockett SD**, Nagtegaal ID. Terminology, Molecular Features, Epidemiology, and Management of Serrated Colorectal Neoplasia. *Gastroenterology* 2019; **157**: 949-966.e4 [PMID: 31323292 DOI: 10.1053/j.gastro.2019.06.041]
12 **Song YR**, Song SZ, Gong AX. [Advancement in the study of carcinogenesis mechanism and endoscopic diagnosis of colorectal serrated lesions]. *Chinese Journal of Digestive Endoscopy* 2021; 38(5): 412-415 [DOI: 10.3760/cma.j.cn321463-20200420-00093]
13 **Jung YS**, Park JH, Park CH. Serrated Polyps and the Risk of Metachronous Colorectal Advanced Neoplasia: A Systematic Review and Meta-Analysis. *Clin Gastroenterol Hepatol* 2022; **20**: 31-43.e1 [PMID: 33007512 DOI: 10.1016/j.cgh.2020.09.051]
14 **Gao Q**, Tsoi KK, Hirai HW, Wong MC, Chan FK, Wu JC, Lau JY, Sung JJ, Ng SC. Serrated polyps and the risk of synchronous colorectal advanced neoplasia: a systematic review and meta-analysis. *Am J Gastroenterol* 2015; **110**: 501-9; quiz 510 [PMID: 25756237 DOI: 10.1038/ajg.2015.49]
15 **Gupta S**, Lieberman D, Anderson JC, Burke CA, Dominitz JA, Kaltenbach T, Robertson DJ, Shaukat A, Syngal S, Rex DK. Recommendations for Follow-Up After Colonoscopy and Polypectomy: A Consensus Update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 2020; **158**: 1131-1153.e5 [PMID: 32044092 DOI: 10.1053/j.gastro.2019.10.026]
16 **Erichsen R**, Baron JA, Hamilton-Dutoit SJ, Snover DC, Torlakovic EE, Pedersen L, Frøslev T, Vyberg M, Hamilton SR, Sørensen HT. Increased Risk of Colorectal Cancer Development Among Patients With Serrated Polyps. *Gastroenterology* 2016; **150**: 895-902.e5 [PMID: 26677986 DOI: 10.1053/j.gastro.2015.11.046]
17 **IJspeert JE**, Vermeulen L, Meijer GA, Dekker E. Serrated neoplasia-role in colorectal carcinogenesis and clinical implications. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 401-409 [PMID: 25963511 DOI: 10.1038/nrgastro.2015.73]
18 **Deng JW**, Fang JW. [Pathway and clinical characteristics of serrated colorectal cancer]. *National Medical Journal of China* 2020; 100 (34): 2716-2720 [DOI: 10.3760/cma.j.cn112137-20191230-02854]
19 **Omori K**, Yoshida K, Tamiya S, Daa T, Kan M. Endoscopic Observation of the Growth Process of a Right-Side Sessile Serrated Adenoma/Polyp with Cytological Dysplasia to an Invasive Submucosal Adenocarcinoma. *Case Rep Gastrointest Med* 2016; **2016**: 6576351 [PMID: 27437153 DOI: 10.1155/2016/6576351]
20 **Kinoshita S**, Nishizawa T, Uraoka T. Progression to invasive cancer from sessile serrated adenoma/polyp. *Dig Endosc* 2018; **30**: 266 [PMID: 29168233 DOI: 10.1111/den.12988]
21 **Amemori S**, Yamano HO, Tanaka Y, Yoshikawa K, Matsushita HO, Takagi R, Harada E, Yoshida Y, Tsuda K, Kato B, Tamura E, Eizuka M, Sugai T, Adachi Y, Yamamoto E, Suzuki H, Nakase H. Sessile serrated adenoma/polyp showed rapid malignant transformation in the final 13 months. *Dig Endosc* 2020; **32**: 979-983 [PMID: 31677187 DOI: 10.1111/den.13572]
22 **Yu-hu L**, Lei-lei Z, Gui-quan C, Dong P, Jin C, Shi-hao, Z. The analysis of detection rate and risk factors of colorectal sessile serrated adenoma. *Journal of Tropical Medicine* 2019; 19 (1): 65-67 [DOI: 10.3969/j.issn.1672-3619.2019.01.016]
23 **Li QY**, Xiao P, Ling TS, Sun YY, Luo LJ, Liang R, Deng ZJ, Situ WJ. [Detection of proximal serrated polyps:a single-center retrospective analysis]. *Chinese Journal of Digestive Endoscopy* 2019; **36** (2): 86-90 [DOI: 10.3760/cma.j.issn.1007-5232.2019.02.002]
24 **Murakami T**, Sakamoto N, Ritsuno H, Shibuya T, Osada T, Mitomi H, Yao T, Watanabe S. Distinct endoscopic characteristics of sessile serrated adenoma/polyp with and without dysplasia/carcinoma. *Gastrointest Endosc* 2017; **85**: 590-600 [PMID: 27663716 DOI: 10.1016/j.gie.2016.09.018]
25 **Hazewinkel Y**, López-Cerón M, East JE, Rastogi A, Pellisé M, Nakajima T, van Eeden S, Tytgat KM, Fockens P, Dekker E. Endoscopic features of sessile serrated adenomas: validation by international experts using high-resolution white-light

endoscopy and narrow-band imaging. *Gastrointest Endosc* 2013; **77**: 916-924 [PMID: 23433877 DOI: 10.1016/j.gie.2012.12.018]

26 **Cassese G**, Amendola A, Maione F, Giglio MC, Pagano G, Milone M, Aprea G, Luglio G, De Palma GD. Serrated Lesions of the Colon-Rectum: A Focus on New Diagnostic Tools and Current Management. *Gastroenterol Res Pract* 2019; **2019**: 9179718 [PMID: 30774654 DOI: 10.1155/2019/9179718]

27 **Murakami T**, Sakamoto N, Nagahara A. Endoscopic diagnosis of sessile serrated adenoma/polyp with and without dysplasia/carcinoma. *World J Gastroenterol* 2018; **24**: 3250-3259 [PMID: 30090005 DOI: 10.3748/wjg.v24.i29.3250]

28 **Yamamoto S**, Varkey J, Hedenström P. Acetic acid spray for better delineation of recurrent sessile serrated adenoma in the colon. *VideoGIE* 2019; **4**: 547-548 [PMID: 31840127 DOI: 10.1016/j.vgie.2019.08.001]

29 **Tribonias G**, Theodoropoulou A, Stylianou K, Giotis I, Mpitouli A, Moschovis D, Komeda Y, Manola ME, Paspatis G, Tzouvala M. Irrigating Acetic Acid Solution During Colonoscopy for the Detection of Sessile Serrated Neoplasia: A Randomized Controlled Trial. *Dig Dis Sci* 2022; **67**: 282-292 [PMID: 33515378 DOI: 10.1007/s10620-021-06858-x]

30 **Kono Y**, Higashi R, Mizushima H, Shimizu D, Katayama T, Kosaka M, Hirata I, Hirata T, Gotoda T, Miyahara K, Moritou Y, Kunihiro M, Nakagawa M, Ichimura K, Okada H. Usefulness of Acetic Acid Spray with Narrow-Band Imaging for Identifying the Margin of Sessile Serrated Lesions. *Dig Dis Sci* 2023 [PMID: 36609732 DOI: 10.1007/s10620-022-07816-x]

31 **Suzuki Y**, Ohata K, Matsuhashi N. Delineating sessile serrated adenomas/polyps with acetic acid spray for a more accurate piecemeal cold snare polypectomy. *VideoGIE* 2020; **5**: 519-521 [PMID: 33204905 DOI: 10.1016/j.vgie.2020.05.030]

32 **Tadepalli US**, Feihel D, Miller KM, Itzkowitz SH, Freedman JS, Kornacki S, Cohen LB, Bamji ND, Bodian CA, Aisenberg J. A morphologic analysis of sessile serrated polyps observed during routine colonoscopy (with video). *Gastrointest Endosc* 2011; **74**: 1360-1368 [PMID: 22018553 DOI: 10.1016/j.gie.2011.08.008]

33 **Yamashina T**, Takeuchi Y, Uedo N, Aoi K, Matsuura N, Nagai K, Matsui F, Ito T, Fujii M, Yamamoto S, Hanaoka N, Higashino K, Ishihara R, Tomita Y, Iishi H. Diagnostic features of sessile serrated adenoma/polyps on magnifying narrow band imaging: a prospective study of diagnostic accuracy. *J Gastroenterol Hepatol* 2015; **30**: 117-123 [PMID: 25088839 DOI: 10.1111/jgh.12688]

34 **Saito S**, Tajiri H, Ikegami M. Serrated polyps of the colon and rectum: Endoscopic features including image enhanced endoscopy. *World J Gastrointest Endosc* 2015; **7**: 860-871 [PMID: 26240687 DOI: 10.4253/wjge.v7.i9.860]

35 **Wang Y**, Ren YB, Yang XS, Huang YH, Zhang L, Li X, Bai P, Wang L, Fan X, Ding YM, Li HL, Lin XC. [Comparison of endoscopic features between colorectal sessile serrated adenoma/polyp with or without cytologic dysplasia and hyperplastic polyp]. *Zhonghua Yi Xue Za Zhi* 2019; **99**: 2214-2220 [PMID: 31434395 DOI: 10.3760/cma.j.issn.0376-2491.2019.28.012]

36 **Yamada M**, Sakamoto T, Otake Y, Nakajima T, Kuchiba A, Taniguchi H, Sekine S, Kushima R, Ramberan H, Parra-Blanco A, Fujii T, Matsuda T, Saito Y. Investigating endoscopic features of sessile serrated adenomas/polyps by using narrow-band imaging with optical magnification. *Gastrointest Endosc* 2015; **82**: 108-117 [PMID: 25840928 DOI: 10.1016/j.gie.2014.12.037]

37 **Uraoka T**, Higashi R, Horii J, Harada K, Hori K, Okada H, Mizuno M, Tomoda J, Ohara N, Tanaka T, Chiu HM, Yahagi N, Yamamoto K. Prospective evaluation of endoscopic criteria characteristic of sessile serrated adenomas/polyps. *J Gastroenterol* 2015; **50**: 555-563 [PMID: 25270966 DOI: 10.1007/s00535-014-0999-y]

38 **Kimura T**, Yamamoto E, Yamano HO, Suzuki H, Kamimae S, Nojima M, Sawada T, Ashida M, Yoshikawa K, Takagi R, Kato R, Harada T, Suzuki R, Maruyama R, Kai M, Imai K, Shinomura Y, Sugai T, Toyota M. A novel pit pattern identifies the precursor of colorectal cancer derived from sessile serrated adenoma. *Am J Gastroenterol* 2012; **107**: 460-469 [PMID: 22233696 DOI: 10.1038/ajg.2011.457]

39 **Takamaru H**, Wu SYS, Saito Y. Endocytoscopy: technology and clinical application in the lower GI tract. *Transl Gastroenterol Hepatol* 2020; **5**: 40 [PMID: 32632391 DOI: 10.21037/tgh.2019.12.04]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 April 24; 14(4): 179-189

DOI: 10.5306/wjco.v14.i4.179

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Interaction between age and gender on survival outcomes in extramedullary multiple myeloma over the past two decades

Ayrton I Bangolo, Pierre Fwelo, Chinmay Trivedi, Sowmya Sagireddy, Hamed Aljanaahi, Auda Auda, Maryama Mohamed, Sonia Onyeka, Miriam Fisher, Jyoti Thapa, Erwin J Tabucanon, Lyuben Georgiev, Annetta Wishart, Shilpee Kumari, Conrad Erikson, Mary Bangura, Orent Paddy, Rashmi Madhukar, Eugenio L Gomez, Joshua Rathod, Mansi Naria, Basel Hajal, Mohammad Awadhalla, David Siegel, Harsh Parmar, Noa Biran, David H Vesole, Pooja Phull, Simcha Weissman

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: van Hest N, United Kingdom; Solimando AG, Italy

Received: December 17, 2022
Peer-review started: December 17, 2022
First decision: February 20, 2023
Revised: February 20, 2023
Accepted: March 17, 2023
Article in press: March 17, 2023
Published online: April 24, 2023

Ayrton I Bangolo, Chinmay Trivedi, Sowmya Sagireddy, Hamed Aljanaahi, Auda Auda, Maryama Mohamed, Sonia Onyeka, Miriam Fisher, Jyoti Thapa, Erwin J Tabucanon, Lyuben Georgiev, Annetta Wishart, Shilpee Kumari, Conrad Erikson, Mary Bangura, Orent Paddy, Rashmi Madhukar, Eugenio L Gomez, Joshua Rathod, Mansi Naria, Basel Hajal, Mohammad Awadhalla, Simcha Weissman, Department of Medicine, Palisades Medical Center, North Bergen, NJ 07047, United States

Pierre Fwelo, Department of Epidemiology, Human Genetics, and Environmental Sciences, UTHealth School of Public Health, Houston, TX 77204, United States

David Siegel, Harsh Parmar, Noa Biran, David H Vesole, Pooja Phull, Division of Myeloma, John Theurer Cancer Center, Hackensack, NJ 07601, United States

Corresponding author: Ayrton I Bangolo, MBBS, MD, Doctor, Department of Medicine, Palisades Medical Center, 7600 River Road, North Bergen, NJ 07047, United States. ayrtonbangolo@yahoo.com

Abstract

BACKGROUND

Extramedullary multiple myeloma (MM) (EMM) is a rare and aggressive sub-entity of MM that can be present at diagnosis or develop anytime during the disease course. There is a paucity of data on the clinical characteristics and overall epidemiology of EMM. Furthermore, there is a scarcity of data on how the interaction of age and gender influences the survival of EMM.

AIM

To evaluate the clinical characteristics of patients with EMM over the past 2 decades and to identify epidemiologic characteristics that may impact overall prognosis.

METHODS

A total of 858 patients diagnosed with EMM, between 2000 and 2017, were ultimately enrolled in our study by retrieving the Surveillance, Epidemiology, and

End Results database. We analyzed demographics, clinical characteristics, and overall mortality (OM) as well as cancer-specific mortality (CSM) of EMM. Variables with a *P* value < 0.1 in the univariate Cox regression were incorporated into the multivariate Cox model to determine the independent prognostic factors, with a hazard ratio (HR) of greater than 1 representing adverse prognostic factors.

RESULTS

From a sample of 858 EMM, the male gender (63.25%), age range 60-79 years (51.05%), and non-Hispanic whites (66.78%) were the most represented. Central Nervous System and the vertebral column was the most affected site (33.10%). Crude analysis revealed higher OM in the age group 80+ [HR = 6.951, 95% confidence interval (95%CI): 3.299-14.647, *P* = 0], Non-Hispanic Black population (HR = 1.339, 95%CI: 1.02-1.759, *P* = 0.036), Bones not otherwise specified (NOS) (HR = 1.74, 95%CI: 1.043-2.902, *P* = 0.034), and widowed individuals (HR = 2.107, 95%CI: 1.511-2.938, *P* = 0). Skin involvement (HR = 0.241, 95%CI: 0.06-0.974, *P* = 0.046) and a yearly income of $75000+ (HR = 0.259, 95%CI: 0.125-0.538, *P* = 0) had the lowest OM in the crude analysis. Crude analysis revealed higher CSM in the age group 80+, Non-Hispanic Black, Bones NOS, and widowed. Multivariate cox proportional hazard regression analyses only revealed higher OM in the age group 80+ (HR = 9.792, 95%CI: 4.403-21.774, *P* = 0) and widowed individuals (HR = 1.609, 95%CI: 1.101-2.35, *P* = 0.014). Multivariate cox proportional hazard regression analyses of CSM also revealed higher mortality of the same groups. Eyes, mouth, and ENT involvement had the lowest CSM in the multivariate analysis. There was no interaction between age and gender in the adjusted analysis for OM and CSM.

CONCLUSION

EMM is a rare entity. To our knowledge, there is a scarcity of data on the clinical characteristics and prognosis factors of patients with extramedullary multiple myeloma. In this retrospective cohort, using a United States-based population, we found that age, marital status, and tumor site were independent prognostic factors. Furthermore, we found that age and gender did not interact to influence the mortality of patients with EMM.

Key Words: Multiple myeloma; Age; Gender; Mortality; Plasmacytoma

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Very little is known about extramedullary multiple myeloma (EMM), owing to its rarity and scarcity of data on the subject. So far it was found that advanced age was the single most important prognostic value for poor outcome in EMM. However, how age interacts with gender to affect mortality in EMM remains unknown. We found that age did not interact with gender to affect mortality in EMM.

Citation: Bangolo AI, Fwelo P, Trivedi C, Sagireddy S, Aljanaahi H, Auda A, Mohamed M, Onyeka S, Fisher M, Thapa J, Tabucanon EJ, Georgiev L, Wishart A, Kumari S, Erikson C, Bangura M, Paddy O, Madhukar R, Gomez EL, Rathod J, Naria M, Hajal B, Awadhalla M, Siegel D, Parmar H, Biran N, Vesole DH, Phull P, Weissman S. Interaction between age and gender on survival outcomes in extramedullary multiple myeloma over the past two decades. *World J Clin Oncol* 2023; 14(4): 179-189

URL: https://www.wjgnet.com/2218-4333/full/v14/i4/179.htm

DOI: https://dx.doi.org/10.5306/wjco.v14.i4.179

INTRODUCTION

Multiple myeloma (MM) is a rare cancer with the hallmark of monoclonal plasma cell proliferation in the bone marrow[1]. MM accounts for approximately 1%-2% of all cancers. A subclone can thrive and grow independent of the bone marrow microenvironment resulting in extramedullary MM (EMM) which is an aggressive subentity of MM[2]. Affecting up to 30% of patients with MM, EMM can be present either at diagnosis or anytime during the disease process[1,2].

EMM is frequently associated with high-risk cytogenetics. As evidenced by a pilot study, which revealed an association of chromosome 1 abnormalities in bone marrow myeloma cells with extramedullary progression. Optical mapping showed the potential for refining the complex genomic architecture in MM and its phenotypes[3]. Only few studies in the literature have addressed the clinical characteristics of patients with EMM[4-8]. Age at the diagnosis of MM and the site of extramedullary

disease have been shown to be independent prognostic factors[4,9]. Furthermore, there is some data associating the male gender with MM[5]. However, to the best of our knowledge, there is a lack of studies addressing the interaction between age and gender in EMM, which makes our study the first of its kind.

To fill in the gaps in the literature, we conducted a retrospective cohort study amongst patients with EMM using the Surveillance, Epidemiology, and End Results (SEER) database, to evaluate the interaction of age and gender in regard to mortality of EMM as well as independent prognostic factors of patients with EMM over the past 2 decades.

MATERIALS AND METHODS

Study design

A population-based retrospective cohort study of patients with EMM was conducted using the SEER research. In addition, 18 registries in the November 2020 submission database were also utilized (http://www.seer.cancer.gov). The SEER Program is one of the largest and most authoritative sources of the cancer-related dataset in the United States, which is sponsored by the United States National Cancer Institute. The SEER 18 database collects cancer incidence, patients' clinicopathological features, and survival data from 18 population-based cancer registries and covers nearly 28% of the United States population[9]. This dataset is de-identified and publicly available, thus, the study is exempt from an Institutional Review Board's review. A detailed description of the database and data collection can be found elsewhere[10].

Patient selection

Inclusion criteria: All patients with EMM diagnosed from 2000 to 2017 were identified following criteria from previous studies[11]. We used site and morphology ICD-O-3 histology/behavior, malignant variables codes 9731/3 (*i.e.*, solitary plasmacytoma of bone) and 9734/3 (*i.e.*, extraosseous plasmacytoma) to identify patients with EMM. We also restricted our cohort to patients with 2 tumors and diagnostic confirmation through positive histology, immunotherapy, or genetic studies. Thus, increasing the accuracy of our findings and eliminating possible false-positive diagnoses.

Exclusion criteria: We excluded patients with unknown age at diagnosis, tumor stage, tumor site, or race. Lastly, we excluded patients diagnosed through autopsy.

Study variables

Main exposures: Gender (male and female), age (0-39, 40-59, 60-79, and 80+), and their interaction were the main exposures of interest.

Sociodemographic and tumor characteristics: Gender, year of diagnosis, extramedullary site of the tumor, location, annual salary, Civil status, year of diagnosis, surgical resection, as well as chemotherapy, were assessed for the purpose of the study.

Statistical analysis

We performed a crude and adjusted Cox proportional hazard regression to investigate the impact of the interaction between age and gender on EMM mortality. Variables with a value < 0.1 in the univariate Cox regression model were incorporated into the multivariate Cox proportional analysis to determine the independent prognostic factors associated with overall mortality (OM) and cancer-specific mortality (CSM), with a hazard ratio (HR) > 1 representing adverse prognostic factors. All tests were two-sided, with a confidence interval set as 95% and P value < 0.05 deemed statistically significant. All statistical tests were performed by using Software STATA16.1.

RESULTS

We enrolled 858 patients with EMM in our study. The baseline characteristics of our study are summarized in Table 1. The male gender (63.25%), age range 60-79 at diagnosis (51.05%), Non-Hispanic Whites (66.78 %), and married patients (66.32%) were the most represented groups. The Central Nervous System and vertebral column were the most affected location (33.10%). Most patients were living in metropolitan areas with a population of at least 1 million people (56.06%). Most patients did not receive chemotherapy (81.47%).

A crude analysis of factors associated with all-cause mortality and EMM-related mortality among United States patients between 2000 and 2017 is demonstrated in Table 2. Crude analysis revealed higher OM in the age group 80+ [HR = 6.951, 95% confidence interval (95%CI): 3.299-14.647, P = 0], Non-Hispanic Black population (HR = 1.339, 95%CI: 1.02-1.759, P = 0.036), other bones (HR = 1.74,

Table 1 Demographic and Clinicopathologic characteristics of United States patients with extramedullary multiple myeloma between 2000 and 2017

Characteristics	*n*	%
Total	858	100
Gender		
Female	311	36.25
Male	547	63.25
Age at diagnosis, yr		
0-39	41	4.78
40-59	309	36.01
60-79	438	51.05
80+	70	8.16
Race		
Non-Hispanic white	573	66.78
Non-Hispanic black	133	15.50
Hispanic	110	12.82
Other	42	4.90
Extramedullary site		
CNS and vertebral column	284	33.10
Bones, subcutaneous tissues, connective tissues, and soft tissues of the trunk	108	12.59
Bones, soft tissues, subcutaneous tissues, and connective tissues of the pelvis and sacrum	97	11.31
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the upper extremities	64	7.46
Bones, soft tissues, subcutaneous tissues, and connective tissues of the lower extremities	45	5.24
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the face and skull	60	6.99
Other bones, NOS	37	4.31
Eyes, mouth, and ENT	101	11.77
Lung, breast, and mediastinum	26	3.03
Gastrointestinal tract	18	2.10
Skin	12	1.40
Kidney, suprarenal glands, and retroperitoneum	6	0.70
Living area		
Counties in metropolitan areas of 1 million persons	481	56.06
Counties in metropolitan areas of 250000 to 1 million persons	173	20.16
Counties in metropolitan areas of 250000 persons	76	8.86
Nonmetropolitan counties adjacent to a metropolitan area	77	8.97
Nonmetropolitan counties not adjacent to a metropolitan area	51	5.94
Income per year		
< $35000	12	1.40
$35000-44999	74	8.62
$45000-54999	154	17.95
$55000-64999	232	27.04
$65000-74999	183	21.33
$75000+	203	23.66

Marital Status		
Married	569	66.32
Single	106	12.35
Divorced/separated	79	9.21
Widowed	62	7.23
Unknown	42	4.90

CNS: Central Nervous System; NOS: Not otherwise specified.

95%CI: 1.043-2.902, P = 0.034), and widowed individuals (HR = 2.107, 95%CI: 1.511-2.938, P = 0). Skin involvement (HR = 0.241, 95%CI: 0.06-0.974, P = 0.046) and a yearly income of $75000+ (HR = 0.259, 95%CI: 0.125-0.538, P = 0) had the lowest OM in the crude analysis. Crude analysis revealed higher CSM in age group 80+ (HR = 10.111, 95%CI: 3.083-33.159, P = 0), Non-Hispanic Black (HR = 1.446, 95%CI: 1.017-2.055, P = 0.04), other bones (HR = 1.887, 95%CI: 1.044-3.411, P = 0.035) and widowed individuals (HR = 2.463, 95%CI: 1.612-3.765, P = 0).

Multivariate cox proportional hazard regression analyses of factors affecting all-cause mortality and EMM-related mortality among United States patients between 2000 and 2017 are demonstrated in Table 3. Multivariate cox proportional hazard regression analyses only revealed higher OM in the age group 80+ (HR = 9.792, 95%CI: 4.403-21.774, P = 0) and widowed individuals (HR = 1.609, 95%CI: 1.101-2.35, P = 0.014). Multivariate cox proportional hazard regression analyses of CSM showed similar findings revealing higher mortality in the age group 80+ (HR = 13.672, 95%CI: 3.915-47.746, P = 0) and widowed individuals (HR = 2.085, 95%CI: 1.275-3.409, P = 0.003). Involvement of eyes, mouth and ENT sites (HR = 0.425, 95%CI: 0.235-0.768, P = 0.005) had the lowest CSM in the multivariate analysis. Importantly, the study also revealed that the interaction between age and gender was not a statistically significant predictor of mortality in patients with EMM as shown in Table 4.

DISCUSSION

In this large SEER data-based retrospective cohort study, we demonstrated that EMM was associated with a higher OM and CSM in patients greater than 80 years of age and those patients who had been widowed. However, interestingly, the interaction between age and gender was not found to be statistically significant in predicting mortality in EMM patients.

EMM is a highly aggressive entity of MM, with clinical behavior distinct from marrow-restricted myeloma[12]. EMM is historically known to bear a worse prognosis compared to marrow-restricted myeloma[13]. Several studies have been carried out to investigate clinical characteristics and prognostic factors of EMM[4-8,12]. However, there is a paucity of data investigating the interaction of age and gender in regard to the mortality of EMM.

The interaction between gender and race and its influence on survival disparities in head and neck cancers has been well-documented[13]. Furthermore, gender was found to be the most important predictor with young and middle-aged females having the most favorable prognosis in non-smokers with oral squamous cell carcinoma[14]. However, no study has evaluated the impact of these interactions in the EMM population subgroup.

Our study did not reveal any interaction between age, gender, and race in regard to adjusted mortality in patients with EMM. Age was found to be the single most important prognostic factor for OM and CSM. Age was also found to be an important prognostic factor for the survival of EMM in a study by Li *et al*[5]. Gender and race were not of prognostic value in our cohort reaffirming the similar results found in the Li series[5].

Several retrospective studies have found marital status to be an independent prognostic factor in the survival of oncologic patients[15-19]. Patients that were married had better survival compared to their nonmarried counterparts[20-24]. This was also true in our study, where widowed patients had the highest OM and CSM, followed by single and divorced patients. This is perhaps due to the lack of psychological and emotional support as well as the increased incidence of depression and other mood disorders amongst these individuals, which could directly, or indirectly influence the treatment and regular oncology follow-up.

We hope that the results of this study will shed some light on the clinical presentation of this rare and aggressive manifestation of MM. In better understanding EMM, we hope to inspire larger prospective studies on the management of this subset of patients, which is particularly important in the era of novel agents including immunomodulatory agents, proteasome inhibitors, monoclonal antibodies, and, more recently, the advent of chimeric antigen receptor T-cell therapy and bispecific agents. This can be especially important with the new emergence of microRNAs that help prevent drug resistance when

Table 2 Crude analysis of factors associated with all-cause mortality and extramedullary multiple myeloma; related mortality among United States patients between 2000 and 2017

Characteristics	Overall mortality	EMD MM mortality
	Crude proportional-hazard ratio (95% confidence interval)	
Gender		
Female	1 (reference)	1 (reference)
Male	1.02 (0.826-1.259)	0.804 (0.611-1.056)
Age at diagnosis, yr		
0-39	1 (reference)	1 (reference)
40-59	1.683 (0.82-3.452)	2.409 (0.754-7.696)
60-79	3.271 (1.615-6.627)[c]	4.918 (1.565-15.461)[c]
80+	6.951 (3.299-14.647)[c]	10.111 (3.083-33.159)[c]
Race		
Non-Hispanic white	1 (reference)	1 (reference)
Non-Hispanic black	1.339 (1.02-1.759)[b]	1.446 (1.017-2.055)[b]
Hispanic	0.991 (0.719-1.365)	0.791 (0.495-1.263)
Other	1.016 (0.63-1.639)	1.209 (0.67-2.179)
Extramedullary site		
CNS and vertebral column	1 (reference)	1 (reference)
Bones, subcutaneous tissues, connective tissues, and soft tissues of the trunk	1.53 (1.113-2.102)[c]	1.187 (0.774-1.82)
Bones, soft tissues, subcutaneous tissues, and connective tissues of the pelvis and sacrum	1.027 (0.716-1.475)	1.15 (0.746-1.772)
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the upper extremities	1.036 (0.682-1.573)	0.718 (0.391-1.32)
Bones, soft tissues, subcutaneous tissues, and connective tissues of the lower extremities	1.668 (1.058-2.63)[b]	1.466 (0.814-2.642)
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the face and skull	1.135 (0.756-1.702)	0.919 (0.529-1.598)
Other bones, NOS	1.74 (1.043-2.902)[b]	1.887 (1.044-3.411)[b]
Eyes, mouth, and ENT	0.929 (0.668-1.293)	0.451 (0.259-0.783)[c]
Lung, breast, and mediastinum	1.588 (0.895-2.816)	1.278 (0.589-2.773)
Gastrointestinal tract	0.787 (0.367-1.688)	0.55 (0.174-1.745)
Skin	0.241 (0.06-0.974)[b]	0.195 (0.027-1.403)
Kidney, suprarenal glands, and retroperitoneum	1.861 (0.591-5.86)	0.907 (0.126-6.531)
Living area		
Counties in metropolitan areas of 1 million persons	1 (reference)	1 (reference)
Counties in metropolitan areas of 250000 to 1 million persons	1.021 (0.789-1.322)	0.803 (0.556-1.158)
Counties in metropolitan areas of 250000 persons	0.794 (0.538-1.17)	0.8 (0.482-1.327)
Nonmetropolitan counties adjacent to a metropolitan area	1.099 (0.775-1.559)	0.937 (0.578-1.518)
Nonmetropolitan counties not adjacent to a metropolitan area	1.191 (0.798-1.779)	1.105 (0.647-1.888)
Income per year		
< $35000	1 (reference)	1 (reference)
$35000-44999	0.457 (0.213-0.984)[b]	0.412 (0.167-1.014)[a]
$45000-54999	0.356 (0.17-0.745)[c]	0.33 (0.139-0.782)[b]
$55000-64999	0.358 (0.174-0.737)[c]	0.236 (0.101-0.554)[c]

$65000-74999	0.328 (0.158-0.681)[c]	0.292 (0.124-0.685)[c]
$75000+	0.259 (0.125-0.538)[c]	0.24 (0.102-0.563)[c]
Marital status		
Married	1 (reference)	1 (reference)
Single	1.305 (0.967-1.763)[a]	1.515 (1.027-2.236)[b]
Divorced/separated	1.531 (1.094-2.142)[b]	1.681 (1.084-2.605)[b]
Widowed	2.107 (1.511-2.938)[c]	2.463 (1.612-3.765)[c]

[a]$P < 0.1$.
[b]$P < 0.05$.
[c]$P < 0.01$.
EMD: Extramedullary disease; MM: Multiple myeloma; CNS: Central Nervous System; NOS: Not otherwise specified.

Table 3 Multivariate cox proportional hazard regression analyses of factors affecting all-cause mortality and extramedullary disease multiple myeloma related mortality among United States patients between 2000 and 2017

Characteristics	Overall mortality	EMD MM mortality
	Adjusted proportional hazard ratio (95% confidence interval)	
Gender		
Female	1 (reference)	1 (reference)
Male	1.256 (0.989-1.594)[a]	1.022 (0.748-1.397)
Age at diagnosis, yr		
0-39	1 (reference)	1 (reference)
40-59	2.206 (1.047-4.647)[b]	3.154 (0.957-10.395)[a]
60-79	4.129 (1.974-8.635)[c]	5.667 (1.738-18.48)[c]
80+	9.792 (4.403-21.774)[c]	13.672 (3.915-47.746)[c]
Race		
Non-Hispanic white	1 (reference)	1 (reference)
Non-Hispanic black	1.315 (0.96-1.802)[a]	1.34 (0.884-2.03)
Hispanic	1.034 (0.734-1.457)	0.833 (0.506-1.371)
Other	1.25 (0.743-2.104)	1.741 (0.916-3.308)[a]
Extramedullary site		
CNS and vertebral column	1 (reference)	1 (reference)
Bones, subcutaneous tissues, connective tissues, and soft tissues of the trunk	1.401 (0.996-1.972)[a]	1.046 (0.664-1.649)
Bones, soft tissues, subcutaneous tissues, and connective tissues of the pelvis and sacrum	0.98 (0.671-1.432)	1.091 (0.689-1.729)
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the upper extremities	1.024 (0.661-1.586)	0.672 (0.353-1.279)
Bones, soft tissues, subcutaneous tissues, and connective tissues of the lower extremities	1.488 (0.909-2.436)	1.382 (0.733-2.605)
Bones, soft tissues, subcutaneous tissues, connective tissues, and lymph nodes of the face and skull	0.99 (0.641-1.53)	0.76 (0.414-1.394)
Other bones, NOS	1.195 (0.694-2.058)	1.199 (0.629-2.284)
Eyes, mouth, and ENT	0.902 (0.631-1.29)	0.425 (0.235-0.768)[c]
Lung, breast, and mediastinum	1.187 (0.628-2.246)	0.959 (0.392-2.346)
Gastrointestinal tract	0.677 (0.303-1.512)	0.383 (0.114-1.283)
Skin	0.327 (0.08-1.34)	0.325 (0.044-2.394)

Kidney, suprarenal glands, and retroperitoneum	3.055 (0.881-10.601)[a]	0.865 (0.108-6.901)
Living area		
Counties in metropolitan areas of 1 million persons	1 (reference)	1 (reference)
Counties in metropolitan areas of 250000 to 1 million persons	0.957 (0.715-1.282)	0.778 (0.512-1.181)
Counties in metropolitan areas of 250000 persons	0.819 (0.523-1.282)	0.85 (0.47-1.539)
Nonmetropolitan counties adjacent to a metropolitan area	0.997 (0.653-1.522)	0.836 (0.461-1.516)
Nonmetropolitan counties not adjacent to a metropolitan area	0.877 (0.533-1.442)	0.757 (0.379-1.512)
Income per year		
< $35000	1 (reference)	1 (reference)
$35000-44999	0.48 (0.21-1.095)[a]	0.381 (0.14-1.036)[a]
$45000-54999	0.452 (0.196-1.04)[a]	0.375 (0.135-1.044)[a]
$55000-64,999	0.446 (0.192-1.036)[a]	0.275 (0.096-0.788)[b]
$65000-74999	0.413 (0.173-0.983)[b]	0.381 (0.129-1.121)[a]
$75000+	0.324 (0.134-0.783)[b]	0.29 (0.095-0.878)[b]
Marital status		
Married	1 (reference)	1 (reference)
Single	1.5 (1.079-2.086)[b]	1.668 (1.089-2.556)[b]
Divorced/separated	1.49 (1.037-2.139)[b]	1.463 (0.908-2.355)
Widowed	1.609 (1.101-2.35)[b]	2.085 (1.275-3.409)[c]

[a]$P < 0.1$.
[b]$P < 0.05$.
[c]$P < 0.01$.
EMD: Extramedullary disease; MM: Multiple myeloma; CNS: Central Nervous System; NOS: Not otherwise specified.

Table 4 Joint test analysis of the predictors of extramedullary multiple myeloma and overall mortality among United States extramedullary multiple myeloma patients, 2000-2017

Variables	MM mortality			Overall mortality	
	DF	χ^2	*P* value	χ^2	*P* value
Race/ethnicity	3	5.7436	0.1248	3.2403	0.3560
Age at diagnosis	3	21.2193	< 0.0001	49.2869	< 0.0001
Gender	1	0.5044	0.4776	0.5168	0.4722
Extramedullary Site	11	16.6070	0.1200	15.6578	0.1543
Living area	4	2.1023	0.7169	0.8175	0.9361
Income	5	7.3539	0.1956	7.4157	0.1915
Marital status	3	10.8183	0.0128	11.7967	0.0081
chemotherapy	1	2.3104	0.1285	1.4536	0.2280
Year of diagnosis	17	25.3142	0.0879	16.1848	0.5108
Interaction between age and gender	3	2.1285	0.5462	1.0296	0.7941

DF: Degree of freedom; MM: Multiple myeloma.

combined with anti-MM drug regimens and improve the patient's management[25].

Our study has several strengths. Firstly, the database used is the largest cancer database in the United States. The sample size of the study is non-negligible. Also, owing to the stringent inclusion criteria and the fact that we used patients with only confirmed EMM for our diagnosis, we eliminated false positive results which increase the accuracy of our study findings. However, a few limitations should be

considered in our study. Information could not be obtained on radiotherapy and Hematopoietic Stem Cell Transplant. The information on chemotherapy was unfulfilled. Furthermore, the SEER database publicly available lacks information on comorbidities, which could lead to missing data on potential confounders owing to the retrospective nature of the study.

CONCLUSION

EMM is a rare entity of MM that can be present at diagnosis or develop during the disease course. In this large retrospective SEER database-based study, we found that age and gender do not interact to influence the mortality of patients with EMM. Age was the single most important prognostic factor. We hope that the results of this study will shed light on this important non-significant interaction between age and gender in regard to mortality amongst EMM patients and perhaps inspire larger prospective studies on this subject.

ARTICLE HIGHLIGHTS

Research background

Age has been established as the single most important prognostic factor of extramedullary multiple myeloma (EMM). However, the interaction between age and gender in the mortality of EMM has yet to be studied.

Research motivation

The main motivation of this study was to identify independent predictors of outcomes, as well as how age and gender interact to affect mortality in EMM.

Research objectives

This study has the objective to establish the overall epidemiology of EMM, as well as the interaction between age and gender on mortality.

Research methods

This is a retrospective study involving 858 patients diagnosed with EMM, between 2000 and 2017 using the Surveillance, Epidemiology, and End Results database.

Research results

Patients older than 80 years and widowed had higher overall mortality (OM) and cancer-specific mortality (CSM). Eyes, mouth, and ENT involvement were protective factors regarding CSM. There was no interaction between age and gender in the adjusted analysis for OM and CSM.

Research conclusions

Although age is the single most important prognostic value of mortality in EMM, it does not interact with gender to affect mortality in patients with EMM.

Research perspectives

Future prospective studies are needed to better understand the impact of newer agents in the management of this aggressive subset of MM.

FOOTNOTES

Author contributions: Bangolo AI searched the literature, wrote, and revised the manuscript; Fwelo P extracted and analysed the data, revised, and edited the manuscript; Trivedi C, Sagireddy S, Aljanaahi H, Auda A, Mohamed M, Onyeka S, Fisher M, Thapa J, Tabucanon EJ, Georgiev L, Wishart A, Kumari S, Erikson C, Bangura M, Paddy O, Madhukar R, Gomez EL, Rathod J, Naria M, Hajal B, Awadhalla M, Siegel D, Parmar H, Biran N, and Vesole DH revised and edited the manuscript; Phull P and Weissman S revised and approved the final version and are the article's guarantors; All authors certify that they contributed sufficiently to the intellectual content and data analysis; Each author has reviewed the final version of the manuscript and approved it for publication.

Institutional review board statement: The study protocol was reviewed by the Ethics Committee at Palisades Medical Center and the need for IRB approval was waived as the SEER database is a public-use dataset.

Informed consent statement: The Surveillance, Epidemiology, and End Results (SEER) database was a public-use

dataset, of which the informed consent was waived.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The data used and/or analyzed in this study are available in the Surveillance, Epidemiology, and End Results (SEER) Database of the National Cancer Institute (http://seer.cancer.gov).

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: United States

ORCID number: Ayrton I Bangolo 0000-0002-2133-2480; Simcha Weissman 0000-0002-0796-6217.

S-Editor: Fan JR
L-Editor: A
P-Editor: Fan JR

REFERENCES

1 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* 2022; **72**: 7-33 [PMID: 35020204 DOI: 10.3322/caac.21708]
2 **Bhutani M**, Foureau DM, Atrash S, Voorhees PM, Usmani SZ. Extramedullary multiple myeloma. *Leukemia* 2020; **34**: 1-20 [PMID: 31776467 DOI: 10.1038/s41375-019-0660-0]
3 **Kriegova E**, Fillerova R, Minarik J, Savara J, Manakova J, Petrackova A, Dihel M, Balcarkova J, Krhovska P, Pika T, Gajdos P, Behalek M, Vasinek M, Papajik T. Whole-genome optical mapping of bone-marrow myeloma cells reveals association of extramedullary multiple myeloma with chromosome 1 abnormalities. *Sci Rep* 2021; **11**: 14671 [PMID: 34282158 DOI: 10.1038/s41598-021-93835-z]
4 **Mohan M**, Yarlagadda N, Szabo A, Singh A, Pina Oviedo S, Schinke C. Clinical characteristics of testicular extramedullary involvement in multiple myeloma. *Am J Hematol* 2021; **96**: E77-E81 [PMID: 33338289 DOI: 10.1002/ajh.26072]
5 **Li G**, Song YP, Lv Y, Li ZZ, Zheng YH. Clinical Characteristics and Prognostic Analysis of Multiple Myeloma with Extramedullary Disease: A SEER-Based Study. *J Oncol* 2021; **2021**: 6681521 [PMID: 33603785 DOI: 10.1155/2021/6681521]
6 **Tian C**, Wang L, Wu L, Zhu L, Xu W, Ye Z, Zhao Z, Wang Y, Zhang Y. Clinical characteristics and prognosis of multiple myeloma with bone-related extramedullary disease at diagnosis. *Biosci Rep* 2018; **38** [PMID: 29685954 DOI: 10.1042/BSR20171697]
7 **Gao S**, Li Q, Dong F, Yang P, Chen Y, Wang J, Wang Y, Jing H. Clinical characteristics and survival outcomes of newly diagnosed multiple myeloma patients presenting with extramedullary disease: A retrospective study. *Leuk Res* 2022; **115**: 106793 [PMID: 35248783 DOI: 10.1016/j.leukres.2022.106793]
8 **Janjetovic S**, Lohneis P, Nogai A, Balci D, Rasche L, Jähne D, Bokemeyer C, Schilling G, Blau IW, Schmidt-Hieber M. Clinical and Biological Characteristics of Medullary and Extramedullary Plasma Cell Dyscrasias. *Biology (Basel)* 2021; **10** [PMID: 34356484 DOI: 10.3390/biology10070629]
9 **Wu D**, Wang WJ, Zhang M. [Bone Marrow Morphology of Multiple Myeloma with Non-bone-related Extramedullary Disease]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2018; **26**: 807-811 [PMID: 29950224 DOI: 10.7534/j.issn.1009-2137.2018.03.029]
10 **Duggan MA**, Anderson WF, Altekruse S, Penberthy L, Sherman ME. The Surveillance, Epidemiology, and End Results (SEER) Program and Pathology: Toward Strengthening the Critical Relationship. *Am J Surg Pathol* 2016; **40**: e94-e102 [PMID: 27740970 DOI: 10.1097/PAS.0000000000000749]
11 **Jagosky MH**, Usmani SZ. Extramedullary Disease in Multiple Myeloma. *Curr Hematol Malig Rep* 2020; **15**: 62-71 [PMID: 32198576 DOI: 10.1007/s11899-020-00568-3]
12 **Weinstock M**, Ghobrial IM. Extramedullary multiple myeloma. *Leuk Lymphoma* 2013; **54**: 1135-1141 [PMID: 23210572 DOI: 10.3109/10428194.2012.740562]
13 **Mazul AL**, Naik AN, Zhan KY, Stepan KO, Old MO, Kang SY, Nakken ER, Puram SV. Gender and race interact to influence survival disparities in head and neck cancer. *Oral Oncol* 2021; **112**: 105093 [PMID: 33232879 DOI: 10.1016/j.oraloncology.2020.105093]
14 **Oh LJ**, Asher R, Veness M, Smee R, Goldstein D, Gopalakrishna Iyer N, Balasubramanian D, Low TH, Palme CE, Gupta R, Clark J. Effect of age and gender in non-smokers with oral squamous cell carcinoma: Multi-institutional study. *Oral Oncol* 2021; **116**: 105210 [PMID: 33618102 DOI: 10.1016/j.oraloncology.2021.105210]
15 **Tang L**, Pan Z, Zhang X. The effect of marital status on the survival of patients with multiple myeloma. *Hematology*

2022; **27**: 187-197 [PMID: 35068385 DOI: 10.1080/16078454.2022.2026027]

16 **Wang S**, Chen L, Chen D, Chao J, Shao Y, Tang K, Chen W. Effect of Marital Status on the Survival of Patients With Adenocarcinoma of the Esophagogastric Junction: A Population-Based, Propensity-Matched Study. *Cancer Control* 2021; **28**: 10732748211066309 [PMID: 34910613 DOI: 10.1177/10732748211066309]

17 **Zhou C**, Zhang Y, Hu X, Fang M, Xiao S. The effect of marital and insurance status on the survival of elderly patients with stage M1b colon cancer: a SEER-based study. *BMC Cancer* 2021; **21**: 891 [PMID: 34353300 DOI: 10.1186/s12885-021-08627-5]

18 **Alyabsi M**, Ramadan M, Algarni M, Alshammari K, Jazieh AR. The effect of marital status on stage at diagnosis and survival in Saudis diagnosed with colorectal cancer: cancer registry analysis. *Sci Rep* 2021; **11**: 8603 [PMID: 33883627 DOI: 10.1038/s41598-021-88042-9]

19 **Xiao K**, Zhao Y, Cai Y, Chen P, Chen J, Ye R, Yuan B, Liu X. The effect of marital status on the survival of patients with colorectal neuroendocrine neoplasms: an analysis of the SEER database. *Rev Esp Enferm Dig* 2020; **112**: 109-117 [PMID: 31830797 DOI: 10.17235/reed.2019.6183/2019]

20 **Dong J**, Dai Q, Zhang F. The effect of marital status on endometrial cancer-related diagnosis and prognosis: a Surveillance Epidemiology and End Results database analysis. *Future Oncol* 2019; **15**: 3963-3976 [PMID: 31746633 DOI: 10.2217/fon-2019-0241]

21 **Hinyard L**, Wirth LS, Clancy JM, Schwartz T. The effect of marital status on breast cancer-related outcomes in women under 65: A SEER database analysis. *Breast* 2017; **32**: 13-17 [PMID: 28012410 DOI: 10.1016/j.breast.2016.12.008]

22 **Xie JC**, Yang S, Liu XY, Zhao YX. Effect of marital status on survival in glioblastoma multiforme by demographics, education, economic factors, and insurance status. *Cancer Med* 2018; **7**: 3722-3742 [PMID: 30009575 DOI: 10.1002/cam4.1688]

23 **Liang Y**, Wu X, Lu C, Xiao F. Impact of marital status on the prognosis of liver cancer patients without surgery and the critical window. *Ann Palliat Med* 2021; **10**: 2990-2999 [PMID: 33615813 DOI: 10.21037/apm-20-1885]

24 **Feng Y**, Dai W, Li Y, Mo S, Li Q, Cai S. The effect of marital status by age on patients with colorectal cancer over the past decades: a SEER-based analysis. *Int J Colorectal Dis* 2018; **33**: 1001-1010 [PMID: 29546559 DOI: 10.1007/s00384-018-3017-7]

25 **Desantis V**, Saltarella I, Lamanuzzi A, Melaccio A, Solimando AG, Mariggiò MA, Racanelli V, Paradiso A, Vacca A, Frassanito MA. MicroRNAs-Based Nano-Strategies as New Therapeutic Approach in Multiple Myeloma to Overcome Disease Progression and Drug Resistance. *Int J Mol Sci* 2020; **21** [PMID: 32349317 DOI: 10.3390/ijms21093084]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 May 24; 14(5): 190-214

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 5 May 24, 2023

MINIREVIEWS

190 Acute left-sided malignant colonic obstruction: Is there a role for endoscopic stenting?

Russo S, Conigliaro R, Coppini F, Dell'Aquila E, Grande G, Pigò F, Mangiafico S, Lupo M, Marocchi M, Bertani H, Cocca S

198 Tyrosine kinase inhibitors and human epidermal growth factor receptor-2 positive breast cancer

Abunada A, Sirhan Z, Thyagarajan A, Sahu RP

ORIGINAL ARTICLE

Basic Study

203 Thymoquinone enhances the antioxidant and anticancer activity of Lebanese propolis

AlDreini S, Fatfat Z, Abou Ibrahim N, Fatfat M, Gali-Muhtasib H, Khalife H

World Journal of Clinical Oncology

Monthly Volume 14 Number 5 May 24, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Oncology*, Xiang Wang, MD, PhD, Associate Professor, Associate Chief Physician, Department of Oral Medicine, Nanjing Stomatological Hospital, Medical School of Nanjing University, No. 30 Zhongyang Road, Nanjing 210008, Jiangsu Province, China. wangxiang@nju.edu.cn

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 edition of Journal Citation Reports® cites the 2021 Journal Citation Indicator (JCI) for *WJCO* as 0.35.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Yu-Jie Ma*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
May 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 May 24; 14(5): 190-197

DOI: 10.5306/wjco.v14.i5.190

ISSN 2218-4333 (online)

MINIREVIEWS

Acute left-sided malignant colonic obstruction: Is there a role for endoscopic stenting?

Salvatore Russo, Rita Conigliaro, Francesca Coppini, Emanuela Dell'Aquila, Giuseppe Grande, Flavia Pigò, Santi Mangiafico, Marinella Lupo, Margherita Marocchi, Helga Bertani, Silvia Cocca

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Chow WK, Taiwan; Gu GL, China

Received: September 18, 2022
Peer-review started: September 18, 2022
First decision: November 11, 2022
Revised: November 23, 2022
Accepted: April 25, 2023
Article in press: April 25, 2023
Published online: May 24, 2023

Salvatore Russo, Rita Conigliaro, Giuseppe Grande, Flavia Pigò, Santi Mangiafico, Marinella Lupo, Margherita Marocchi, Helga Bertani, Silvia Cocca, Gastroenterology and Digestive Endoscopy Unit, Modena University Hospital, Modena 41126, Italy

Francesca Coppini, Gastroenterology and Digestive Endoscopy Unit, Azienda USL, IRCCs di Reggio Emilia, Reggio Emilia 42122, Italy

Emanuela Dell'Aquila, Medical Oncology 1, IRCCS Regina Elena National Cancer Institute, Rome 0144, Italy

Corresponding author: Salvatore Russo, MD, Doctor, Gastroenterology and Digestive Endoscopy Unit, Modena University Hospital, Via Pietro Giardini 1355, Baggiovara, Modena 41126, Italy. russo.salvatore@aou.mo.it

Abstract

The therapy of left-sided malignant colonic obstruction continues to be one of the largest problems in clinical practice. Numerous studies on colonic stenting for neoplastic colonic obstruction have been reported in the last decades. Thereby the role of self-expandable metal stents (SEMS) in the treatment of malignant colonic obstruction has become better defined. However, numerous prospective and retrospective investigations have highlighted serious concerns about a possible worse outcome after endoscopic colorectal stenting as a bridge to surgery, particularly in case of perforation. This review analyzes the most recent evidence in order to highlight pros and cons of SEMS placement in left-sided malignant colonic obstruction.

Key Words: Colorectal neoplasm; Intestinal obstruction; Endoscopy; Self expandable metallic stents; Colorectal surgery; Chemotherapy

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Self-expandable metal stents (SEMS) should be considered as a primary option in palliative treatment of malignant left-sided colonic obstruction. In patients with conceivably curable left-sided colon cancer, SEMS placement as a bridge to surgery should be carefully discussed, specifically focusing on lower risk and lower permanent stoma rates, but potentially higher recurrence rates when compared to surgery. In this scenario the endoscopic expertise has a significant impact on the complication rate.

Citation: Russo S, Conigliaro R, Coppini F, Dell'Aquila E, Grande G, Pigò F, Mangiafico S, Lupo M, Marocchi M, Bertani H, Cocca S. Acute left-sided malignant colonic obstruction: Is there a role for endoscopic stenting? *World J Clin Oncol* 2023; 14(5): 190-197
URL: https://www.wjgnet.com/2218-4333/full/v14/i5/190.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i5.190

INTRODUCTION

Colorectal cancer (CRC) is the third most frequently diagnosed malignancy in the world and the second cause of cancer-related mortality[1]. CRC is still among the most common reason for large bowel obstruction in adults and about 20% of patients with CRC are admitted with emergency[2-4]. Obstructive CRC most frequently develops in the sigmoid colon, with 75% of tumors located distal to the splenic flexure[5]. Emergency surgery (ES) is the standard approach for obstructive right-sided colon cancer, along with primary resection and ileocolic anastomosis[6]. However, it is debatable whether emergency or radical surgery following stenting as a bridge to surgery (BTS) should be considered for obstructive left-sided colorectal cancer[7]. Self- expandable metal stents (SEMS) for BTS (Figure 1) have shown excellent short-term results, but related complications such as perforations may be disastrous and long-term outcomes are still a matter of debate[8-11].

STENT AS A BRIDGE-TO-SURGERY

Clinical aspects

Over the last decades, many papers have been published on colonic stenting for neoplastic obstruction, including randomized controlled trials (RCT), post-hoc analysis and systematic reviews. Moreover, in 2020 the European Society of Gastrointestinal Endoscopy (ESGE) released updated guidelines on this topic[7]. Even though the role of SEMSs in the management of malignant colonic obstruction has been better defined, several issues still remain. Although screening programs are widespread in developed countries, large bowel obstruction is one of the most common causes of ES in patients with CRC[7,12]. For example, in the United Kingdom, the rate of colorectal cancer presenting as an emergency remains at 20%[13]. Colonic SEMS placement is mainly suggested for patients who have obstructive symptoms and CT-results compatible with obstructing CRC. Acute colorectal obstruction (ACRO) is a medical emergency related to CRC that occurs more frequently in patients with advanced disease, in whom ES is responsible of significant morbidity and mortality than elective surgery, particularly in aged patients[14,15]. These patients usually present to the emergency department with nausea, vomiting, constipation and/or abdominal distention, often combined with poor intake of food from the previous days[16].

In ACRO, the main therapeutic aim is to decrease colonic distension and to prevent complications (i.e. necrosis, perforation), generally associated with pneumoperitoneum and systemic inflammatory response syndrome. Therefore, colonic stenting is an interesting option to obtain this goal in ACRO, as a BTS and for palliative purposes in patients with advanced and/or unfit for surgery CRC[7,15].

Effective stent placement makes it feasible to perform non-surgical intestinal decompression and prepare the colon for a forthcoming elective oncologic resection. Furthermore, in CRC obstruction, the proximal colon is frequently dilated with vascular insufficiency, with an increased risk of colostomy/ileostomy in case of ES. As shown in many studies, in this situation SEMSs may decompress the dilated proximal colon, thus obviating the requirement of ES with colostomy/ileostomy[17].

To evaluate the severity of obstruction, in Japan a modified point score system called ColoRectal Obstruction Scoring System (CROSS) (Table 1) is widely used. CROSS 0 patients need ES or SEMS placement. CROSS 1 or 2 patients are candidates for elective surgery. In CROSS 3 and 4 patients SEMS placement is not required because they can receive food. A post hoc analysis of two prospective, observational, single-arm multicenter clinical trials demonstrated the short-term high efficacy and safety of SEMS placement as a BTS for patients with obstructive CRC classified as CROSS 0, 1, and 2[18].

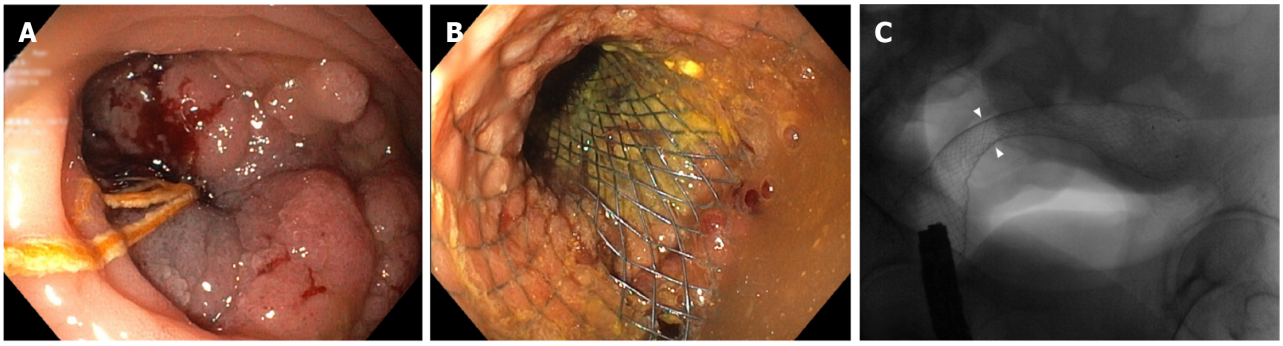
Clinical success and adverse events

In a large cohort prospective study, the clinical success rate of SEMS placement was 95.5% and the

Table 1 ColoRectal Obstruction Scoring System adapted from Ohki *et al*[18]

Level of oral intake	Score
Requiring continuous decompression	0
No oral intake	1
Liquid or enteral nutrient intake	2
Soft solids, low-residue, and full diet with symptoms of stricure[1]	3
Soft solids, low-residue, and full diet without symptoms of stricure[1]	4

[1]Symptoms of stricture include abdominal pain/cramps, abdominal distension, nausea, vomiting, constipation, and diarrhea, which are related to gastrointestinal transit.



DOI: 10.5306/wjco.v14.i5.190 **Copyright** ©The Author(s) 2023.

Figure 1 Left-sided colorectal cancer obstruction treated with self-expandable metal stents. A: Obstructing cancer of the sigmoid colon; B: Endoscopic view after self-expandable metal stents (SEMS) deployment; C: Radiological view of the deployed SEMS.

technical success rate 97.9%. Major adverse events included perforation (2.1%), stent migration (1.0%), and stent occlusion (0.8%)[19]. The primary cause of perforation was the procedure itself (0.8%) followed by comorbidities (impending perforation, obstructive colitis) not manifest prior to SEMS insertion (0.6%). In a retrospective study, the technical success rate for stent placement for left-sided malignant colonic obstruction (LS-MCO) and rectal obstruction did not differ, but the clinical success rate was lower in patients with rectal obstruction (85.4% *vs* 92.1%; $P = 0.02$). In addition, the latter group of patients had a higher complication rate (37.4% *vs* 25.1%; $P = 0.01$), due to an increased risk of extra-intestinal cancer[20]. Furthermore, it is well established from the literature that expertise, method, lesion characteristics, and the location of the obstruction or architecture of the colon, such as tortuosity, have a significant impact on the technical and clinical failure rates for colonic stenting[7,21]. Since there have been growing concerns about protracted and technically challenging stent placement in complex patients, the Colonic Stent Safe Procedure Research Group, in collaboration with the Japan Gastroenterological Endoscopy Society, has developed mini-guidelines to ensure the procedural safety and efficacy for colonic stent placement. A post-hoc analysis[22] of a large multicenter clinical trial identified the risk factors for difficult colonic stenting cases such as a CROSS score of 0 before SEMS placement, evidence of peritoneal carcinomatosis, tumor site in the right colon, stricture length ≥5 cm and placement of multiple SEMSs[22]. In light of this evidence, Kuwai *et al*[22] concluded that before attempting SEMS placement for obstructive CRC clinicians must anticipate technical challenges.

The choice of the stent

Various SEMS have been developed, but they can be classified as covered and uncovered. A recent meta-analysis examined the effectiveness of uncovered vs covered stents in treating colonic obstruction either as a curative BTS or palliative option. Uncovered SEMSs presented less complications (e.g. tumor overgrowth and displacement), longer SEMS patency (mean duration 18 mo), while the risk of tumor ingrowth was higher, as expected. Rates of technical success, clinical success, perforation, stool impaction and stent obstruction were similar in both groups[21].

It is difficult to make recommendations regarding the SEMS length or diameter, as few studies have shown conflicting results. When selecting a stent after fluoroscopic measurement of colonic stricture length, it is widely accepted in clinical practice to follow a simple rule: to prepare for stent foreshortening, the distal edge of the SEMS should be placed proximal to the obstruction. Furthermore, the SEMS length should include 1-2 cm on each side beyond the stricture, considering the extent of shortening once deployed[7,17,21,23].

Is bridge-to-surgery stenting a safe alternative to emergency surgery?

Emergency surgery is burdened by high anastomotic leakage rates, up to 33%[12]. Furthermore a recent study suggests that emergency presentation remains an independent poor prognostic indicator after curative colorectal resection[24]. The optimal management of left-sided malignant large bowel obstruction is less clear than the right-sided cancer where the surgical approach is highly recommended [25].

Several surgical options exist for left-sided bowel obstruction including primary resection (with or without anastomosis), subtotal colectomy (with or without anastomosis) or unfunctioning ileostomy/colostomy with interval resection[24,25].

For the first time in 1994 Tejero *et al*[26] described the technique of SEMS placement in 2 patients with ACRO as a BTS. Nearly twenty years after this initial description, the debate is still open regarding the role of SEMSs as a BTS for symptomatic LS-MCO because interpretation of the literature on this subject is still challenging.

The fundamental hypotheses driving the growing interest in SEMS placement are that it can turn ES into elective surgery, reducing preoperative morbidity. Webster *et al*[25] analyzed 19 international guidelines for the treatment of LS-MCO from 2010 to 2018 and asked whether ES or stent placement as a bridge to surgery was the best procedure in terms of morbidity, mortality and long-term oncological outcomes**.** They concluded that there was a lack of high-quality evidence[25]. The more recent guidelines of the European Society of Gastrointestinal Endoscopy recommend to reserve colonic stenting in case of clinical symptoms and radiological signs of obstructing CRC, without evidence of perforation (strong recommendation, low quality evidence)[7].

In 2011, one of the first multicenter randomized trials comparing ES with colonic stenting as a BTS for left-sided CRC showed that colonic stenting had no decisive clinical advantages for global health status, mortality, morbidity and stoma rates. Moreover their results raised concerns about overt and silent perforations responsible for tumor spread[27].

A systematic review and meta-analysis of RCTs on colonic stenting as a BTS *vs* ES for acute symptomatic malignant left sided colonic obstruction[12] showed that patients treated with SEMS as a BTS had less short-term overall morbidity and reduced rates of both permanent and transient stoma. Albeit influenced by local expertise, level of obstruction and patient's clinical status, stenting as a BTS for LS-MCO showed lower risk than ES in the short-term morbidity (60 d after surgery). However, recurrence rate data between the two groups showed a clear trend in favour of ES over stenting as a BTS (26% *vs* 40%), although this was not statistically significant.

In a subsequent multicenter randomized controlled trial (ESCO trial) comparing stenting as a BTS to ES for malignant colonic obstruction, Arezzo *et al*[28] reported a similar short term complications rate between the two groups but a higher stoma rate in the ES group (P = 0.031). Looking at the long term oncologic results of the ESCO trial, no difference was observed between the two groups in terms of overall survival, time to progression and disease free survival[29]. These results have also been confirmed in a more recent meta-analysis by Cirocchi *et al*[30].

While the majority of studies tried to understand if SEMS placement is more convenient than ES[12,31,32], there are few studies comparing the bridge to elective surgery approach such as decompressive stoma (DS) *vs* SEMS placement. Creation of a DS is a quite simple procedure with a near 100% success rate and can be performed in almost all patients while, as mentioned above, colonic stenting is an intervention requiring specific technical skills and expertise (in both colonoscopy and fluoroscopic techniques), including the ability to select correctly the patient based on stricture's length and location, and carries risks of adverse events. A population-based cohort study[33] comparing the two bridge to elective surgery approaches showed that SEMS appears to be a safest procedure, with a shorter hospital admission, as well as in palliative care. In a recent meta-analysis of seven studies (1 prospective, 6 retrospective), involving 646 and 712 patients who underwent SEMS and DS approaches respectively, Zhang *et al* found a significantly lower complication rate in the SEMS group than in the DS group (8.68 *vs* 16.85%; P = 0.004), without differences in short-term mortality and permanent stoma rates. In line with the previously cited study[33], the authors concluded that SEMSs may be a better alternative to DS for obstructive CRC, but highlighted the lack of high-quality RCTs[34].

Finally, a newly published randomized trial with a longer follow-up (3 y) and larger population compared to prior studies, randomized patients with left-sided obstructive colon cancer to colonic stenting or surgical decompression. The authors showed that among patients undergoing potentially curative treatment, there were no significant differences in 30-d postoperative mortality or duration of hospital stay between stenting followed by delayed elective surgery and emergency surgery group. Moreover the use of a stoma resulted more frequent in patients treated with immediate surgery than in patients treated with SEMS (67.9% *vs* 47.5%; P = 0.003), without substantial differences in peri-operative morbidity, intensive care use, quality of life and 3-y recurrence or mortality[35].

Timing of surgery

The proper timing of surgery subsequent to SEMS placement as a BTS is not clear yet. Adequate radial stent expansion, ischemia reversibility of the colon proximal to the stricture and colon cleansing require sufficient time after SEMS deployment. In order to reduce the risk of stoma and postoperative complications, such as anastomotic leaks, abscesses, and wound's problems, surgery should be postponed for at

least 2 wk after SEMS placement. However, long delays in surgery could increase the complications rate related to SEMS. Therefore, surgery is suggested approximately 14 d after SEMS insertion[7,17].

STENT AS PALLIATIVE TREATMENT

Three randomized controlled trials compared SEMS and decompressive stoma as palliative treatments for malignant bowel obstructions[36-38]. Palliative situations included patients unfit for surgery, as well as patients with inoperable primary lesions or metastatic disease. Given its effectiveness and the enhanced quality of life (QoL) that comes from avoiding a stoma, colonic stenting has been judged to be superior in both investigations. In a randomized prospective trial, Fiori *et al*[37,38] found that the mortality and morbidity rates following palliative stenting and colostomies were comparable. However, in the stenting group a shorter hospital stay, a faster return to oral intake, and a shorter operating time were recorded. On the other hand, a Dutch trial with a similar study design was prematurely stopped because of the unacceptable high mortality rate due to perforations in the stenting group. The authors hypothesized that the unpredictable high frequency of perforation in the nonsurgical arm could be associated with the type of stent used at that time[39].

Stent and chemotherapy

Data about the effects and safety of systemic chemotherapy alone or in association with biological agents (anti-VEGF or anti-EGFR) combined with palliative stenting in metastatic colorectal cancer (mCRC) patients are lacking.

In a metanalysis including 837 mCRC patients, patients treated with SEMS had similar overall survival compared to surgery-treated patients (7.64 mo *vs* 7.88 mo respectively), shorter time before starting chemotherapy (33.36 d *vs* 15.53 d, P < 0.00001) and lower 30-d mortality (4.2% *vs* 10.5%, P = 0.01)[40]. Tumor response to chemotherapy could increase the rate of complications related to stent placement, such as stent migration or late perforation, but, on the other hand, could reduce the risk of obstruction by maintaining its luminal patency, especially in a palliative setting. A multicenter retrospective study included 38 mCRC patients treated with only chemotherapy; major complications related to stenting were: Perforation (8%), stent migration (5%), and re-obstruction secondary to tumor ingrowth (13%)[41]. A retrospective trial including 72 mCRC patients compared long-term outcomes of palliative SEMS in patients treated with chemotherapy or with best supportive care. In the chemotherapy group, there was a higher rate of late migration (20% *vs* 2.4%, P = 0.018, for chemotherapy and best supportive care group respectively); patients refractory to chemotherapy reported a higher rate of late obstruction in comparison to patients who reached disease control during treatment (35.7% in disease progression, 0% in disease control, P = 0.014)[42]. A recent metanalysis evaluated the impact of systemic treatment (chemotherapy alone or in association with targeted therapy) on the risk of complications after SEMS deployment and on outcome in terms of survival rates. Chemotherapy was shown to not be related to a higher risk of SEMS-related complications nor a reduction in the survival rates[43].

The introduction of bevacizumab improved outcome of mCRC patients[44], although data about its effect on stent placement are still controversial. Moreover, some authors raised the hypothesis of an increased risk to develop SEMS-related complications (such as perforation) in patients on bevacizumab [45,46]. Conversely, other authors demonstrated that the addition of bevacizumab to chemotherapy was not related to a higher perforation rate in comparison to chemotherapy alone[47,48]. In an Italian retrospective, multicenter study including 91 mCRC patients treated with chemotherapy plus anti-VEGF or anti-EGFR agents, no correlation between chemotherapy with or without biological therapy, K-RAS status or risk of SEMS-related complications was shown[46].

These studies had several limitations: Retrospective nature, different outcomes and small sample size, patients with heterogeneous characteristics and different settings. At the state of the art more prospective and randomized trials to define the outcome and safety of the association of SEMS placement and systemic treatment are needed.

CONCLUSION

Colonic stenting is a well-recognized palliative approach for treating malignant left-sided colonic obstruction, with high rates of technical and clinical success. Especially in patients with poor general condition and limited life expectancy, it may allow for an early hospital discharge, an improved QoL and prolonged survival in comparison to surgery.

SEMS placement as a BTS has the advantage to convert an ES into an elective one, reducing preoperative morbidity, allowing for adequate oncological staging, good colonic preparation and faster initiation of chemotherapy. Although numerous prospective and retrospective investigations have highlighted serious concerns about tumor seeding after endoscopic colorectal stent placement, partic-

ularly in cases of perforation, recent high quality studies displayed encouraging results. Operator expertise remains a key element to ensure accurate stent placement and restoration of bowel function with a low rate of complications. For this reason, this approach should be considered a standard practice only in experienced high-volume referral centers and clinicians should carefully select the patients fit for an endoscopic decompressing approach before starting the procedure.

In conclusion, further evidence from prospective, ideally randomized trials on the probability of tumor recurrence following stenting is necessary to show the long-term safety of stenting as a BTS. Until then, the evident short-term advantages, combined with the high mortality rate in frail and elderly patients, should be weighed against the potential long-term threats of tumor recurrence.

FOOTNOTES

Author contributions: Russo S, Cocca S, Coppini F and Dell'Aquila E drafted the manuscript; Conigliaro R, Pigò F, Mangiafico S and Bertani H performed critical revision of the manuscript; Grande G, Marocchi M and Lupo M edited the manuscript and collected the bibliography; All authors have read and approve the final manuscript.

Conflict-of-interest statement: There is no conflict of interest associated with any of the authors or co-authors contributed their efforts in this manuscript.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Italy

ORCID number: Salvatore Russo 0000-0001-8537-1852; Rita Conigliaro 0000-0003-3463-7862; Francesca Coppini 0000-0001-5369-2838; Emanuela Dell'Aquila 0000-0002-7258-1364; Giuseppe Grande 0000-0003-3907-9740; Flavia Pigò 0000-0002-0917-0802; Santi Mangiafico 0000-0003-4760-4162; Marinella Lupo 0000-0002- 9209-1389; Margherita Marocchi 0000-0002-3576-4620; Helga Bertani 0000-0003-0818-7167; Silvia Cocca 0000-0002-0642-8054.

Corresponding Author's Membership in Professional Societies: European Society of Gastrointestinal Endoscopy (ESGE), 61320133; Italian Society of Digestive Endoscopy (SIED); Italian Society of Neurogastroenterology and Motility (SINGEM).

S-Editor: Gong ZM
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Sagar J**. Colorectal stents for the management of malignant colonic obstructions. *Cochrane Database Syst Rev* 2011; **2011**: CD007378 [PMID: 22071835 DOI: 10.1002/14651858.CD007378.pub2]

3 **Baer C**, Menon R, Bastawrous S, Bastawrous A. Emergency Presentations of Colorectal Cancer. *Surg Clin North Am* 2017; **97**: 529-545 [PMID: 28501245 DOI: 10.1016/j.suc.2017.01.004]

4 **Shimura T**, Joh T. Evidence-based Clinical Management of Acute Malignant Colorectal Obstruction. *J Clin Gastroenterol* 2016; **50**: 273-285 [PMID: 26796083 DOI: 10.1097/mcg.0000000000000475]

5 **Frago R**, Ramirez E, Millan M, Kreisler E, del Valle E, Biondo S. Current management of acute malignant large bowel obstruction: a systematic review. *Am J Surg* 2014; **207**: 127-138 [PMID: 24124659 DOI: 10.1016/j.amjsurg.2013.07.027]

6 **Pisano M**, Zorcolo L, Merli C, Cimbanassi S, Poiasina E, Ceresoli M, Agresta F, Allievi N, Bellanova G, Coccolini F, Coy C, Fugazzola P, Martinez CA, Montori G, Paolillo C, Penachim TJ, Pereira B, Reis T, Restivo A, Rezende-Neto J, Sartelli M, Valentino M, Abu-Zidan FM, Ashkenazi I, Bala M, Chiara O, De' Angelis N, Deidda S, De Simone B, Di Saverio S, Finotti E, Kenji I, Moore E, Wexner S, Biffl W, Coimbra R, Guttadauro A, Leppäniemi A, Maier R, Magnone S, Mefire AC, Peitzmann A, Sakakushev B, Sugrue M, Viale P, Weber D, Kashuk J, Fraga GP, Kluger I, Catena F, Ansaloni L. 2017 WSES guidelines on colon and rectal cancer emergencies: obstruction and perforation. *World J Emerg Surg* 2018; **13**: 36 [PMID: 30123315 DOI: 10.1186/s13017-018-0192-3]

7 **van Hooft JE**, Veld JV, Arnold D, Beets-Tan RGH, Everett S, Götz M, van Halsema EE, Hill J, Manes G, Meisner S, Rodrigues-Pinto E, Sabbagh C, Vandervoort J, Tanis PJ, Vanbiervliet G, Arezzo A. Self-expandable metal stents for obstructing colonic and extracolonic cancer: European Society of Gastrointestinal Endoscopy (ESGE) Guideline - Update 2020. *Endoscopy* 2020; **52**: 389-407 [PMID: 32259849 DOI: 10.1055/a-1140-3017]

8 **Kim HJ**, Choi GS, Park JS, Park SY, Jun SH. Higher rate of perineural invasion in stent-laparoscopic approach in

comparison to emergent open resection for obstructing left-sided colon cancer. *Int J Colorectal Dis* 2013; **28**: 407-414 [PMID: 22885839 DOI: 10.1007/s00384-012-1556-x]

9 **Kim SJ**, Kim HW, Park SB, Kang DH, Choi CW, Song BJ, Hong JB, Kim DJ, Park BS, Son GM. Colonic perforation either during or after stent insertion as a bridge to surgery for malignant colorectal obstruction increases the risk of peritoneal seeding. *Surg Endosc* 2015; **29**: 3499-3506 [PMID: 25676202 DOI: 10.1007/s00464-015-4100-6]

10 **Sabbagh C**, Browet F, Diouf M, Cosse C, Brehant O, Bartoli E, Mauvais F, Chauffert B, Dupas JL, Nguyen-Khac E, Regimbeau JM. Is stenting as "a bridge to surgery" an oncologically safe strategy for the management of acute, left-sided, malignant, colonic obstruction? *Ann Surg* 2013; **258**: 107-115 [PMID: 23324856 DOI: 10.1097/sla.0b013e31827e30ce]

11 **Maruthachalam K**, Lash GE, Shenton BK, Horgan AF. Tumour cell dissemination following endoscopic stent insertion. *Br J Surg* 2007; **94**: 1151-1154 [PMID: 17541987 DOI: 10.1002/bjs.5790]

12 **Arezzo A**, Passera R, Lo Secco G, Verra M, Bonino MA, Targarona E, Morino M. Stent as bridge to surgery for left-sided malignant colonic obstruction reduces adverse events and stoma rate compared with emergency surgery: results of a systematic review and meta-analysis of randomized controlled trials. *Gastrointest Endosc* 2017; **86**: 416-426 [PMID: 28392363 DOI: 10.1016/j.gie.2017.03.1542]

13 **Golder AM**, McMillan DC, Horgan PG, Roxburgh CSD. Determinants of emergency presentation in patients with colorectal cancer: a systematic review and meta-analysis. *Sci Rep* 2022; **12**: 4366 [PMID: 35288664 DOI: 10.1038/s41598-022-08447-y]

14 **Bakker IS**, Snijders HS, Grossmann I, Karsten TM, Havenga K, Wiggers T. High mortality rates after nonelective colon cancer resection: results of a national audit. *Colorectal Dis* 2016; **18**: 612-621 [PMID: 26749028 DOI: 10.1111/codi.13262]

15 **Bonin EA**, Baron TH. Update on the indications and use of colonic stents. *Curr Gastroenterol Rep* 2010; **12**: 374-382 [PMID: 20703837 DOI: 10.1007/s11894-010-0136-x]

16 **van Halsema EE**, van Hooft JE. Does short-term morbidity and stoma reduction outweigh a potential long-term risk of colonic stent placement? *Gastrointest Endosc* 2017; **86**: 427-428 [PMID: 28826546 DOI: 10.1016/j.gie.2017.04.021]

17 **Lee JM**, Byeon JS. Colorectal Stents: Current Status. *Clin Endosc* 2015; **48**: 194-200 [PMID: 26064818 DOI: 10.5946/ce.2015.48.3.194]

18 **Ohki T**, Yoshida S, Yamamoto M, Isayama H, Yamada T, Matsuzawa T, Saito S, Kuwai T, Tomita M, Shiratori T, Shimada M, Hirakawa T, Koizumi K, Saida Y. Determining the difference in the efficacy and safety of self-expandable metallic stents as a bridge to surgery for obstructive colon cancer among patients in the CROSS 0 group and those in the CROSS 1 or 2 group: a pooled analysis of data from two Japanese prospective multicenter trials. *Surg Today* 2020; **50**: 984-994 [PMID: 32025817 DOI: 10.1007/s00595-020-01970-3]

19 **Matsuzawa T**, Ishida H, Yoshida S, Isayama H, Kuwai T, Maetani I, Shimada M, Yamada T, Saito S, Tomita M, Koizumi K, Hirata N, Sasaki T, Enomoto T, Saida Y. A Japanese prospective multicenter study of self-expandable metal stent placement for malignant colorectal obstruction: short-term safety and efficacy within 7 days of stent procedure in 513 cases. *Gastrointest Endosc* 2015; **82**: 697-707.e1 [PMID: 25975529 DOI: 10.1016/j.gie.2015.03.1978]

20 **Lee HJ**, Hong SP, Cheon JH, Kim TI, Kim WH, Park SJ. Clinical Outcomes of Self-Expandable Metal Stents for Malignant Rectal Obstruction. *Dis Colon Rectum* 2018; **61**: 43-50 [PMID: 29215476 DOI: 10.1097/DCR.0000000000000910]

21 **Mashar M**, Mashar R, Hajibandeh S. Uncovered versus covered stent in management of large bowel obstruction due to colorectal malignancy: a systematic review and meta-analysis. *Int J Colorectal Dis* 2019; **34**: 773-785 [PMID: 30903271 DOI: 10.1007/s00384-019-03277-3]

22 **Kuwai T**, Yamaguchi T, Imagawa H, Yoshida S, Isayama H, Matsuzawa T, Yamada T, Saito S, Shimada M, Hirata N, Sasaki T, Koizumi K, Maetani I, Saida Y. Factors related to difficult self-expandable metallic stent placement for malignant colonic obstruction: A post-hoc analysis of a multicenter study across Japan. *Dig Endosc* 2019; **31**: 51-58 [PMID: 30113095 DOI: 10.1111/den.13260]

23 **Park JK**, Lee MS, Ko BM, Kim HK, Kim YJ, Choi HJ, Hong SJ, Ryu CB, Moon JH, Kim JO, Cho JY, Lee JS. Outcome of palliative self-expanding metal stent placement in malignant colorectal obstruction according to stent type and manufacturer. *Surg Endosc* 2011; **25**: 1293-1299 [PMID: 20976501 DOI: 10.1007/s00464-010-1366-6]

24 **Dahdaleh FS**, Sherman SK, Poli EC, Vigneswaran J, Polite BN, Sharma MR, Catenacci DV, Maron SB, Turaga KK. Obstruction predicts worse long-term outcomes in stage III colon cancer: A secondary analysis of the N0147 trial. *Surgery* 2018; **164**: 1223-1229 [PMID: 30297240 DOI: 10.1016/j.surg.2018.06.044]

25 **Webster PJ**, Aldoori J, Burke DA. Optimal management of malignant left-sided large bowel obstruction: do international guidelines agree? *World J Emerg Surg* 2019; **14**: 23 [PMID: 31139245 DOI: 10.1186/s13017-019-0242-5]

26 **Tejero E**, Mainar A, Fernández L, Tobío R, De Gregorio MA. New procedure for the treatment of colorectal neoplastic obstructions. *Dis Colon Rectum* 1994; **37**: 1158-1159 [PMID: 7956588 DOI: 10.1007/BF02049822]

27 **van Hooft JE**, Bemelman WA, Oldenburg B, Marinelli AW, Lutke Holzik MF, Grubben MJ, Sprangers MA, Dijkgraaf MG, Fockens P; collaborative Dutch Stent-In study group. Colonic stenting versus emergency surgery for acute left-sided malignant colonic obstruction: a multicentre randomised trial. *Lancet Oncol* 2011; **12**: 344-352 [PMID: 21398178 DOI: 10.1016/S1470-2045(11)70035-3]

28 **Arezzo A**, Balague C, Targarona E, Borghi F, Giraudo G, Ghezzo L, Arroyo A, Sola-Vera J, De Paolis P, Bossotti M, Bannone E, Forcignanò E, Bonino MA, Passera R, Morino M. Colonic stenting as a bridge to surgery versus emergency surgery for malignant colonic obstruction: results of a multicentre randomised controlled trial (ESCO trial). *Surg Endosc* 2017; **31**: 3297-3305 [PMID: 27924392 DOI: 10.1007/s00464-016-5362-3]

29 **Arezzo A**, Forcignanò E, Bonino MA, Balagué C, Targarona E, Borghi F, Giraudo G, Ghezzo L, Passera R, Morino M; collaborative ESCO study group. Long-term Oncologic Results After Stenting as a Bridge to Surgery Versus Emergency Surgery for Malignant Left-sided Colonic Obstruction: A Multicenter Randomized Controlled Trial (ESCO Trial). *Ann Surg* 2020; **272**: 703-708 [PMID: 32833762 DOI: 10.1097/SLA.0000000000004324]

30 **Cirocchi R**, Arezzo A, Sapienza P, Crocetti D, Cavaliere D, Solaini L, Ercolani G, Sterpetti AV, Mingoli A, Fiori E. Current Status of the Self-Expandable Metal Stent as a Bridge to Surgery Versus Emergency Surgery in Colorectal Cancer:

Results from an Updated Systematic Review and Meta-Analysis of the Literature. *Medicina (Kaunas)* 2021; **57** [PMID: 33804232 DOI: 10.3390/medicina57030268]

31 **Wang X**, He J, Chen X, Yang Q. Stenting as a bridge to resection versus emergency surgery for left-sided colorectal cancer with malignant obstruction: A systematic review and meta-analysis. *Int J Surg* 2017; **48**: 64-68 [PMID: 29024743 DOI: 10.1016/j.ijsu.2017.10.004]

32 **Foo CC**, Poon SHT, Chiu RHY, Lam WY, Cheung LC, Law WL. Is bridge to surgery stenting a safe alternative to emergency surgery in malignant colonic obstruction: a meta-analysis of randomized control trials. *Surg Endosc* 2019; **33**: 293-302 [PMID: 30341649 DOI: 10.1007/s00464-018-6487-3]

33 **Veld JV**, Amelung FJ, Borstlap WAA, van Halsema EE, Consten ECJ, Siersema PD, Ter Borg F, van der Zaag ES, de Wilt JHW, Fockens P, Bemelman WA, van Hooft JE, Tanis PJ; Dutch Snapshot Research Group. Comparison of Decompressing Stoma vs Stent as a Bridge to Surgery for Left-Sided Obstructive Colon Cancer. *JAMA Surg* 2020; **155**: 206-215 [PMID: 31913422 DOI: 10.1001/jamasurg.2019.5466]

34 **Zhang J**, Zhu H, Yang W, Liu X, Zhang D, Jiang X, Yang L, Zhou Z. Endoscopic stent versus diverting stoma as a bridge to surgery for obstructive colorectal cancer: a systematic review and meta-analysis. *Langenbecks Arch Surg* 2022; **407**: 3275-3285 [PMID: 35666309 DOI: 10.1007/s00423-022-02517-5]

35 **CReST Collaborative Group**. Colorectal Endoscopic Stenting Trial (CReST) for obstructing left-sided colorectal cancer: randomized clinical trial. *Br J Surg* 2022; **109**: 1073-1080 [PMID: 35986684 DOI: 10.1093/bjs/znac141]

36 **Xinopoulos D**, Dimitroulopoulos D, Theodosopoulos T, Tsamakidis K, Bitsakou G, Plataniotis G, Gontikakis M, Kontis M, Paraskevas I, Vassilobpoulos P, Paraskevas E. Stenting or stoma creation for patients with inoperable malignant colonic obstructions? *Surg Endosc* 2004; **18**: 421-426 [PMID: 14735348 DOI: 10.1007/s00464-003-8109-x]

37 **Fiori E**, Lamazza A, De Cesare A, Bononi M, Volpino P, Schillaci A, Cavallaro A, Cangemi V. Palliative management of malignant rectosigmoidal obstruction. Colostomy vs. endoscopic stenting. A randomized prospective trial. *Anticancer Res* 2004; **24**: 265-268 [PMID: 15015606]

38 **Fiori E**, Lamazza A, Schillaci A, Femia S, Demasi E, Decesare A, Sterpetti AV. Palliative management for patients with subacute obstruction and stage IV unresectable rectosigmoid cancer: colostomy versus endoscopic stenting: final results of a prospective randomized trial. *Am J Surg* 2012; **204**: 321-326 [PMID: 22575396 DOI: 10.1016/j.amjsurg.2011.11.013]

39 **van Hooft JE**, Fockens P, Marinelli AW, Timmer R, van Berkel AM, Bossuyt PM, Bemelman WA; Dutch Colorectal Stent Group. Early closure of a multicenter randomized clinical trial of endoscopic stenting versus surgery for stage IV left-sided colorectal cancer. *Endoscopy* 2008; **40**: 184-191 [PMID: 18322873 DOI: 10.1055/s-2007-995426]

40 **Zhao XD**, Cai BB, Cao RS, Shi RH. Palliative treatment for incurable malignant colorectal obstructions: a meta-analysis. *World J Gastroenterol* 2013; **19**: 5565-5574 [PMID: 24023502 DOI: 10.3748/wjg.v19.i33.5565]

41 **Cézé N**, Charachon A, Locher C, Aparicio T, Mitry E, Barbieux JP, Landi B, Dorval E, Moussata D, Lecomte T. Safety and efficacy of palliative systemic chemotherapy combined with colorectal self-expandable metallic stents in advanced colorectal cancer: A multicenter study. *Clin Res Hepatol Gastroenterol* 2016; **40**: 230-238 [PMID: 26500200 DOI: 10.1016/j.clinre.2015.09.004]

42 **Han JP**, Hong SJ, Kim SH, Choi JH, Jung HJ, Cho YH, Ko BM, Lee MS. Palliative self-expandable metal stents for acute malignant colorectal obstruction: clinical outcomes and risk factors for complications. *Scand J Gastroenterol* 2014; **49**: 967-973 [PMID: 24874189 DOI: 10.3109/00365521.2014.920914]

43 **Scotti GB**, Sapienza P, Lapolla P, Crocetti D, Tarallo M, Brachini G, Mingoli A, Fiori E. Endoscopic Stenting and Palliative Chemotherapy in Advanced Colorectal Cancer: Friends or Foes? *In Vivo* 2022; **36**: 1053-1058 [PMID: 35478131 DOI: 10.21873/invivo.12802]

44 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342 [PMID: 15175435 DOI: 10.1056/nejmoa032691]

45 **Bong JW**, Lee JL, Kim CW, Yoon YS, Park IJ, Lim SB, Yu CS, Kim TW, Kim JC. Risk Factors and Adequate Management for Complications of Bevacizumab Treatment Requiring Surgical Intervention in Patients With Metastatic Colorectal Cancer. *Clin Colorectal Cancer* 2018; **17**: e639-e645 [PMID: 30031634 DOI: 10.1016/j.clcc.2018.06.005]

46 **Fuccio L**, Correale L, Arezzo A, Repici A, Manes G, Trovato C, Mangiavillano B, Manno M, Cortelezzi CC, Dinelli M, Cennamo V, de Bellis M; KRASTENT Study Group. Influence of K-ras status and anti-tumour treatments on complications due to colorectal self-expandable metallic stents: a retrospective multicentre study. *Dig Liver Dis* 2014; **46**: 561-567 [PMID: 24630948 DOI: 10.1016/j.dld.2014.02.006]

47 **Park YE**, Park Y, Park SJ, Cheon JH, Kim WH, Kim TI. Outcomes of stent insertion and mortality in obstructive stage IV colorectal cancer patients through 10 year duration. *Surg Endosc* 2019; **33**: 1225-1234 [PMID: 30167945 DOI: 10.1007/s00464-018-6399-2]

48 **Imbulgoda A**, MacLean A, Heine J, Drolet S, Vickers MM. Colonic perforation with intraluminal stents and bevacizumab in advanced colorectal cancer: retrospective case series and literature review. *Can J Surg* 2015; **58**: 167-171 [PMID: 25799132 DOI: 10.1503/cjs.013014]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 May 24; 14(5): 198-202

DOI: 10.5306/wjco.v14.i5.198

ISSN 2218-4333 (online)

MINIREVIEWS

Tyrosine kinase inhibitors and human epidermal growth factor receptor-2 positive breast cancer

Aya Abunada, Zaid Sirhan, Anita Thyagarajan, Ravi P Sahu

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kegyes D, Romania; Merrett ND, Australia

Received: January 9, 2023
Peer-review started: January 9, 2023
First decision: January 31, 2023
Revised: February 28, 2023
Accepted: April 21, 2023
Article in press: April 21, 2023
Published online: May 24, 2023

Aya Abunada, Department of Pharmacy, Sidra Medicine, Doha 0000, Qatar

Zaid Sirhan, Anita Thyagarajan, Ravi P Sahu, Department of Pharmacology and Toxicology, Boonshoft School of Medicine Wright State University, Dayton, OH 45435, United States

Corresponding author: Ravi P Sahu, BSc, MSc, PhD, Assistant Professor, Department of Pharmacology and Toxicology, Boonshoft School of Medicine Wright State University, 230 Health Sciences Bldg, 3640 Colonel Glenn Hwy, Dayton, OH 45435, United States. ravi.sahu@wright.edu

Abstract

The body of evidence investigating human epidermal growth factor receptor-2 (HER2) directed therapy in patients with breast cancer (BC) has been growing within the last decade. Recently, the use of tyrosine kinase inhibitors (TKIs) has been of particular interest in the treatment of human malignancies. This literature commentary is intended to highlight the most recent findings associated with the widely-studied TKI agents and their clinical significance in improving the outcomes of HER2 positive BC.

Key Words: Human epidermal growth factor receptor-2 positive breast cancer; Tyrosine kinase inhibitors; Lapatinib; Pyrotinib; Tucatinib; Trastuzumab

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Newly published randomized controlled trials within the past two years have provided compelling evidence on the use of tyrosine kinase inhibitors (TKIs) such as Lapatinib, Pyrotinib, Neratinib, Tucatinib, Ruxolitinib, and Afatinib. Several of these agents were found to offer better outcomes in terms of progression-free survival when combined with other agents. While some TKIs, namely Lapatinib, and Neratinib, are supported with a large amount of data than others, the medical literature still lacks substantial evidence to draw a clinical conclusion that could modify/add to the present recommendations in human epidermal growth factor receptor-2 positive breast cancer treatment guidelines.

Citation: Abunada A, Sirhan Z, Thyagarajan A, Sahu RP. Tyrosine kinase inhibitors and human epidermal growth factor receptor-2 positive breast cancer. *World J Clin Oncol* 2023; 14(5): 198-202
URL: https://www.wjgnet.com/2218-4333/full/v14/i5/198.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i5.198

INTRODUCTION

In 2022, breast cancer (BC) has been the most common cause of cancer-related mortality in women in the United States[1]. Amongst all confirmed BC cases, human epidermal growth factor receptor-2 (HER2) positive BC is estimated to comprise around 15%-20%[2]. Thus, the emergence of HER2-directed therapy, namely, humanized monoclonal antibodies (mAbs), has transformed the path of BC outcomes. The first agent, Trastuzumab, was approved by the United States Food and Drug Administration (FDA) in the past two decades and has revolutionized the treatment modalities[3]. Soon after the approval of other mAbs such as Pertuzumab, and ado-Trastuzumab emtansine, several tyrosine kinase inhibitors (TKIs) have also been approved as targeted therapies[4]. Figure 1 illustrates various TKIs and their targets. Within the last two years (2021 and 2022), significant additions to the literature were made on the use of TKIs in HER2 positive BC. This commentary aims to highlight the most recent findings published in the literature up to this date. Furthermore, since all TKIs, (*e.g.*, Lapatinib, Neratinib, Pyrotinib, and Tucatinib) can be used to treat both early stages and metastatic BC (mBC), either in combination or as monotherapy, their addition to hospital formularies can be of benefit from a pharmacoeconomic perspective[5]. The summary highlighting the ongoing and completed/terminated clinical trials on TKIs in HER2 positive BC patients is given in Table 1.

In a recent phase III randomized controlled trials, dual HER2 blockade with Lapatinib, Trastuzumab, and an aromatase inhibitor (AI) was found to be superior compared to a single HER2 blockade with AI plus Lapatinib alone or Trastuzumab alone in terms of progression-free survival (PFS) in postmenopausal women [hazard ratio: 0.62 (95%CI: 0.45-0.88); $P = 0.0063$][6]. However, this trial was intended to offer an alternative regimen for patients not receiving chemotherapy, a scenario typically followed when chemotherapy is contraindicated[6]. Nevertheless, the question of whether dual blockade with Lapatinib + Trastuzumab combination can be superior to first-line chemotherapy in terms of PFS remained unanswered.

Conversely, in another phase III trial, Pyrotinib + Capecitabine combination was found to yield longer PFS [12.5 mo (95%CI: 9.7–not reached)] as compared to the arm receiving Lapatinib + Capecitabine treatment [6.8 mo (5.4–8.1); hazard ratio 0.39 (95%CI: 0.27–0.56); one-sided $P < 0.0001$][7]. However, unlike the above-mentioned trial, the patient population in this trial was comprised of mBC patients.

Along similar lines, when Neratinib + Capecitabine (N + C) treatment was compared to Lapatinib + Capecitabine (L + C) combination, N + C resulted in longer PFS (Median PFS = 7 mo compared to 5.4 mo; $P = 0.0011$)[8]. Besides, the duration of response (DoR) in N + C *vs* L + C was 11.1 mo *vs* 4.2 mo ($P < 0.0001$), and time to intervention for central nervous system (CNS) illness was 27.9% *vs* 33.8% ($P = 0.039$) in Asian patients with mBC who had previously received at least two HER2-directed regimens[8]. The effectiveness and safety profiles of the N + C combination in the Asian group matched those of the general population. The studies indicated that Neratinib may provide further advantages for HER2+ mBC patients treated with Trastuzumab-only regimens for their metastatic illnesses such as CNS[8].

With the scarcity of published evidence comparing the efficacy of Tucatinib to other TKIs, the question of whether it offers additional PFS benefit was investigated through one network meta-analysis[9]. The data demonstrated that the combination of Tucatinib + Trastuzumab + Capecitabine is regarded as the most effective option in improving both overall survival (OS) and PFS ($P = 0.003$ and $P < 0.0001$). With OS, the choices of Trastuzumab emtansine ($P < 0.004$) and Pertuzumab + Trastuzumab + Capecitabine ($P = 0.011$) are comparatively superior. On the other hand, Neratinib and Lapatinib resulted in greater improvement in PFS ($P = 0.001$) when combined with Capecitabine[9].

However, despite the promising efficacy of Tucatinib over other TKIs, it was associated with increased levels of serum creatinine, which was concerning regarding its effect on renal function. However, the increase in serum creatinine level was found to be attributed to the inhibition of tubular secretion of creatinine[10]. Importantly, one study evaluated the use of Tucatinib *vs* placebo when both were combined with Trastuzumab and Capecitabine. It was concluded that Tucatinib can significantly improve OS (9.1 mo longer in the Tucatinib group) and delay the progression of brain metastasis [hazard ratio, 0.55 (95%CI: 0.36-0.85)][11].

Of note, within the last two years, no additional data regarding Afatinib's use in HER2 positive BC was published. Notably, only one study reported the benefits of Afatinib but the subjects included were not limited to BC, and those included BC patients were not HER2 positive[12]. Thus, there is no significant update regarding Afatinib's role in HER2 positive BC treatment.

Table 1 Main ongoing and completed phase 3 trials evaluating tyrosine kinase inhibitors with HER2+ breast cancer

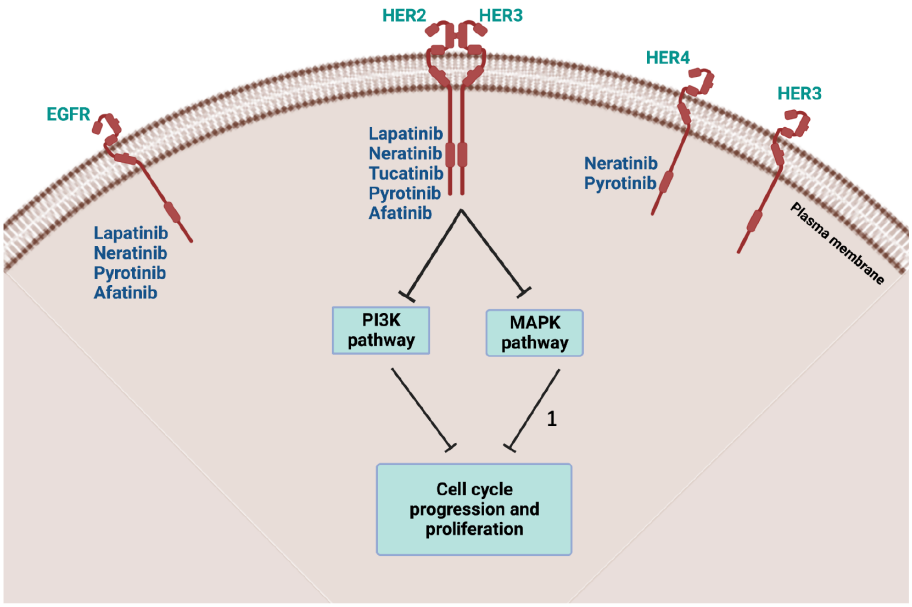
Study title	Conditions	Interventions	Outcome measures	NCT number
Pyrotinib rechallenge in HER2-positive metastatic breast cancer pretreated with Pyrotinib and Trastuzumab	HER2-positive breast cancer, metastatic breast cancer	Trastuzumab plus chemotherapy: Trastuzumab in combination with Pyrotinib plus chemotherapy	PFS, ORR, AEs	NCT05346861 [14]
A study of Pyrotinib plus Capecitabine in patients with HER2+ metastatic breast cancer	HER2 positive metastatic breast cancer	Pyrotinib, Capecitabine	PFS, ORR, AEs, SAEs, DoR, CBR, OS	NCT02973737 [15]
A randomized controlled trial of HER2 positive breast cancer patients treated with Lapatinib *vs* herceptin	HER2-positive breast cancer	Lapatinib/Herceptin	DFS, OS	NCT03085368 [16]
Tykerb evaluation after chemotherapy (TEACH): Lapatinib versus placebo in women with early-stage breast cancer	Neoplasms, breast	Lapatinib	This clinical trial has several outcomes measures to be evaluated including DFS, OS, MDFS	NCT00374322 [17]
Neo altto (neoadjuvant Lapatinib and/or Trastuzumab treatment optimization) study	Neoplasms, breast	Lapatinib, Trastuzumab, Paclitaxel	This clinical trial has several outcomes measures to be evaluated including OS, Par with pCR at the ToS, OR at the ToS	NCT00553358 [18]
Lapatinib in combination with Trastuzumab versus Lapatinib monotherapy in subjects with HER2-positive metastatic breast cancer	Neoplasms, breast	Lapatinib, Trastuzumab	PFS, OS, OR, CBR, TTR, DR, change from baseline in FACT-B scores at week 4, week 12, week 16, week 24, and conclusion or withdrawal from study	NCT00320385 [19]
Paclitaxel with/without GW572016 (Lapatinib) as first line therapy for women with advanced or metastatic breast cancer	Neoplasms, breast	Paclitaxel, GW572016 (Lapatinib)	This clinical trial has several outcomes measures to be evaluated including PFS, OS, DoR	NCT00075270 [20]
Continued HER2 suppression with Lapatinib plus Trastuzumab *vs* Trastuzumab alone (terminated)	Cancer	Lapatinib, Trastuzumab	PFS, OS, Best overall response, CBR (CR, PR or SD ≥ 24 wk), AE	NCT00968968 [21]

PFS: Progression-free survival; ORR: overall response rate; AEs: Adverse events; SAE; serious adverse events; DoR: Duration of response; OS: Overall survival; CBR: Clinical benefit rate; MDFS; Modified disease-free survival; Par: Number of participants; TTR: Time in the therapeutic range; DR: Duration of response; pCR: Pathological complete response; DFS: Disease-free Survival; FACT-B: Functional assessment of cancer therapy-breast cancer; OR: Overall response; ToS: Time of surgery; NCT: National clinical trial.

With Ruxolitinib, a class of the Janus kinase inhibitors, the first and only study performed so far with a Trastuzumab combination indicated that the tolerability data is appealing[12]. However, there was no difference in the PFS than that of Trastuzumab alone in mBC patients as compared to the historical control[13]. To draw a more robust conclusion regarding Ruxolitinib and explore its implications with TKIs, more interventional studies are warranted with larger power using randomized and prospective designs since these aspects are lacking in Ruxolitinib studies.

CONCLUSION

In conclusion, while the body of evidence currently available in the literature is still insufficient to offer recommendations in the treatment guidelines of HER2 positive BC, the existing studies concluding the benefits of TKIs promise hope for patients resistant to conventional first- and second-line treatments.



DOI: 10.5306/wjco.v14.i5.198 **Copyright** ©The Author(s) 2023.

Figure 1 Schematic representation of TKIs targeting EGFR and various HER family receptors, leading to the inhibition of downstream PI3K and MAPK pathway, resulting in the regulation of cell cycle progression and proliferation. [1]The sign denotes inhibition. The authors would like to acknowledge Biorender.com software that was used to create Figure 1.

FOOTNOTES

Author contributions: All the authors were involved in writing, editing, and approving the final version of the manuscript.

Supported by the Elsa U. Pardee Foundation Grant, No. 671432 (to Sahu RP); and NIH R21 Grant, No. ES033806 (to Sahu RP).

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: United States

ORCID number: Ravi P Sahu 0000-0003-3857-9073.

S-Editor: Li L
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Miller KD**, Fidler-Benaoudia M, Keegan TH, Hipp HS, Jemal A, Siegel RL. Cancer statistics for adolescents and young adults, 2020. *CA Cancer J Clin* 2020; **70**: 443-459 [PMID: 32940362 DOI: 10.3322/caac.21637]

2 **American Cancer Society**. Breast cancer HER2 status (2021) What is HER2 Status? American Cancer Society. [cited 17 April 2023]. Available from: https://www.cancer.org/cancer/breast-cancer/understanding-a-breast-cancer-diagnosis/breast-cancer-her2-status.html

3 **Collins DM**, Madden SF, Gaynor N, AlSultan D, Le Gal M, Eustace AJ, Gately KA, Hughes C, Davies AM, Mahgoub T, Ballot J, Toomey S, O'Connor DP, Gallagher WM, Holmes FA, Espina V, Liotta L, Hennessy BT, O'Byrne KJ, Hasmann M, Bossenmaier B, O'Donovan N, Crown J. Effects of HER Family-targeting Tyrosine Kinase Inhibitors on Antibody-dependent Cell-mediated Cytotoxicity in HER2-expressing Breast Cancer. *Clin Cancer Res* 2021; **27**: 807-818 [PMID: 33122343 DOI: 10.1158/1078-0432.CCR-20-2007]

4 **Yang X**, Wu D, Yuan S. Tyrosine Kinase Inhibitors in the Combination Therapy of HER2 Positive Breast Cancer. *Technol*

Cancer Res Treat 2020; **19**: 1533033820962140 [PMID: 33034269 DOI: 10.1177/1533033820962140]

5 **Schlam I**, Swain SM. HER2-positive breast cancer and tyrosine kinase inhibitors: the time is now. *NPJ Breast Cancer* 2021; **7**: 56 [PMID: 34016991 DOI: 10.1038/s41523-021-00265-1]

6 **Johnston SRD**, Hegg R, Im SA, Park IH, Burdaeva O, Kurteva G, Press MF, Tjulandin S, Iwata H, Simon SD, Kenny S, Sarp S, Izquierdo MA, Williams LS, Gradishar WJ. Phase III, Randomized Study of Dual Human Epidermal Growth Factor Receptor 2 (HER2) Blockade With Lapatinib Plus Trastuzumab in Combination With an Aromatase Inhibitor in Postmenopausal Women With HER2-Positive, Hormone Receptor-Positive Metastatic Breast Cancer: ALTERNATIVE. *J Clin Oncol* 2018; **36**: 741-748 [PMID: 29244528 DOI: 10.1200/JCO.2017.74.7824]

7 **Xu B**, Yan M, Ma F, Hu X, Feng J, Ouyang Q, Tong Z, Li H, Zhang Q, Sun T, Wang X, Yin Y, Cheng Y, Li W, Gu Y, Chen Q, Liu J, Cheng J, Geng C, Qin S, Wang S, Lu J, Shen K, Liu Q, Wang H, Luo T, Yang J, Wu Y, Yu Z, Zhu X, Chen C, Zou J; PHOEBE Investigators. Pyrotinib plus capecitabine versus lapatinib plus capecitabine for the treatment of HER2-positive metastatic breast cancer (PHOEBE): a multicentre, open-label, randomised, controlled, phase 3 trial. *Lancet Oncol* 2021; **22**: 351-360 [PMID: 33581774 DOI: 10.1016/S1470-2045(20)30702-6]

8 **Dai MS**, Feng YH, Chen SW, Masuda N, Yau T, Chen ST, Lu YS, Yap YS, Ang PCS, Chu SC, Kwong A, Lee KS, Ow S, Kim SB, Lin J, Chung HC, Ngan R, Kok VC, Rau KM, Sangai T, Ng TY, Tseng LM, Bryce R, Bebchuk J, Chen MC, Hou MF. Analysis of the pan-Asian subgroup of patients in the NALA Trial: a randomized phase III NALA Trial comparing neratinib+capecitabine (N+C) *vs* lapatinib+capecitabine (L+C) in patients with HER2+metastatic breast cancer (mBC) previously treated with two or more HER2-directed regimens. *Breast Cancer Res Treat* 2021; **189**: 665-676 [PMID: 34553296 DOI: 10.1007/s10549-021-06313-5]

9 **DeBusk K**, Abeysinghe S, Vickers A, Nangia A, Bell J, Ike C, Forero-Torres A, Blahna MT. Efficacy of tucatinib for HER2-positive metastatic breast cancer after HER2-targeted therapy: a network meta-analysis. *Future Oncol* 2021; **17**: 4635-4647 [PMID: 34463120 DOI: 10.2217/fon-2021-0742]

10 **Topletz-Erickson AR**, Lee AJ, Mayor JG, Rustia EL, Abdulrasool LI, Wise AL, Dailey B, DeChenne S, Walker LN, Alley SC, Endres CJ. Tucatinib Inhibits Renal Transporters OCT2 and MATE Without Impacting Renal Function in Healthy Subjects. *J Clin Pharmacol* 2021; **61**: 461-471 [PMID: 32989831 DOI: 10.1002/jcph.1750]

11 **Lin NU**, Murthy RK, Abramson V, Anders C, Bachelot T, Bedard PL, Borges V, Cameron D, Carey LA, Chien AJ, Curigliano G, DiGiovanna MP, Gelmon K, Hortobagyi G, Hurvitz SA, Krop I, Loi S, Loibl S, Mueller V, Oliveira M, Paplomata E, Pegram M, Slamon D, Zelnak A, Ramos J, Feng W, Winer E. Tucatinib vs Placebo, Both in Combination With Trastuzumab and Capecitabine, for Previously Treated ERBB2 (HER2)-Positive Metastatic Breast Cancer in Patients With Brain Metastases: Updated Exploratory Analysis of the HER2CLIMB Randomized Clinical Trial. *JAMA Oncol* 2023; **9**: 197-205 [PMID: 36454580 DOI: 10.1001/jamaoncol.2022.5610]

12 **Salawu A**, Hansen AR, Spreafico A, Al-Ezzi E, Webster S, Bedard PL, Doi J, Wang L, Siu LL, Abdul Razak AR. A Phase 2 Trial of Afatinib in Patients with Solid Tumors that Harbor Genomic Aberrations in the HER family: The MOBILITY3 Basket Study. *Target Oncol* 2022; **17**: 271-281 [PMID: 35635640 DOI: 10.1007/s11523-022-00884-z]

13 **Kearney M**, Franks L, Lee S, Tiersten A, Makower DF, Cigler T, Mundi P, Chi DC, Goel A, Klein P, Andreopoulou E, Sparano J, Trivedi M, Accordino M, Califano A, Hershman DL, Silva J, Kalinsky K. Phase I/II trial of ruxolitinib in combination with trastuzumab in metastatic HER2 positive breast cancer. *Breast Cancer Res Treat* 2021; **189**: 177-185 [PMID: 34169393 DOI: 10.1007/s10549-021-06306-4]

14 **Zhang J**. Pyrotinib Rechallenge in Her2-positive Metastatic Breast Cancer Pretreated With Pyrotinib and Trastuzumab. [accessed 2023 Apr 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: http://clinicaltrials.gov/show/NCT05346861 ClinicalTrials.gov Identifier: NCT05346861

15 **Jiangsu HengRui Medicine Co**. A Study of Pyrotinib Plus Capecitabine in Patients With HER2+ Metastatic Breast Cancer. [accessed 2023 Apr 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT02973737 ClinicalTrials.gov Identifier: NCT02973737

16 **Peking Union Medical College Hospital**. A Randomized Controlled Trial of HER-2 Positive Breast Cancer Patients Treated With Lapatinib vs Herceptin. [accessed 2023 Apr 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT03085368 ClinicalTrials.gov Identifier: NCT03085368

17 **GlaxoSmithKline**. Tykerb Evaluation After Chemotherapy (TEACH): Lapatinib Versus Placebo In Women With Early-Stage Breast Cancer. [accessed 2023 Apr 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT00374322 ClinicalTrials.gov Identifier: NCT00374322

18 **Novartis (Novartis Pharmaceuticals)**. Neo ALTTO (Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimisation) Study (Neo ALTTO). [accessed 2023 Apr 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT00553358 ClinicalTrials.gov Identifier: NCT00553358

19 **GlaxoSmithKline**. Lapatinib In Combination With Trastuzumab Versus Lapatinib Monotherapy In Subjects With HER2-positive Metastatic Breast Cancer. [accessed 2023 Apr 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT00320385 ClinicalTrials.gov Identifier: NCT00320385

20 **GlaxoSmithKline**. Paclitaxel With / Without GW572016 (Lapatinib) As First Line Therapy For Women With Advanced Or Metastatic Breast Cancer. [accessed 2023 Apr 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT00075270 ClinicalTrials.gov Identifier: NCT00075270

21 **Novartis (Novartis Pharmaceuticals)**. Continued HER2 Suppression With Lapatinib Plus Trastuzumab Versus Trastuzumab Alone. [accessed 2023 Apr 15]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://clinicaltrials.gov/ct2/show/NCT00968968 ClinicalTrials.gov Identifier: NCT00968968

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 May 24; 14(5): 203-214

DOI: 10.5306/wjco.v14.i5.203

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

Thymoquinone enhances the antioxidant and anticancer activity of Lebanese propolis

Sima AlDreini, Zaynab Fatfat, Najwa Abou Ibrahim, Maamoun Fatfat, Hala Gali-Muhtasib, Hala Khalife

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Chen BH, Taiwan; Selamoglu Z, Turkey

Received: December 28, 2022
Peer-review started: December 28, 2022
First decision: March 15, 2023
Revised: March 24, 2023
Accepted: April 12, 2023
Article in press: April 12, 2023
Published online: May 24, 2023

Sima AlDreini, Najwa Abou Ibrahim, Hala Khalife, Rammal Rammal Laboratory (ATAC Group), Faculty of Sciences I, Hadath 1003, Lebanon

Zaynab Fatfat, Maamoun Fatfat, Hala Gali-Muhtasib, Department of Biology, American University of Beirut, Beirut 1107-2020, Lebanon

Hala Khalife, Applied Biochemistry Laboratory, School of Pharmacy, Camerino University, Camerino 62032, Italy

Corresponding author: Hala Khalife, PhD, Professor, Rammal Rammal Laboratory (ATAC Group), Faculty of Sciences I, Rafic Hariri University Campus, Hadath 1003, Lebanon and Applied Biochemistry Laboratory, School of Pharmacy, Camerino University, Camerino 62032, Italy. hala-khalifeh@hotmail.com

Abstract

BACKGROUND

Reactive oxygen species (ROS) are produced by multiple cellular processes and are maintained at optimal levels in normal cells by endogenous antioxidants. In recent years, the search for potential exogenous antioxidants from dietary sources has gained considerable attention to eliminate excess ROS that is associated with oxidative stress related diseases including cancer. Propolis, a resinous honeybee product, has been shown to have protective effects against oxidative stress and anticancer effects against several types of neoplasms.

AIM

To investigate the antioxidant and anticancer potential of Lebanese propolis when applied alone or in combination with the promising anticancer compound Thymoquinone (TQ) the main constituent of *Nigella sativa* essential oil.

METHODS

Crude extracts of Lebanese propolis collected from two locations, Rashaya and Akkar-Danniyeh, were prepared in methanol and the total phenolic content was determined by Folin–Ciocalteu method. The antioxidant activity was assessed by the ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and to inhibit H_2O_2-induced oxidative hemolysis of human erythrocytes. The anticancer activity was evaluated by [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] MTT assay against HCT-116 human colorectal cancer cells and MDA-MB-231 human breast cancer cells.

RESULTS

The total phenolic content of propolis extract from Rashaya and Akkar-Danniyeh were 56.81 μg and 83.503 μg of gallic acid equivalent /mg of propolis, respectively. Both natural agents exhibited strong antioxidant activities as evidenced by their ability to scavenge DPPH free radical and to protect erythrocytes against H_2O_2-induced hemolysis. They also dose-dependently decreased the viability of both cancer cell lines. The IC_{50} value of each of propolis extract from Rashaya and Akkar-Danniyeh or TQ was 22.3, 61.7, 40.44 μg/mL for breast cancer cells at 72 h and 33.3, 50.9, 33.5 μg/mL for colorectal cancer cells at the same time point, respectively. Importantly, the inhibitory effects of propolis on DPPH radicals and cancer cell viability were achieved at half its concentration when combined with TQ.

CONCLUSION

Our results indicate that Lebanese propolis extract has antioxidant and anticancer potential and its combination with TQ could possibly prevent ROS- mediated diseases.

Key Words: Lebanese propolis; Thymoquinone; Combination; Antioxidant activity; Anticancer activity; Phenolic compounds

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Combining Thymoquinone with Lebanese propolis enhanced its antioxidant activity and its anticancer effects against breast and colorectal cancer cells. The combination of these natural products could have potential health benefits and could possibly prevent oxidative stress mediated diseases including cancer.

Citation: AlDreini S, Fatfat Z, Abou Ibrahim N, Fatfat M, Gali-Muhtasib H, Khalife H. Thymoquinone enhances the antioxidant and anticancer activity of Lebanese propolis. *World J Clin Oncol* 2023; 14(5): 203-214
URL: https://www.wjgnet.com/2218-4333/full/v14/i5/203.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i5.203

INTRODUCTION

Oxidative stress refers to the imbalance between the generation of reactive oxygen species (ROS) and their neutralization by endogenous antioxidant systems resulting in an excess of ROS which has detrimental effects on key cellular components[1,2]. There are two types of ROS: Free radicals and nonradicals. Free radicals are highly reactive molecules because they have at least one unpaired electron in their structure and react with different biological macromolecules[3]. Although nonradical species are less reactive than free radicals, they can easily cause free radical reactions in living organisms[3,4]. The accumulation of ROS causes the peroxidation of cell membrane lipids and cell membrane disintegration, alters the configuration of proteins resulting in loss of biochemical functionality in addition to inducing DNA mutations and replication errors[2]. Ample evidence shows that ROS-mediated oxidative stress is associated with the pathogenesis of various diseases including cancer, cardiovascular diseases, neurodegenerative disorders, and diabetes[5].

Removing excessive ROS by exogenous antioxidants supplementation has long been considered a potential strategy to prevent diseases. Over the last decade, there has been considerable interest in the intake of natural antioxidants from food and diets to strengthen cell antioxidant defense in humans. A recent pilot study demonstrated that a healthy mixed diet rich in antioxidant micronutrients reduced the concentration of ROS in the blood of healthy subjects[6]. Another study showed that regular consumption of an antioxidant-rich juice increased plasma antioxidant capacity and reduced plasma lipid oxidation in healthy individuals[7]. In addition, several clinical trials showed that intake of foods rich in antioxidants can potentiate plasma antioxidant capacity and reduce oxidative stress markers in subjects with diabetes, obesity, and dyslipidemia[8]. Interestingly, the combination of several antioxidants has been suggested to be more potent than the application of single antioxidants given the diverse chemistry and biochemistry of ROS, and the interactions that could arise from antioxidants that have different modes of action[9].

Propolis is a glue-like resinous material produced by honeybees from various plant sources and used in the construction and maintenance of their hives[10,11]. Propolis possesses numerous health-promoting potentials including anti-inflammatory[12], antioxidant[13], anticancer[14] and antidiabetic effects[15]. The chemical composition and therefore the biological effects of propolis vary depending on

several factors such as the geographical region, botanical source, and the bee species[16]. The bioactive compounds of propolis were reported to effectively scavenge free radicals[17]. Different *in vivo* studies reported the protective effects of propolis against the oxidative stress induced by several exogenous oxidants such as cisplatin[18], isoproterenol[19], nicotine[20], UV[21], and carbon tetrachloride[22]. In addition, propolis was demonstrated to reduce the blood pressure and suppress oxidative stress in heart, liver, and renal tissues in animal models of hypertension[23-25].

Thymoquinone (TQ), the major bioactive constituent of *Nigella sativa* (black seed) essential oil, was extensively studied for its diverse therapeutic benefits including antioxidant, anti-inflammatory, anticancer, antibacterial, antifungal and anticonvulsant activity[26]. TQ was reported as a strong scavenger of different ROS and was found to inhibit non-enzymatic lipid peroxidation[27]. TQ was demonstrated to have a protective effect against oxidative stress induced in rats by different agents such as radiation[28], lead[29] and acrylamide[30]. In addition, it reduced the oxidative stress in rat models of myocardial infarction[31], diabetes mellitus[32], lung injury[33] and dopaminergic neurodegeneration [34].

Although the antioxidant potential of propolis and TQ has been well investigated in previous studies, there are no studies that have evaluated the antioxidant and anticancer effects of the combination of both natural agents. Thus, we aimed to test the antioxidant and anticancer activities of combining TQ and propolis that was collected from two locations in Lebanon (Rashaya and Akkar-Danniyeh). We evaluated the total phenolic content of both propolis extract and determined the antihemolytic and antioxidant activity of propolis and TQ in addition to their anticancer effects against HCT-116 human colorectal cancer cells and MDA-MB-231 human breast cancer cells.

MATERIALS AND METHODS

Preparation of thymoquinone

Fresh stocks of the purified synthetic compound TQ (Sigma-Aldrich) were prepared in methanol directly before use.

Preparation of methanol extracts of propolis

Two samples of raw propolis material were collected, the first from Rashaya district in the Beqaa governorate of Lebanon and the second from Akkar-Danniyeh in the north of the country. A mass of 10 g of raw propolis from each sample was chopped into small pieces and extracted with 100 mL distilled water. The extraction was carried at 80°C for 3 h and the obtained solution was subsequently filtered through a Buchner funnel. Residues were then extracted with 100 mL methanol. The extraction was carried at room temperature for 4 h then at 50°C for 15 min. The propolis extracts were subsequently filtered three times by Buchner funnel. The obtained filtrate was evaporated by nitrogen gas to obtain the methanol propolis extract (MPE). MPE-R denotes MPE from Rashaya and MPE-D denotes MPE from Akkar-Danniyeh.

Total phenolic content

The relative content in phenols was determined according to the Folin Ciocalteu method. Briefly, 100 µL of MPE-R or MPE-D (1 mg/mL of methanol) from each sample were mixed with 500 µL of Folin Ciocalteu's phenol reagent 10%. After 5 min, 1.5 mL of 2% sodium bicarbonate were added to the solution. The mixture was maintained at room temperature in the dark for 30 min after which the absorbance was recorded at 760 nm using a spectrophotometer. The total phenolic content was calculated using the calibration curve generated from standard solutions of gallic acid ranging from 0 to 50 µg/mL ($y = 0.2811x - 0.3266$; $R^2 = 0.956$). Total phenolic content was expressed as the average of 3 independent experiments performed in triplicates and as µg of gallic acid equivalents (GAE)/mg of propolis.

DPPH assay

Free radical-scavenger activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Briefly, 1 mL of MPE-R or MPE-D (10-100 µg/mL) were mixed with 1 mL of DPPH (0.052 mg/mL methanol). The reaction mixtures were homogenized and incubated in the dark at room temperature for 30 min and the absorbance (Abs) was measured at 515 nm by a Gene Quant 1300 UV-Vis spectrophotometer. The ascorbic acid was used as a reference antioxidant and a mixture of 1 mL DPPH with 1 mL methanol was used as a control. For combination treatments, TQ (12.5-100 µg/mL) was combined with MPE-R or MPE-D (10-50 µg/mL) and the experiment was carried as described above. The DPPH scavenging ability of the different agents was calculated using the following equation: % DPPH inhibition = [(Abs control − Abs sample)]/ (Abs control)] × 100.

H_2O_2- induced hemolysis

Fresh human blood was washed three times with 1X phosphate-buffered saline (PBS). With every wash,

the sample was centrifuged for 12 min at 4°C and 2500 rpm, the supernatant was discarded, and the pellet was resuspended in PBS. Then, the pellet was resuspended in Dulbecco's PBS and 1 mL of the cell suspension was mixed with 100 µL of each of MPE-R, MPE-D or TQ at 10, 50, and 100 µg/mL. After 5 min, 1 mL of 10% H_2O_2 was added, and the mixture was incubated at 37°C for 90 min and shaken every 30 min. This was followed by centrifugation at 4°C and 2500 rpm for 10 min and measurement of the absorbance of the supernatant at 540 nm. The positive control consisted of a mixture of blood with 10% H_2O_2. The results were expressed as percentage of inhibition of hemolysis. % inhibition of hemolysis= [(Abs control − Abs sample)]/(Abs control)] × 100

Hemolytic activity

Fresh human blood was washed three times with 1X PBS. With every wash, the sample was centrifuged at 4°C and 2500 rpm for 12 min, the supernatant was discarded, and the pellet was resuspended in PBS. The washed blood was mixed with each of MPE-R or MPE-D (10, 100, 200 µg/mL), TQ (20, 50, 100 µg/mL) or their combinations. The mixture was kept at 37°C for 90 min and was shaken every 30 min. The samples were then centrifuged at 4°C, 2500 rpm, for 10 min after which the absorbance of the supernatant was recorded at 540 nm. The positive control consisted of a mixture of blood with 1% SDS which is known to cause hemolysis. The results were expressed as the percentage of hemolysis. % hemolysis= [Abs sample/ Abs control] × 100

Cell culture conditions

HCT-116 human colorectal cancer cells and MDA-MB-231 human breast cancer cells were maintained in RPMI 1640 (Lonza; Cat.N: BE12-115F) supplemented with 10% fetal bovine serum (Sigma F9665) and 1% penicillin/ streptomycin (Sigma, P4333) in a humidified atmosphere at 37°C in 5% CO_2.

MTT cell viability assay

HCT-116 and MDA-MB-231 cells were seeded overnight in 96-well microtiter plates at a density of 10^4 cells/well. After 24 h, cells were treated with MPE-R, MPE-D or TQ at a concentration ranging from 1-15 µg/mL or with the combination of MPE-R or MPE-D (0.5-7.5 µg/mL) with TQ (0.5-7.5 µg/mL). After 24, 48 and 72 h of treatment, cells in each well were incubated with 20 µL of [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] MTT for 3 to 4 h, then with 100 µL of DMSO for about 1 h. The MTT optical density (O.D.) was then measured by a microplate spectrophotometer at 515 nm. The results are expressed as percentage of viable cells with respect to the untreated control using this formula: % viability = [mean O.D. treatment/mean O.D. control] × 100.

Statistical analysis

Data are presented as means ± SD. Two tailed Student's *t*-test was performed to evaluate the statistical significance of the difference between the groups using GraphPad Prism V.9.5.0 software. Statistical significance was set with a 95% confidence interval at $P < 0.05$, $P < 0.01$ and $P < 0.0001$.

RESULTS

Total phenolic content of propolis varies depending on location

The total phenolic content of propolis extracts was determined by Folin Ciocalteu method and is reported as gallic acid equivalents by reference to a standard curve (y = 0.2811x - 0.3266; $R^2 = 0.956$). The phenolic content was variable depending on location such that the total phenolic content of MPE- D in 1 mg of propolis was 47% higher than that of MPE-R(Table 1).

TQ enhanced the antioxidant activity of propolis

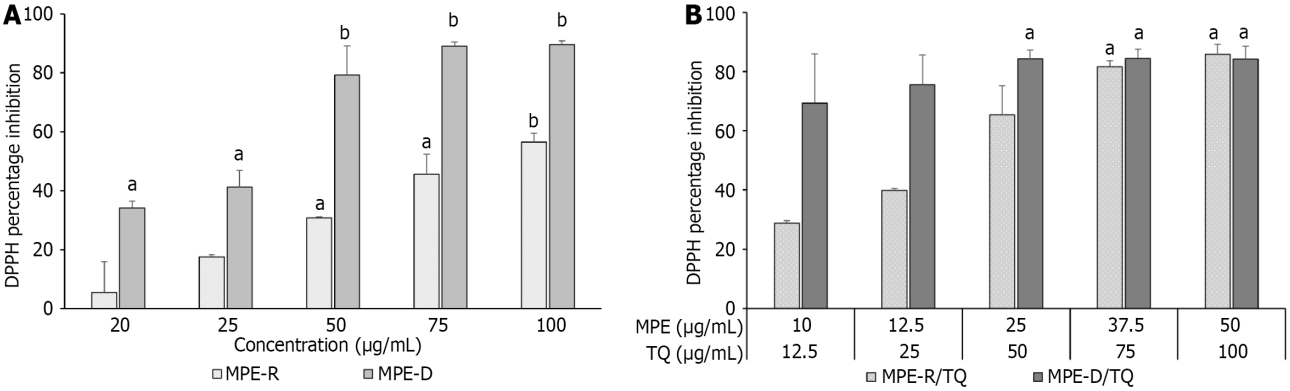
We then evaluated the ability of propolis extracts to scavenge free radicals using DPPH radical scavenging assay. Both propolis extract exhibited a dose-dependent DPPH inhibition efficiency suggesting antioxidant potential. MPE-D had higher antioxidant activity than MPE-R as reflected by the higher percentages of inhibition recorded at all the concentrations ranging from 20-100 µg/mL. MPE-R showed maximum inhibition of DPPH of 56.5% at 100 µg/mL, while inhibition by MPE-D reached 89% at 75 and 100 µg/mL (Figure 1A).

To determine whether the antioxidant effects of propolis extracts could be potentiated by TQ, we combined each of MPE-R or MPE-D (10-50 µg/mL) with TQ (12.5-100 µg/mL) and evaluated their antioxidant activity in comparison to single treatments. Results showed that the combination with TQ enhanced the antioxidant activity of propolis extracts. While a dose of 100 µg/mL of MPE-R induced 56.5% inhibition of DPPH, the combination of 50 µg/mL of MPE-R with 100 µg/mL TQ caused 85.7% inhibition. MPE-D alone showed maximal inhibitory effects of 89% at 75-100 µg/mL, while combination with 50-100 µg/mL TQ resulted in 84% inhibition at lower concentrations of 25-50 µg/mL (Figure 1B).

Table 1 Total phenolic content of methanol propolis extract from Rashaya and Akkar-Danniyeh in µg of gallic acid equivalents/mg of propolis and µg/mL of methanol propolis extract

	TPC (µg GAE/mg)	TPC (µg GAE/mL of MPE)
MPE-R	56.81	2.3
MPE-D	83.503	3.997

TPC: Total phenolic content; MPE: Methanol propolis extract; MPE-R: Methanol propolis extract from Rashaya; MPE-D: Methanol propolis extract from Akkar-Danniyeh; GAE: Gallic acid equivalents.



DOI: 10.5306/wjco.v14.i5.203 **Copyright** ©The Author(s) 2023.

Figure 1 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of methanol propolis extract alone or in combination with Thymoquinone. A: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of each of methanol propolis extract from Rashaya (MPE-R) and from Akkar-Danniyeh (MPE-D; 20-100 µg/mL) alone; B: DPPH free radical scavenging activity of each of MPE-R and MPE-D (10-50 µg/mL) in combination with Thymoquinone (TQ; 12.5-100 µg/mL). The samples were mixed with DPPH and the absorbance of the mixture was measured after 30 min. The values are expressed as percentage of DPPH percentage inhibition relative to the control. Each value represents the mean ± SD of $n = 2$ experiments. $^{a}P < 0.05$ and $^{b}P < 0.01$ are significantly different from control using two-tailed Student's *t*-test.

Propolis extracts and TQ protected human red blood cells against oxidative hemolysis

We then evaluated the biological relevance of the antioxidant activity of propolis extracts and of TQ by testing the protective effects of single treatments against oxidative hemolysis induced by H_2O_2 in human red blood cells. Treatment with MPE-R, MPE-D or TQ exhibited good antihemolytic potential against H_2O_2-induced hemolysis. A dose of 10 µg/mL of each of MPE-R, MPE-D and TQ induced 46, 49 and 51% decrease in hemolysis, respectively (Figure 2).

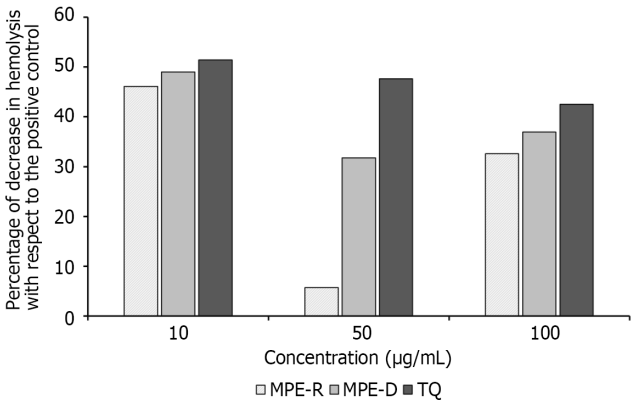
The combination of propolis extracts with TQ had no hemolytic activity at low concentrations

To investigate if propolis extracts or TQ are toxic to human red blood cells, we evaluated their hemolytic potential at concentrations ranging from 10- 200 µg/mL and 20- 100 µg/mL, respectively. Both MPE-R and MPE-D produced less than 5% hemolysis at 10 µg/mL, suggesting that these extracts are not toxic to red blood cells at this concentration. Increasing concentrations of MPE-D up to 100 or 200 µg/mL also showed low hemolytic activity of 7.8%. Similarly, hemolysis by TQ was less than 5% at all the tested concentrations. However, MPE-R induced higher hemolytic response that reached 20% at 200 µg/mL (Figure 3A).

Combining 5 µg/mL MPE-R or MPE-D with 10 µg/mL TQ produced less than 5% hemolysis suggesting that combinations at these low doses have low hemolytic effects. However, increasing concentrations to 50 µg/mL MPE-R and 25 µg/mL TQ or 100 µg/mL MPE-R and 50 µg/mL TQ produced 12.7% and 21.9% hemolysis, respectively. Similar concentrations of MPE-D and TQ produced 7.3% and 13.7% hemolysis, respectively (Figure 3B), suggesting that MPE-R had higher hemolytic effects when combined with TQ at higher doses.

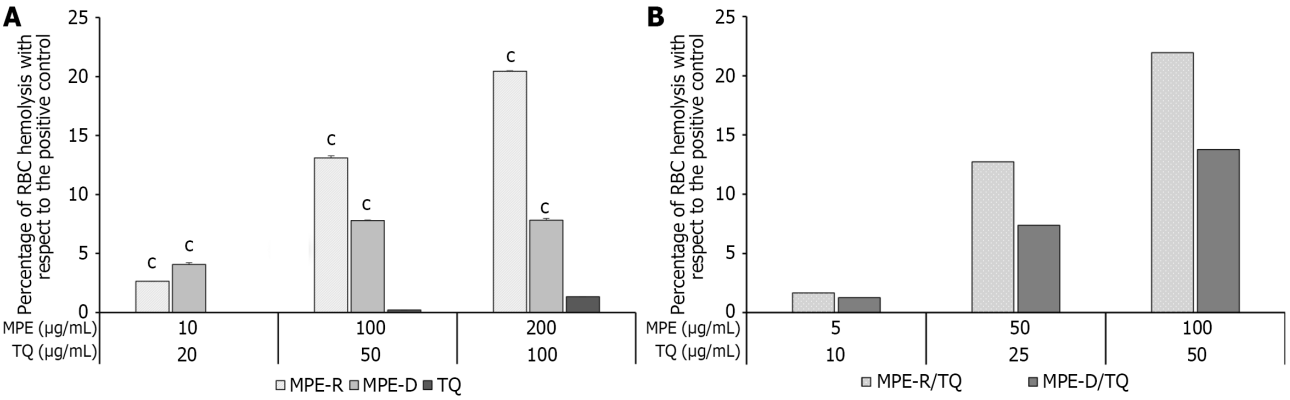
TQ potentiated the inhibitory effects of propolis extracts on cancer cell viability

Next, we tested the anticancer activity of propolis extracts when applied alone or in combination with TQ. MDA-MB-231 human breast cancer cells and HCT-116 human colorectal cancer cells were treated with different concentrations of the natural products for 24, 48 and 72 h after which cell viability was assessed by MTT assay. Single treatments with MPE-R or MPE-D (1-15 µg/mL) reduced the viability of both cell lines in a dose dependent manner to almost similar levels. Treatment of MDA-MB 231 cells with 15 µg/mL of MPE-R, MPE-D or TQ for 72 h caused 34.6%, 18.5% and 24.52% reduction in cell



DOI: 10.5306/wjco.v14.i5.203 **Copyright** ©The Author(s) 2023.

Figure 2 *In vitro* antihemolytic/cytoprotective activity of each of Thymoquinone and methanol propolis extract from Rashaya and Akkar-Danniyeh against H_2O_2- induced oxidative hemolysis. Human red blood cells suspension was preincubated with methanol propolis extract from Rashaya (MPE-R), methanol propolis extract from Akkar-Danniyeh (MPE-D), or Thymoquinone (TQ; 10-100 µg/mL) for 5 min. The cell suspension was then incubated with 10% H_2O_2 for 90 min at 37°C. The samples were then centrifuged, and the absorbance of the supernatant was measured. The values are expressed as percentage of decrease in hemolysis with respect to the positive control (10 % H_2O_2). Each value is obtained from n = 1 experiment performed in monoplicate.



DOI: 10.5306/wjco.v14.i5.203 **Copyright** ©The Author(s) 2023.

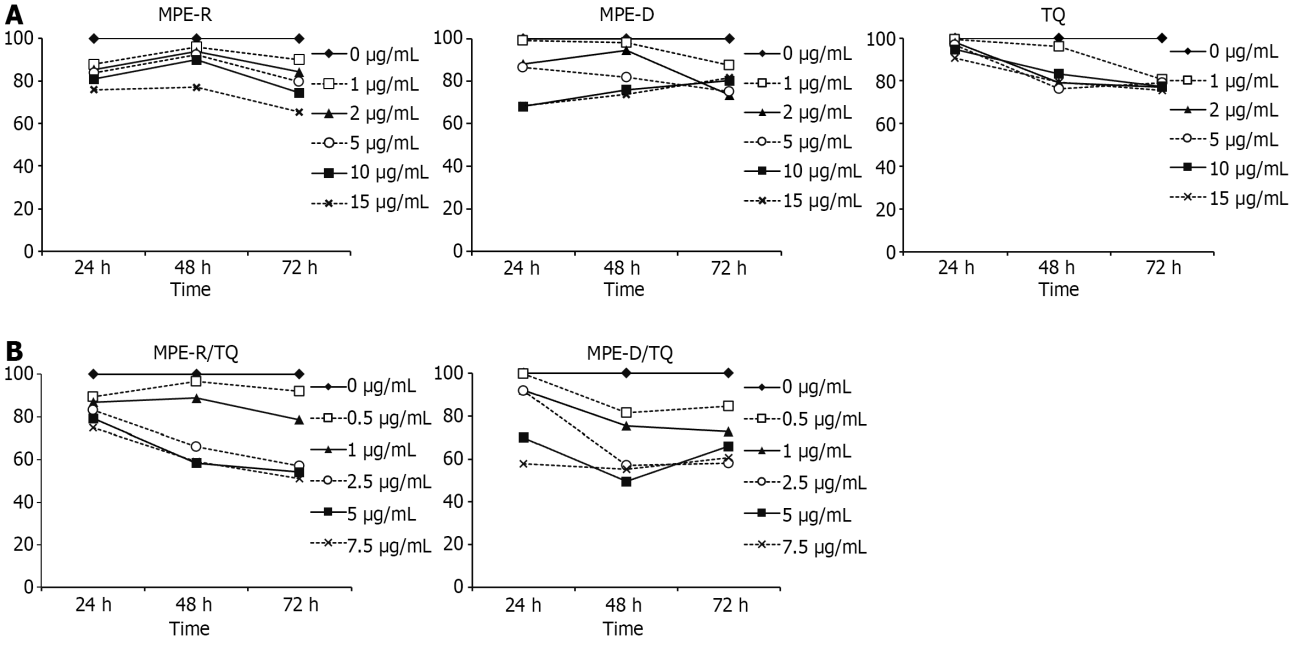
Figure 3 *In vitro* hemolytic activity of each of methanol propolis extract from Rashaya and Akkar-Danniyeh alone or in combination with Thymoquinone. A: Hemolytic activity of each of methanol propolis extract from Rashaya (MPE-R) and methanol propolis extract from Akkar-Danniyeh (MPE-D; 10-200 µg/mL) and Thymoquinone (TQ; 20-100 µg/mL); B: hemolytic activity of the combination of MPE- R or- D (5-100 µg/mL) and TQ (10-50 µg/mL). Washed fresh human blood was incubated with the natural products for 90 min. The samples were then centrifuged, and the absorbance of the supernatant was measured. The values are expressed as percentages of red blood cells hemolysis with respect to the positive control (SDS 1%). Each value represents the mean ± SD of n = 3 experiments for MPE-R and MPE-D single treatments and n = 1 for TQ single treatment and combination treatments. $^cP < 0.0001$ is significantly different from positive control using two-tailed Student's t-test.

viability, respectively. The IC_{50} value of each of MPE-R, MPE-D or TQ at 72 h was 22.3, 61.7, 40.44 µg/mL, respectively. Combining lower doses of 7.5 µg/mL MPE-R or MPE-D with 7.5 µg/mL TQ decreased cell viability by 48.9% and 39.3%, respectively (Figure 4A and B), suggesting enhanced efficacy by combination treatment.

Treatment of HCT-116 cells for 72 h with 15 µg/mL of MPE-R, MPE-D or TQ decreased cell viability by 18.6, 14.3 and 26%, respectively. The IC_{50} value of each of MPE-R, MPE-D or TQ at 72 h was 33.3, 50.9, 33.5 µg/mL, respectively. Interestingly, the combination of half doses of MPE-R or MPE-D (7.5 µg/mL) with 7.5 µg/mL TQ caused a respective decrease in viability of 40.9% and 34.4% at 72 h (Figure 5A and B). Thus, combining propolis extracts with TQ enhanced their anticancer activities against breast and colorectal cancer cells.

DISCUSSION

The intake of dietary antioxidants is known to support the endogenous antioxidant system and prevent oxidative stress-mediated diseases[35]. Studies have shown that combining dietary antioxidants from different sources produces more potent antioxidant effects and possibly more effective therapeutic potential than single agents. Combining *Nigella sativa* oil with honey was shown to augment its



DOI: 10.5306/wjco.v14.i5.203 **Copyright** ©The Author(s) 2023.

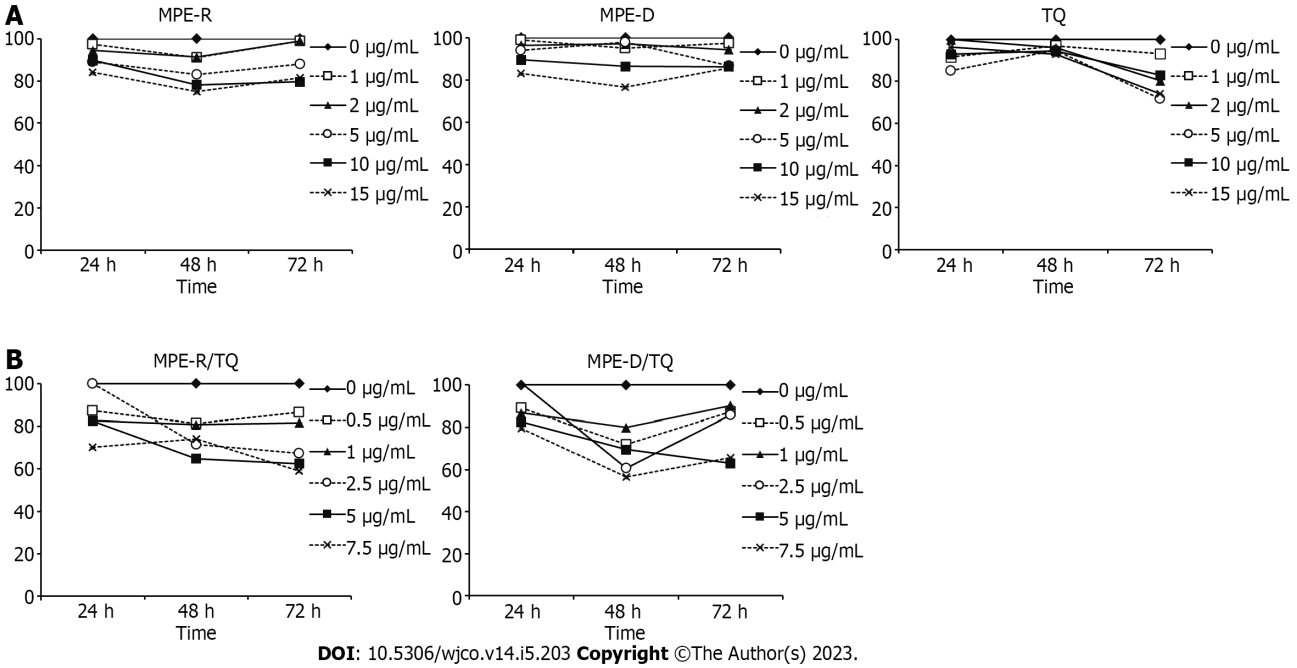
Figure 4 Anticancer activity of Thymoquinone and methanol propolis extract from Rashaya and Akkar-Danniyeh against MDA-MB-231 human breast cancer cells. A: Cells were treated with each of methanol propolis extract from Rashaya (MPE-R), methanol propolis extract from Akkar-Danniyeh (MPE-D) and Thymoquinone (TQ; 0-15 μg/mL) alone for 24, 48 and 72 h; B: Cells were treated with the combination of each of MPEs (0-7.5 μg/mL) with TQ (0-7.5 μg/mL) for the same time point. Cell viability was then determined using MTT assay. The values are expressed as percentage of viable cells relative to untreated control. Each value represents the mean ± SD of n = 1 experiment performed in duplicates.

antioxidant capacity[36]. In addition, the combination of *Nigella sativa* seeds and honey exhibited antioxidant effects and decreased the viability of ovarian cancer cells[37]. Interestingly, oral intake of honey potentiated the protective effect of *Nigella sativa* grains against methylnitrosourea-induced oxidative stress and carcinogenesis in Sprague Dawely rats[38]. Here, we evaluated the antioxidant and the anticancer potential of combining propolis, the third most important component of bee products [39], with TQ as the major bioactive constituent of *Nigella sativa* essential oil. The key finding of the present study is that combining TQ with Lebanese propolis at half its concentration resulted in an enhanced antioxidant and anticancer effects in comparison to propolis alone as demonstrated by the improved DPPH radical scavenging activity and inhibitory effects against breast and colorectal cancer cell lines.

First, we assessed the total phenolic content of propolis collected from two different Lebanese regions Rashaya and Akkar- Danniyeh. The phenolic content is the most widely investigated among all the components of propolis because it was reported to be mainly responsible for its biological activity[40]. According to the results reported by El-Ali *et al*[41], the total phenolic content of ethanol extract of propolis collected from the two Lebanese regions Debaal and Wadi Faara were similar to our study's finding. On the other hand, higher phenolic content values were recorded in the ethanol extract of propolis collected from the Lebanese regions Fakeha and Berqayel and the citrus groves of the Lebanese coast[41,42]. This variation in total phenolic content of propolis collected from different Lebanese regions could be attributed to several factors including the botanical origin of the raw material, mode of collection, collecting season, or the solvent used in the extraction method[40].

Next, we assessed the antioxidant activity of MPE-R and MPE-D alone or in combination with TQ using DPPH free radical scavenging test. DPPH is a stable nitrogen-centered free radical which color changes from violet to yellow when it receives a hydrogen- or electron- from an antioxidant[43]. MPE-R and MPE-D exhibited significant DPPH scavenging efficacy reflecting the presence of antioxidants within their constituents. Numerous studies reported a positive correlation between antioxidant activity of propolis extracts and their contents of phenolic compounds suggesting that they are responsible of the antioxidant activity of the extracts[41,44,45]. Phenolics are known to have a hydroxyl group attached to their aromatic ring which can donate electron to free radicals and therefore stabilize them[46]. As for TQ which is a non-phenolic compound, a recent computational study reported that it attacks free radical preferentially at its 3CH position and preferably *via* hydrogen atom transfer[47].

After demonstrating the antioxidant efficacy of each of MPE-R, MPE-D and TQ, we assessed their potential to protect red blood cells from oxidative damage and hemolysis induced by H_2O_2. Red blood cells are highly prone to oxidative damage due to its high membrane concentration of polyunsaturated fatty acids[48]. When the membrane lipids of red blood cells are subjected to ROS attack, they lose a hydrogen atom from an unsaturated fatty acyl chain. This initiates lipid peroxidation that propagates as



DOI: 10.5306/wjco.v14.i5.203 **Copyright** ©The Author(s) 2023.

Figure 5 Anticancer activity of Thymoquinone and methanol propolis extract from Rashaya and Akkar-Danniyeh against HCT-116 human colorectal cancer cells. A: Cells were treated with each of methanol propolis extract from Rashaya (MPE–R), methanol propolis extract from Akkar-Danniyeh (MPE-D) and Thymoquinone (TQ; 0-15 μg/mL) alone for 24, 48 and 72 h; B: Cells were treated with the combination of each of MPEs (0-7.5 μg/mL) with TQ (0-7.5 μg/mL) for the same time point. Cell viability was then determined using MTT assay. The values are expressed as percentage of viable cells relative to untreated control. Each value represents the mean ± SD of n = 1 experiment performed in duplicates.

a chain reaction and lead to membrane damage and consequently hemolysis[49,50]. Our findings are in the same line with previous research that has shown the anti-hemolytic activity of propolis or TQ under oxidative stress conditions[40,51]. The antihemolytic activity of MPE-R and MPE-D could be associated with their phenolic content. Phenolic compounds are supposed to donate electrons to hydrogen peroxide, neutralize it to water and prevent it to induce hemolysis[52].

The assessment of hemolytic activity of blood-contacting compounds is of high importance for their future application *in vivo*[53]. Our results are in agreement with those reported by Shubharani *et al*[54] who showed that low concentrations of ethanolic extract of Indian propolis did not have hemolytic activity. Although high concentrations of Lebanese propolis showed low to moderate hemolysis, same concentrations of Polish or Brazilian propolis extract did not cause hemolysis[40,55].

Cancer cells exhibit elevated levels of ROS which promote cell cycle progression and lead to an increase in cell proliferation[56]. By-products of oxidative damage such as 8-hydroxy-2-deoxy-guanosine, malondialdehyde, 4-hydroxy-2-nonenal, and carbonylated proteins were speculated to play a mutagenic role[57]. In addition, oxidative stress was found to be responsible for inactivation of several key proteins such as caspases, phosphatases, and phosphatase and tensin homologue, and inhibits p53 binding to gene promoters which reduce apoptosis and increase cell survival[58]. Dietary antioxidants have been demonstrated to have chemopreventive and anticancer effects *in vitro* and *in vivo*[59]. Numerous studies demonstrated the anticancer effect of each of TQ, propolis and its phenolic compounds in different types of cancer[60,61]. To our knowledge, this is the first study that demonstrates the promising anticancer effect of the combination of these agents. Only one study demonstrated the anticancer effect of Lebanese propolis collected from the south of the country on leukemic T cells[10]. Although MPE-D had higher antioxidant activity than MPE-R, the inhibitory effect of both extracts on the cell viability of cancer cell lines was almost the same. This result suggests that phenolic compounds may not be responsible for this inhibitory effect of the extracts.

CONCLUSION

In summary, the Lebanese propolis from Rashaya and Akkar-Danniyeh exhibited promising therapeutic potential as reflected by their potent DPPH radical scavenging activity, protective effects against H_2O_2 induced hemolysis and inhibitory effects against breast and colorectal cancer cell lines. The combination of TQ with propolis resulted in enhanced antioxidant and anticancer activities in comparison to single treatments. Thus, this combination could have potential health benefits and holds promise for the prevention of oxidative stress related diseases. Further studies should be conducted to analyze the

chemical composition of propolis, decipher the antioxidant and anticancer mechanism of its combination with TQ in addition to evaluating the effects of TQ and propolis in animal models of oxidative stress.

ARTICLE HIGHLIGHTS

Research background

Oxidative stress is implicated in the pathogenesis of numerous diseases including cancer. Propolis, the third most important component of bee products, and Thymoquinone (TQ), the main constituent of *Nigella sativa* essential oil, were extensively reported to have antioxidant and anticancer effects. However, the antioxidant potential of the combination of these natural products as well as their anticancer activity against breast and colorectal cancer cells have not been investigated yet.

Research motivation

To establish a new therapeutic approach for oxidative stress induced cancers using a combination of natural agents from food and diets.

Research objectives

To investigate the antioxidant and anticancer potential of Lebanese propolis and TQ alone and in combination.

Research methods

Folin–Ciocalteu method was used to determine the total phenolic content of the methanolic extract of propolis. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay and the H_2O_2-induced oxidative hemolysis of human erythrocytes *in vitro* assay were employed to assess the antioxidant activity of TQ and Lebanese propolis. The MTT assay was used to evaluate the anticancer activity of these natural agents in single and dual treatment against HCT-116 human colorectal cancer cells and MDA-MB-231 human breast cancer cells *in vitro*.

Research results

Combination of TQ with Lebanese propolis at half its concentration improved the antioxidant and anticancer activity of propolis as reflected by the enhanced DPPH radical scavenging activity and inhibitory effects against breast and colorectal cancer cells.

Research conclusions

Our results suggest the use of a combination of TQ and Lebanese propolis as potential therapy for the management of oxidative stress and treatment of breast and colorectal cancer. This is the first study to report the promising enhancement in Lebanese propolis antioxidant and anticancer activity when combined with TQ.

Research perspectives

Further research on the antioxidant and anticancer mechanisms of the combination of these natural agents and its therapeutic effects in animal models of oxidative stress should be performed in the future.

FOOTNOTES

Author contributions: AlDreini S carried out lab work as part of her MSc thesis, performed analysis and interpretation of data; Fatfat Z drafted the manuscript; Abou Ibrahim N provided propolis, and contributed intellectually to the study; Fatfat M supervised the experimental work; Khalife H reviewed the manuscript and contributed in the critical appraisal of data; Gali-Muhtasib H conceived the project, supervised the work, and edited the manuscript draft; All authors have read and approved the final manuscript.

Supported by the Lebanese University and the Undergraduate Research Experience funds at the American University of Beirut.

Institutional review board statement: There was no need to provide an Institutional Review Board Approval Form or Document since no human or animal subjects were involved in this study.

Conflict-of-interest statement: All the authors declare no conflicts of interest.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Lebanon

ORCID number: Sima AlDreini 0000-0002-8463-6780; Zaynab Fatfat 0000-0003-1254-0648; Abou Ibrahim Najwa 0000-0001-6051-0174; Maamoun Fatfat 0000-0001-9642-6708; Hala Gali-Muhtasib 0000-0001-6840-3015; Hala Khalife 0000-0001-8565-3585.

S-Editor: Liu JH
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Zhu L**, Yuan C, Zhang D, Ma Y, Ding X, Zhu G. BHV-1 induced oxidative stress contributes to mitochondrial dysfunction in MDBK cells. *Vet Res* 2016; **47**: 47 [PMID: 27000063 DOI: 10.1186/s13567-016-0332-2]
2 **Pisoschi AM**, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *Eur J Med Chem* 2021; **209**: 112891 [PMID: 33032084 DOI: 10.1016/j.ejmech.2020.112891]
3 **Phaniendra A**, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem* 2015; **30**: 11-26 [PMID: 25646037 DOI: 10.1007/s12291-014-0446-0]
4 **Ali AT**, Al-Swayeh OA, Al-Rashed RS, Al-Mofleh IA, Al-Dohayan AD, Al-Tuwaijri AS. Role of oxygen-derived free radicals on gastric mucosal injury induced by ischemia-reperfusion. *Saudi J Gastroenterol* 1996; **2**: 19-28 [PMID: 19864838]
5 **Sharifi-Rad M**, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Calina D, Cho WC, Sharifi-Rad J. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front Physiol* 2020; **11**: 694 [PMID: 32714204 DOI: 10.3389/fphys.2020.00694]
6 **Zeng Z**, Zdzieblik D, Centner C, Brauchle C, Gollhofer A, König D. Changing dietary habits increases the intake of antioxidant vitamins and reduces the concentration of reactive oxygen species in blood: A pilot study. *Int J Food Prop* 2020; **23**: 1337-1346 [DOI: 10.1080/10942912.2020.1800727]
7 **Díaz-Rubio ME**, Pérez-Jiménez J, Martínez-Bartolomé MÁ, Álvarez I, Saura-Calixto F. Regular consumption of an antioxidant-rich juice improves oxidative status and causes metabolome changes in healthy adults. *Plant Foods Hum Nutr* 2015; **70**: 9-14 [PMID: 25481643 DOI: 10.1007/s11130-014-0455-4]
8 **Ávila-Escalante ML**, Coop-Gamas F, Cervantes-Rodríguez M, Méndez-Iturbide D, Aranda-González II. The effect of diet on oxidative stress and metabolic diseases-Clinically controlled trials. *J Food Biochem* 2020; **44**: e13191 [PMID: 32160647 DOI: 10.1111/jfbc.13191]
9 **Bai H**, Liu R, Chen HL, Zhang W, Wang X, Zhang XD, Li WL, Hai CX. Enhanced antioxidant effect of caffeic acid phenethyl ester and Trolox in combination against radiation induced-oxidative stress. *Chem Biol Interact* 2014; **207**: 7-15 [PMID: 24211618 DOI: 10.1016/j.cbi.2013.10.022]
10 **Noureddine H**, Hage-Sleiman R, Wehbi B, Fayyad-Kazan H, Hayar S, Traboulssi M, Alyamani OA, Faour WH, ElMakhour Y. Chemical characterization and cytotoxic activity evaluation of Lebanese propolis. *Biomed Pharmacother* 2017; **95**: 298-307 [PMID: 28850929 DOI: 10.1016/j.biopha.2017.08.067]
11 **Braakhuis A**. Evidence on the Health Benefits of Supplemental Propolis. *Nutrients* 2019; **11** [PMID: 31717277 DOI: 10.3390/nu11112705]
12 **Inui S**, Hosoya T, Yoshizumi K, Sato H, Kumazawa S. Phytochemical and anti-inflammatory properties of Senegalese propolis and isolated compounds. *Fitoterapia* 2021; **151**: 104861 [PMID: 33631223 DOI: 10.1016/j.fitote.2021.104861]
13 **Kocot J**, Kiełczykowska M, Luchowska-Kocot D, Kurzepa J, Musik I. Antioxidant Potential of Propolis, Bee Pollen, and Royal Jelly: Possible Medical Application. *Oxid Med Cell Longev* 2018; **2018**: 7074209 [PMID: 29854089 DOI: 10.1155/2018/7074209]
14 **Forma E**, Bryś M. Anticancer Activity of Propolis and Its Compounds. *Nutrients* 2021; **13** [PMID: 34444754 DOI: 10.3390/nu13082594]
15 **El Adaouia Taleb R**, Djebli N, Chenini H, Sahin H, Kolayli S. In vivo and in vitro anti-diabetic activity of ethanolic propolis extract. *J Food Biochem* 2020; **44**: e13267 [PMID: 32378224 DOI: 10.1111/jfbc.13267]
16 **El Menyiy N**, Bakour M, El Ghouizi A, El Guendouz S, Lyoussi B. Influence of Geographic Origin and Plant Source on Physicochemical Properties, Mineral Content, and Antioxidant and Antibacterial Activities of Moroccan Propolis. *Int J Food Sci* 2021; **2021**: 5570224 [PMID: 33791359 DOI: 10.1155/2021/5570224]
17 **Syed Salleh SNA**, Mohd Hanapiah NA, Ahmad H, Wan Johari WL, Osman NH, Mamat MR. Determination of Total Phenolics, Flavonoids, and Antioxidant Activity and GC-MS Analysis of Malaysian Stingless Bee Propolis Water

Extracts. *Scientifica (Cairo)* 2021; **2021**: 3789351 [PMID: 34721923 DOI: 10.1155/2021/3789351]
18 **Yuluğ E**, Türedi S, Yıldırım Ö, Yenilmez E, Aliyazıcıoğlu Y, Demir S, Özer-Yaman S, Menteşe A. Biochemical and morphological evaluation of the effects of propolis on cisplatin induced kidney damage in rats. *Biotech Histochem* 2019; **94**: 204-213 [PMID: 30512970 DOI: 10.1080/10520295.2018.1543895]
19 **Ahmed R**, Tanvir EM, Hossen MS, Afroz R, Ahmmed I, Rumpa NE, Paul S, Gan SH, Sulaiman SA, Khalil MI. Antioxidant Properties and Cardioprotective Mechanism of Malaysian Propolis in Rats. *Evid Based Complement Alternat Med* 2017; **2017**: 5370545 [PMID: 28261310 DOI: 10.1155/2017/5370545]
20 **Khaled S**, Makled MN, Nader MA. Protective effects of propolis extract against nicotine-evoked pulmonary and hepatic damage. *Environ Sci Pollut Res Int* 2022; **29**: 5812-5826 [PMID: 34431048 DOI: 10.1007/s11356-021-16093-6]
21 **Fonseca YM**, Marquele-Oliveira F, Vicentini FT, Furtado NA, Sousa JP, Lucisano-Valim YM, Fonseca MJ. Evaluation of the Potential of Brazilian Propolis against UV-Induced Oxidative Stress. *Evid Based Complement Alternat Med* 2011; **2011** [PMID: 20953396 DOI: 10.1155/2011/863917]
22 **Badr G**, Sayed EA, Waly H, Hassan KA, Mahmoud MH, Selamoglu Z. The Therapeutic Mechanisms of Propolis Against CCl(4) -Mediated Liver Injury by Mediating Apoptosis of Activated Hepatic Stellate Cells and Improving the Hepatic Architecture through PI3K/AKT/mTOR, TGF-β/Smad2, Bcl2/BAX/P53 and iNOS Signaling Pathways. *Cell Physiol Biochem* 2019; **53**: 301-322 [PMID: 31343125 DOI: 10.33594/000000140]
23 **Ozdemir B**, Gulhan MF, Sahna E, Selamoglu Z. The investigation of antioxidant and anti-inflammatory potentials of apitherapeutic agents on heart tissues in nitric oxide synthase inhibited rats *via* Nω-nitro-L-arginine methyl ester. *Clin Exp Hypertens* 2021; **43**: 69-76 [PMID: 32799699 DOI: 10.1080/10641963.2020.1806294]
24 **Selamoglu ZS**, Ozdemir I, Ciftci O, Gulhan MF, Savci A. Antioxidant Effect of Ethanolic Extract of Propolis in Liver of L-NAME Treated Rats. *Adv Clin Exp Med* 2015; **24**: 227-232 [PMID: 25931353 DOI: 10.17219/acem/40461]
25 **Salmas RE**, Gulhan MF, Durdagi S, Sahna E, Abdullah HI, Selamoglu Z. Effects of propolis, caffeic acid phenethyl ester, and pollen on renal injury in hypertensive rat: An experimental and theoretical approach. *Cell Biochem Funct* 2017; **35**: 304-314 [PMID: 28833317 DOI: 10.1002/cbf.3277]
26 **Badary OA**, Hamza MS, Tikamdas R. Thymoquinone: A Promising Natural Compound with Potential Benefits for COVID-19 Prevention and Cure. *Drug Des Devel Ther* 2021; **15**: 1819-1833 [PMID: 33976534 DOI: 10.2147/DDDT.S308863]
27 **Cobourne-Duval MK**, Taka E, Mendonca P, Bauer D, Soliman KF. The Antioxidant Effects of Thymoquinone in Activated BV-2 Murine Microglial Cells. *Neurochem Res* 2016; **41**: 3227-3238 [PMID: 27585756 DOI: 10.1007/s11064-016-2047-1]
28 **Demir E**, Taysi S, Ulusal H, Kaplan DS, Cinar K, Tarakcioglu M. Nigella sativa oil and thymoquinone reduce oxidative stress in the brain tissue of rats exposed to total head irradiation. *Int J Radiat Biol* 2020; **96**: 228-235 [PMID: 31638880 DOI: 10.1080/09553002.2020.1683636]
29 **Mabrouk A**. Protective effect of thymoquinone against lead-induced antioxidant defense system alteration in rat liver. *Acta Biol Hung* 2017; **68**: 248-254 [PMID: 28901799 DOI: 10.1556/018.68.2017.3.2]
30 **Abdel-Daim MM**, Abo El-Ela FI, Alshahrani FK, Bin-Jumah M, Al-Zharani M, Almutairi B, Alyousif MS, Bungau S, Aleya L, Alkahtani S. Protective effects of thymoquinone against acrylamide-induced liver, kidney and brain oxidative damage in rats. *Environ Sci Pollut Res Int* 2020; **27**: 37709-37717 [PMID: 32608003 DOI: 10.1007/s11356-020-09516-3]
31 **Khalifa AA**, Rashad RM, El-Hadidy WF. Thymoquinone protects against cardiac mitochondrial DNA loss, oxidative stress, inflammation and apoptosis in isoproterenol-induced myocardial infarction in rats. *Heliyon* 2021; **7**: e07561 [PMID: 34355083 DOI: 10.1016/j.heliyon.2021.e07561]
32 **Faisal Lutfi M**, Abdel-Moneim AH, Alsharidah AS, Mobark MA, Abdellatif AAH, Saleem IY, Al Rugaie O, Mohany KM, Alsharidah M. Thymoquinone Lowers Blood Glucose and Reduces Oxidative Stress in a Rat Model of Diabetes. *Molecules* 2021; **26** [PMID: 33920728 DOI: 10.3390/molecules26082348]
33 **Alzohairy MA**, Khan AA, Alsahli MA, Almatroodi SA, Rahmani AH. Protective Effects of Thymoquinone, an Active Compound of Nigella sativa, on Rats with Benzo(a)pyrene-Induced Lung Injury through Regulation of Oxidative Stress and Inflammation. *Molecules* 2021; **26** [PMID: 34072086 DOI: 10.3390/molecules26113218]
34 **Dong J**, Zhang X, Wang S, Xu C, Gao M, Liu S, Li X, Cheng N, Han Y, Wang X. Thymoquinone Prevents Dopaminergic Neurodegeneration by Attenuating Oxidative Stress Via the Nrf2/ARE Pathway. *Front Pharmacol* 2020; **11**: 615598 [PMID: 33519481 DOI: 10.3389/fphar.2020.615598]
35 **Hegazy AM**, El-Sayed EM, Ibrahim KS, Abdel-Azeem AS. Dietary antioxidant for disease prevention corroborated by the Nrf2 pathway. *J Complement Integr Med* 2019; **16** [PMID: 30726190 DOI: 10.1515/jcim-2018-0161]
36 **Briga M**, Odobasic A, Sestan I, Begic S. Black seed oil as an additive to honey. *Eur Food Res Technol* 2019; **7**: 15-25
37 **Rathi B**, Devanesan S, AlSalhi MS, Ranjith Singh AJ. In-vitro free radical scavenging effect and cytotoxic analysis of Black Cummins and Honey formulation. *Saudi J Biol Sci* 2021; **28**: 1576-1581 [PMID: 33732043 DOI: 10.1016/j.sjbs.2020.12.051]
38 **Mabrouk GM**, Moselhy SS, Zohny SF, Ali EM, Helal TE, Amin AA, Khalifa AA. Inhibition of methylnitrosourea (MNU) induced oxidative stress and carcinogenesis by orally administered bee honey and Nigella grains in Sprague Dawely rats. *J Exp Clin Cancer Res* 2002; **21**: 341-346 [PMID: 12385575]
39 **Pasupuleti VR**, Sammugam L, Ramesh N, Gan SH. Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. *Oxid Med Cell Longev* 2017; **2017**: 1259510 [PMID: 28814983 DOI: 10.1155/2017/1259510]
40 **Woźniak M**, Mrówczyńska L, Kwaśniewska-Sip P, Waśkiewicz A, Nowak P, Ratajczak I. Effect of the Solvent on Propolis Phenolic Profile and its Antifungal, Antioxidant, and In Vitro Cytoprotective Activity in Human Erythrocytes Under Oxidative Stress. *Molecules* 2020; **25** [PMID: 32957629 DOI: 10.3390/molecules25184266]
41 **El-Ali M**, Jaber A, Dorra Z, El Riachi M, Ibrahim G, Cheble E. Chemical Analysis and Antioxidant Activity of Four Propolis Samples Collected from Different Regions of Lebanon. *Journal of Apitherapy and Nature* 2021; **4**: 1-21 [DOI: 10.35206/jan.828140]
42 **Zeitoun R**, Hayar S, JanbeiH Z, Elmakhour Y. Analysis of the chemical composition of Lebanese propolis. *Asian J Appl*

Sci Technol 2018; **9**: 8735-8742

43 **Alinezhad H**, Azimi R, Zare M, Ebrahimzadeh MA, Eslami S, Nabavi SF, Nabavi SM. Antioxidant and antihemolytic activities of ethanolic extract of flowers, leaves, and stems of hyssopus officinalis L. var. angustifolius. *Int J Food Prop* 2013; **16**: 1169-1178 [DOI: 10.1080/10942912.2011.578319]

44 **Nina N**, Quispe C, Jiménez-Aspee F, Theoduloz C, Giménez A, Schmeda-Hirschmann G. Chemical profiling and antioxidant activity of Bolivian propolis. *J Sci Food Agric* 2016; **96**: 2142-2153 [PMID: 26138367 DOI: 10.1002/jsfa.7330]

45 **Asem N**, Abdul Gapar NA, Abd Hapit NH, Omar EA. Correlation between total phenolic and flavonoid contents with antioxidant activity of malaysian stingless bee propolis extract. *J Apic Res* 2020; **59**: 437-442 [DOI: 10.1080/00218839.2019.1684050]

46 **Afsar T**, Razak S, Khan MR, Mawash S, Almajwal A, Shabir M, Haq IU. Evaluation of antioxidant, anti-hemolytic and anticancer activity of various solvent extracts of Acacia hydaspica R. Parker aerial parts. *BMC Complement Altern Med* 2016; **16**: 258 [PMID: 27473625 DOI: 10.1186/s12906-016-1240-8]

47 **Hossen J**, Ali MA, Reza S. Theoretical investigations on the antioxidant potential of a non-phenolic compound thymoquinone: a DFT approach. *J Mol Model* 2021; **27**: 173 [PMID: 34014420 DOI: 10.1007/s00894-021-04795-0]

48 **Pichai N**, Durairaj B. Antiplatelet and antihemolytic activities of hydroethanolic extract of Annona muricata leavesa. *Int J Pharm Sci Res* 2020; **11**: 5125-5128 [DOI: 10.13040/IJPSR.0975-8232.11(10).5125-28]

49 **Mendes L**, de Freitas V, Baptista P, Carvalho M. Comparative antihemolytic and radical scavenging activities of strawberry tree (Arbutus unedo L.) leaf and fruit. *Food Chem Toxicol* 2011; **49**: 2285-2291 [PMID: 21703325 DOI: 10.1016/j.fct.2011.06.028]

50 **Zhao H**, Zhang H, Yang S. Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of chinese jujube. *Food Sci Hum Wellness* 2014; **3**: 183-190 [DOI: 10.1016/j.fshw.2014.12.005]

51 **Aman S**, Moin S. Antioxidant Activity of Thymoquinone and its Protective Effect Against Oxidative Hemolysis. *Int J Sci Res* 2012; **2**: 28-30 [DOI: 10.15373/22778179/APR2013/108]

52 **Ebrahimzadeh MA**, Nabavi SF, Nabavi SM, Eslami B. Antihemolytic and antioxidant activities of allium paradoxum. *Cent Eur J Biol* 2010; **5**: 338-345 [DOI: 10.2478/s11535-010-0013-5]

53 **Weber M**, Steinle H, Golombek S, Hann L, Schlensak C, Wendel HP, Avci-Adali M. Blood-Contacting Biomaterials: In Vitro Evaluation of the Hemocompatibility. *Front Bioeng Biotechnol* 2018; **6**: 99 [PMID: 30062094 DOI: 10.3389/fbioe.2018.00099]

54 **Shubharani R**, Sivaram V, Kishore BR. In-vitro cytotoxicity of indian bee propolis on cancer cell lines. *Int J Pharma Bio Sci* 2014; **5**: 698-706

55 **Campos JF**, Dos Santos UP, da Rocha Pdos S, Damião MJ, Balestieri JB, Cardoso CA, Paredes-Gamero EJ, Estevinho LM, de Picoli Souza K, Dos Santos EL. Antimicrobial, Antioxidant, Anti-Inflammatory, and Cytotoxic Activities of Propolis from the Stingless Bee Tetragonisca fiebrigi (Jataí). *Evid Based Complement Alternat Med* 2015; **2015**: 296186 [PMID: 26185516 DOI: 10.1155/2015/296186]

56 **Liou GY**, Storz P. Reactive oxygen species in cancer. *Free Radic Res* 2010; **44**: 479-496 [PMID: 20370557 DOI: 10.3109/10715761003667554]

57 **Zahra KF**, Lefter R, Ali A, Abdellah EC, Trus C, Ciobica A, Timofte D. The Involvement of the Oxidative Stress Status in Cancer Pathology: A Double View on the Role of the Antioxidants. *Oxid Med Cell Longev* 2021; **2021**: 9965916 [PMID: 34394838 DOI: 10.1155/2021/9965916]

58 **Shan W**, Zhong W, Swanlund J, Oberley TD. Oxidative stress in prostate cancer. In: Spitz DR, Dornfeld KJ, Krishnan K, Gius D. Oxidative stress in cancer biology and therapy. Humana Press, 2012: 301-331 [DOI: 10.1007/978-1-61779-397-4_15]

59 **Merlin JPJ**, Rupasinghe HPV, Dellaire G, Murphy K. Role of Dietary Antioxidants in p53-Mediated Cancer Chemoprevention and Tumor Suppression. *Oxid Med Cell Longev* 2021; **2021**: 9924328 [PMID: 34257824 DOI: 10.1155/2021/9924328]

60 **Valente MJ**, Baltazar AF, Henrique R, Estevinho L, Carvalho M. Biological activities of Portuguese propolis: protection against free radical-induced erythrocyte damage and inhibition of human renal cancer cell growth in vitro. *Food Chem Toxicol* 2011; **49**: 86-92 [PMID: 20934479 DOI: 10.1016/j.fct.2010.10.001]

61 **Gomathinayagam R**, Ha JH, Jayaraman M, Song YS, Isidoro C, Dhanasekaran DN. Chemopreventive and Anticancer Effects of Thymoquinone: Cellular and Molecular Targets. *J Cancer Prev* 2020; **25**: 136-151 [PMID: 33033708 DOI: 10.15430/JCP.2020.25.3.136]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 June 24; 14(6): 215-229

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 6 June 24, 2023

ORIGINAL ARTICLE

Observational Study

215 Hyperthermia combined with chemotherapy *vs* chemotherapy in patients with advanced pancreatic cancer: A multicenter retrospective observational comparative study

Fiorentini G, Sarti D, Mambrini A, Hammarberg Ferri I, Bonucci M, Sciacca PG, Ballerini M, Bonanno S, Milandri C, Nani R, Guadagni S, Dentico P, Fiorentini C

CORRECTION

227 Correction: Propensity-matched analysis of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma undergoing a liver transplant

Brandão ABM, Rodriguez S, Fleck Jr AM, Marroni CA, Wagner MB, Hörbe A, Fernandes MV, Cerski CT, Coral GP

World Journal of Clinical Oncology

Monthly Volume 14 Number 6 June 24, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Oncology*, Rania Mohammed Hathout, PhD, Professor, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, African Union Organization Street, Abbassia, Cairo 11566, Egypt. rania.hathout@pharma.asu.edu.eg

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2022 edition of Journal Citation Reports® cites the 2021 Journal Citation Indicator (JCI) for *WJCO* as 0.35.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Yu-Jie Ma*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
June 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 June 24; 14(6): 215-226

DOI: 10.5306/wjco.v14.i6.215

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Observational Study

Hyperthermia combined with chemotherapy *vs* chemotherapy in patients with advanced pancreatic cancer: A multicenter retrospective observational comparative study

Giammaria Fiorentini, Donatella Sarti, Andrea Mambrini, Ivano Hammarberg Ferri, Massimo Bonucci, Paola Giordano Sciacca, Marco Ballerini, Salvatore Bonanno, Carlo Milandri, Roberto Nani, Stefano Guadagni, Patrizia Dentico, Caterina Fiorentini

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Lee L, South Korea; Ma X

Received: April 27, 2023
Peer-review started: April 27, 2023
First decision: May 12, 2023
Revised: May 18, 2023
Accepted: June 6, 2023
Article in press: June 6, 2023
Published online: June 24, 2023

Giammaria Fiorentini, Ivano Hammarberg Ferri, Integrative Oncology, Integrative Oncology Outpatient Clinic, Bologna 40121, Italy

Donatella Sarti, Department of Oncology, Santa Maria della Misericordia Hospital, Urbino 60129, Italy

Andrea Mambrini, Department of Oncology, Azienda Sanitaria Locale Toscana Nord Ovest, Massa Carrara Hospital, Massa 54100, Italy

Massimo Bonucci, Integrative Oncology, Association Research Center for Integrative Oncology Treatments, Roma 00166, Italy

Paola Giordano Sciacca, Complementary Medicine Service, District Hospital of Merano, Merano 39012, Italy

Marco Ballerini, Hyperthermia Unit, Bellessere Medical Center, Terni 05100, Italy

Salvatore Bonanno, Radiotherapy Unit, A. Rizza Hospital, Siracusa 96100, Italy

Carlo Milandri, Medical Oncology, San Donato Hospital, Arezzo 52100, Italy

Roberto Nani, Interventional Radiology Unit, Humanitas Gavazzeni, Bergamo 24121, Italy

Stefano Guadagni, Applied Clinical Sciences and Biotechnology, Section of General Surgery, University of L'Aquila, L'Aquila 67100, Italy

Patrizia Dentico, Hyperthermia Service, Medical Oncology Unit, San Giuseppe Hospital, Empoli 50053, Italy

Caterina Fiorentini, Prevention and Sports Medicine, University Hospital Klinikum rechts der Isar, Technical University of Munich, Munich 80331, Germany

Corresponding author: Giammaria Fiorentini, MD, Adjunct Professor, Chief Doctor, Director, Integrative Oncology, Integrative Oncology Outpatient Clinic, Via dell'Indipendenza, 20, Bologna 40121, Italy. g.fiorentini@alice.it

Abstract

BACKGROUND

Several studies report the useful therapeutic results of regional hyperthermia in association with chemotherapy (CHT) and radiotherapy for the treatment of pancreatic cancer. Modulated electro-hyperthermia (mEHT) is a new hyperthermia technique that induces immunogenic death or apoptosis of pancreatic cancer cells in laboratory experiments and increases tumor response rate and survival in pancreatic cancer patients, offering beneficial therapeutic effects against this severe type of cancer.

AIM

To assess survival, tumor response and toxicity of mEHT alone or combined with CHT compared with CHT for the treatment of locally advanced or metastatic pancreatic cancer.

METHODS

This was a retrospective data collection on patients affected by locally advanced or metastatic pancreatic cancer (stage III and IV) performed in 9 Italian centers, members of International Clinical Hyperthermia Society-Italian Network. This study included 217 patients, 128 (59%) of them were treated with CHT (no-mEHT) and 89 (41%) patients received mEHT alone or in association with CHT. mEHT treatments were performed applying a power of 60-150 watts for 40-90 min, simultaneously or within 72 h of administration of CHT.

RESULTS

Median patients' age was 67 years (range 31-92 years). mEHT group had a median overall survival greater than non-mEHT group (20 mo, range 1.6-24, *vs* 9 mo, range 0.4-56.25, $P < 0.001$). mEHT group showed a higher number of partial responses (45% *vs* 24%, $P = 0.0018$) and a lower number of progressions (4% *vs* 31%, $P < 0.001$) than the no-mEHT group, at the three months follow-up. Adverse events were observed as mild skin burns in 2.6% of mEHT sessions.

CONCLUSION

mEHT seems safe and has beneficial effects on survival and tumor response of stage III-IV pancreatic tumor treatment. Further randomized studies are warranted to confirm or not these results.

Key Words: Modulated electro hyperthermia; Locally advanced pancreatic tumor; Overall survival; Tumor response; Gemcitabine; Apoptosis; Immunogenic cell death

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Pancreatic cancer has very poor prognosis with a 5-year overall survival of 5% and a median overall survival (OS) time of 8-12 mo. The concomitant use of modulated electro-hyperthermia (mEHT) in addition to chemotherapy has been introduced. mEHT has specific antitumor effects, increasing survival and tumor response. This was a retrospective data collection on 217 patients affected by locally advanced pancreatic cancer performed in 9 Italian centres, aiming to assess survival, tumour response and toxicity. The mEHT group had a greater OS (20 mo *vs* 9 mo, $P < 0.001$), higher number of partial responses (45% *vs* 24%, $P = 0.0018$) and a lower number of progressions (4% *vs* 31%, $P < 0.001$) than no-mEHT group. Adverse events were observed in 2.6% of mEHT sessions. mEHT have beneficial effects on survival and tumor response of stage III-IV pancreatic tumor patients, without adding toxicity.

Citation: Fiorentini G, Sarti D, Mambrini A, Hammarberg Ferri I, Bonucci M, Sciacca PG, Ballerini M, Bonanno S, Milandri C, Nani R, Guadagni S, Dentico P, Fiorentini C. Hyperthermia combined with chemotherapy *vs* chemotherapy in patients with advanced pancreatic cancer: A multicenter retrospective observational comparative study. *World J Clin Oncol* 2023; 14(6): 215-226

URL: https://www.wjgnet.com/2218-4333/full/v14/i6/215.htm

DOI: https://dx.doi.org/10.5306/wjco.v14.i6.215

INTRODUCTION

Pancreatic cancer (PC) is a particularly serious disease with poor prognosis[1,2]. Main risk factors of

pancreatic cancer are non-hereditary and environmental factors, such as increased age, diabetes, obesity, smoking, alcohol intake and diet high in fat and low in vegetables. Only 10% of PC are familial or hereditary. Numerous molecular biology studies are underway the genes and pathological conditions involved in PC onset, in particular, the most studied are: Lynch syndrome, hereditary pancreatitis, Peutz-Jeghers syndrome, cystic fibrosis and breast cancer gene (BRCA)[2].

The Lynch syndrome is an inherited condition that is associated with 5% of colon cancer cases. Patients with this syndrome have about 10-fold increased risk of developing PC[3].

Hereditary pancreatitis is another rare inherited condition that is usually diagnosed in young individuals (< 20 years); its main symptoms are frequent episodes of severe inflammation of the pancreatic gland, leading to chronic pancreatitis. This pathology increases of about a 50% the risk of developing PC; this risk is increased when associated to smoke[4].

Peutz-Jeghers Syndrome is characterized by polyps in the small intestine and pigmented spots on nose and lips. Patients with this syndrome have a 10%-35% risk of developing PC[5]. Cystic fibrosis induces pancreatic insufficiency and chronic pancreatitis and increases of 5 to 6 times the risk of developing pancreatic cancer[6].

BRCA 1 and 2 mutations are often related to inherited ovarian and breast cancer. It is well known that the BRCA1 mutation can also induce an increased risk (4-9 times) of developing PC[7].

Over the past twenty years, PC prognosis has been improved by early diagnosis, the use of neoadjuvant, adjuvant and palliative therapies and the increased number of centres expert in hepatic-pancreatic surgery.

Today PC is the seventh cause of cancer-related death worldwide with a median overall survival < 1 year[1,2]. Incidence and mortality of this cancer have increased significantly in the last two decades[2]. The most common PC histology is adenocarcinoma that accounts for about 90% of cases. Radical surgery followed by adjuvant radiotherapy (RT) and/or chemotherapy (CHT) is considered the gold standard treatment for this tumor; however, surgery is indicated only in 10% of patients at the first diagnosis, whereas, 90% are non-resectable because they are locally advanced or metastatic. Neoadjuvant CHT or a combination of RT and chemo-therapy can be performed in locally advanced PC without evidence of distant metastases, in order to allow surgical approach[8,9].

FOLFIRINOX (leucovorin, fluorouracil, irinotecan, oxaliplatin) and the combination of nab-paclitaxel and gemcitabine are the more suitable upfront CHT for PC patients with good performance status (PS). These protocols have partial activity, resulting in similar overall survival (32-54 mo), but they have frequently severe side effects[8-10].

Recent studies on cisplatin therapeutic use in PC patients with BRCA 1 and 2 mutations report clinical benefit, although superiority over other chemotherapeutic treatments and optimal dosing and combination therapies are still ongoing. PARP inhibitors such as niraparib and olaparib have also promising results in this setting. They prevent single-strand DNA break repair, resulting in double-stranded DNA breaks that cannot to be repaired by homologous recombination deficiency tumors, causing cell cycle arrest and apoptosis. Olaparib increases progression-free survival, when used as a maintenance therapy for PC patients responding to first-line platinum-based therapy. Although these drugs show interesting results, however their efficacy is undermined by drug resistance and high cost [7].

PC is generally refractory to CHT and RT because of its low perfused and hypoxic microenvironment that is mainly due to large accumulation of non-tumor cells (stroma), obstructing vascularization and the delivery systemic chemotherapeutics. This also reduces oxygen delivery and hence the sensitivity to CHT and RT, impacting negatively on prognosis. In vitro experiments have shown that hyperthermia can increase the effectiveness of conventional treatments[15-19].

Regional hyperthermia (RHT) optimize the distribution of drugs in cancer cells, improves blood circulation, increases district oxygen level, reduces DNA repair and promotes cancer cell death[16]. RHT increases temperature inside the tumor up to 39.5/43 °C, using an external radiofrequency equipment. RHT is often associated to CHT and RT, in order to increase responses and overall survival (OS) in PC [20-24]. A systematic review shows the benefits of hyperthermia added to CHT and/or RT for PC patients from 14 clinical trials, resulting in median OS of 6-18.6 mo that is longer than that observed in the control cohorts and improved tumor response with 31.3% overall response rate that is greater than the control cohort. These data suggest that this combination positively affect tumor response and OS for PC patients[24-28].

Modulated Electro- Hyperthermia (mEHT) is obtained with a 13.56 MHz capacitive equipment and is a relatively new RHT technique (introduced in clinical practice in the last decade)[25,27,28-30]. This type of RHT is more selective in killing tumor cells and overcoming the limited tissue permeation of radio-frequency. There is no direct way to measure the temperature inside the tissues in vivo, however it can be predicted from input power applied, because of the high efficacy of the electric field[29-34]. mEHT increases the temperature of the targeted malignant cells over 3 °C higher than surrounding environment[29].

The high heterogeneity of PC cells is counteracted by mEHT, which selectively damages the external membrane of cancer cells that have different energy uptake on the membrane rafts from healthy tissue and different electron impedance compared to normal cells[17-19].

Data from the literature show that mEHT is safe and feasible and has not only palliative but also therapeutic effects in several advanced cancer and also in pancreatic adenocarcinoma in monotherapy or in combination with CHT and/or RT[25,27,28,32-35]. This method seems to re-sensitize patients who are refractory to CHT and RT and improve palliation, increasing survival time, tumor response and quality of life (QoL), prolonging OS and improving QoL[25,27,28,32-34,36-39].

The purpose of this multicenter observational comparative study was to assess the advantages of mEHT in association to CHT compared to CHT alone in locally advanced or metastatic pancreatic tumors. This study was carried out by members of International Clinical Hyperthermia Society-Italian Network and created the platform for a large randomized phase III trial to compare outcomes with or without mEHT application.

MATERIALS AND METHODS

Sample selection

This study was multicenter, retrospective, observational, comparative and case-control, aiming to assess survival and tumor response of CHT or mEHT plus CHT for the treatment of locally advanced or metastatic pancreatic tumors. Inclusion criteria were: Older than 18 years, informed consent signed, histological diagnosis of locally advanced or metastatic pancreatic tumor, PS of 0-2, treatment with CHT, mEHT or mEHT plus CHT, data on tumor response and survival. From 2003 to 2021, 628 patients with locally advanced or metastatic pancreatic tumor were treated in nine Italian centers, 217 of them were included in this study, 89 (41%) of them were treated with mEHT plus CHT (mETH group) and 128 (59%) with only CHT (no-mEHT group). CHT regimen was mostly gemcitabine-based in both study groups (Table1). The majority (95%) of gemcitabine-based treatments were administered on the same day of mEHT treatment. In a minority of patients (5%), it was administered the following day or within the following 72 h because of precarious clinical conditions and geographic accessibility. Even if gemcitabine had a half-life of 42-94 min and was eliminated within 5-11 h after infusion, the pharmacokinetic elimination half-life for dFdU varies between 2 and 24 h, and it is still present systemically in concentrations greater than 1 μmol/L up to 1 wk after infusion.

This was a retrospective data collection, CHT type was chosen by singular physician, we collected the data when the treatments (CHT and mEHT) were already done.

mEHT treatment protocols

mEHT was performed with EHY-2000 plus device (CE0123 Oncotherm, Torisdorf, Germany) and a radiofrequency current of 13.56 MHz as previously described[28-31]. mEHT treatments were performed 1-3 times/week for a total of 4-6 wk, starting at 60 W/40 min and increasing up to 150 W/ 90 min in 2 wk[28]. Power increase was performed in the initial mEHT sessions, in order to assess patient treatment tolerance. Starting with the increased potency could have caused a feeling of heat and pain on the scars, as indicated in previous protocols that we have set up[32,36]. Power increase is performed in the initial mEHT sessions and was not used for efficacy purpose, the efficacy was measured after reaching the final power of 150 W/90 min. The computer connected to the hyperthermia machine has a program that calculates and converts the Kilojoules dispensed by the machine into degrees of temperature at the treatment site. This was represented on the screen and printed on graphs. Temperature on the target was 41-42.5°C during mEHT sessions as they were assessed in previous publications from the manufacturer of the device[38]. As concerning metastases, most patients had disease located in the upper abdomen and for this reason the treatment was suitable because we used an applicator-antenna of 30 cm diameter that allowed to treat the entire upper abdomen and multiple liver or nodal metastases at the same time.

Outcome measures

The primary objective was OS that was measured from date of diagnosis to date of death or last available follow up date. The analysis of OS was made for the whole mEHT and no-mEHT group or by age ≥ 70 years or < 70 years. Secondary objectives were tumor response and treatment tolerability. Progression free survival (PFS) was computed from date of treatment start to progression.

Tumor response was measured with the Response Evaluation Criteria in Solid Tumors version 1.4 at three months follow up imaging. In case of multiple tumors, the target lesion was considered that with the largest diameter in outcome measures. Complete response was obtained if every target lesion disappeared; partial response (PR) if tumor diameter decreased by > 30%; progressive disease (PD) was observed if tumor size increased by > 20%, or one or more new lesions appeared. Stable disease (SD) was considered in all the other cases.

Since no data were available on cardiac toxicity due to radiofrequency disturbance from mEHT applied on upper abdomen; twenty-one patients out 89 treated with mEHT were monitored with electrocardiogram and echocardiogram before and after treatment in order to evaluate any alterations in cardiac rhythm and morphology.

As concerning the other adverse events were all monitored according to clinical practice and classified using the Common Terminology Criteria for Adverse Events version 5.0.

Statistical analysis

Survival and patients' age were indicated as median and range values, whereas frequencies were indicated as percentages. Kaplan-Meier method and log-rank test were used or OS analysis, with survival probability on the Y axis and time (months) on the X axis. Mann-Whitney test and Student's test for proportions were used to assess statistical significance ($P \leq 0.05$) among differences of patient characteristics. χ^2 test was used to assess statistical significance ($P \leq 0.05$) among differences of tumor response.

RESULTS

Sample description

The sample included 217 pancreatic patients: 122 (56%) were males and 95 (44%) were females, 89 (41%) of these were treated with the combination of CHT and mEHT (mETH group) and 128 (59%) with only CHT (no-mEHT group). Their median age was 67 years (37-89 years, range). Most patients were metastatic (65%). In the 217 patients, 235 metastatic sites were observed, with liver being the most frequent metastatic site (132/235, 57%). Previous CHT was administered to 136 (63%) patients, previous RT to 10 (5%) and surgery to 51 (24%). Most frequent CHT regimen was gemcitabine-based regimens: Gemcitabine-oxaliplatin (35%), gemcitabine (29%) and gemcitabine-abraxane (9%). Inclusion criteria indicated both radio and CHT in concomitant used with mEHT, however, none of the patients included in the study received RT in association to mEHT. The two groups had similar characteristics (Table 1).

Overall survival and progression free survival

mEHT group had a median OS greater than non-mEHT group (20 mo, range 1.6-24, *vs* 9 mo, range 0.4-56.25, $P < 0.001$) (Figure 1). mEHT group had a median PFS greater than non-mEHT group (7 mo, range 2-24, *vs* 5 mo, range 0.4-41, $P < 0.05$) (Figure 2). The analysis of OS by age ≥ 70 years or < 70 years showed that there was no difference in OS between mEHT ≥ 70 years (20 mo, 2-43 mo range) and < 70 years (20 mo 3-27 mo range) $P = 0.235$, whereas no-mEHT < 70 years had a higher OS than no-mEHT ≥ 70 years group (12 mo range 1-56 *vs* 8 mo range 1-47, $P = 0.01$) (Figure 3). mEHT had a longer OS than no-mEHT group both among ≥ 70 years (20 mo range 3-27 *vs* 8 mo range 1-47, $P < 0.01$) and < 70 years (20 mo range 2-43 *vs* 12 mo range 1-56, $P < 0.01$).

Tumor response

Tumor response at three months follow-up was available for 87 (98%) of mEHT and 111 (88%) patients for non-mEHT group. mEHT patients showed a higher number of PR (45% *vs* 24%, $P = 0.0018$) and a lower number of progressions (PD) (4% *vs* 31%, $P < 0.01$) than no-mEHT group. SD had similar value in both groups: 51% for mEHT and 45% for no-mEHT (Table 2).

mEHT safety

Median mEHT sessions was 16.8 (range 6-25), resulting 1495 mEHT delivered sessions. Adverse events were observed in 2.6% of cases and included: Low grade (G1) skin pain in 22 (1.5%) sessions and low-mild grade (G1-2) burns in 16 (1.1%) cases that resolved in a few days. Hyperthermia did not increase haematological, hepatic, pulmonary and metabolic toxicity due to CHT.

In particular no increased blood pressure or any other cardiac changes were observed for mEHT sessions in patients who received adequate cardiological monitoring including clinical examination, electrocardiogram and echocardiogram.

DISCUSSION

Hyperthermia involves the application of heat-generating energy to tumors, increasing their temperature to 39-43.5 ◦C and improving the response to systemic therapies. Hyperthermia has been studied for cancer treatment since the 80s, especially for the benefits observed in laboratory experiments, clinical case series and phase II studies. The results of these studies show that hyperthermia enhances CHT, RT and immunotherapy efficacy (tumor response and survival) for several cancers and also in pancreatic adenocarcinoma[16-20,24-28,32-36].

Hyperthermia can be performed with different electromagnetic devices/techniques that can be classified as loco-regional or whole-body methods, according to the size of the body treated[26,31,40-44]. First studies on hyperthermia effects in PC were made mainly using whole-body hyperthermia, whereas, more recent clinical studies apply mainly loco-RHT, such as mEHT that is non-invasive technique that balances low-power thermal effects and non-thermal electric processes, operating with

Table 1 The sample, *n* (%)

	Whole sample, *n* = 217		mEHT, *n* = 89		No-mEHT, *n* = 128		*P* value[1]
	Median	Range	Median	Range	Median	Range	
Age (yr)	67	34-89	64	38-82	69	34-89	NS
Student's test for proportions							
M	122	56	58	65	64	50	NS
F	95	44	31	35	64	50	NS
Metastatic	142	65	70	79	72	56	0.004
Previous chemotherapy	136	63	68	76	68	53	0.005
Previous Radiotherapy	10	5	1	1	9	7	NS
Current chemotherapy type							
Gemcitabine/oxaliplatin	76	35	34	38	42	33	NS
Gemcitabine	62	29	26	29	36	28	NS
Gemcitabine/abraxane	39	18	9	10	30	23	NS
Gemcitabine/5-fluorouracil	5	2	4	4	1	1	NS
Gemcitabine/cisplatin	10	5	2	2	8	6	NS
Gemcitabine/Nab-paclitaxel	2	1	0	0	2	2	NS
FOLFIRINOX	5	2	1	1	4	3	NS
Other	10	5	5	6	5	4	NS
None	8	4	8	9	0	0	NS
Site of metastases	(*N* = 235)		(*N* = 132)		(*N* = 103)		
Liver	132	57	70	53	63	61	NS
Peritoneum	55	23	35	27	20	19	NS
Lymph nodes	37	16	22	17	15	15	NS
Other	10	4	5	4	5	5	NS

[1]Mann-whitney test.
FOLFIRINOX: Folinic acid/fluorouracil/irinotecan/oxaliplatin; NS: Not significative.

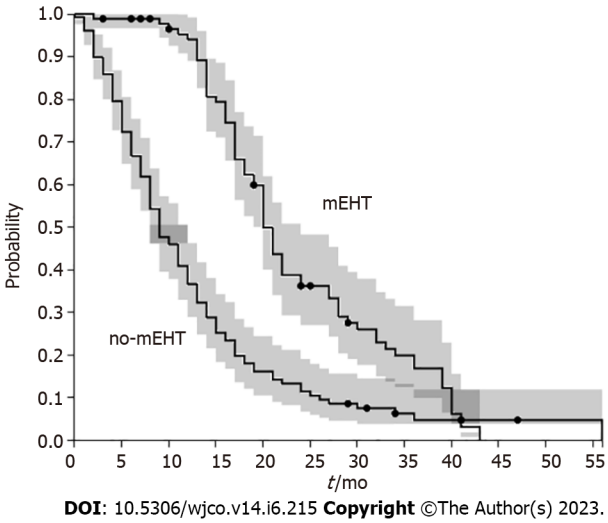
Table 2 Tumor response, *n* (%)

	mEHT	*n* = 87	no-mEHT	*n* = 111	*P* value
PR	39	45	27	24	0.0018
SD	44	51	50	45	0.8430
PD	4	4	34	31	< 0.001

PR: Partial response; PD: Progression disease; SD: Stable disease; mEHT: Modulated electro-hyperthermia.

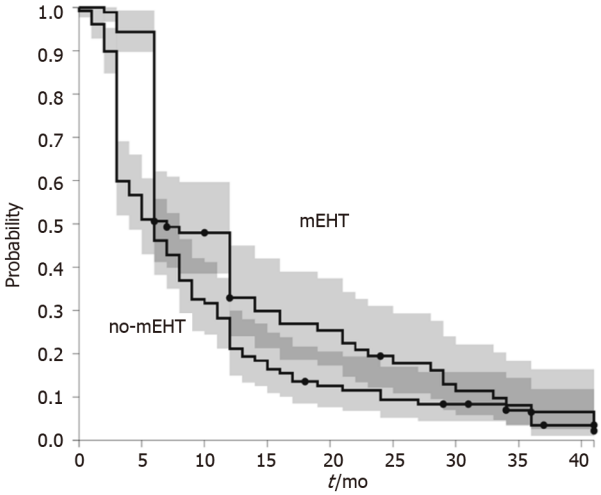
capacitive coupled impedance on a radiofrequency of 13.56 MHz[25-31,37-38]. Current mEHT protocols show that optimal treatment is obtained when the mEHT is performed two or three-times a week, resulting in improvements of OS, disease control and QoL and PFS[25-28,33,39,40]. mEHT has also benefits in tumor control when used as neoadjuvant therapy in combination with CHT and RT[42].

mEHT can counteract heterogenicity of PC and its resistance to systemic therapy, because it targets selectively tumor cells (heating homogeneously the target tissues), exploiting their several biophysical differences from normal cells, such as energy absorption and damage-associated molecular patterns[16-19,37,38]. mEHT induces programmed or immunogenic apoptosis of tumor cells, increasing DNA fragmentation, MAPK/ERK signaling pathways and pro-apoptotic Bcl-2 activation, low mitochondrial membrane potential, the concentration of intracellular $Ca2^{+}$, Fas and c-Jun N-terminal kinases and the expression of pro-apoptotic genes (EGR1, JUN, and CDKN1A), while silencing other genes that are



DOI: 10.5306/wjco.v14.i6.215 Copyright ©The Author(s) 2023.

Figure 1 Overall survival of modulated electro-hyperthermia (mEHT) and no-mEHT groups. Dots represent censors, cloud area represent 95%CI. mEHT: Modulated electro-hyperthermia.



DOI: 10.5306/wjco.v14.i6.215 Copyright ©The Author(s) 2023.

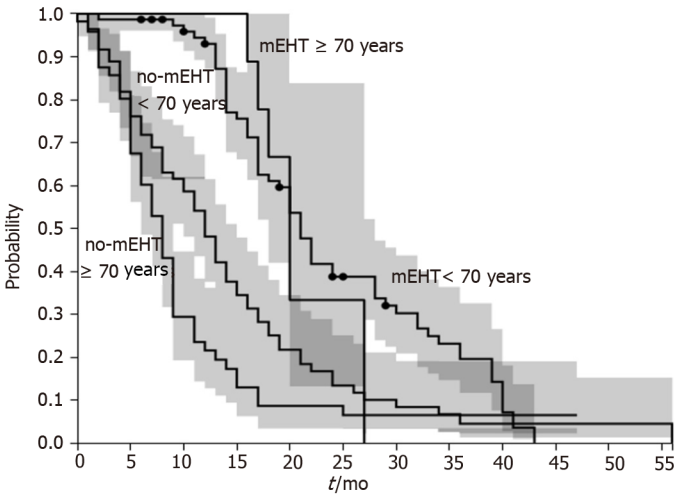
Figure 2 Progression free survival of Modulated electro-hyperthermia (mEHT) and no-mEHT groups. Dots represent censors, cloud area represent 95%CI. mEHT: Modulated electro-hyperthermia.

associated with cytoprotective functions[16-19,30].

In our study, mEHT group had a longer OS than the no-mEHT group (20 mo *vs* 9 mo, $P < 0.001$). This effect was present also among ≥ 70 years patients (20 mo, range 3-27 *vs* 8 mo, range 1-47, $P < 0.01$). mEHT group had also a median PFS greater than non-mEHT group (7 mo, range 2-24, *vs* 5 mo, range 0.4-41, $P < 0.05$). OS improvement was observed in other studies when mEHT was associated with CHT, resulting in an OS of 12.9 mo (95%CI: 9.9-15.9) and disease control rate of 50% in pancreatic cancer treatment[35-38]. Other studies on mEHT reported an OS of 8.9-19 mo and a PFS of 3.9-12.9 mo in advanced pancreatic adenocarcinoma[25-28,32,33]. The results of our study are in agreement with the above data, even if they used different types of deep hyperthermia devices but similar CHT regimens.

OS improvement was of particular interest for elderly patients who suffer most from side effects of CHT. The analysis of OS by age ≥ 70 years or < 70 years showed that there was no difference in OS between mEHT ≥ 70 years and < 70 years, whereas no-mEHT < 70 years had a higher OS than no-mEHT ≥ 70 years group (12 mo range 1-56 *vs* 8 mo range 1-47, $P = 0.01$). These data would recommend to use mEHT in elderly patients instead of a second or third line of CHT with heavy side effects.

The combination mEHT with CHT also improved tumor response, disease control rate (DCR = 96%), as it was reported in other studies (DCR = 71%-96%)[21,23,24,29]. mEHT group had a higher number of PR (45% *vs* 24%, $P = 0.0018$) and a lower number of progressions (PD) (4% *vs* 31%, $P < 0.001$) than the no-mEHT group. This was also reported in other studies, showing higher DCR of mEHT group than that of no-mEHT group (96% *vs* 77%, $P < 0.05$)[16-19].



DOI: 10.5306/wjco.v14.i6.215 **Copyright** ©The Author(s) 2023.

Figure 3 Overall survival of modulated electro-hyperthermia (mEHT) and no-mEHT groups divided by age. Dots represent censors, cloud area represent 95%CI. mEHT: Modulated electro-hyperthermia.

Adverse events that were related to hyperthermia were observed in 2.6% of the sample, showing G1-2 pain and a skin burns. These data agree with toxicity reported in other studies (5%) and suggest that mEHT-related toxicity is low[25,28,33]. Little has been published on the possible cardiotoxicity of radiofrequencies produced by mEHT when applied to the upper abdomen, in locations close to the heart. Twenty-one patients out 89 treated with mEHT were evaluated by electrocardiogram, echocardiogram before and after treatment with mEHT. We observed that upper abdominal mEHT does not generate rhythm disturbance, changes in electrocardiogram, cardiac morphology and blood pressure elevation.

The novelty of this study is the accurate reporting of a significant number of patients treated with mEHT compared with an equally large number of patients who received second and third-line CHT. The data on such a large total number of patients (217) and on the therapeutic effect of mEHT against PC has not been published before in the literature, of particular interest is also the improvement the of older patients' prognosis of older patients that reaches values comparable to that observed in younger patients.

The observational and retrospective nature of the study is the main limitation together with the long period of observation and inclusion from 2003 to 2021 during which many diagnostic and therapeutic approaches were modified and the still low number of patients treated with mEHT due the limited number of centers using mEHT in Italy. Future immunological research for PC treatment should be directed on circulating tumor cells (CTCs). It should be of interest characterize the transcriptomes of human pancreatic ductal adenocarcinoma CTCs, primary, and metastatic lesions at single-cell scale. Cell-interaction analysis and functional studies *in vitro* and *in vivo* reveal that CTCs and natural killer (NK) cells interact *via* the immune checkpoint molecule pair HLA-E:CD94-NKG2A. The breakdown of this interaction by blockade of NKG2A or knockdown of HLA-E expression could enhance NK-mediated tumor cell killing *in vitro* and prevents tumor metastasis *in vivo*[43].

Other methods of hyperthermia when the tumor is metastatic are currently being studied such as whole body hyperthermia but still seem to be at an early stage of application in clinical practice or burdened with excessive complexity[44].

Our study would like to open a new avenue in the treatment of locally advanced pancreatic cancer including mEHT in therapeutic options, since the results of CHT alone are not satisfactory. It is also necessary to know that the centers that practice hyperthermia at a good level are few both in Europe and in the World. Therefore, it is not easy to reach case studies with numerous patients to implement randomized trials. Future studies with a greater number of patients and randomized protocols are needed to confirm these results.

CONCLUSION

Skilled doctors who use RHT on a daily basis argue that the reasons for the progress hampered in this field are not the lack of effectiveness of technique but the paucity of qualified hyperthermia centres, lack of quality assurance processes, poor temperature monitoring and heterogeneous practices, lack of funding, poorer tolerance of older technology, limited access for the patients.

To overcome these limitations, we performed this study on a disease with high lethality where current therapeutic options in advanced stages are still unsatisfactory.

Therefore, our observations do not have the value of a prospective randomized study with defined and adequate times and methods of unfolding but, this study, despite many limitations, showed that mEHT group had improved OS and disease control rate in stage III-IV pancreatic cancer. These data are also reported for elderly patients. These data suggest that the association of mEHT to CHT is effective for the treatment of pancreatic tumors. mEHT was safe and had only mild toxicity. Notably not adding any toxicity compared to CHT. We hope that the results of our study will orient the scientific community to perform prospective, randomized, clinical study to further define the mEHT safety and efficacy in pancreatic cancer patients.

ARTICLE HIGHLIGHTS

Research background

Modulated Electro- Hyperthermia (mEHT) optimize the distribution of drugs in cancer cells, improves blood circulation, increases district oxygen level, reduces DNA repair and promotes cancer cell death. mEHT is effective for different types of tumors and also in pancreatic adenocarcinoma resulting in better tumor response and longer survival, as reported in clinical case series and phase II studies.

Research motivation

Pancreatic cancer is generally refractory to chemotherapy (CHT) and radiotherapy because of its low perfused and hypoxic microenvironment that reduces the efficacy and sensitivity to these treatments. The high heterogeneity of pancreatic cancer cells is counteracted by mEHT, which selectively damages the external membrane of cancer cells that have different energy uptake on the membrane rafts from healthy tissue, inducing immunogenic death of cancer cells or apoptosis.

Research objectives

The aim of this study was to assess the advantages of mEHT in association to CHT compared to CHT alone in locally advanced or metastatic pancreatic tumors.

Research methods

This was a retrospective data collection on patients affected by metastatic or locally advanced pancreatic cancer performed in 9 Italian centres. This study included 217 patients, 128 (59%) of them were treated with CHT (no-mEHT) and 89 (41%) patients received mEHT alone or in association with CHT. mEHT treatments were performed applying a power of 60-150 watts for 40-90 min two or three times a week for 4-6 wk.

Research results

mEHT group had a median overall survival (OS) greater than non-mEHT group (20 mo, range 1.6-24, *vs* 9 mo, range 0.4-56.25, $P < 0.001$). mEHT group showed a higher number of partial responses (45% *vs* 24%, $P = 0.0018$) and a lower number of progressions (PD) (4% *vs* 31%, $P < 0.001$) than the no-mEHT group, at the three months follow-up. Adverse events were observed in 2.6% of mEHT sessions.

Research conclusions

The results obtained in this study provided new evidence that mEHT is safe and has beneficial effects on survival and tumor response of stage III-IV pancreatic tumor treatment. Further studies are warranted.

Research perspectives

This multicentre retrospective observational comparative study on 217 patients provides further evidence that mEHT improved OS and disease control rate in stage III-IV pancreatic cancer. This study would like to open a new avenue in the treatment of locally advanced pancreatic cancer including mEHT in therapeutic options, since the results of CHT alone are not satisfactory.

FOOTNOTES

Author contributions: Fiorentini G, Sarti D and Guadagni S wrote the paper; Fiorentini G and Sarti D performed the formal analysis; Fiorentini G, Sarti D, Bonucci M, Hammarberg Ferri I, Mambini A, Sciacca P, Ballerini M, Bonanno S, Milandri C, Nani R, Guadagni S, Dentico P and Fiorentini C collected the data; Fiorentini G, Sarti D and Bonucci M administered and supervised the project administration; Fiorentini G, Sarti D, Bonucci M, Ferri I, Mambini A, Sciacca P, Ballerini M, Bonanno S, Milandri C, Nani R, Guadagni S, Dentico P and Fiorentini C reviewed and edited the final manuscript.

Institutional review board statement: The study was approved by our Institutional Review Board.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous data that were collected retrospectively after each patient agreed and performed the treatment after signing the written consent for the treatment.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Data sharing statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Italy

ORCID number: Giammaria Fiorentini 0000-0002-5615-889X; Donatella Sarti 0000-0002-8031-7846; Andrea Mambrini 0000-0001-9736-4991; Carlo Milandri 0000-0001-6932-8977; Roberto Nani 0000-0002-0923-5151; Stefano Guadagni 0000-0001-8525-084X; Caterina Fiorentini 0000-0002-9346-1409.

S-Editor: Ma YJ
L-Editor: A
P-Editor: Ma YJ

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 7-30 [PMID: 31912902 DOI: 10.3322/caac.21590]
2 **International Agency for Research on Cancer**. World Health Organization. Global Cancer Observatory 2018. Available from: https://gco.iarc.fr/
3 **Zalevskaja K**, Mecklin JP, Seppälä TT. Clinical characteristics of pancreatic and biliary tract cancers in Lynch syndrome: A retrospective analysis from the Finnish National Lynch Syndrome Research Registry. *Front Oncol* 2023; **13**: 1123901 [PMID: 36816932 DOI: 10.3389/fonc.2023.1123901]
4 **Panchoo AV**, VanNess GH, Rivera-Rivera E, Laborda TJ. Hereditary pancreatitis: An updated review in pediatrics. *World J Clin Pediatr* 2022; **11**: 27-37 [PMID: 35096544 DOI: 10.5409/wjcp.v11.i1.27]
5 **Tacheci I**, Kopacova M, Bures J. Peutz-Jeghers syndrome. *Curr Opin Gastroenterol* 2021; **37**: 245-254 [PMID: 33591027 DOI: 10.1097/MOG.0000000000000718]
6 **Archangelidi O**, Cullinan P, Simmonds NJ, Mentzakis E, Peckham D, Bilton D, Carr SB. Incidence and risk factors of cancer in individuals with cystic fibrosis in the UK; a case-control study. *J Cyst Fibros* 2022; **21**: 302-308 [PMID: 34348871 DOI: 10.1016/j.jcf.2021.07.004]
7 **Kindler HL**, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, Park JO, Hochhauser D, Arnold D, Oh DY, Reinacher-Schick A, Tortora G, Algül H, O'Reilly EM, Bordia S, McGuinness D, Cui K, Locker GY, Golan T. Overall Survival Results From the POLO Trial: A Phase III Study of Active Maintenance Olaparib Versus Placebo for Germline BRCA-Mutated Metastatic Pancreatic Cancer. *J Clin Oncol* 2022; **40**: 3929-3939 [PMID: 35834777 DOI: 10.1200/JCO.21.01604]
8 **Chin V**, Nagrial A, Sjoquist K, O'Connor CA, Chantrill L, Biankin AV, Scholten RJ, Yip D. Chemotherapy and radiotherapy for advanced pancreatic cancer. *Cochrane Database Syst Rev* 2018; **3**: CD011044 [PMID: 29557103 DOI: 10.1002/14651858.CD011044.pub2]
9 **Martin-Perez E**, Domínguez-Muñoz JE, Botella-Romero F, Cerezo L, Matute Teresa F, Serrano T, Vera R. Multidisciplinary consensus statement on the clinical management of patients with pancreatic cancer. *Clin Transl Oncol* 2020; **22**: 1963-1975 [PMID: 32318964 DOI: 10.1007/s12094-020-02350-6]
10 **Gourgou-Bourgade S**, Bascoul-Mollevi C, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Boige V, Bérille J, Conroy T. Impact of FOLFIRINOX compared with gemcitabine on quality of life in patients with metastatic pancreatic cancer: results from the PRODIGE 4/ACCORD 11 randomized trial. *J Clin Oncol* 2013; **31**: 23-29 [PMID: 23213101 DOI: 10.1200/JCO.2012.44.4869]
11 **Ye LF**, Ren C, Bai L, Liang JY, Hu MT, Yang H, Wang ZQ, Wang FH, Xu RH, Li YH, Wang DS. Efficacy and safety of modified FOLFIRINOX as salvage therapy for patients with refractory advanced biliary tract cancer: a retrospective study. *Invest New Drugs* 2021; **39**: 836-845 [PMID: 33411209 DOI: 10.1007/s10637-020-01045-7]
12 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris

M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]

13 **Feliu J**, Jorge Fernández M, Macarulla T, Massuti B, Albero A, González González JF, Quintero-Aldana G, Delgado-Mingorance JI, Fernández Montes A, García Piernavieja C, Valladares-Ayerbes M, López Muñoz AM, Mondéjar Solís R, Vicente P, Casado Gonzalez E, González Cebrián I, López-Vivanco G. Phase II clinical trial of nab-paclitaxel plus gemcitabine in elderly patients with previously untreated locally advanced or metastatic pancreatic adenocarcinoma: the BIBABRAX study. *Cancer Chemother Pharmacol* 2021; **87**: 543-553 [PMID: 33452559 DOI: 10.1007/s00280-020-04214-w]

14 **Oba A**, Ho F, Bao QR, Al-Musawi MH, Schulick RD, Del Chiaro M. Neoadjuvant Treatment in Pancreatic Cancer. *Front Oncol* 2020; **10**: 245 [PMID: 32185128 DOI: 10.3389/fonc.2020.00245]

15 **Weniger M**, Honselmann KC, Liss AS. The Extracellular Matrix and Pancreatic Cancer: A Complex Relationship. *Cancers (Basel)* 2018; **10** [PMID: 30200666 DOI: 10.3390/cancers10090316]

16 **Maurici CE**, Colenbier R, Wylleman B, Brancato L, van Zwol E, Van den Bossche J, Timmermans JP, Giovannetti E, Mori da Cunha MGMC, Bogers J. Hyperthermia Enhances Efficacy of Chemotherapeutic Agents in Pancreatic Cancer Cell Lines. *Biomolecules* 2022; **12** [PMID: 35625581 DOI: 10.3390/biom12050651]

17 **Forika G**, Balogh A, Vancsik T, Zalatnai A, Petovari G, Benyo Z, Krenacs T. Modulated Electro-Hyperthermia Resolves Radioresistance of Panc1 Pancreas Adenocarcinoma and Promotes DNA Damage and Apoptosis In Vitro. *Int J Mol Sci* 2020; **21** [PMID: 32707717 DOI: 10.3390/ijms21145100]

18 **Forika G**, Kiss E, Petovari G, Danko T, Gellert AB, Krenacs T. Modulated Electro-Hyperthermia Supports the Effect of Gemcitabine Both in Sensitive and Resistant Pancreas Adenocarcinoma Cell Lines. *Pathol Oncol Res* 2021; **27**: 1610048 [PMID: 34955688 DOI: 10.3389/pore.2021.1610048]

19 **Krenacs T**, Meggyeshazi N, Forika G, Kiss E, Hamar P, Szekely T, Vancsik T. Modulated Electro-Hyperthermia-Induced Tumor Damage Mechanisms Revealed in Cancer Models. *Int J Mol Sci* 2020; **21** [PMID: 32872532 DOI: 10.3390/ijms21176270]

20 **Ohguri T**, Imada H, Yahara K, Narisada H, Morioka T, Nakano K, Korogi Y. Concurrent chemoradiotherapy with gemcitabine plus regional hyperthermia for locally advanced pancreatic carcinoma: initial experience. *Radiat Med* 2008; **26**: 587-596 [PMID: 19132489 DOI: 10.1007/s11604-008-0279-y]

21 **Ishikawa T**, Kokura S, Sakamoto N, Ando T, Imamoto E, Hattori T, Oyamada H, Yoshinami N, Sakamoto M, Kitagawa K, Okumura Y, Yoshida N, Kamada K, Katada K, Uchiyama K, Handa O, Takagi T, Yasuda H, Sakagami J, Konishi H, Yagi N, Naito Y, Yoshikawa T. Phase II trial of combined regional hyperthermia and gemcitabine for locally advanced or metastatic pancreatic cancer. *Int J Hyperthermia* 2012; **28**: 597-604 [PMID: 22838644 DOI: 10.3109/02656736.2012.695428]

22 **Maebayashi T**, Ishibashi N, Aizawa T, Sakaguchi M, Sato T, Kawamori J, Tanaka Y. Treatment outcomes of concurrent hyperthermia and chemoradiotherapy for pancreatic cancer: Insights into the significance of hyperthermia treatment. *Oncol Lett* 2017; **13**: 4959-4964 [PMID: 28588736 DOI: 10.3892/ol.2017.6066]

23 **Datta NR**, Pestalozzi B, Clavien PA, Siebenhüner A, Puric E, Khan S, Mamot C, Riesterer O, Knuchel J, Reiner CS, Bodis S; members of the HEATPAC Trial Group. "HEATPAC" - a phase II randomized study of concurrent thermochemoradiotherapy versus chemoradiotherapy alone in locally advanced pancreatic cancer. *Radiat Oncol* 2017; **12**: 183 [PMID: 29162142 DOI: 10.1186/s13014-017-0923-8]

24 **van der Horst A**, Versteijne E, Besselink MGH, Daams JG, Bulle EB, Bijlsma MF, Wilmink JW, van Delden OM, van Hooft JE, Franken NAP, van Laarhoven HWM, Crezee J, van Tienhoven G. The clinical benefit of hyperthermia in pancreatic cancer: a systematic review. *Int J Hyperthermia* 2018; **34**: 969-979 [PMID: 29168401 DOI: 10.1080/02656736.2017.1401126]

25 **Fiorentini G**, Sarti D, Casadei V, Milandri C, Dentico P, Mambrini A, Nani R, Fiorentini C, Guadagni S. Modulated Electro-Hyperthermia as Palliative Treatment for Pancreatic Cancer: A Retrospective Observational Study on 106 Patients. *Integr Cancer Ther* 2019; **18**: 1534735419878505 [PMID: 31561722 DOI: 10.1177/1534735419878505]

26 **Rogers SJ**, Datta NR, Puric E, Timm O, Marder D, Khan S, Mamot C, Knuchel J, Siebenhüner A, Pestalozzi B, Guckenberger M, Bodis S, Riesterer O. The addition of deep hyperthermia to gemcitabine-based chemoradiation may achieve enhanced survival in unresectable locally advanced adenocarcinoma of the pancreas. *Clin Transl Radiat Oncol* 2021; **27**: 109-113 [PMID: 33598571 DOI: 10.1016/j.ctro.2021.01.008]

27 **Sarti D**, Milandri C, Fiorentini C, Mambrini A, Fiorentini G. Modulated electro-hyperthermia for the treatment of elderly pancreatic cancer patients. *Argum Geriatr Oncol* 2020; **5**: 1-7

28 **Fiorentini G**, Sarti D, Ranieri G, Gadaleta CD, Fiorentini C, Milandri C, Mambrini A, Guadagni S. Modulated electro-hyperthermia in stage III and IV pancreatic cancer: Results of an observational study on 158 patients. *World J Clin Oncol* 2021; **12**: 1064-1071 [PMID: 34909400 DOI: 10.5306/wjco.v12.i11.1064]

29 **Szasz O**. Bioelectromagnetic Paradigm of Cancer Treatment—Modulated Electro-Hyperthermia (mEHT). *Open J Biophys* 2019; **9**: 98-109 [DOI: 10.4236/ojbiphy.2019.92008]

30 **Szasz A**. Thermal and nonthermal effects of radiofrequency on living state and applications as an adjuvant with radiation therapy. *J Radiat Cancer Res* 2019; **10**: 1-17. [DOI: 10.4103/jrcr.jrcr_25_18]

31 **Lee SY**, Szigeti GP, Szasz AM. Oncological hyperthermia: The correct dosing in clinical applications. *Int J Oncol* 2019; **54**: 627-643 [PMID: 30483754 DOI: 10.3892/ijo.2018.4645]

32 **Alshaibi HF**, Al-Shehri B, Hassan B, Al-Zahrani R, Assiss T. Modulated Electrohyperthermia: A New Hope for Cancer Patients. *Biomed Res Int* 2020; **2020**: 8814878 [PMID: 33274226 DOI: 10.1155/2020/8814878]

33 **Petenyi FG**, Garay T, Muhl D, Izso B, Karaszi A, Borbenyi E, Herold M, Herold Z, Szasz AM, Dank M. Modulated Electro-Hyperthermic (mEHT) Treatment in the Therapy of Inoperable Pancreatic Cancer Patients-A Single-Center Case-Control Study. *Diseases* 2021; **9** [PMID: 34842668 DOI: 10.3390/diseases9040081]

34 **Szasz AM**, Minnaar CA, Szentmártoni G, Szigeti GP, Dank M. Review of the Clinical Evidences of Modulated Electro-Hyperthermia (mEHT) Method: An Update for the Practicing Oncologist. *Front Oncol* 2019; **9**: 1012 [PMID: 31737558

DOI: 10.3389/fonc.2019.01012]

35 **Peters GJ**, Clavel M, Noordhuis P, Geyssen GJ, Laan AC, Guastalla J, Edzes HT, Vermorken JB. Clinical phase I and pharmacology study of gemcitabine (2', 2'-difluorodeoxycytidine) administered in a two-weekly schedule. *J Chemother* 2007; **19**: 212-221 [PMID: 17434832 DOI: 10.1179/joc.2007.19.2.212]

36 **Fiorentini G**, Sarti D, Casadei V, Minnaar C, Szász MA. Modulated Electro-Hyperthermia (mEHT)[oncothermia®] Protocols as Complementary Treatment. *Oncothermia J* 2019; **25**: 85-115

37 **Szasz A**. Challenges and Solutions of Oncological Hyperthermia. *Thermal Medicine* 2013; **29**: 1-23 [DOI: 10.3191/thermalmed.29.1]

38 **Szasz O**, Szasz A. Heating, efficacy and dose of local hyperthermia. *Open J Bioph* 2016; **6**: 10-18 [DOI: 10.4236/ojbiphy.2016.61002]

39 **Tschoep-Lechner KE**, Milani V, Berger F, Dieterle N, Abdel-Rahman S, Salat C, Issels RD. Gemcitabine and cisplatin combined with regional hyperthermia as second-line treatment in patients with gemcitabine-refractory advanced pancreatic cancer. *Int J Hyperthermia* 2013; **29**: 8-16 [PMID: 23245336 DOI: 10.3109/02656736.2012.740764]

40 **Maluta S**, Schaffer M, Pioli F, Dall'oglio S, Pasetto S, Schaffer PM, Weber B, Giri MG. Regional hyperthermia combined with chemoradiotherapy in primary or recurrent locally advanced pancreatic cancer : an open-label comparative cohort trial. *Strahlenther Onkol* 2011; **187**: 619-625 [PMID: 21932025 DOI: 10.1007/s00066-011-2226-6]

41 **Fiorentini G**, Sarti D, Gadaleta CD, Ballerini M, Fiorentini C, Garfagno T, Ranieri G, Guadagni S. A Narrative Review of Regional Hyperthermia: Updates From 2010 to 2019. *Integr Cancer Ther* 2020; **19**: 1534735420932648 [PMID: 33054425 DOI: 10.1177/1534735420932648]

42 **Kim S**, Lee JH, Cha J, You SH. Beneficial effects of modulated electro-hyperthermia during neoadjuvant treatment for locally advanced rectal cancer. *Int J Hyperthermia* 2021; **38**: 144-151 [PMID: 33557636 DOI: 10.1080/02656736.2021.1877837]

43 **Liu X**, Song J, Zhang H, Liu X, Zuo F, Zhao Y, Yin X, Guo X, Wu X, Xu J, Hu J, Jing J, Ma X, Shi H. Immune checkpoint HLA-E:CD94-NKG2A mediates evasion of circulating tumor cells from NK cell surveillance. *Cancer Cell* 2023; **41**: 272-287.e9 [PMID: 36706761 DOI: 10.1016/j.ccell.2023.01.001]

44 **Dbouk M**, Katona BW, Brand RE, Chak A, Syngal S, Farrell JJ, Kastrinos F, Stoffel EM, Blackford AL, Rustgi AK, Dudley B, Lee LS, Chhoda A, Kwon R, Ginsberg GG, Klein AP, Kamel I, Hruban RH, He J, Shin EJ, Lennon AM, Canto MI, Goggins M. The Multicenter Cancer of Pancreas Screening Study: Impact on Stage and Survival. *J Clin Oncol* 2022; **40**: 3257-3266 [PMID: 35704792 DOI: 10.1200/JCO.22.00298]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 June 24; 14(6): 227-229

DOI: 10.5306/wjco.v14.i6.227

ISSN 2218-4333 (online)

CORRECTION

Correction: Propensity-matched analysis of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma undergoing a liver transplant

Ajacio Bandeira de Mello Brandão, Santiago Rodriguez, Alfeu de Medeiros Fleck Jr, Claudio Augusto Marroni, Mário B Wagner, Alex Hörbe, Matheus V Fernandes, Carlos TS Cerski, Gabriela Perdomo Coral

Specialty type: Transplantation

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A, A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): E

P-Reviewer: Rizzo A, Italy; Wei W, China; Zharikov YO, Russia

Received: October 25, 2022
Peer-review started: October 25, 2022
First decision: November 25, 2022
Revised: December 6, 2022
Accepted: February 1, 2023
Article in press: February 1, 2023
Published online: June 24, 2023

Ajacio Bandeira de Mello Brandão, Santiago Rodriguez, Claudio Augusto Marroni, Matheus V Fernandes, Gabriela Perdomo Coral, Graduate Program in Medicine: Hepatology, School of Medicine, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre 90050170, RS, Brazil

Ajacio Bandeira de Mello Brandão, Alfeu de Medeiros Fleck Jr, Claudio Augusto Marroni, Liver Transplantation Group, Santa Casa de Misericórdia de Porto Alegre, Porto Alegre 90020090, RS, Brazil

Santiago Rodriguez, Department of Hepatology, Hospital Vozandes Quito-HVQ, Quito 170521, Ecuador

Mário B Wagner, School of Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre 90035002, RS, Brazil

Alex Hörbe, Interventional Radiology Unit, Santa Casa de Misericórdia de Porto Alegre, Porto Alegre 90020090, RS, Brunei Darussalam

Carlos TS Cerski, Department of Pathology, School of Medicine, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre 90035002, RS, Brazil

Corresponding author: Ajacio Bandeira de Mello Brandão, PhD, Researcher, Graduate Program in Medicine: Hepatology, School of Medicine, Universidade Federal de Ciências da Saúde de Porto Alegre, R. Eng. Álvaro Nunes Pereira, 400/402, Porto Alegre 90050170, RS, Brazil. ajaciob@gmail.com

Abstract

Rereading the article "Propensity-matched analysis of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma undergoing a liver transplant" (DOI: 10.5306/wjco.v13.i8.688), published on August 24, we observe, with concern, that figures 3 and 4 are wrong. The authors have attached the correct figures for correction.

Key Words: Cholangiocarcinoma; Hepatocellular carcinoma; Liver; Prognosis; Recurrence; Survival analysis; Correction

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The authors have attached the correct figures for correction rereading the article "Propensity-matched analysis of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma undergoing a liver transplant" (DOI: 10.5306/wjco.v13.i8.688).

Citation: Brandão ABM, Rodriguez S, Fleck Jr AM, Marroni CA, Wagner MB, Hörbe A, Fernandes MV, Cerski CT, Coral GP. Correction: Propensity-matched analysis of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma undergoing a liver transplant. *World J Clin Oncol* 2023; 14(6): 227-229
URL: https://www.wjgnet.com/2218-4333/full/v14/i6/227.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i6.227

TO THE EDITOR

Rereading our article "Propensity-matched analysis of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma undergoing a liver transplant" [1] (DOI: 10.5306/wjco.v13.i8.688), published on August 24, we observe, with concern, that figures 3 and 4 are wrong. At some point, we have mistakenly forwarded a wrong file. Therefore, we attach the correct figures for correction (Figures 1 and 2). We hope that it will be possible to replace the wrong figures with the correct ones and that this process will not be difficult. We apologize in advance for this unfortunate mistake. Of note, correction does not change the interpretation of results or conclusion.

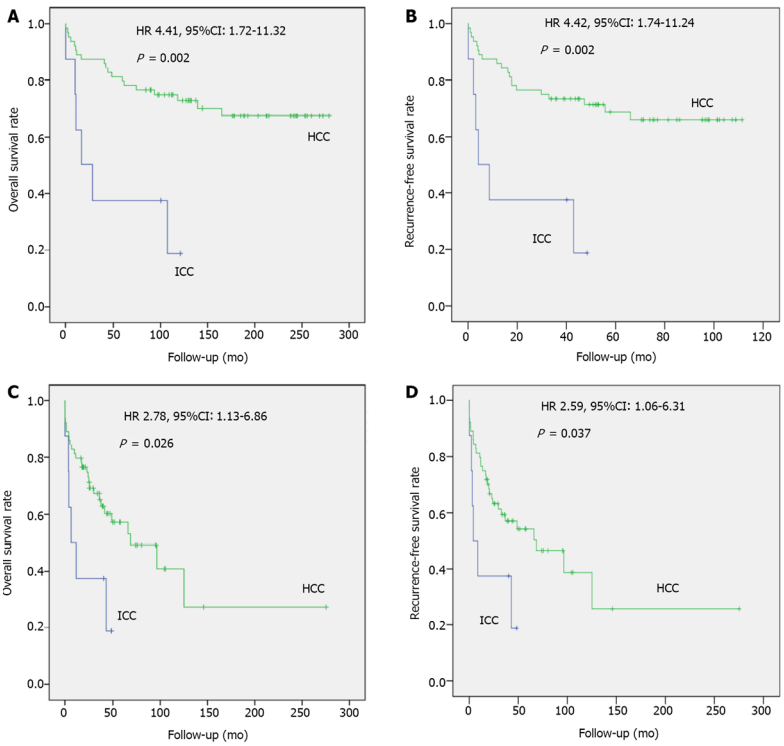


Figure 1 Corrected "Figure 3". A-D: Kaplan-Meier curves representing post-liver transplant overall survival and recurrence-free survival in patients with intrahepatic cholangiocarcinoma (ICC) compared with patients with hepatocellular carcinoma (HCC), matched 1:8 for pretransplant tumor characteristics (A and B) and posttransplant tumor characteristics (C and D). HRs: Hazard ratios; CI: Confidence interval.

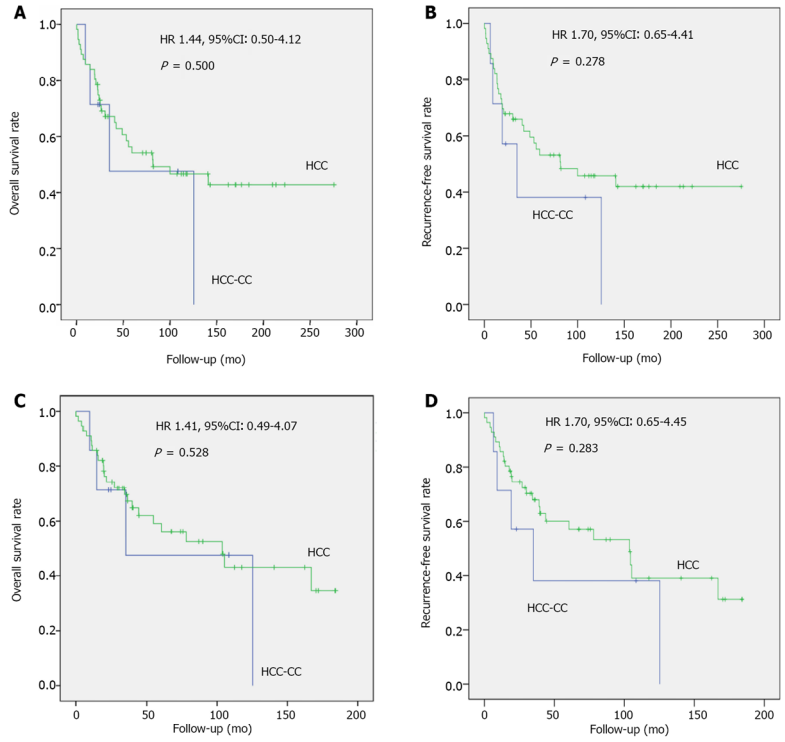


Figure 2 Corrected "Figure 4". A-D: Kaplan-Meier curves representing post-liver transplant overall survival and recurrence-free survival in patients with mixed hepatocellular-cholangiocarcinoma (HCC-CC) compared with patients with HCC, matched 1:8 for pretransplant tumor characteristics (A and B) and posttransplant tumor characteristics (C and D). HRs: Hazard ratios; CI: Confidence interval.

FOOTNOTES

Author contributions: All the authors solely contributed to this paper.

Conflict-of-interest statement: All authors have no conflicts of interest to disclose.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Brazil

ORCID number: Ajacio Bandeira de Mello Brandão 0000-0001-8411-5654; Santiago Rodriguez 0000-0001-8610-3622; Alfeu de Medeiros Fleck Jr 0000-0002-0424-6919; Claudio Augusto Marroni 0000-0002-1718-6548; Mário B Wagner 0000-0002-3661-4851; Alex Hörbe 0000-0002-6550-2947; Matheus V Fernandes 0000-0002-1179-0912; Carlos TS Cerski 0000-0002-2757-4974; Gabriela Perdomo Coral 0000-0003-4318-2871.

S-Editor: Chen YL
L-Editor: A
P-Editor: Chen YL

REFERENCES

1 **Brandão ABM**, Rodriguez S, Fleck Jr AM, Marroni CA, Wagner MB, Hörbe A, Fernandes MV, Cerski CT, Coral GP. Propensity-matched analysis of patients with intrahepatic cholangiocarcinoma or mixed hepatocellular-cholangiocarcinoma and hepatocellular carcinoma undergoing a liver transplant. *World J Clin Oncol* 2022; **13**: 688-701 [PMID: 36160465 DOI: 10.5306/wjco.v13.i8.688]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 July 24; 14(7): 230-284

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 7 July 24, 2023

REVIEW

230 Stromal inflammation, fibrosis and cancer: An old intuition with promising potential

Oey O, Sunjaya AF, Khan Y, Redfern A

MINIREVIEWS

247 Role of prophylactic central neck lymph node dissection for papillary thyroid carcinoma in the era of de-escalation

Pavlidis ET, Pavlidis TE

ORIGINAL ARTICLE

Retrospective Study

259 Relationship between anal cancer recurrence and cigarette smoking

McMahon KR, Gemma N, Clapp M, Sanchez-Montejo P, Dibello J, Laipply E

SYSTEMATIC REVIEWS

265 Cancer screening and management in the transgender population: Review of literature and special considerations for gender affirmation surgery

Panichella JC, Araya S, Nannapaneni S, Robinson SG, You S, Gubara SM, Gebreyesus MT, Webster T, Patel SA, Hamidian Jahromi A

World Journal of Clinical Oncology

Monthly Volume 14 Number 7 July 24, 2023

Contents

ABOUT COVER

Peer Reviewer of *World Journal of Clinical Oncology*, Jorge Gutiérrez-Cuevas, PhD, Professor, Department of Molecular Biology and Genomics, Institute for Molecular Biology in Medicine and Gene Therapy, CUCS, University of Guadalajara, Guadalajara 44340, Jalisco, Mexico. gutierrezcj05@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJCO* as 2.8; IF without journal self cites: 2.8; 5-year IF: 3.0; Journal Citation Indicator: 0.36.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Mai-Xin Ji*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Jia-Ping Yan*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
July 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 July 24; 14(7): 230-246

DOI: 10.5306/wjco.v14.i7.230

ISSN 2218-4333 (online)

REVIEW

Stromal inflammation, fibrosis and cancer: An old intuition with promising potential

Oliver Oey, Angela Felicia Sunjaya, Yasir Khan, Andrew Redfern

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Agostino SD, Italy; Gutiérrez-Cuevas J, Mexico

Received: May 16, 2023
Peer-review started: May 16, 2023
First decision: May 25, 2023
Revised: June 7, 2023
Accepted: June 21, 2023
Article in press: June 21, 2023
Published online: July 24, 2023

Oliver Oey, Faculty of Medicine, University of Western Australia, Perth 6009, Crawley NA, Australia

Oliver Oey, Department of Medical Oncology, Sir Charles Gardner Hospital, Nedlands 6009, Australia

Angela Felicia Sunjaya, Institute of Cardiovascular Science, University College London, London WC1E 6DD, United Kingdom

Yasir Khan, Department of Medical Oncology, St John of God Midland Public and Private Hospital, Midland 6056, WA, Australia

Andrew Redfern, Department of Medical Oncology, Fiona Stanley Hospital, Murdoch 6150, WA, Australia

Corresponding author: Angela Felicia Sunjaya, BMed, MD, Master's Student, Researcher, Institute of Cardiovascular Science, University College London, 62 Huntley St, London WC1E 6DD, United Kingdom. angelafelicia.s@gmail.com

Abstract

It is now well established that the biology of cancer is influenced by not only malignant cells but also other components of the tumour microenvironment. Chronic inflammation and fibrosis have long been postulated to be involved in carcinogenesis. Chronic inflammation can promote tumorigenesis *via* growth factor/cytokine-mediated cellular proliferation, apoptotic resistance, immunosuppression; and free-radical-induced oxidative deoxyribonucleic acid damage. Fibrosis could cause a perturbation in the dynamics of the tumour microenvironment, potentially damaging the genome surveillance machinery of normal epithelial cells. In this review, we will provide an in-depth discussion of various diseases characterised by inflammation and fibrosis that have been associated with an increased risk of malignancy. In particular, we will present a comprehensive overview of the impact of alterations in stromal composition on tumorigenesis, induced as a consequence of inflammation and/or fibrosis. Strategies including the application of various therapeutic agents with stromal manipulation potential and targeted cancer screening for certain inflammatory diseases which can reduce the risk of cancer will also be discussed.

Key Words: Inflammation; Fibrosis; Tumour microenvironment; Stroma; Cancer

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Chronic inflammation and fibrosis have long been postulated to be involved in carcinogenesis *via* numerous mechanisms including but not limited to growth factor/cytokine-mediated cellular proliferation, apoptotic resistance, immunosuppression; and free-radical-induced oxidative deoxyribonucleic acid damage. In this review, we discuss various inflammatory and/or fibrotic conditions that have been associated with increased cancer risk, with particular emphasis on their pathophysiology. We also review various therapeutic agents and specific cancer screening that could be applicable in reducing the incidence of cancers developing from the corresponding inflammatory and/or fibrotic conditions, thereby reducing morbidity and mortality.

Citation: Oey O, Sunjaya AF, Khan Y, Redfern A. Stromal inflammation, fibrosis and cancer: An old intuition with promising potential. *World J Clin Oncol* 2023; 14(7): 230-246
URL: https://www.wjgnet.com/2218-4333/full/v14/i7/230.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i7.230

INTRODUCTION

In recent years, there is growing consensus that the biology of cancer is not solely defined by malignant cells, but also by the surrounding tumour microenvironment (TME). The TME consists of cellular and non-cellular stroma. The concept that the TME may influence cancer biology was inspired by the observation of immune cells surrounding the tumour by Rudolf Virchow in 1863, and "the seed and soil theory" by Stephen Paget in 1889, in which he hypothesised that the metastatic destination of a certain cancer is dependent on similarities between the TME of primary tumour and the microenvironment at the site of metastases[1,2]. Since then, there have been significant advancements in the understanding of the impact of the TME on the behaviour of malignant cells, from initial tumorigenesis, through progression to therapy resistance[3-5]. This review will focus on the impact of both the physiological and pathological tissue microenvironment, particularly stromal fibrosis and inflammation, on tumorigenesis.

In this context, stroma refers to the component of an organ which provides biomechanical and nutritional support to the corresponding parenchyma. Specifically, it comprises of immune cells, fibroblasts, mesenchymal stromal cells, endothelial cells, pericytes, adipocytes, and the extracellular matrix (ECM). The ECM, consisting of collagen, proteoglycans, glycosaminoglycans and other macromolecules, provides structural and biochemical support for cellular components in the surrounding parenchyma. Of note, some authors do not include immune cells as a component of stroma, however, immune cells such as macrophages, neutrophils and lymphocytes, play an integral role to the function of parenchymal cells and can have far-reaching effects on tumour biology and consequent behaviour, as such they will be classified as a stromal component in this review.

Many stromal components have been shown through various in vitro and animal studies to influence the behaviour and fate of normal cells, including altering the risk of malignant transformation[6-8]. Inflammation and fibrosis are both common processes that significantly alter the cellular and ECM components of normal stroma and so may influence or underlie such behavioural shifts. Both processes have been seen to upregulate the expression of several tumorigenic signalling pathways including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), signal transducers and activators of transcription (STAT), wingless-related integration site (Wnt) and phosphatidylinositide 3-kinase (PI3K) *via* the release of pro inflammatory cytokines[9-12]. Hence, several inflammatory and fibrotic conditions have been linked as triggers for tumour development in the organ involved, whether due to autoimmune responses (inflammatory bowel disease and colorectal cancer[13]), bacterial or viral infections (pneumonia or tuberculosis with lung cancer[14]) and environmental factors (silica and lung cancer[15]).

Often, these pathological processes appear to be required for tumorigenesis rather than simply an overrepresentation of certain otherwise normal stromal components. For instance, inflamed adipose mammary tissue in the context of obese mice, increases myofibroblasts number, promoting fibrosis and transformation of normal to malignant breast tissue[6], whereas normal mouse fibroblasts have been shown to prevent clonal proliferation of polyoma virus-transformed cells *in vitro*[7]. However, there are less frequent precedents where normal stromal components may also contribute to tumorigenesis. Normal fibroblasts have been demonstrated to promote the generation of breast cancer stem cells[8]. Additionally, high mammographic breast density, which results from a higher density of stromal and glandular breast components and a lower proportion of adipocytes, is a potent risk factor for breast cancer development.

In this review we will discuss various medical conditions substantively characterised by inflammation and fibrosis, specifically those known to be linked to increased cancer risk. Furthermore, we will look to whether scenarios exist where physiological variations in stromal composition correlate with differing cancer incidence. In doing so, we will discuss the biological contribution of the various stromal components to tumorigenesis known to date and discuss interventions that may influence these processes to achieve therapeutic advantage.

PATHOLOGICAL INFLAMMATION, FIBROSIS AND CANCER RISK

A range of medical conditions exist that involve one or both of these processes. A common evolution pathogenically is of initial inflammation with subsequent fibrosis. However, each of the processes may occur in isolation. Here we look across a range of scenarios at whether each may affect cancer risk in isolation or whether both appear to be required for tumorigenesis (Table 1).

INFLAMMATORY BOWEL DISEASE AND COLORECTAL CANCER

Inflammatory bowel disease (IBD) is sub-divided into ulcerative colitis (UC), which affects only the large bowel, and Crohn's disease (CD) which can involve any area of the gut from mouth to rectum[16]. The risk of developing colorectal cancer in UC patients is elevated compared to the disease-free population, with an overall risk of 4.8[13]. Similarly, in CD risk is elevated although to a more moderate degree, by 2-3 times[17]. In keeping with the small bowel involvement in CD, small intestinal tumours are also increased, by a relative risk of 18.75%[17]. The risk in both conditions is associated with duration and extent of inflammation[18]. Beyond inflammation, both conditions can also result in fibrosis although the pattern differs. Fibrosis leading to eventual stricture and potential obstruction is more common in CD than UC, with around 25% of CD sufferers eventually destined to develop a stricture over the course of the illness[19]. On initial consideration this appears at odds with the risk of colorectal cancer, but may be explained by the distribution of fibrotic change. In UC fibrosis is often superficial, affecting only the mucosal and sub-mucosal layers[20] but still, therefore, able to impact the epithelial layer from which neoplasms arise, and generally impacting a longer continuous length of colon. In contrast, Crohn's disease is characterized by patchy change and skip lesions such that the total area of involved epithelium is often less[16].

Considering these patterns and parallel links in other organs between inflammation, fibrosis and neoplastic transformation, the development of colitis-associated carcinoma (CAC) appears highly likely to be directly attributable to chronic inflammation and consequent fibrosis[21,22]. There is a biological rationale, with previous studies showing that certain inflammatory cytokines prominent in UC, namely TNF-α, IL-6 and TGF-β can promote a pro-tumorigenic microenvironment by stimulating essential cancer stem cell pathways, evading growth suppressors, and resisting apoptosis[23-25]. This occurs *via* induction of various molecular signalling pathways including NF-κB[9], STAT[26] and Wnt pathways[27]. Incidentally, these cytokines can also promote fibrosis. TNF-α has been demonstrated to induce IL-6 production, which is partly responsible for proliferation of fibroblasts[28,29]. In addition, TGF-β, highly expressed in intestinal epithelial cells, inflammatory cells and fibroblasts is known to induce fibrogenesis and ultimately the deposition of ECM such as collagen, *via* the Wnt/β-catenin pathway, which is also often activated early in dysplastic and surrounding non-dysplastic intestinal epithelial cells, in the setting of CAC carcinogenesis[10,30,31]. This concurs with the upregulation of type 1 collagen, revealed by proteomic analysis in the early stages of colorectal carcinogenesis[32]. Whether or not collagen promotes CAC carcinogenesis remains ambiguous, however increase in collagen may disrupt the polarity of healthy intestinal epithelial cells and stimulate cellular proliferation, thereby promoting malignant transformation.

While it is generally understood that fibrosis occurs as a result of chronic inflammation, it is now understood that fibrosis in IBD may occur without inflammation[33], and further that not all people with IBD develop fibrosis[34]. This prompts the question as to whether either fibrosis or inflammation without the companion process can also trigger carcinogenesis – a question which remains unanswered today due to a lack of cohorts with data that allow the linking degrees of inflammation and fibrosis to cancer risk.

CHRONIC PANCREATITIS AND PANCREATIC DUCTAL ADENOCARCINOMA

Chronic pancreatitis (CP) is a major risk factor for the development of pancreatic ductal adenocarcinoma (PDAC), increasing the risk of PDAC by 20-fold relative to disease-free population[35]. Both CP and PDAC share a common pathological feature – abundant desmoplastic and inflammatory stroma[36]. Hence, the link between the former and the latter could be attributed to the events occurring in the surrounding inflammatory milieu. This was proven in an animal study involving the insertion of K-ras oncogenes within the endogenous K-ras locus, in which mice without pancreatitis did not develop PDAC, while those with pancreatitis did[37]. Thus, it could be deduced that inflammation is a critical factor in PDAC carcinogenesis, at least in response to this, the commonest of oncogenes implicated in pancreatic cancer. In chronic pancreatitis, the release of inflammatory cytokines such as TNF-α and TGF-β and growth factors such as vascular endothelial growth factor (VEGF) and PDGF trigger the proliferation of fibroblasts and the activation of pancreatic stellate cells (PSC) towards a more myofibroblast-like phenotype[38,39]. Activated PSC have a number of functions, including sustaining proliferative signalling in pancreatic epithelial cells; the release of growth factors; and the synthesis of ECM proteins, notably collagen, fibronectin and laminin[40,41]. The deposition of various ECM proteins could cause a perturbation in the dynamics of the ECM, potentially damaging the genome surveillance machinery of normal epithelial cells. Supportive of a role for certain ECM components in PDAC progression is the finding that collagen 1, 4 and hyaluronic acid which promotes cell survival, proliferation and invasion, with higher levels associated with reduced survival[42-44]. This is further supported by the therapeutic benefit derived from the administration of PEGylated Recombinant Human Hyaluronidase in addition to chemotherapy in PDAC patients[45,46].

Table 1 Summary of various inflammatory and fibrotic conditions and relevant malignancies

Disease	Associated cancer	Mechanism	Risk ratio	Possible therapeutic targets
Inflammatory bowel disease	Colorectal cancer	Increased pro-inflammatory cytokines (TNF-α, IL-6 and TGF-β)[24,30,34]; Increased signalling of pro-tumorigenic molecular pathways, apoptosis resistance, fibrogenesis (NF-κB and Wnt/β-catenin)[9,27,30,34]	Ulcerative colitis – 4.8-fold increase[13]; Crohn's disease – 2-3-fold increase[17]	Thiopurines[173] and anti-inflammatory such as mesalazine [174] and NSAID[149]
Chronic pancreatitis	Pancreatic ductal adenocarcinoma	Increased cytokines (TNF-α and TGF-β), growth factors (VEGF, PDGF)[38]; Fibroblast and pancreatic epithelial cell proliferation[40]; Activation of pancreatic stellate cells [39,40]; Increased ECM protein (collagen 1 and 4, laminin, fibronectin) and hyaluronic acid deposition[38]	20-fold increase[35]	PEGylated Recombinant Human Hyaluronidase[45,46]; NSAID[149]
Idiopathic pulmonary fibrosis	Lung cancer	Cellular morphological abnormalities (metaplasia, dysplasia) in fibrotic areas[59]; Reduced immune expression (monocytes, lymphocytes, macrophages) in fibrotic areas[50]; Mutations in tumour-suppressor genes [54]; Upregulated gene expression of ECM components such as collagen and MMP (MMP9 and 11)[57]	3.5-7.3 fold increase [51]	Anti-fibrotic drugs (pirfenidone and nindetanib)[175]
Pneumoconiosis	Lung cancer	Silicosis: Chronic increased release of pro-inflammatory cytokines (IL-12, IL-23 and TNFα) results in DNA damage [66]; Immunosuppression through increased expression of inhibitory immune markers (PD-1, LAG3, FOXP30)[70]. Asbestosis: Increased inflammation (IL-1*β*, TGF-β and PDGF) and fibrosis through expression of NLRP3[70]; Increased ROS and RNS[64,68]; Increased expression of proliferation signalling pathways (EGFR-ERK)[73]	Silicosis – 3-fold increase[15]; Asbestosis – 1.5-6.8-fold increase[65,65]	Anti-fibrotic drugs (pirfenidone and nindetanib)[152]
TB	Lung cancer	Upregulation of anti-apoptotic protein expression *via* inflammatory cytokines (TNF-α and IL-6)[59,76,78]	Pneumonia – 1.4-fold increase[14]; TB – 1.9-fold increase[14]	NSAID[176]
Liver cirrhosis	Hepato-cellular carcinoma	Cellular proliferation, telomere shortening *via* inflammatory cytokines (TGF-β, TNF-α and interleukins)[83,84]; Genomic instability (p53, Ras, mTOR, Wnt signalling pathways)[11,84]; Reduced expression of CD4+ and CD8+ cytotoxic T cell[85]; Increased regulatory T-cell response [86]; Activation of hepatic stellate cells increase myofibroblast and ECM production[11,87]; Hypoxia in fibrosis leads to genotoxicity (ROS, RNO) and angiogenesis (VEGF)[92]	Hepatitis B related – 1.17-fold increase [81]; Hepatitis C related - 1.15-fold increase[81]; NAFLD-related – 1.6-23.7-fold increase[161]	LOX/LOXL2 inhibitors[161,162]; NSAID, Pentoxifylline [177,178]
Primary biliary cholangitis	Cholangiocarcinoma	Increased proliferative signalling *via* inflammatory cytokines (IL-1β, IL-6 and HGF)[96-98]; IL-6 activates p38-MAPK, increases DNA methyltransferase (DNMT) Mcl-1 and telomerase expression[96]; DNA damage (BRAF, K-ras, cyclin d-1, c-myc, COX-2 and p53) due to dysregulated NO production[98]; Fibroblast proliferation and ECM production (collagen type 1 and 3)[103]	9-fold increase[94]	Natural anti-inflammatory products (Curcumin)[102]
GERD and Barrett's oesophagus	Oesophageal cancer	Increased inflammatory cell recruitment (macrophages T, B, dendritic cells)[107]; Inflammatory cytokine release (TNF-α, IL-6, IL-1β, IL-8) activates pro tumorigenic signalling pathways (NF-Kb, STAT-3, HIF-1a)[107,108]; Reduced immune response due to immunosuppressive cytokines (IL-10)[112]; Oxidative stress (ROS and RNS) induce mutagenesis of oncogenes and tumor suppressor genes[110]	30-125-fold increase [106]	NSAID[149]
OSF	Oral squamous cell carcinoma	Increased inflammatory cell recruitment[118]; Oxidative stress induces p53 mutation, decreased DMNT and increased HSP70 and MDM2-P2 promoter[120,122]; Increased prostaglandins, cytokines and growth factors (IL-6, TNF-α, PDGF and TGF-β)[118,119]; Fibrogenesis *via* IL-6 and TGF-β leads to increased ECM protein production (collagen, fibonectin) and inhibit ECM breakdown (PAI-1, TIMP)[124,125]; OSF-associated fibroblast promote dysplastic keratinocyte proliferation *via* GRO-α release and EGFR/ERK activation[128]	19-fold increase[114]	Anti-oxidants, steroids and hyaluronidase [178]
Physiological breast stromal density, breast conditions – chronic mastitis, sclerosing adenosis	Breast cancer	Mammographically dense breast have higher ECM proportion (collagen, immune cells)[131,133]; Mammographically dense breast have higher proportion of glandular epithelial components and lower proportion of adipocytes[132-134]	Physiological higher MBD: 4-6-fold increase[130]; Chronic mastitis: 3-fold increase[137]; Sclerosing adenosis: 2-fold increase[138]	Anti-estrogens (tamoxifen, raloxifene, exemestane and anastrozole)[154-157]; NSAID[149]; LOX-like inhibitors[159,160,163]

GERD: Gastroesophageal reflux disease; OSF: Oral submucosal fibrosis; TNF-α: Tumor necrosis factor-alpha; TGF-β: Transforming growth factor beta; NF-κB: Nuclear factor κB; VEGF: Vascular endothelial growth factor; NSAID: Anti-inflammatory; GRO-α: Regulated oncogene-α; MBD: Mammographic breast density; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal–regulated kinase; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; TB: Tuberculosis; ECM: Extracellular matrix; PDGF: Platelet-derived growth factor; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; HGF: Hepatocyte growth factor; NO: Nitric oxide; BRAF: Proto oncogene B-Raf or v-raf murine sarcoma viral oncogene homolog B1; COX-2: Cyclooxygenase-2; NAFLD: Non alcoholic fatty liver disease.

However, certain alterations in the ECM can be tumour-inhibitory rather than promoting. Quantitative analysis of stroma density in PDAC samples from patients' autopsy revealed that tissue stroma density was substantially lower in samples from patients with metastatic PDAC and that higher stromal content was associated with a more favourable outcome[47]. This finding was further supported by Rhim *et al*[48] who demonstrated that diminished stromal density induced by knocking out sonic hedgehog in an established PDAC mouse model significantly enhanced tumour vascularity and proliferation. Furthermore, another study by Erkan *et al*[49] in which resected PDAC tumors were analysed for PSC activity and collagen deposition showed that the combination of high collagen deposition and low stromal activity was associated with a better prognosis than low collagen deposition and high stromal activity. While these studies relate to the effect of stroma on tumour progression/regression, considering the similarities between carcinogenesis and organ development, it is likely that these findings apply to PDAC carcinogenesis. Combining findings from these studies, the role of chronic inflammation and fibrosis in influencing PDAC risk remains ambiguous.

INTERSTITIAL LUNG DISEASE AND LUNG CANCER

Idiopathic pulmonary fibrosis (IPF) is the most common subtype of interstitial lung disease which is characterised by aberrant accumulation of fibrotic tissue in the lung parenchyma[50]. While the pathophysiology of IPF remains to be fully elucidated, the disease is thought to be mainly fibrosis-driven with minimal involvement of inflammation cascade[50]. Over the past decade, many studies have shown that IPF is linked to development of lung cancer, with a relative risk of 3.5-7.3 compared to healthy population[51]. One of the main reasons for this association is that IPF and lung cancer could have similarities in their pathophysiology, in terms of cellular morphological anomalies, dysregulated cytokine signalling and genetic mutations[52]. A study by Kawasaki *et al*[53] established that morphological aberrations in the lung epithelial layer, ranging from metaplasia and dysplasia to carcinoma, have been identified in fibrotic lung regions of IPF patients. This could be related to microsatellite instability and loss of heterozygosity, including mutations in tumour-suppressor genes such as fragile histidine triad gene, that are present at higher frequency in lung epithelial cells of IPF patients relative to healthy population[54,55]. Genetic alterations like these could be attributed to fibrosis, mainly mediated by TGF-ß released by various immune cells, and other changes in the stroma in IPF patients[56]. Using publicly available datasets, Saito *et al*[57] confirmed that 10% of the genes upregulated in lung cancer stroma, which include those coding for ECM components, mainly collagen (COL1A2, COL3A1, and COL5A2), and matrix metalloproteinases (MMP9 and 11), are also elevated in IPF. Furthermore, while increased immune cell infiltrates releasing cytokines, which promote epithelial proliferation and resist apoptosis are noted in the early stages of IPF, reduced number of lymphocytes, macrophages and monocytes were reported in fibrotic-predominant areas compared to epithelial-predominant ones in the later stages[57-61]. This implies that lung epithelial cells undergoing malignant transformation in the former are more likely to evade immune surveillance and progress to invasive malignancies in the latter. This observation concurs with the fact that lung cancers associated with IPF tend to develop in the peripheral and lower lobes – the fibrotic-predominant regions[62].

While IPF is mainly driven by fibrosis, other subtypes of ILD such as pneumoconiosis involve an inflammatory-driven condition that has been associated with lung cancer[50,63,64]. Patients with silicosis and asbestosis are about 3 times and 1.5 times more likely to develop lung cancer than the general population[15,65]. Chronic inflammation triggered as a result of the continuous activation of macrophages in an attempt to clear the silica particles is thought to mediate lung carcinogenesis in patients with silicosis[63]. Consequently, there is massive release of cytokines such as IL-12, IL-23, and TNFα which place lung epithelial cells at an increased risk of DNA damage and thus their susceptibility to malignant transformation[66]. This is demonstrated unequivocally by Wang *et al*[66] in Gprc5a-knockout mice exposed to silica where neoplastic epithelial cells were found in areas of intense lung damage and fibrosis which were thought to be a consequence of chronic inflammation. Furthermore, Freire *et al*[67] demonstrated increased lung adenocarcinomas in mice treated with the combination of the carcinogen N-nitrosodimethylamine and silica. On histopathological analysis, there was increased expression of various inhibitory immune markers including programmed cell death protein 1, lymphocyte-activation gene 3, and forkhead box P3, as well as the presence of regulatory T cells in mice treated with NMDA and silica compared to silica alone[67]. This produces marked immunosuppression which increases the risk of carcinogenesis, providing another plausible explanation for the link between silicosis and lung cancer.

Similarly, in the case of asbestosis – linked with a 6.8-times and increased incidence of lung cancer respectively compared with the general population – the pathogenesis by which it causes malignancy appears to be a combination of inflammation and the direct genotoxic effect of asbestos fibres on the genome[68,69]. Alveolar macrophages have been known to play a major role in handling asbestosis fibres[68]. The entrapment of asbestos stimulates the activation of NOD-like receptor family, the pyrin domain containing 3 expressed in alveolar macrophages which promotes the activation of IL-1*β*, along with other cytokines such as TGF-β and PDGF which are responsible for the formation of

fibrotic nodules[68,70]. In addition, macrophages increase the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby stimulating genotoxicity, chronic inflammation and thus malignancy transformation[71]. More specifically, numerous studies have demonstrated that chronic inflammation as a result of asbestos exposure affected several cell signalling pathways that are likely responsible for the development of lung cancer including the epidermal growth factor receptor (EGFR)-related extracellular signal–regulated kinase (ERK) signaling that promote lung epithelial cell and fibroblast proliferation[71-73]. While these studies have established the effect of chronic inflammation on development of lung cancer and mesothelioma, there is still a need to ascertain the relevance of fibrosis and lung cancer in vivo.

PNEUMONIA, TUBERCULOSIS AND LUNG CANCER

Infections of the lung have been previously linked with the future development of lung cancer. A meta-analysis by Brenner *et al*[14] demonstrated that pneumonia and tuberculosis was linked with a 1.4- and 1.9-times increased risk of developing lung cancer in the future. While both pneumonia and tuberculosis constitute as infection of the lung parenchyma, the degree of pulmonary inflammation and subsequent fibrosis likely explains the variation in the risk of developing lung cancer[14]. In regards to the former, pulmonary inflammation occurs for a shorter duration and thus the resulting fibrosis is less if not negligible compared to the latter, where a significant level of inflammation and fibrosis is involved[74,75]. Furthermore, in the setting of further tuberculosis (TB) recurrences which can occur in up to 47% of TB patients, repeated inflammatory response will increase the risk of lung cancer each time, with high cumulative risk associated with more frequent recurrences[76,77]. The mechanism by which inflammation increases cancer risk relates to the action of ROS and RNS produced by immune cells on the genome of lung epithelial cells and the ability of pro-inflammatory cytokines such as TNF-α and IL-6 to upregulate the expression of anti-apoptotic proteins[76,78]. Additionally, recurrent bouts of inflammation results in fibrosis in the surrounding lung parenchyma, which increases the risk of cancer associated with poor lymph drainage[79]. Further supporting the link between inflammation and lung cancer risk is a meta-analysis by Khuder *et al*[80] which demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) conferred a protective benefit in reducing lung cancer risk following adjustment for smoking (OR: 0.68; 95%CI: 0.55–0.85). These studies reaffirm the association between inflammation, fibrosis and lung cancer risk.

LIVER CIRRHOSIS AND HEPATOCELLULAR CARCINOMA

The link between hepatic cirrhosis and hepatocellular carcinoma (HCC) is well-established, with the 5-year HCC cumulative risk of 17% and 15% respectively for hepatitis B-related and hepatitis C-related cirrhosis respectively[81]. NAFLD-related cirrhosis is also associated with the development of HCC, with multi-centre cohort studies showing 1.6 to 23.7 times increased risk[82]. Chronic inflammation and fibrosis are thought to be the major mechanisms explaining this association. In chronic hepatitis, a multitude of immune cells release various cytokines, most notably, TGF-β, TNF-α and interleukins, which lead to an increase in cellular proliferation, telomere shortening and genomic instability involving signalling pathways such as mechanistic target of rapamycin and Wnt signalling[83,84]. Additionally, previous studies revealed that CD4+ cells – involved in activation of the tumour-killing CD8+ cytotoxic T cells – and regulatory T cells – responsible for suppressing immune response – are diminished and increased respectively in cirrhosis[85,86]. Furthermore, chronic inflammation leads to fibrosis. Specifically, TGF-β released by Kupffer cells (macrophages) promote the activation of quiescent hepatic stellate cells (HSCs), analogous to PSCs in the pancreas, becoming myofibroblasts which are the primary source of ECM proteins including collagen, undulin, fibronectin and elastin[11,87]. More recently, others have identified additional cytokines, growth factors and lipid signals produced by other stromal components including endothelial cells, Kupffer cells and adipocytes are involved in HSC activation[88-90]. Fibrosis impairs the hepatic vasculature and produces a hypoxic environment, triggering the production of reactive oxygen, nitrogen species (ROS and RNO). ROS and RNO in turn can cause oxidative DNA damage among hepatocytes, predisposing them to malignant transformation[91]. Additionally, hypoxia induces the transcription of pro-angiogenic factors such as VEGF which is responsible for angiogenesis[92]. Further exacerbating this tumorigenic environment, neo-angiogenesis promotes the recruitment of immune cells like macrophages which results in further inflammation driving a vicious cycle. Today, the relationship between cirrhosis and HCC is extremely robust, that liver stiffness, a hallmark of hepatic cirrhosis is being studied as a means of assessing HCC risk[93].

PRIMARY BILIARY CHOLANGITIS AND CHOLANGIOCARCINOMA

Primary biliary cholangitis (PBC) is one of the most common risk factors for cholangiocarcinoma, with ninefold increased risk of developing cholangiocarcinoma[94]. The pathogenesis of cholangiocarcinogenesis in patients with PBC is multifactorial. Apart from the biliary constituent in PBC patients, chronic inflammation involving cytokines and growth factors, notably IL-6, hepatocyte growth factor, and IL-1β, released by various stromal and immune cells have been implicated in sustaining proliferative signalling in biliary cells[95-98]. IL-6 is believed to be a predominant contributor in cholangiocarcinogenesis, with the potential to promote cellular proliferation, survival and immortalisation *via* different mechanisms – p38MAPK activation[99], increasing DNA methyltransferase[96], Mcl-1 and telomerase expression[100]. In

addition, the inflammatory milieu in the surrounding bile duct raises the production of NO which increases the probability of DNA damage, affecting genes such as BRAF, K-ras, cyclin d-1, c-myc, COX-2 and p53[98,101]. Using a hamster model of cholangiocarcinoma, Prakobwong *et al*[102] demonstrated a decrease in incidence of cholangiocarcinoma, accompanied by decline in pro-inflammatory, growth signalling and anti-apoptotic protein expression including COX-2, cyclin-d1, c-myc, bcl-2 and bcl-xL following administration of curcumin, traditional anti-inflammatory agent derived from turmeric. This highlights the crucial role of inflammation in cholangiocarcinogenesis. Thirdly, fibrosis, instigated by the release of cytokines like IL-6 and TGF-β by immune cells, has also been shown to be involved in the neoplastic transformation of biliary cells. Using a liver cirrhosis mouse model, Farazi *et al*[103] showed that increased levels of fibroblasts along with type 1 and 3 collagen stimulate intrahepatic cholangiocyte proliferation and subsequent malignant transformation in p53-deficient mice. In another study, Ling *et al*[104] demonstrated that cholangiocarcinoma was induced in a rat model of thioacetamide (TAA)-induced hepatic fibrosis. The association between inflammation, fibrosis and cholangiocarcinogenesis is sufficiently convincing to stimulate interest in agents such as curcurmin that may diminish the two are being investigated to reduce the risk of cholangiocarcinoma[102,105].

GASTROESOPHAGEAL REFLUX DISEASE, BARRETT'S OESOPHAGUS AND OESOPHAGEAL CANCER

For a long time, chronic gastroesophageal reflux disease (GERD) patients have been known to be at risk of oesophageal cancer (OC), with 10%-20% developing Barrett's oesophagus (BO), making them 30-125 times more likely than the general population to develop OC[106]. Unlike HCC and cholangiocarcinoma where fibrosis is thought to be crucial to carcinogenesis, the pathophysiology of OC is inflammation-predominant. In GERD patients, chronic inflammation and oesophageal injury initiated by reflux of gastric acid bile and salt, result in BO, which is an intermediate step to progression to OC. More specifically, reflux promotes the recruitment of inflammatory cells, notably macrophages T, B and dendritic cells which release various pro-inflammatory cytokines such as TNF-α, IL-6, IL-1B and IL-8 that are responsible for NF-Kb, STAT-3, and HIF-1a activation[12,107,108]. This in turn leads to cellular proliferation and de-differentiation as part of a metaplastic process, a frequent precursor to neoplastic transformation. Further, immunosuppressive cytokines, notably IL-10 are found at higher levels in BO, and thus, could render healthy squamous epithelial cells undergoing malignant transformation less susceptible to destruction as a result of immune surveillance[109]. Furthermore, chronic inflammation creates a state of oxidative stress, evident by the increased levels of ROS and RNS present in BO[110]. The heightened level of oxidative stress in turn induces mutagenesis of oncogenes and tumour-suppressor genes, including TP53, K-ras, FBXW7 and PI3KCA, thereby contributing to OC carcinogenesis[110]. While chronic inflammation contributes significantly to OC carcinogenesis, the role of other aspects of stroma, including fibrosis on OC carcinogenesis remains unexplored. Interestingly, fibrosis is not apparent in BO, hence providing evidence of an inflammatory condition increasing cancer risk without the need for progression to fibrosis. Considering the reverse situation, we can hypothesise regarding the role of fibrosis on carcinogenesis from studies on eosinophilic oesophagitis, where both inflammation and fibrosis are prominent features but were not found to be associated with increased risk of OC[111]. Several mediators appear to be involved in this fibrosis, namely TGF-β, Th-2 type cytokines and ROS[112,113]. We could hypothesise that fibrosis may suppress neoplastic transformation in this scenario[111]. At this stage, while chronic inflammation substantially elevates OC cancer risk, fibrosis may have differing context specific effects on OC risk.

ORAL SUBMUCOSAL FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA

Apart from tobacco smoking, oral submucosal fibrosis (OSF) is the major risk factor for the development of oral squamous cell carcinoma (OSCC), increasing the likelihood by up to 19-fold compared to a healthy population[114]. The aetiology for OSF has long been established, with increasing incidence attributed to daily consumption of areca nut and betel quid[115,116]. In addition to the carcinogenic potential of constituents of areca nut and betel quid on activating oncogenes and inhibiting tumour-suppressor genes, they are also known to be inflammatory[117]. This promotes the recruitment of immune cells, predominantly, macrophages, T cells and lymphocytes to the oral mucosa, which in turn release ROS, prostaglandins, cytokines and growth factors, notably IL-6, TNF-α, PDGF and TGF-β[118]. These biological mediators, present in the surrounding oral squamous epithelium, promote oral squamous cell proliferation and survival [118]. Additionally, ROS promotes oxidative damage and mutagenesis, resulting in p53 mutations, decreased levels of DNA-methyltransferase repair enzyme and upregulated levels of HSP70 and MDM2-P2 promoter, which ultimately lead to neoplastic transformation in areas of OSF[119-123]. Interestingly, some of the aforementioned biological mediators, namely IL-6 and TGF-ß are significantly involved in fibrogenesis – synthesising ECM proteins like collagen and fibronectin and simultaneously producing plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor of metalloprotease which inhibit ECM breakdown[124-126]. This produces extensive fibrosis, particularly in the lamina propria, a hallmark feature of OSF. Recently, in an immunohistochemical study involving tissues obtained from patients with normal mucosa and OSF, Gadbail *et al*[127] demonstrated that Ki67 expression, a marker for cell proliferation, was directly proportional to α-SMA expression, a marker for myofibroblast formation, potentially highlighting that fibrosis may be directly involved in neoplastic transformation. The effect of fibrosis on malignant transformation of oral squamous epithelial cells is further stressed in an *in-vitro* study by Ye *et al*[128], who showed that growth-regulated oncogene-α from OSF-associated fibroblasts promote dysplastic keratinocyte cell line proliferation *via* activation of the EGFR/ERK signalling pathway. The potential of inflammation and fibrosis in OSF to cause neoplastic transformation to OSCC is regarded as high, justifying the ongoing search for anti-inflammatory and anti-fibrotic agents to suppress these

processes in OSF[76,129].

BREAST CANCER, PHYSIOLOGICAL MAMMOGRAPHIC DENSITY, PATHOLOGICAL INFLAMMATION AND CANCER RISK

Up to this point the breast appears to be a unique case in considering links between stromal composition and cancer risk. The differentiator is the strong established link between mammographic breast density (MBD), as assessed on mammographic images, which ties to the stromal composition of the normal breast, and breast cancer risk. Women with MBD lying in the highest quartile have a 4-6-fold higher risk of developing breast cancer than those in the lowest quartile [130,131]. Dense tissue has been found to correlate with higher proportions of ECM, particularly collagen[132], immune cells[133] and glandular epithelial components, and lower proportions of adipocytes[134]. As well as promoting initial carcinogenesis, higher mammographic density has been found to correlate with a higher risk of local relapse, a lower rate for complete response to chemotherapy[135] and a higher rate of relapse after treatment in locally advanced tumours [136].

This raises the question as to whether higher 'physiological' tissue stromal density carries higher risks of cancer in other organs, as well as whether pathological inflammatory and fibrotic processes impact cancer risk in the breast. Considering the latter, inflammatory conditions that result in a sustained inflammatory environment in the breast are relatively rare. Chronic mastitis is a condition whereby there is sustained inflammation usually relating to chronic infection. A retrospective cohort study by Chen *et al*[137] revealed that patients aged ≥ 40 with a history of mastitis have 3-fold increased risk of developing breast cancer aHR = 3.71, 95%CI = 1.9–7.02) compared to those without a history of mastitis. On the same note, fibrotic condition of the breast such as sclerosing adenosis has also been associated with an approximate doubling of breast cancer risk in a US retrospective cohort[138]. This further highlights the significance of inflammation and fibrosis in influencing cancer risk and emphasises consideration of more rigorous screening for these conditions and therapeutics which could manipulate the stroma and reduce cancer risk.

STROMAL MANIPULATION TO THERAPEUTIC ADVANTAGE

The abundant evidence for multiple robust links between inflammation, fibrosis and carcinogenesis (Figure 1), as well as the frequently overlapping spectrum of implicated signalling mediators and pathways, suggest that there may be substantial therapeutic benefit to be achieved by detecting and targeting these processes across many cancer types (Table 1).

Knowledge of the links between inflammation and malignancy are widely exploited in the screening of at-risk individuals with a variety of conditions. First there is promise in the assessment of stromal characteristics to predict cancer risk, thereby allowing identification of individuals suitable for screening or for whom screening could be adjusted. For instance, the strong relationship between MBD and breast risk has been described above. Initiatives are already in progress to use MBD levels to tailor screening, both considering the age at which to start screening and the frequency as well as whether other modalities should be considered such as ultrasound or MRI[139,140]. Additionally, robust link between liver cirrhosis and HCC has prompted surveillance quantification of alpha-feto protein and liver as a means to diagnose HCC earlier[141]. Furthermore, there are screening recommendations for patients with BO and IBD to undergo surveillance gastroscopy and colonoscopy to detect the relevant malignancies at early stages[142,143].

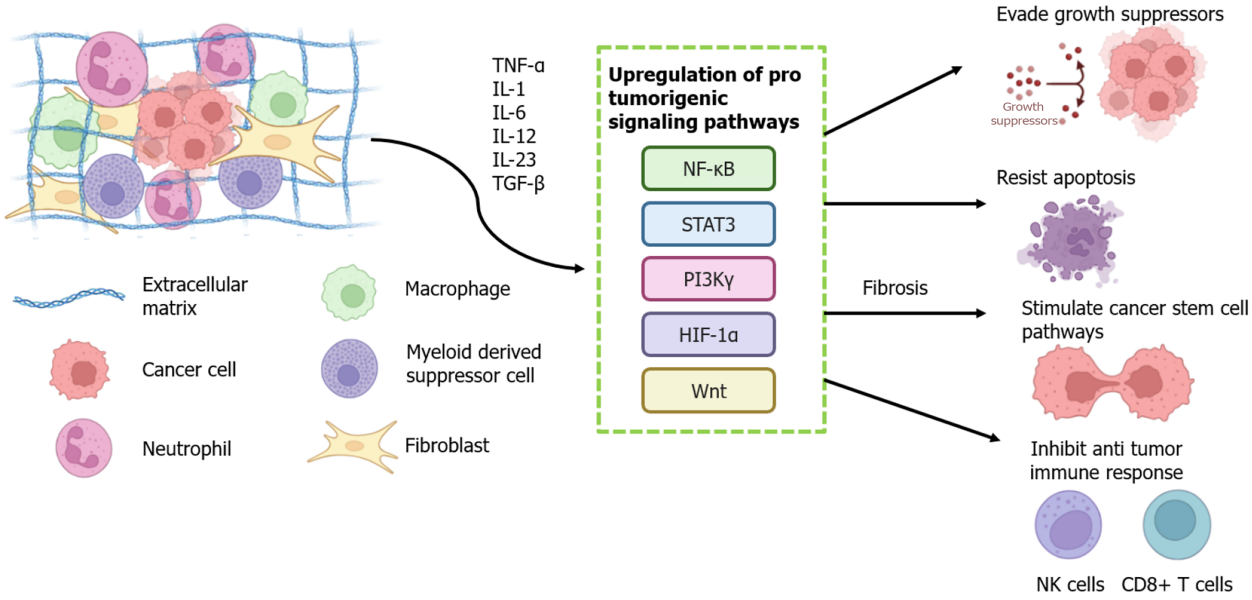
Beyond detection, the common mechanisms underlying links between tissue inflammation, fibrosis and malignancy have led to development of a number of strategies to target these underlying processes including the application of therapeutics including anti-proliferatives, anti-inflammatories, anti-estrogens and anti-fibrotics which will be discussed below.

Anti-proliferative

Thiopurines (azathioprine, mercaptopurine and thioguanine) has been a mainstay drug for IBD patients over the last 50 years. Its main drug effect is derived from the production of its metabolites 6-thioguaninenucleotides (6-TGN) and 6-methylmercaptopurine (6-MMP)[144]. These metabolites exert an immunosuppressive and anti-proliferative effect by binding Ras-related C3 botulinum toxin substrate 1 (Rac1) to thioguanosine triphosphate thus mitigating chronic gut inflammation in IBD. This blockade of Rac1 signalling results in decreased anti-apoptotic protein Bcl-xL expression and subsequent promotion of pro-inflammatory T-cell apoptosis[145,146]. A meta-analysis by Zhu *et al*[147] involving 95397 IBD patients, found that thiopurine use is associated with reduced risk of colorectal neoplasia (case control OR = 0.49, 95%CI: 0.34–0.70; cohort RR = 0.96, 95%CI: 0.94–0.98). While effective as a chemopreventive agent, thiopurine use should be balanced with potential adverse effects such as risk of myelosuppression and in the long term, development of lymphoproliferative disorders[146,148].

Anti-inflammatory

NSAID used widely in the treatment of chronic pain syndromes have been studied as a chemopreventive agent in a wide range of cancers. NSAIDs reduce inflammation by reversibly and non-selectively inhibiting cyclooxygenase (COX) enzymes which in turn lead to decreased production of prostaglandins and leukotrienes, mediators which have been implicated in carcinogenesis. A meta-analysis by Qiao *et al*[149] comprising of 218 studies demonstrated that aspirin use



DOI: 10.5306/wjco.v14.i7.230 **Copyright** ©The Author(s) 2023.

Figure 1 Schematic showing the links between inflammation, fibrosis and cancer in the tumour microenvironment. NK: Natural killer; HIF-1α: Hypoxia-inducible factor 1alpha; PI3K: Phosphatidylinositide 3-kinase; STAT3: Signal transducer and activator of transcription 3; NF-κB: Nuclear factor qB; Wnt: Wingless-related integration site.

was associated with a significant reduction in risk of gastric, esophageal, colorectal, pancreatic, ovarian, endometrial, breast and prostate cancer with rates ranging from 6%-25%. Another meta-analysis investigating the link between NSAID and skin cancer risk has also shown positive results, with significant reduction in risk of developing basal cell carcinoma, squamous cell carcinoma and non-melanoma skin cancer, but not melanoma. Interestingly, no significant chemopreventive effect is observed for COX-2 selective-NSAIDs and NSAID use among European populations[150].

5-aminosalicylates (5-ASA) is a drug class with anti-inflammatory and immunosuppressive properties, generally utilized in treatment of IBD and various rheumatologic conditions which has recently been found to possess chemopreventive properties. It works *via* multifactorial mechanisms but two well-understood mechanisms are the inhibition of prostaglandins and leukotrienes synthesis and scavenging of reactive oxygen species[151]. Previous systematic review of 31 independent observational studies in IBD has demonstrated that 5-ASA use is associated with a 43% reduction in risk of colorectal malignancy among patients with IBD. Of note, the reduction in risk of colorectal malignancy of 50% was more prominent in UC as compared to CD, where the risk reduction was non-significant. Furthermore, the incidence of IBD-related colorectal cancer have significantly declined in recent years and whilst numerous factors could cause this, the role of 5-ASA and other immunomodulatory agents are likely to have contributed to the decrease in cancer incidence[13].

Anti-fibrotic

Nintedanib and pirfenidone are two anti-fibrotic agents which have been approved for the management of IPF. Both work *via* modulation of fibrogenic growth factors, thereby decreasing fibroblast proliferation, myofibroblast differentiation, collagen and fibronectin synthesis, and extracellular matrix deposition[152]. Recent retrospective study by Naoi *et al*[153] demonstrated that the cumulative incidence of lung cancer in patients with IPF treated with antifibrotic agent was significantly lower than those who were not (2.2% *vs* 4.4% at 1 year, 2.2% *vs* 6.7% at 3 years, and 3.3% *vs* 9.7% at 5 years, respectively; $P = 0.004$)[153]. Interestingly, the use of anti-fibrotic agent was also associated with lower lung-cancer related mortality (1.6% *vs* 15.2%, respectively; $P = 0.0001$)[153]. With established benefits in terms of slowing progression, possibly improving survival in IPF and more recently, preventing lung cancer development, the use of anti-fibrotic agents should be strongly considered in all IPF patients provided that there are no contraindications.

Anti-estrogens in breast cancer

Anti-estrogens inhibit the synthesis or antagonise action of estrogen in target organs. Anti-estrogens encompass selective estrogen receptor modulators (SERMs), selective estrogen receptor degrader, aromatase inhibitors, gonadotrophin release hormone agonists and antagonists. Previous studies have shown that tamoxifen, raloxifene, exemestane and anastrozole have significantly reduced the incidence of breast cancer in high-risk women by 49%[154], 76%[155], 65%[156], 49%[157] respectively. Currently, two SERMs, tamoxifen and raloxifene, are approved by the FDA for breast cancer chemoprevention, with anastrozole and exemestane pending approval. The mechanism of action by which antiestrogens prevent breast cancer remains unclear, however, the reduction of breast stromal density brought about by antiestrogen use is thought to confer a less pro-tumorigenic environment and hence lowering breast cancer risk.

Stromal disruption

Lysyl oxidase (LOX) and LOX-like inhibitors are another drug class targeting the stroma of immense chemopreventive potential. LOXL is amine oxidase which catalyse the cross-linking of collagen and elastin in normal tissue and extracellular matrix, facilitating carcinogenesis, cell proliferation, migration and metastases[158]. Whilst previous studies have mainly investigated LOXL inhibitors as an anti-cancer agent, the preliminary results have been promising and LOX role in carcinogenesis make it a particularly interesting target to prevent carcinogenesis. Anti-GS341, antibody targeting LOXL-2 has been shown to significantly reduce tumour volume and lung metastases in a breast cancer xenograft model using MDA-MB-231 cells into immunocompromised SCID mice[159]. Additionally, an orally bioavailable LOX/LOXL2 inhibitor, CCT365623, developed by Leung *et al*[160] produced significant diminution in tumor growth and metastases in an in vivo model of transgenic LOX-dependent breast tumor mice[160]. These promising preclinical findings have translated to clinical trials exploring LOX/LOXL inhibitor in numerous diseases including myelofibrosis, cirrhosis, and breast cancer[161-164].

Another potential stromal disruption agent targets the extracellular matrix, particularly degradation of hyaluronic acid (HA), an important component of the ECM known to participate in carcinogenesis, tumor progression and metastasis in various cancers[165]. PEGPH20 is a PEGylated human hyaluronidase that showed promise both as single agent or in combination, in numerous preclinical studies[165-167]. Thompson *et al*[168] showed that repetitive PEGPH20 administration significantly inhibited tumor growth by 70% in high-HA prostate PC3 tumors and improved both docetaxel and liposomal doxorubicin activity in PC3 tumors. Additionally, using HA synthase 3-overexpressing and wild-type SKOV3 ovarian cancer model and in the BxPC3 pancreas xenograft tumour model, Morosi *et al*[166] showed that PEGPH20 enhanced the antitumor activity of paclitaxel by modifying the tumour tissue architecture. Despite the promising potential of PEGPH20 in preclinical studies, clinical trials of PEGPH20 in various advanced solid tumours have been disappointing with PEGPH20 failing to meet its primary end point of improvement in overall survival[169]. However, it is crucial to note that PEGPH20 has not been explored in preventing carcinogenesis such as in the context of IBD, cirrhosis and IPF. Considering the significance of the ECM in carcinogenesis, future studies should study the effect of ECM-degrading agents such as PEGPH20 in carcinogenesis.

In addition to targeting the ECM, agents targeting other components of the ECM have been studied. Most notably, agents targeting myofibroblasts which produce pathological fibrosis and thus a pro-carcinogenic environment have shown promising results in previous studies. Depletion of myofibroblasts by targeting its marker, fibroblast activation protein-α, has been shown to inhibit tumor growth by augmenting anti-tumor immunity[170,171]. Additionally, agents targeting TGF-β, an important cytokine in myofibroblast activation have also been studied as TGF-β inhibition has been demonstrated to prevent myofibroblast activation and prevent immunosuppression and thus cancer progression[172]. Again while these agents are studied as anti-cancer therapies, these drugs have immense potential to be utilised as chemopreventive agents in disorders of chronic inflammation and fibrosis to prevent carcinogenesis.

CONCLUSION

In conclusion, the correlation between chronic inflammation, fibrosis and cancer risk is complex, with the former being more straightforward. Chronic inflammation in the stroma of different body tissues promotes carcinogenesis *via* different mechanisms – growth factor/cytokine-mediated cellular proliferation, apoptotic resistance and immunosuppression; and free-radical-induced oxidative DNA damage. However, certain immune cells, involved in tumour-surveillance may be depleted, as seen in IPF and hepatic cirrhosis, thereby raising cancer risk by compromising immune surveillance of tumours. The relationship between stromal fibrosis and cancer risk varies in different organs, implying that the effects of fibrosis could be tissue-specific. Increased stromal fibrosis is associated with an increased cancer risk in organs like the lung, liver, biliary tract and colorectal region. Conversely, in other organs such as pancreas and potentially, oesophagus, increased stromal fibrosis may confer a lower cancer risk.

At this current time, the mechanism by which fibrosis influences cancer risk is still ambiguous. We propose two hypotheses. Firstly, a fibrotic environment contributes to an aberration in ECM dynamics which affects normal cellular behaviour and ultimately neoplastic transformation. Secondly, we hypothesise that fibrosis may present as a safe alternative to cellular regeneration which has the potential to produce aberrant DNA mutations, resulting in tumour formation. What determines the former or the latter are a multitude of factors which could include fibroblast heterogeneity and plasticity; extent of fibrosis; inflammation; and the predominance of certain mediators over others. Therefore, future studies, especially *in-vitro* and animal studies, should investigate the mechanisms by which fibrosis contributes to carcinogenesis in various organs in further depth and determine if fibrosis, alone or only in conjunction with inflammation would promote carcinogenesis. Furthermore, the role of surveillance screening and therapeutic agents with stroma manipulation potential in patients with diseases which involve chronic inflammation and fibrosis should be further studied to reduce the incidence of relevant cancers.

FOOTNOTES

Author contributions: Oey O and Redfern A conceptualized the research idea; Oey O, Sunjaya AF, Khan Y, Redfern A all contributed towards study methodology, analysis, writing and editing of the manuscript; Redfern A and Yasir K supervised the manuscript preparation; All authors have read and agreed to the published version of the manuscript.

Conflict-of-interest statement: No conflicts of interest to be reported.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Australia

ORCID number: Angela Felicia Sunjaya 0000-0001-8831-0449.

S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

REFERENCES

1 **Paget S**. The distribution of secondary growths in cancer of the breast. 1889. *Cancer Metastasis Rev* 1989; **8**: 98-101 [PMID: 2673568]
2 **Virchow R**. Cellular pathology. As based upon physiological and pathological histology. Lecture XVI--Atheromatous affection of arteries. 1858. *Nutr Rev* 1989; **47**: 23-25 [PMID: 2649802 DOI: 10.1111/j.1753-4887.1989.tb02747.x]
3 **Franco M**, Bustuoabad OD, di Gianni PD, Goldman A, Pasqualini CD, Ruggiero RA. A serum-mediated mechanism for concomitant resistance shared by immunogenic and non-immunogenic murine tumours. *Br J Cancer* 1996; **74**: 178-186 [PMID: 8688319 DOI: 10.1038/bjc.1996.335]
4 **Wyckoff J**, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J, Condeelis J. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* 2004; **64**: 7022-7029 [PMID: 15466195 DOI: 10.1158/0008-5472.can-04-1449]
5 **Zaynagetdinov R**, Sherrill TP, Polosukhin VV, Han W, Ausborn JA, McLoed AG, McMahon FB, Gleaves LA, Degryse AL, Stathopoulos GT, Yull FE, Blackwell TS. A critical role for macrophages in promotion of urethane-induced lung carcinogenesis. *J Immunol* 2011; **187**: 5703-5711 [PMID: 22048774 DOI: 10.4049/jimmunol.1100558]
6 **Seo**, BR, Bhardwaj P, Choi S, Gonzalez J, Eguiluz RCA, Wang K, Mohanan S, Morris PG, Du B, Zhou XK, Vahdat LT, Verma A, Elemento O, Hudis CA, Williams RM, Gourdon D, Dannenberg AJ, Fischbach C. Obesity-dependent changes in interstitial ECM mechanics promote breast tumorigenesis. *Sci Transl Med* 2015 [DOI: 10.1126/scitranslmed.3010467]
7 **Stoker MG**, Shearer M, O'Neill C. Growth inhibition of polyoma-transformed cells by contact with static normal fibroblasts. *J Cell Sci* 1966; **1**: 297-310 [PMID: 4291022 DOI: 10.1242/jcs.1.3.297]
8 **Wang**, B, Xi C, Liu M, Sun H, Liu S, Song L, Kang H. Breast fibroblasts in both cancer and normal tissues induce phenotypic transformation of breast cancer stem cells: a preliminary study. *Peer J* 2018; e4805 [DOI: 10.7287/peerj.4805v0.2/reviews/1]
9 **Burkitt MD**, Hanedi AF, Duckworth CA, Williams JM, Tang JM, O'Reilly LA, Putoczki TL, Gerondakis S, Dimaline R, Caamano JH, Pritchard DM. NF-κB1, NF-κB2 and c-Rel differentially regulate susceptibility to colitis-associated adenoma development in C57BL/6 mice. *J Pathol* 2015; **236**: 326-336 [PMID: 25727407 DOI: 10.1002/path.4527]
10 **Wu B**, Crampton SP, Hughes CC. Wnt signaling induces matrix metalloproteinase expression and regulates T cell transmigration. *Immunity* 2007; **26**: 227-239 [PMID: 17306568 DOI: 10.1016/j.immuni.2006.12.007]
11 **Cai CX**, Buddha H, Castelino-Prabhu S, Zhang Z, Britton RS, Bacon BR, Neuschwander-Tetri BA. Activation of Insulin-PI3K/Akt-p70S6K Pathway in Hepatic Stellate Cells Contributes to Fibrosis in Nonalcoholic Steatohepatitis. *Dig Dis Sci* 2017; **62**: 968-978 [PMID: 28194671 DOI: 10.1007/s10620-017-4470-9]
12 **Dvorak K**, Chavarria M, Payne CM, Ramsey L, Crowley-Weber C, Dvorakova B, Dvorak B, Bernstein H, Holubec H, Sampliner RE, Bernstein C, Prasad A, Green SB, Garewal H. Activation of the interleukin-6/STAT3 antiapoptotic pathway in esophageal cells by bile acids and low pH: relevance to barrett's esophagus. *Clin Cancer Res* 2007; **13**: 5305-5313 [PMID: 17875759 DOI: 10.1158/1078-0432.ccr-07-0483]
13 **Jess T**, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. *Clin Gastroenterol Hepatol* 2012; **10**: 639-645 [PMID: 22289873 DOI: 10.1016/j.cgh.2012.01.010]
14 **Brenner DR**, McLaughlin JR, Hung RJ. Previous lung diseases and lung cancer risk: a systematic review and meta-analysis. *PLoS One* 2011; **6**: e17479 [PMID: 21483846 DOI: 10.1371/journal.pone.0017479]
15 **Partanen T**, Pukkala E, Vainio H, Kurppa K, Koskinen H. Increased incidence of lung and skin cancer in Finnish silicotic patients. *J Occup Med* 1994; **36**: 616-622 [PMID: 8071722]
16 **Fakhoury M**, Negrulj R, Mooranian A, Al-Salami H. Inflammatory bowel disease: clinical aspects and treatments. *J Inflamm Res* 2014; **7**: 113-120 [PMID: 25075198 DOI: 10.2147/JIR.S65979]
17 **Laukoetter MG**, Mennigen R, Hannig CM, Osada N, Rijcken E, Vowinkel T, Krieglstein CF, Senninger N, Anthoni C, Bruewer M. Intestinal cancer risk in Crohn's disease: a meta-analysis. *J Gastrointest Surg* 2011; **15**: 576-583 [PMID: 21152994 DOI: 10.1007/s11605-010-1402-9]
18 **Lutgens MW**, van Oijen MG, van der Heijden GJ, Vleggaar FP, Siersema PD, Oldenburg B. Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm Bowel Dis* 2013; **19**: 789-799 [PMID: 23448792 DOI: 10.1097/MIB.0b013e31828029c0]
19 **Louis E**, Collard A, Oger AF, Degroote E, Aboul Nasr El Yafi FA, Belaiche J. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. *Gut* 2001; **49**: 777-782 [PMID: 11709511 DOI: 10.1136/gut.49.6.777]
20 **LUMB G**, PROTHEROE RH. Ulcerative colitis; a pathologic study of 152 surgical specimens. *Gastroenterology* 1958; **34**: 381-407 [PMID: 13512610 DOI: 10.1016/S0016-5085(58)80002-5]
21 **Gordon IO**, Agrawal N, Willis E, Goldblum JR, Lopez R, Allende D, Liu X, Patil DY, Yerian L, El-Khider F, Fiocchi C, Rieder F. Fibrosis in

ulcerative colitis is directly linked to severity and chronicity of mucosal inflammation. *Aliment Pharmacol Ther* 2018; **47**: 922-939 [PMID: 29411405 DOI: 10.1111/apt.14526]

22 **Boldeanu MV**, Siloşi I, Ghiluşi M, Cojocaru M, Biciuşcă V, Avrămescu CS, Cojocaru IM, Ciurea T, Albu DF, Siloşi CA. Investigation of inflammatory activity in ulcerative colitis. *Rom J Morphol Embryol* 2014; **55**: 1345-1351 [PMID: 25611265]

23 **Atreya R**, Mudter J, Finotto S, Müllberg J, Jostock T, Wirtz S, Schütz M, Bartsch B, Holtmann M, Becker C, Strand D, Czaja J, Schlaak JF, Lehr HA, Autschbach F, Schürmann G, Nishimoto N, Yoshizaki K, Ito H, Kishimoto T, Galle PR, Rose-John S, Neurath MF. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo. *Nat Med* 2000; **6**: 583-588 [PMID: 10802717 DOI: 10.1038/75068]

24 **Francescone R**, Hou V, Grivennikov SI. Cytokines, IBD, and colitis-associated cancer. *Inflamm Bowel Dis* 2015; **21**: 409-418 [PMID: 25563695 DOI: 10.1097/MIB.0000000000000236]

25 **Korkaya H**, Liu S, Wicha MS. Regulation of cancer stem cells by cytokine networks: attacking cancer's inflammatory roots. *Clin Cancer Res* 2011; **17**: 6125-6129 [PMID: 21685479 DOI: 10.1158/1078-0432.CCR-10-2743]

26 **Grivennikov S**, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell* 2009; **15**: 103-113 [PMID: 19185845 DOI: 10.1016/j.ccr.2009.01.001]

27 **Xing Y**, Chen X, Cao Y, Huang J, Xie X, Wei Y. Expression of Wnt and Notch signaling pathways in inflammatory bowel disease treated with mesenchymal stem cell transplantation: evaluation in a rat model. *Stem Cell Res Ther* 2015; **6**: 101 [PMID: 25998108 DOI: 10.1186/s13287-015-0092-3]

28 **Li C**, Iness A, Yoon J, Grider JR, Murthy KS, Kellum JM, Kuemmerle JF. Noncanonical STAT3 activation regulates excess TGF-β1 and collagen I expression in muscle of stricturing Crohn's disease. *J Immunol* 2015; **194**: 3422-3431 [PMID: 25740948 DOI: 10.4049/jimmunol.1401779]

29 **Okuno T**, Andoh A, Bamba S, Araki Y, Fujiyama Y, Fujiyama M, Bamba T. Interleukin-1beta and tumor necrosis factor-alpha induce chemokine and matrix metalloproteinase gene expression in human colonic subepithelial myofibroblasts. *Scand J Gastroenterol* 2002; **37**: 317-324 [PMID: 11916194 DOI: 10.1080/003655202317284228]

30 **Claessen**, MM, Schipper MEI, Oldenburg B, Siersema PD, Offerhaus GJA, Vleggaar fp. WNT-pathway activation in IBD-associated colorectal carcinogenesis: potential biomarkers for colonic surveillance. *Cell Oncol* 2010; **32**: 303-310 [DOI: 10.1155/2010/957698]

31 **Babyatsky MW**, Rossiter G, Podolsky DK. Expression of transforming growth factors alpha and beta in colonic mucosa in inflammatory bowel disease. *Gastroenterology* 1996; **110**: 975-984 [PMID: 8613031 DOI: 10.1053/gast.1996.v110.pm8613031]

32 **Zou X**, Feng B, Dong T, Yan G, Tan B, Shen H, Huang A, Zhang X, Zhang M, Yang P, Zheng M, Zhang Y. Up-regulation of type I collagen during tumorigenesis of colorectal cancer revealed by quantitative proteomic analysis. *J Proteomics* 2013; **94**: 473-485 [PMID: 24332065 DOI: 10.1016/j.jprot.2013.10.020]

33 **Hünerwadel A**, Fagagnini S, Rogler G, Lutz C, Jaeger SU, Mamie C, Weder B, Ruiz PA, Hausmann M. Severity of local inflammation does not impact development of fibrosis in mouse models of intestinal fibrosis. *Sci Rep* 2018; **8**: 15182 [PMID: 30315190 DOI: 10.1038/s41598-018-33452-5]

34 **Latella G**, Di Gregorio J, Flati V, Rieder F, Lawrance IC. Mechanisms of initiation and progression of intestinal fibrosis in IBD. *Scand J Gastroenterol* 2015; **50**: 53-65 [PMID: 25523556 DOI: 10.3109/00365521.2014.968863]

35 **Krejs GJ**. Pancreatic cancer: epidemiology and risk factors. *Dig Dis* 2010; **28**: 355-358 [PMID: 20814212 DOI: 10.1159/000319414]

36 **Haeberle L**, Steiger K, Schlitter AM, Safi SA, Knoefel WT, Erkan M, Esposito I. Stromal heterogeneity in pancreatic cancer and chronic pancreatitis. *Pancreatology* 2018; **18**: 536-549 [PMID: 29778400 DOI: 10.1016/j.pan.2018.05.004]

37 **Guerra C**, Schuhmacher AJ, Cañamero M, Grippo PJ, Verdaguer L, Pérez-Gallego L, Dubus P, Sandgren EP, Barbacid M. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. *Cancer Cell* 2007; **11**: 291-302 [PMID: 17349585 DOI: 10.1016/j.ccr.2007.01.012]

38 **Luttenberger T**, Schmid-Kotsas A, Menke A, Siech M, Beger H, Adler G, Grünert A, Bachem MG. Platelet-derived growth factors stimulate proliferation and extracellular matrix synthesis of pancreatic stellate cells: implications in pathogenesis of pancreas fibrosis. *Lab Invest* 2000; **80**: 47-55 [PMID: 10653002 DOI: 10.1038/Labinvest.3780007]

39 **Mews P**, Phillips P, Fahmy R, Korsten M, Pirola R, Wilson J, Apte M. Pancreatic stellate cells respond to inflammatory cytokines: potential role in chronic pancreatitis. *Gut* 2002; **50**: 535-541 [PMID: 11889076 DOI: 10.1136/gut.50.4.535]

40 **Apte MV**, Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. *Gut* 1999; **44**: 534-541 [PMID: 10075961 DOI: 10.1136/gut.44.4.534]

41 **Marzoq AJ**, Mustafa SA, Heidrich L, Hoheisel JD, Alhamdani MSS. Impact of the secretome of activated pancreatic stellate cells on growth and differentiation of pancreatic tumour cells. *Sci Rep* 2019; **9**: 5303 [PMID: 30923340 DOI: 10.1038/s41598-019-41740-x]

42 **Cheng XB**, Kohi S, Koga A, Hirata K, Sato N. Hyaluronan stimulates pancreatic cancer cell motility. *Oncotarget* 2016; **7**: 4829-4840 [PMID: 26684359 DOI: 10.18632/oncotarget.6617]

43 **Linder S**, Castaños-Velez E, von Rosen A, Biberfeld P. Immunohistochemical expression of extracellular matrix proteins and adhesion molecules in pancreatic carcinoma. *Hepatogastroenterology* 2001; **48**: 1321-1327 [PMID: 11677955]

44 **Whatcott CJ**, Diep CH, Jiang P, Watanabe A, LoBello J, Sima C, Hostetter G, Shepard HM, Von Hoff DD, Han H. Desmoplasia in Primary Tumors and Metastatic Lesions of Pancreatic Cancer. *Clin Cancer Res* 2015; **21**: 3561-3568 [PMID: 25695692 DOI: 10.1158/1078-0432.CCR-14-1051]

45 **Hingorani SR**, Harris WP, Beck JT, Berdov BA, Wagner SA, Pshevlotsky EM, Tjulandin SA, Gladkov OA, Holcombe RF, Korn R, Raghunand N, Dychter S, Jiang P, Shepard HM, Devoe CE. Phase Ib Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer. *Clin Cancer Res* 2016; **22**: 2848-2854 [PMID: 26813359 DOI: 10.1158/1078-0432.CCR-15-2010]

46 **Hingorani SR**, Zheng L, Bullock AJ, Seery TE, Harris WP, Sigal DS, Braiteh F, Ritch PS, Zalupski MM, Bahary N, Oberstein PE, Wang-Gillam A, Wu W, Chondros D, Jiang P, Khelifa S, Pu J, Aldrich C, Hendifar AE. HALO 202: Randomized Phase II Study of PEGPH20 Plus Nab-Paclitaxel/Gemcitabine Versus Nab-Paclitaxel/Gemcitabine in Patients With Untreated, Metastatic Pancreatic Ductal Adenocarcinoma. *J Clin Oncol* 2018; **36**: 359-366 [DOI: 10.3410/f.732284626.793571311]

47 **Torphy RJ**, Wang Z, True-Yasaki A, Volmar KE, Rashid N, Yeh B, Anderson JM, Johansen JS, Hollingsworth MA, Yeh JJ, Collisson EA. Stromal Content Is Correlated With Tissue Site, Contrast Retention, and Survival in Pancreatic Adenocarcinoma. *JCO Precis Oncol* 2018; **2018** [PMID: 30506016 DOI: 10.1200/PO.17.00121]

48 **Rhim AD**, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA, Dekleva EN, Saunders T, Becerra CP, Tattersall IW, Westphalen CB, Kitajewski J, Fernandez-Barrena MG, Fernandez-Zapico ME, Iacobuzio-Donahue C, Olive, KP, Stanger BZ. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell 2014; 25: 735-747 [DOI: 10.3410/f.718415427.793498285]

49 **Erkan M**, Michalski CW, Rieder S, Reiser-Erkan C, Abiatari I, Kolb A, Giese NA, Esposito I, Friess H, Kleeff J. The activated stroma index is a novel and independent prognostic marker in pancreatic ductal adenocarcinoma. *Clin Gastroenterol Hepatol* 2008; **6**: 1155-1161 [PMID: 18639493 DOI: 10.1016/j.cgh.2008.05.006]

50 **Barratt SL**, Creamer A, Hayton C, Chaudhuri N. Idiopathic Pulmonary Fibrosis (IPF): An Overview. *J Clin Med* 2018; **7** [PMID: 30082599 DOI: 10.3390/jcm7080201]

51 **Naccache JM**, Gibiot Q, Monnet I, Antoine M, Wislez M, Chouaid C, Cadranel J. Lung cancer and interstitial lung disease: a literature review. *J Thorac Dis* 2018; **10**: 3829-3844 [PMID: 30069384 DOI: 10.21037/jtd.2018.05.75]

52 **Debes JD**, van Tilborg M, Groothuismink ZMA, Hansen BE, Schulze Zur Wiesch J, von Felden J, de Knegt RJ, Boonstra A. Levels of Cytokines in Serum Associate With Development of Hepatocellular Carcinoma in Patients With HCV Infection Treated With Direct-Acting Antivirals. *Gastroenterology* 2018; **154**: 515-517.e3 [PMID: 29102620 DOI: 10.1053/j.gastro.2017.10.035]

53 **Kawasaki H**, Ogura T, Yokose T, Nagai K, Nishiwaki Y, Esumi H. p53 gene alteration in atypical epithelial lesions and carcinoma in patients with idiopathic pulmonary fibrosis. *Hum Pathol* 2001; **32**: 1043-1049 [PMID: 11679937 DOI: 10.1053/hupa.2001.28246]

54 **Uematsu K**, Yoshimura A, Genma A, Mochimaru H, Hosoya Y, Kunugi S, Matsuda K, Seike M, Kurimoto F, Takenaka K, Koizumi K, Fukuda Y, Tanaka S, Chin K, Jablons DM, Kudoh S. Aberrations in the fragile histidine triad (FHIT) gene in idiopathic pulmonary fibrosis. *Cancer Res* 2001; **61**: 8527-8533 [DOI: 10.2165/00128413-200113160-00017]

55 **Vassilakis DA**, Sourvinos G, Spandidos DA, Siafakas NM, Bouros D. Frequent genetic alterations at the microsatellite level in cytologic sputum samples of patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2000; **162**: 1115-1119 [PMID: 10988139 DOI: 10.1164/ajrccm.162.3.9911119]

56 **Bergeron A**, Soler P, Kambouchner M, Loiseau P, Milleron B, Valeyre D, Hance AJ, Tazi A. Cytokine profiles in idiopathic pulmonary fibrosis suggest an important role for TGF-beta and IL-10. *Eur Respir J* 2003; **22**: 69-76 [PMID: 12882453 DOI: 10.1183/09031936.03.00014703]

57 **Saito A**, Horie M, Micke P, Nagase T. The Role of TGF-β Signaling in Lung Cancer Associated with Idiopathic Pulmonary Fibrosis. *Int J Mol Sci* 2018; **19** [PMID: 30445777 DOI: 10.3390/ijms19113611]

58 **Car BD**, Meloni F, Luisetti M, Semenzato G, Gialdroni-Grassi G, Walz A. Elevated IL-8 and MCP-1 in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1994; **149**: 655-659 [PMID: 8118632 DOI: 10.1164/ajrccm.149.3.8118632]

59 **Yoshida M**, Sakuma J, Hayashi S, Abe K, Saito I, Harada S, Sakatani M, Yamamoto S, Matsumoto N, Kaneda Y. A histologically distinctive interstitial pneumonia induced by overexpression of the interleukin 6, transforming growth factor beta 1, or platelet-derived growth factor B gene. *Proc Natl Acad Sci U S A* 1995; **92**: 9570-9574 [PMID: 7568174 DOI: 10.1073/pnas.92.21.9570]

60 **Furuie H**, Yamasaki H, Suga M, Ando M. Altered accessory cell function of alveolar macrophages: a possible mechanism for induction of Th2 secretory profile in idiopathic pulmonary fibrosis. *Eur Respir J* 1997; **10**: 787-794 [PMID: 9150314 DOI: 10.1183/09031936.97.10040787]

61 **Flavia Greiffo IF**, Marion Frankenberger, Jürgen Behr, Oliver Eickelberg. Circulating monocytes from interstitial lung disease patients show an activated phenotype. *European Respiratory Journal* 2016; 48 [DOI: 10.1183/13993003.congress-2016.pa3894]

62 **Aburto M**, Herráez I, Iturbe D, Jiménez-Romero A. Diagnosis of Idiopathic Pulmonary Fibrosis: Differential Diagnosis. *Med Sci (Basel)* 2018; **6** [PMID: 30181506 DOI: 10.3390/medsci6030073]

63 **Davis GS**. The pathogenesis of silicosis. State of the art. *Chest* 1986; **89**: 166S-169S [PMID: 3004837 DOI: 10.1378/chest.89.3_supplement.166s]

64 **Kamp DW**, Weitzman SA. Asbestosis: clinical spectrum and pathogenic mechanisms. *Proc Soc Exp Biol Med* 1997; **214**: 12-26 [PMID: 9012357 DOI: 10.3181/00379727-214-44065]

65 **Kwak K**, Kang D, Paek D. Environmental exposure to asbestos and the risk of lung cancer: a systematic review and meta-analysis. *Occup Environ Med* 2022; **79**: 207-214 [PMID: 33972375 DOI: 10.1136/oemed-2020-107222]

66 **Wang X**, Xu D, Liao Y, Zhong S, Song H, Sun B, Zhou BP, Deng J, Han B. Epithelial neoplasia coincides with exacerbated injury and fibrotic response in the lungs of Gprc5a-knockout mice following silica exposure. *Oncotarget* 2015; **6**: 39578-39593 [PMID: 26447616 DOI: 10.18632/oncotarget.5532]

67 **Freire J**, Ajona D, de Biurrun G, Agorreta J, Segura V, Guruceaga E, Bleau AM, Pio R, Blanco D, Montuenga LM. Silica-induced chronic inflammation promotes lung carcinogenesis in the context of an immunosuppressive microenvironment. *Neoplasia* 2013; **15**: 913-924 [PMID: 23908592 DOI: 10.1593/neo.13310]

68 **Liu G**, Cheresh P, Kamp DW. Molecular basis of asbestos-induced lung disease. *Annu Rev Pathol* 2013; **8**: 161-187 [PMID: 23347351 DOI: 10.1146/annurev-pathol-020712-163942]

69 **Selikoff IJ**, Churg J, Hammond EC. Classics in Oncology: Asbestos exposure and neoplasia. *CA Cancer J Clin* 1984; **34**: 48-56 [PMID: 6420020 DOI: 10.3322/canjclin.34.1.48]

70 **Dostert C**, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 2008; **320**: 674-677 [PMID: 18403674 DOI: 10.1126/science.1156995]

71 **Heintz NH**, Janssen-Heininger YM, Mossman BT. Asbestos, lung cancers, and mesotheliomas: from molecular approaches to targeting tumor survival pathways. *Am J Respir Cell Mol Biol* 2010; **42**: 133-139 [PMID: 20068227 DOI: 10.1165/rcmb.2009-0206TR]

72 **Mossman BT**, Lippmann M, Hesterberg TW, Kelsey KT, Barchowsky A, Bonner JC. Pulmonary endpoints (lung carcinomas and asbestosis) following inhalation exposure to asbestos. *J Toxicol Environ Health B Crit Rev* 2011; **14**: 76-121 [PMID: 21534086 DOI: 10.1080/10937404.2011.556047]

73 **Shukla A**, Hillegass JM, MacPherson MB, Beuschel SL, Vacek PM, Butnor KJ, Pass HI, Carbone M, Testa JR, Heintz NH, Mossman BT. ERK2 is essential for the growth of human epithelioid malignant mesotheliomas. *Int J Cancer* 2011; **129**: 1075-1086 [PMID: 21710492 DOI: 10.1002/ijc.25763]

74 **Grief SN**, Loza JK. Guidelines for the Evaluation and Treatment of Pneumonia. *Prim Care* 2018; **45**: 485-503 [PMID: 30115336 DOI: 10.1016/j.pop.2018.04.001]

75 **Hunter RL**. The Pathogenesis of Tuberculosis: The Early Infiltrate of Post-primary (Adult Pulmonary) Tuberculosis: A Distinct Disease Entity. *Front Immunol* 2018; **9**: 2108 [PMID: 30283448 DOI: 10.3389/fimmu.2018.02108]

76 **Lin CY**, Hsieh PL, Liao YW, Peng CY, Yu CC, Lu MY. Arctigenin Reduces Myofibroblast Activities in Oral Submucous Fibrosis by

LINC00974 Inhibition. *Int J Mol Sci* 2019; **20** [PMID: 30884781 DOI: 10.3390/ijms20061328]
77 **Mirsaeidi M**, Sadikot RT. Patients at high risk of tuberculosis recurrence. *Int J Mycobacteriol* 2018; **7**: 1-6 [PMID: 29516879 DOI: 10.4103/ijmy.ijmy_164_17]
78 **Waris G**, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog* 2006; **5**: 14 [PMID: 16689993 DOI: 10.1186/1477-3163-5-14]
79 **Ardies CM**. Inflammation as cause for scar cancers of the lung. *Integr Cancer Ther* 2003; **2**: 238-246 [PMID: 15035887 DOI: 10.1177/1534735403256332]
80 **Khuder SA**, Herial NA, Mutgi AB, Federman DJ. Nonsteroidal antiinflammatory drug use and lung cancer: a metaanalysis. *Chest* 2005; **127**: 748-754 [PMID: 15764753 DOI: 10.1378/chest.127.3.748]
81 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101 DOI: 10.1053/j.gastro.2004.09.014]
82 **Kanwal F**, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, Li L, Desiderio R, Thrift AP, Asch SM, Chu J, El-Serag HB. Risk of Hepatocellular Cancer in Patients With Non-Alcoholic Fatty Liver Disease. *Gastroenterology* 2018; **155**: 1828-1837.e2 [PMID: 30144434 DOI: 10.1053/j.gastro.2018.08.024]
83 **Estevez J**, Chen VL, Podlaha O, Li B, Le A, Vutien P, Chang ET, Rosenberg-Hasson Y, Jiang Z, Pflanz S, Ge D, Gaggar A, Nguyen MH. Differential Serum Cytokine Profiles in Patients with Chronic Hepatitis B, C, and Hepatocellular Carcinoma. *Sci Rep* 2017; **7**: 11867 [PMID: 28928388 DOI: 10.1038/s41598-017-11975-7]
84 **Plentz RR**, Caselitz M, Bleck JS, Gebel M, Flemming P, Kubicka S, Manns MP, Rudolph KL. Hepatocellular telomere shortening correlates with chromosomal instability and the development of human hepatoma. *Hepatology* 2004; **40**: 80-86 [PMID: 15239089 DOI: 10.1002/hep.20271]
85 **McGovern BH**, Golan Y, Lopez M, Pratt D, Lawton A, Moore G, Epstein M, Knox TA. The impact of cirrhosis on CD4+ T cell counts in HIV-seronegative patients. *Clin Infect Dis* 2007; **44**: 431-437 [PMID: 17205454 DOI: 10.1086/509580]
86 **Ormandy LA**, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res* 2005; **65**: 2457-2464 [PMID: 15781662 DOI: 10.1158/0008-5472.can-04-3232]
87 **Hellerbrand C**, Stefanovic B, Giordano F, Burchardt ER, Brenner DA. The role of TGFbeta1 in initiating hepatic stellate cell activation in vivo. *J Hepatol* 1999; **30**: 77-87 [PMID: 9927153 DOI: 10.1016/s0168-8278(99)80010-5]
88 **Meng F**, Wang K, Aoyoma T, Grivennikov SI, Paik YH, Scholten D, Cong M, Iwaisako K, Liu X, Zhang M, Osterreicher CH, Stickel F, Ley K, Brenner DA, Kisesleva T. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology* 2012; **143**: 765-776 e763 [DOI: 10.3410/f.717953960.793459481]
89 **Shafiei MS**, Shetty S, Scherer PE, Rockey DC. Adiponectin regulation of stellate cell activation *via* PPARγ-dependent and -independent mechanisms. *Am J Pathol* 2011; **178**: 2690-2699 [PMID: 21641391 DOI: 10.1016/j.ajpath.2011.02.035]
90 **Xie G**, Wang X, Wang L, Atkinson RD, Kanel GC, Gaarde WA, Deleve LD. Role of differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic fibrosis in rats. *Gastroenterology* 2012; **142**: 918-927.e6 [PMID: 22178212 DOI: 10.1053/j.gastro.2011.12.017]
91 **Breimer LH**. Molecular mechanisms of oxygen radical carcinogenesis and mutagenesis: the role of DNA base damage. *Mol Carcinog* 1990; **3**: 188-197 [PMID: 2206282 DOI: 10.1002/mc.2940030405]
92 **Corpechot C**, Barbu V, Wendum D, Kinnman N, Rey C, Poupon R, Housset C, Rosmorduc O. Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis. *Hepatology* 2002; **35**: 1010-1021 [PMID: 11981751 DOI: 10.1053/jhep.2002.32524]
93 **Adler M**, Larocca L, Trovato FM, Marcinkowski H, Pasha Y, Taylor-Robinson SD. Evaluating the risk of hepatocellular carcinoma in patients with prominently elevated liver stiffness measurements by FibroScan: a multicentre study. *HPB (Oxford)* 2016; **18**: 678-683 [PMID: 27485062 DOI: 10.1016/j.hpb.2016.05.005]
94 **Boonstra K**, Bokelaar R, Stadhouders PH, Tuynman HA, Poen AC, van Nieuwkerk KM, Witteman EM, Hamann D, Witteman BJ, Beuers U, Ponsioen CY. Increased cancer risk in a large population-based cohort of patients with primary biliary cirrhosis: follow-up for up to 36 years. *Hepatol Int* 2014; **8**: 266-274 [PMID: 26202508 DOI: 10.1007/s12072-014-9530-z]
95 **Yoon JH**, Canbay AE, Werneburg NW, Lee SP, Gores GJ. Oxysterols induce cyclooxygenase-2 expression in cholangiocytes: implications for biliary tract carcinogenesis. *Hepatology* 2004; **39**: 732-738 [PMID: 14999691 DOI: 10.1002/hep.20125]
96 **Sugawara H**, Yasoshima M, Katayanagi K, Kono N, Watanabe Y, Harada K, Nakanuma Y. Relationship between interleukin-6 and proliferation and differentiation in cholangiocarcinoma. *Histopathology* 1998; **33**: 145-153 [PMID: 9762547 DOI: 10.1046/j.1365-2559.1998.00445.x]
97 **Terada T**, Nakanuma Y, Sirica AE. Immunohistochemical demonstration of MET overexpression in human intrahepatic cholangiocarcinoma and in hepatolithiasis. *Hum Pathol* 1998; **29**: 175-180 [PMID: 9490278 DOI: 10.1016/s0046-8177(98)90229-5]
98 **Jaiswal M**, LaRusso NF, Burgart LJ, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. *Cancer Res* 2000; **60**: 184-190 [PMID: 10646872 DOI: 10.1053/gast.2001.20875]
99 **Wehbe H**, Henson R, Meng F, Mize-Berge J, Patel T. Interleukin-6 contributes to growth in cholangiocarcinoma cells by aberrant promoter methylation and gene expression. *Cancer Res* 2006; **66**: 10517-10524 [PMID: 17079474 DOI: 10.1158/0008-5472.CAN-06-2130]
100 **Yamagiwa Y**, Meng F, Patel T. Interleukin-6 decreases senescence and increases telomerase activity in malignant human cholangiocytes. *Life Sci* 2006; **78**: 2494-2502 [PMID: 16336976 DOI: 10.1016/j.lfs.2005.10.015]
101 **Haswell-Elkins MR**, Satarug S, Tsuda M, Mairiang E, Esumi H, Sithithaworn P, Mairiang P, Saitoh M, Yongvanit P, Elkins DB. Liver fluke infection and cholangiocarcinoma: model of endogenous nitric oxide and extragastric nitrosation in human carcinogenesis. *Mutat Res* 1994; **305**: 241-252 [PMID: 7510035 DOI: 10.1016/0027-5107(94)90244-5]
102 **Prakobwong S**, Khoontawad J, Yongvanit P, Pairojkul C, Hiraku Y, Sithithaworn P, Pinlaor P, Aggarwal BB, Pinlaor S. Curcumin decreases cholangiocarcinogenesis in hamsters by suppressing inflammation-mediated molecular events related to multistep carcinogenesis. *Int J Cancer* 2011; **129**: 88-100 [PMID: 20824699 DOI: 10.1002/ijc.25656]
103 **Farazi PA**, Zeisberg M, Glickman J, Zhang Y, Kalluri R, DePinho RA. Chronic bile duct injury associated with fibrotic matrix microenvironment provokes cholangiocarcinoma in p53-deficient mice. *Cancer Res* 2006; **66**: 6622-6627 [PMID: 16818635 DOI: 10.1158/0008-5472.can-05-4609]
104 **Ling H**, Roux E, Hempel D, Tao J, Smith M, Lonning S, Zuk A, Arbeeny C, Ledbetter S. Transforming growth factor β neutralization ameliorates pre-existing hepatic fibrosis and reduces cholangiocarcinoma in thioacetamide-treated rats. *PLoS One* 2013; **8**: e54499 [PMID:

23349909 DOI: 10.1371/journal.pone.0054499]

105 **Strack I**, Schulte S, Varnholt H, Schievenbusch S, Töx U, Wendland K, Steffen HM, Drebber U, Dienes HP, Odenthal M. β-Adrenoceptor blockade in sclerosing cholangitis of Mdr2 knockout mice: antifibrotic effects in a model of nonsinusoidal fibrosis. *Lab Invest* 2011; **91**: 252-261 [PMID: 20921947 DOI: 10.1038/labinvest.2010.162]

106 **Wild CP**, Hardie LJ. Reflux, Barrett's oesophagus and adenocarcinoma: burning questions. *Nat Rev Cancer* 2003; **3**: 676-684 [PMID: 12951586 DOI: 10.1038/nrc1166]

107 **O'Riordan JM**, Abdel-latif MM, Ravi N, McNamara D, Byrne PJ, McDonald GS, Keeling PW, Kelleher D, Reynolds JV. Proinflammatory cytokine and nuclear factor kappa-B expression along the inflammation-metaplasia-dysplasia-adenocarcinoma sequence in the esophagus. *Am J Gastroenterol* 2005; **100**: 1257-1264 [PMID: 15929754 DOI: 10.1111/j.1572-0241.2005.41338.x]

108 **Kimura S**, Kitadai Y, Tanaka S, Kuwai T, Hihara J, Yoshida K, Toge T, Chayama K. Expression of hypoxia-inducible factor (HIF)-1alpha is associated with vascular endothelial growth factor expression and tumour angiogenesis in human oesophageal squamous cell carcinoma. *Eur J Cancer* 2004; **40**: 1904-1912 [PMID: 15288294 DOI: 10.1016/j.ejca.2004.04.035]

109 **Fitzgerald RC**, Abdalla S, Onwuegbusi BA, Sirieix P, Saeed IT, Burnham WR, Farthing MJ. Inflammatory gradient in Barrett's oesophagus: implications for disease complications. *Gut* 2002; **51**: 316-322 [PMID: 12171950 DOI: 10.1136/gut.51.3.316]

110 **Sihvo EI**, Salminen JT, Rantanen TK, Rämö OJ, Ahotupa M, Färkkilä M, Auvinen MI, Salo JA. Oxidative stress has a role in malignant transformation in Barrett's oesophagus. *Int J Cancer* 2002; **102**: 551-555 [PMID: 12447994 DOI: 10.1002/ijc.10755]

111 **Syed A**, Maradey-Romero C, Fass R. The relationship between eosinophilic esophagitis and esophageal cancer. *Dis Esophagus* 2017; **30**: 1-5 [PMID: 30052901 DOI: 10.1093/dote/dox050]

112 **Rieder F**, Nonevski I, Ma J, Ouyang Z, West G, Protheroe C, DePetris G, Schirbel A, Lapinski J, Goldblum J, Bonfield T, Lopez R, Harnett K, Lee J, Hirano I, Falk G, Biancani P, Fiocchi C. T-helper 2 cytokines, transforming growth factor β1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. *Gastroenterology* 2014; **146**: 1266-77.e1 [PMID: 24486052 DOI: 10.1053/j.gastro.2014.01.051]

113 **Yisireyili M**, Wulamu W, Aili A, Li Y, Alimujiang A, Aipire A, Aizezi M, Zhang W, Cao Z, Mijiti A, Abudureyimu K. Chronic restraint stress induces esophageal fibrosis with enhanced oxidative stress in a murine model. *Exp Ther Med* 2019; **18**: 1375-1383 [PMID: 31316626 DOI: 10.3892/etm.2019.7669]

114 **Merchant A**, Husain SS, Hosain M, Fikree FF, Pitiphat W, Siddiqui AR, Hayder SJ, Haider SM, Ikram M, Chuang SK, Saeed SA. Paan without tobacco: an independent risk factor for oral cancer. *Int J Cancer* 2000; **86**: 128-131 [PMID: 10728606 DOI: 10.1002/(sici)1097-0215(20000401)86:1<128::aid-ijc20>3.0.co;2-m]

115 **Adel M**, Liao CT, Lee LY, Hsueh C, Lin CY, Fan KH, Wang HM, Ng SH, Lin CH, Tsao CK, Huang SF, Kang CJ, Fang KH, Wang YC, Chang KP, Fang TJ, Yang LY, Yen TC. Incidence and Outcomes of Patients With Oral Cavity Squamous Cell Carcinoma and Fourth Primary Tumors: A Long-term Follow-up Study in a Betel Quid Chewing Endemic Area. *Medicine (Baltimore)* 2016; **95**: e2950 [PMID: 27015170 DOI: 10.1097/MD.0000000000002950]

116 **van Wyk CW**, Stander I, Padayachee A, Grobler-Rabie AF. The areca nut chewing habit and oral squamous cell carcinoma in South African Indians. A retrospective study. *S Afr Med J* 1993; **83**: 425-429 [PMID: 8211462]

117 **Shafique K**, Mirza SS, Vart P, Memon AR, Arain MI, Tareen MF, Haq ZU. Areca nut chewing and systemic inflammation: evidence of a common pathway for systemic diseases. *J Inflamm (Lond)* 2012; **9**: 22 [PMID: 22676449 DOI: 10.1186/1476-9255-9-22]

118 **Jeng JH**, Wang YJ, Chiang BL, Lee PH, Chan CP, Ho YS, Wang TM, Lee JJ, Hahn LJ, Chang MC. Roles of keratinocyte inflammation in oral cancer: regulating the prostaglandin E2, interleukin-6 and TNF-alpha production of oral epithelial cells by areca nut extract and arecoline. *Carcinogenesis* 2003; **24**: 1301-1315 [PMID: 12807728 DOI: 10.1093/carcin/bgg083]

119 **Baral R**, Patnaik S, Das BR. Co-overexpression of p53 and c-myc proteins linked with advanced stages of betel- and tobacco-related oral squamous cell carcinomas from eastern India. *Eur J Oral Sci* 1998; **106**: 907-913 [PMID: 9786319 DOI: 10.1046/j.0909-8836.1998.eos106502.x]

120 **Lee HC**, Yin PH, Yu TN, Chang YD, Hsu WC, Kao SY, Chi CW, Liu TY, Wei YH. Accumulation of mitochondrial DNA deletions in human oral tissues -- effects of betel quid chewing and oral cancer. *Mutat Res* 2001; **493**: 67-74 [PMID: 11516716 DOI: 10.1016/s1383-5718(01)00160-7]

121 **Lee SS**, Tsai CH, Ho YC, Yu CC, Chang YC. Heat shock protein 27 expression in areca quid chewing-associated oral squamous cell carcinomas. *Oral Dis* 2012; **18**: 713-719 [PMID: 22490108 DOI: 10.1111/j.1601-0825.2012.01933.x]

122 **Lee SS**, Tsai CH, Yu CC, Ho YC, Hsu HI, Chang YC. The expression of O(6) -methylguanine-DNA methyltransferase in human oral keratinocytes stimulated with arecoline. *J Oral Pathol Med* 2013; **42**: 600-605 [PMID: 23278137 DOI: 10.1111/jop.12037]

123 **Liu TY**, Chen CL, Chi CW. Oxidative damage to DNA induced by areca nut extract. *Mutat Res* 1996; **367**: 25-31 [PMID: 8596543 DOI: 10.1016/s0165-1218(96)90018-x]

124 **Haque MF**, Meghji S, Khitab U, Harris M. Oral submucous fibrosis patients have altered levels of cytokine production. *J Oral Pathol Med* 2000; **29**: 123-128 [PMID: 10738939 DOI: 10.1034/j.1600-0714.2000.290304.x]

125 **Leivonen SK**, Lazaridis K, Decock J, Chantry A, Edwards DR, Kähäri VM. TGF-β-elicited induction of tissue inhibitor of metalloproteinases (TIMP)-3 expression in fibroblasts involves complex interplay between Smad3, p38α, and ERK1/2. *PLoS One* 2013; **8**: e57474 [PMID: 23468994 DOI: 10.1371/journal.pone.0057474]

126 **Yang SF**, Hsieh YS, Tsai CH, Chou MY, Chang YC. The upregulation of type I plasminogen activator inhibitor in oral submucous fibrosis. *Oral Oncol* 2003; **39**: 367-372 [PMID: 12676256 DOI: 10.1016/s1368-8375(02)00123-9]

127 **Gadbail AR**, Chaudhary M, Sarode SC, Gondivkar S, Tekade SA, Zade P, Hande A, Sarode GS, Patil S. Ki67, CD105, and α-SMA expression supports the transformation relevant dysplastic features in the atrophic epithelium of oral submucous fibrosis. *PLoS One* 2018; **13**: e0200171 [PMID: 30001387 DOI: 10.1371/journal.pone.0200171]

128 **Ye MY**, Chen MY, Chang YH, Huang JS, Huang TT, Wong TY, Hong TM, Chen YL. Growth-regulated oncogene-α from oral submucous fibrosis fibroblasts promotes malignant transformation of oral precancerous cells. *J Oral Pathol Med* 2018; **47**: 880-886 [PMID: 30035347 DOI: 10.1111/jop.12768]

129 **Daga D**, Singh RK, Pal US, Gurung T, Gangwar S. Efficacy of oral colchicine with intralesional hyaluronidase or triamcinolone acetonide in the Grade II oral submucous fibrosis. *Natl J Maxillofac Surg* 2017; **8**: 50-54 [PMID: 28761276 DOI: 10.4103/njms.NJMS_5_17]

130 **Boyd NF**, Byng JW, Jong RA, Fishell EK, Little LE, Miller AB, Lockwood GA, Tritchler DL, Yaffe MJ. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. *J Natl Cancer Inst* 1995; **87**: 670-675 [PMID: 7752271 DOI: 10.1093/jnci/87.9.670]

131 **Green AK**, Hankinson SE, Bertone-Johnson ER, Tamimi RM. Mammographic density, plasma vitamin D levels and risk of breast cancer in postmenopausal women. *Int J Cancer* 2010; **127**: 667-674 [PMID: 19960434 DOI: 10.1002/ijc.25075]

132 **Bodelon C**, Mullooly M, Pfeiffer RM, Fan S, Abubakar M, Lenz P, Vacek PM, Weaver DL, Herschorn SD, Johnson JM, Sprague BL, Hewitt S, Shepherd J, Malkov S, Keely PJ, Eliceiri KW, Sherman ME, Conklin MW, Gierach GL. Mammary collagen architecture and its association with mammographic density and lesion severity among women undergoing image-guided breast biopsy. *Breast Cancer Res* 2021; **23**: 105 [PMID: 34753492 DOI: 10.1186/s13058-021-01482-z]

133 **Huo CW**, Chew G, Hill P, Huang D, Ingman W, Hodson L, Brown KA, Magenau A, Allam AH, McGhee E, Timpson P, Henderson MA, Thompson EW, Britt K. High mammographic density is associated with an increase in stromal collagen and immune cells within the mammary epithelium. *Breast Cancer Res* 2015; **17**: 79 [PMID: 26040322 DOI: 10.1186/s13058-015-0592-1]

134 **Engin AB**, Engin A, Gonul II. The effect of adipocyte-macrophage crosstalk in obesity-related breast cancer. *J Mol Endocrinol* 2019; **62**: R201-R222 [PMID: 30620711 DOI: 10.1530/JME-18-0252]

135 **Skarping I**, Förnvik D, Sartor H, Heide-Jørgensen U, Zackrisson S, Borgquist S. Mammographic density is a potential predictive marker of pathological response after neoadjuvant chemotherapy in breast cancer. *BMC Cancer* 2019; **19**: 1272 [PMID: 31888552 DOI: 10.1186/s12885-019-6485-4]

136 **Hwang KT**, Chu AJ, Kim J, Lee JY, Chang JH, Oh S, Kim YA, Jung J, Oh B. Prognostic Influence of Preoperative Mammographic Breast Density in Operable Invasive Female Breast Cancer. *Sci Rep* 2018; **8**: 16075 [PMID: 30375450 DOI: 10.1038/s41598-018-34297-8]

137 **Chen YC**, Chan CH, Lim YB, Yang SF, Yeh LT, Wang YH, Chou MC, Yeh CB. Risk of Breast Cancer in Women with Mastitis: A Retrospective Population-Based Cohort Study. *Medicina (Kaunas)* 2020; **56** [PMID: 32722165 DOI: 10.3390/medicina56080372]

138 **Visscher DW**, Nassar A, Degnim AC, Frost MH, Vierkant RA, Frank RD, Tarabishy Y, Radisky DC, Hartmann LC. Sclerosing adenosis and risk of breast cancer. *Breast Cancer Res Treat* 2014; **144**: 205-212 [PMID: 24510013 DOI: 10.1007/s10549-014-2862-5]

139 **Pinsky RW**, Helvie MA. Mammographic breast density: effect on imaging and breast cancer risk. *J Natl Compr Canc Netw* 2010; **8**: 1157-64; quiz 1165 [PMID: 20971840 DOI: 10.6004/jnccn.2010.0085]

140 **Mann RM**, Athanasiou A, Baltzer PAT, Camps-Herrero J, Clauser P, Fallenberg EM, Forrai G, Fuchsjäger MH, Helbich TH, Killburn-Toppin F, Lesaru M, Panizza P, Pediconi F, Pijnappel RM, Pinker K, Sardanelli F, Sella T, Thomassin-Naggara I, Zackrisson S, Gilbert FJ, Kuhl CK; European Society of Breast Imaging (EUSOBI). Breast cancer screening in women with extremely dense breasts recommendations of the European Society of Breast Imaging (EUSOBI). *Eur Radiol* 2022; **32**: 4036-4045 [PMID: 35258677 DOI: 10.1007/s00330-022-08617-6]

141 **Choi DT**, Kum HC, Park S, Ohsfeldt RL, Shen Y, Parikh ND, Singal AG. Hepatocellular Carcinoma Screening Is Associated With Increased Survival of Patients With Cirrhosis. *Clin Gastroenterol Hepatol* 2019; **17**: 976-987.e4 [PMID: 30616961 DOI: 10.1016/j.cgh.2018.10.031]

142 **Huguet JM**, Ferrer-Barceló L, Suárez P, Sanchez E, Prieto JD, Garcia V, Sempere J. Colorectal cancer screening and surveillance in patients with inflammatory bowel disease in 2021. *World J Gastroenterol* 2022; **28**: 502-516 [PMID: 35316962 DOI: 10.3748/wjg.v28.i5.502]

143 **Spechler SJ**. Barrett esophagus and risk of esophageal cancer: a clinical review. *JAMA* 2013; **310**: 627-636 [PMID: 23942681 DOI: 10.1001/jama.2013.226450]

144 **Van Asseldonk DP**, de Boer NK, Peters GJ, Veldkamp AI, Mulder CJ, Van Bodegraven AA. On therapeutic drug monitoring of thiopurines in inflammatory bowel disease; pharmacology, pharmacogenomics, drug intolerance and clinical relevance. *Curr Drug Metab* 2009; **10**: 981-997 [PMID: 20214590 DOI: 10.2174/138920009790711887]

145 **Tiede I**, Fritz G, Strand S, Poppe D, Dvorsky R, Strand D, Lehr HA, Wirtz S, Becker C, Atreya R, Mudter J, Hildner K, Bartsch B, Holtmann M, Blumberg R, Walczak H, Iven H, Galle PR, Ahmadian MR, Neurath MF. CD28-dependent Rac1 activation is the molecular target of azathioprine in primary human CD4+ T lymphocytes. *J Clin Invest* 2003; **111**: 1133-1145 [PMID: 12697733 DOI: 10.1172/jci16432]

146 **de Boer NKH**, Peyrin-Biroulet L, Jharap B, Sanderson JD, Meijer B, Atreya I, Barclay ML, Colombel JF, Lopez A, Beaugerie L, Marinaki AM, van Bodegraven AA, Neurath MF. Thiopurines in Inflammatory Bowel Disease: New Findings and Perspectives. *J Crohns Colitis* 2018; **12**: 610-620 [PMID: 29293971 DOI: 10.1093/ecco-jcc/jjx181]

147 **Zhu Z**, Mei Z, Guo Y, Wang G, Wu T, Cui X, Huang Z, Zhu Y, Wen D, Song J, He H, Xu W, Cui L, Liu C. Reduced Risk of Inflammatory Bowel Disease-associated Colorectal Neoplasia with Use of Thiopurines: a Systematic Review and Meta-analysis. *J Crohns Colitis* 2018; **12**: 546-558 [PMID: 29370346 DOI: 10.1093/ecco-jcc/jjy006]

148 **Gargallo-Puyuelo CJ**, Laredo V, Gomollón F. Thiopurines in Inflammatory Bowel Disease. How to Optimize Thiopurines in the Biologic Era? *Front Med (Lausanne)* 2021; **8**: 681907 [PMID: 34336887 DOI: 10.3389/fmed.2021.681907]

149 **Qiao Y**, Yang T, Gan Y, Li W, Wang C, Gong Y, Lu Z. Associations between aspirin use and the risk of cancers: a meta-analysis of observational studies. *BMC Cancer* 2018; **18**: 288 [PMID: 29534696 DOI: 10.1186/s12885-018-4156-5]

150 **Ma Y**, Yu P, Lin S, Li Q, Fang Z, Huang Z. The association between nonsteroidal anti-inflammatory drugs and skin cancer: Different responses in American and European populations. *Pharmacol Res* 2020; **152**: 104499 [PMID: 31689521 DOI: 10.1016/j.phrs.2019.104499]

151 **Ehrenpreis ED**, Kruchko DH. Rapid Review: Nonsteroidal Anti-inflammatory Agents and Aminosalicylates in COVID-19 Infections. *J Clin Gastroenterol* 2020; **54**: 602-605 [PMID: 32530870 DOI: 10.1097/MCG.0000000000001371]

152 **Llanos-González AB**, Martínez JB, Peloche GB, Suárez-Cuartín G, Zygmunt VV, Planas-Cerezales L, López JMP, Martín LP, Masanes RJ, Zamora NP, Gil SG, Sargatal JD, Gonzálvez AM, Fernández OA, Molina MM. Antifibrotic treatment in progressive non-IPF fibrotic interstitial lung diseases. *Eur Respir J* 2019; **54**: 1731 [DOI: 10.1183/13993003.congress-2019.pa1731]

153 **Naoi H**, Suzuki Y, Mori K, Aono Y, Kono M, Hasegawa H, Yokomura K, Inoue Y, Hozumi H, Karayama M, Furuhashi K, Enomoto N, Fujisawa T, Nakamura Y, Inui N, Nakamura H, Suda T. Impact of antifibrotic therapy on lung cancer development in idiopathic pulmonary fibrosis. *Thorax* 2022; **77**: 727-730 [PMID: 35354649 DOI: 10.1136/thoraxjnl-2021-218281]

154 **Fisher B**, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Vogel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 1998; **90**: 1371-1388 [PMID: 9747868 DOI: 10.1093/jnci/90.18.1371]

155 **Cummings SR**, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, Norton L, Nickelsen T, Bjarnason NH, Morrow M, Lippman ME, Black D, Glusman JE, Costa A, Jordan VC. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. *JAMA* 1999; **281**: 2189-2197 [PMID: 10376571 DOI: 10.1001/jama.281.23.2189]

156 **Zhang Y**, Simondsen K, Kolesar JM. Exemestane for primary prevention of breast cancer in postmenopausal women. *Am J Health Syst Pharm* 2012; **69**: 1384-1388 [PMID: 22855103 DOI: 10.2146/ajhp110585]

157 **Cuzick J**, Sestak I, Forbes JF, Dowsett M, Cawthorn S, Mansel RE, Loibl S, Bonanni B, Evans DG, Howell A; IBIS-II investigators. Use of

anastrozole for breast cancer prevention (IBIS-II): long-term results of a randomised controlled trial. *Lancet* 2020; **395**: 117-122 [PMID: 31839281 DOI: 10.1016/S0140-6736(19)32955-1]

158 **Ferreira S**, Saraiva N, Rijo P, Fernandes AS. LOXL2 Inhibitors and Breast Cancer Progression. *Antioxidants (Basel)* 2021; **10** [PMID: 33669630 DOI: 10.3390/antiox10020312]

159 **Grossman M**, Ben-Chetrit N, Zhuravlev A, Afik R, Bassat E, Solomonov I, Yarden Y, Sagi I. Tumor Cell Invasion Can Be Blocked by Modulators of Collagen Fibril Alignment That Control Assembly of the Extracellular Matrix. *Cancer Res* 2016; **76**: 4249-4258 [PMID: 27221706 DOI: 10.1158/0008-5472.CAN-15-2813]

160 **Leung L**, Niculescu-Duvaz D, Smitten D, Lopes F, Calens C, McLeary R, Saturno G, Davies L, Aljarah M, Bown M, Johnson L, Zambon A, Chambers T, Menard D, Bayliss N, Knight R, Fish L, Lawrence R, Challinor M, Tang HR, Marais R, Springer C. Anti-metastatic Inhibitors of Lysyl Oxidase (LOX): Design and Structure-Activity Relationships. *Journal of Medicinal Chemistry* 2019; 62: 5863-5884 [DOI: 10.1021/acs.jmedchem.9b00335.s001]

161 **Meissner EG**, McLaughlin M, Matthews L, Gharib AM, Wood BJ, Levy E, Sinkus R, Virtaneva K, Sturdevant D, Martens C, Porcella SF, Goodman ZD, Kanwar B, Myers RP, Subramanian M, Hadigan C, Masur H, Kleiner DE, Heller T, Kottilil S, Kovacs JA, Morse CG. Simtuzumab treatment of advanced liver fibrosis in HIV and HCV-infected adults: results of a 6-month open-label safety trial. *Liver Int* 2016; **36**: 1783-1792 [PMID: 27232579 DOI: 10.1111/liv.13177]

162 **Sciences G**. Simtuzumab (GS-6624) in the Prevention of Progression of Liver Fibrosis in Adults With Primary Sclerosing Cholangitis (PSC). Vol. 2022 (ClinicalTrials.gov, 2012). [DOI: 10.31038/imroj.2017231]

163 **Center MSKC**. Phase II Study of Tetrathiomolybdate (TM) in Patients With Breast Cancer. Vol. 2022 (clinicaltrials.gov, 2005) [DOI: 10.1158/1538-7445.sabcs18-pd9-07]

164 **Pharmacist**. Study to Evaluate Safety, Pharmacokinetic and Pharmacodynamic Dose Escalation and Expansion Study of PXS-5505 in Patients With Primary, Post-polycythemia Vera or Post-essential Thrombocythemia Myelofibrosis. Vol. 2022 (ClinicaTrials.gov, 2020). [DOI: 10.1182/blood-2022-158344]

165 **Caon I**, Bartolini B, Parnigoni A, Caravà E, Moretto P, Viola M, Karousou E, Vigetti D, Passi A. Revisiting the hallmarks of cancer: The role of hyaluronan. *Semin Cancer Biol* 2020; **62**: 9-19 [PMID: 31319162 DOI: 10.1016/j.semcancer.2019.07.007]

166 **Morosi L**, Meroni M, Ubezio P, Fuso Nerini I, Minoli L, Porcu L, Panini N, Colombo M, Blouw B, Kang DW, Davoli E, Zucchetti M, D'Incalci M, Frapolli R. PEGylated recombinant human hyaluronidase (PEGPH20) pre-treatment improves intra-tumour distribution and efficacy of paclitaxel in preclinical models. *J Exp Clin Cancer Res* 2021; **40**: 286 [PMID: 34507591 DOI: 10.1186/s13046-021-02070-x]

167 **Jacobetz MA**, Chan DS, Neesse A, Bapiro TE, Cook N, Frese KK, Feig C, Nakagawa T, Caldwell ME, Zecchini HI, Lolkema MP, Jiang P, Kultti A, Thompson CB, Maneval DC, Jodrell DI, Frost GI, Shepard HM, Skepper JN, Tuveson DA. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. *Gut* 2013; **62**: 112-120 [PMID: 22466618 DOI: 10.1136/gutjnl-2012-302529]

168 **Thompson CB**, Shepard HM, O'Connor PM, Kadhim S, Jiang P, Osgood RJ, Bookbinder LH, Li X, Sugarman BJ, Connor RJ, Nadjsombati S, Frost GI. Enzymatic depletion of tumor hyaluronan induces antitumor responses in preclinical animal models. *Mol Cancer Ther* 2010; **9**: 3052-3064 [PMID: 20978165 DOI: 10.1158/1535-7163.MCT-10-0470]

169 **Doherty GJ**, Tempero M, Corrie PG. HALO-109-301: a Phase III trial of PEGPH20 (with gemcitabine and nab-paclitaxel) in hyaluronic acid-high stage IV pancreatic cancer. *Future Oncol* 2018; **14**: 13-22 [PMID: 29235360 DOI: 10.2217/fon-2017-0338]

170 **Kraman M**, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, Gopinathan A, Tuveson DA, Fearon DT. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. *Science* 2010; **330**: 827-830 [PMID: 21051638 DOI: 10.1126/science.1195300]

171 **Arnold JN**, Magiera L, Kraman M, Fearon DT. Tumoral immune suppression by macrophages expressing fibroblast activation protein-α and heme oxygenase-1. *Cancer Immunol Res* 2014; **2**: 121-126 [PMID: 24778275 DOI: 10.1158/2326-6066.CIR-13-0150]

172 **Belhabib I**, Zaghdoudi S, Lac C, Bousquet C, Jean C. Extracellular Matrices and Cancer-Associated Fibroblasts: Targets for Cancer Diagnosis and Therapy? *Cancers (Basel)* 2021; **13** [PMID: 34298680 DOI: 10.3390/cancers13143466]

173 **Jess T**, Simonsen J, Jørgensen KT, Pedersen BV, Nielsen NM, Frisch M. Decreasing risk of colorectal cancer in patients with inflammatory bowel disease over 30 years. *Gastroenterology* 2012; **143**: 375-81.e1; quiz e13 [PMID: 22522090 DOI: 10.1053/j.gastro.2012.04.016]

174 **Miura Y**, Saito T, Tanaka T, Takoi H, Yatagai Y, Inomata M, Nei T, Saito Y, Gemma A, Azuma A. Reduced incidence of lung cancer in patients with idiopathic pulmonary fibrosis treated with pirfenidone. *Respir Investig* 2018; **56**: 72-79 [PMID: 29325685 DOI: 10.1016/j.resinv.2017.09.007]

175 **Bittoni MA**, Carbone DP, Harris RE. Ibuprofen and fatal lung cancer: A brief report of the prospective results from the Third National Health and Nutrition Examination Survey (NHANES III). *Mol Clin Oncol* 2017; **6**: 917-920 [PMID: 28588790 DOI: 10.3892/mco.2017.1239]

176 **Tao Y**, Li Y, Liu X, Deng Q, Yu Y, Yang Z. Nonsteroidal anti-inflammatory drugs, especially aspirin, are linked to lower risk and better survival of hepatocellular carcinoma: a meta-analysis. *Cancer Manag Res* 2018; **10**: 2695-2709 [PMID: 30147368 DOI: 10.2147/CMAR.S167560]

177 **Gutiérrez-Cuevas J**, Lucano-Landeros S, López-Cifuentes D, Santos A, Armendariz-Borunda J. Epidemiologic, Genetic, Pathogenic, Metabolic, Epigenetic Aspects Involved in NASH-HCC: Current Therapeutic Strategies. *Cancers (Basel)* 2022; **15** [PMID: 36612019 DOI: 10.3390/cancers15010023]

178 **Rai A**, Shrivastava PK, Kumar A, Prasad K, Shakeel S, Ul Haque Z. Comparative effectiveness of medicinal interventions for oral submucous fibrosis: A network meta-analysis. *J Stomatol Oral Maxillofac Surg* 2023; **124**: 101423 [PMID: 36781110 DOI: 10.1016/j.jormas.2023.101423]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 July 24; 14(7): 247-258

DOI: 10.5306/wjco.v14.i7.247

ISSN 2218-4333 (online)

MINIREVIEWS

Role of prophylactic central neck lymph node dissection for papillary thyroid carcinoma in the era of de-escalation

Efstathios T Pavlidis, Theodoros E Pavlidis

Specialty type: Surgery

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Al-Ani RM, Iraq; He XH, China; Şurlin VM, Romania

Received: April 24, 2023
Peer-review started: April 24, 2023
First decision: June 14, 2023
Revised: June 14, 2023
Accepted: July 3, 2023
Article in press: July 3, 2023
Published online: July 24, 2023

Efstathios T Pavlidis, Theodoros E Pavlidis, 2nd Propedeutic Department of Surgery, Hippocration Hospital, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki 54642, Greece

Corresponding author: Theodoros E Pavlidis, Doctor, PhD, Full Professor, Surgeon, 2nd Propedeutic Department of Surgery, Hippocration Hospital, School of Medicine, Aristotle University of Thessaloniki, Konstantinoupoleos 49, Thessaloniki 54642, Greece.
pavlidth@auth.gr

Abstract

Thyroid cancer is the most common endocrine malignancy. While there has been no appreciable increase in the observed mortality of well-differentiated thyroid cancer, there has been an overall rise in its incidence worldwide over the last few decades. Patients with papillary thyroid carcinoma (PTC) and clinical evidence of central (cN1) and/or lateral lymph node metastases require total thyroidectomy plus central and/or lateral neck dissection as the initial surgical treatment. Nodal status in PTC patients plays a crucial role in the prognostic evaluation of the recurrence risk. The 2015 guidelines of the American Thyroid Association (ATA) have more accurately determined the indications for therapeutic central and lateral lymph node dissection. However, prophylactic central neck lymph node dissection (pCND) in negative lymph node (cN0) PTC patients is controversial, as the 2009 ATA guidelines recommended that CND "should be considered" routinely in patients who underwent total thyroidectomy for PTC. Although the current guidelines show clear indications for therapeutic CND, the role of pCND in cN0 patients with PTC is still debated. In small solitary papillary carcinoma (T1, T2), pCND is not recommended unless there are high-risk prediction factors for recurrence and diffuse nodal spread (extrathyroid extension, mutation in the *BRAF* gene). pCND can be considered in cN0 disease with advanced primary tumors (T3 or T4) or clinical lateral neck disease (cN1b) or for staging and treatment planning purposes. The role of the preoperative evaluation is fundamental to minimizing the possible detrimental effect of overtreatment of the types of patients who are associated with low disease-related morbidity and mortality. On the other hand, it determines the choice of appropriate treatment and determines if close monitoring of patients at a higher risk is needed. Thus, pCND is currently recommended for T3 and T4 tumors but not for T1 and T2 tumors without high-risk prediction factors of recurrence.

Key Words: Well differentiated carcinoma; Papillary thyroid cancer; Prophylactic central neck dissection; Thyroid disease; Thyroidectomy; Lymphadenectomy

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Nodal status in papillary thyroid cancer patients plays an important role in the prognosis of risk for recurrence. Preoperative evaluation is crucial for minimizing the possible risk of injury from overtreatment. Undoubtedly, therapeutic central neck dissection in addition to total thyroidectomy should be performed if there is positive lymph node involvement. The role of prophylactic central neck lymph node dissection in patients with papillary thyroid carcinoma with negative lymph nodes has been debated. It is currently recommended for T3 and T4 tumors but not for T1 and T2 tumors without high-risk prediction factors of recurrence.

Citation: Pavlidis ET, Pavlidis TE. Role of prophylactic central neck lymph node dissection for papillary thyroid carcinoma in the era of de-escalation. *World J Clin Oncol* 2023; 14(7): 247-258
URL: https://www.wjgnet.com/2218-4333/full/v14/i7/247.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i7.247

INTRODUCTION

Well-differentiated thyroid cancer is the most common endocrine malignancy, with approximately 570000 new cases annually. Furthermore, papillary thyroid carcinoma constitutes 90% of the new cases of thyroid cancer[1]. In comparison to statistics from the previous decade, there is now an over 100% increase in its incidence worldwide. This upsurge is somewhat due not only to increasing human exposure to defined incriminated factors for the development of thyroid carcinoma but possibly also to increases in health care utilization and imaging practices (ultrasound, fine needle aspiration), which can efficiently detect small asymptomatic nodules that otherwise remain undiagnosed[2,3].

The incidence of thyroid cancer reaches its peak between the fourth and fifth decade of life, with a predominance of women with a mean ratio of 4/1[4]. The overall five-year survival rate of thyroid carcinoma reaches over 95%, which could be characterized as excellent. Thus, it is one of the most amenable malignancies to treatment. The incidence of deaths in the United States is only 0.5 per 100,000 population and has not changed significantly from 1975 to 2009[5]. Despite the increasing incidence and due to widespread high-sensitivity screening practices, there is no described increase in mortality, which supports that well-differentiated thyroid cancer is in fact being overdiagnosed[6,7].

In the last version of the American Thyroid Association (ATA) guidelines, total thyroidectomy remains the preferred management method for tumors with a diameter above 4 cm or with a diameter under 4 cm but with high-risk features. It is widely established that high-risk features, including a family history of thyroid carcinoma, prior neck irradiation, extrathyroid extension, multifocality, and central lymph node involvement, with or without lateral lymph node neck involvement, require more extended surgical resections (total thyroidectomy with or without lymph node dissection)[8].

Papillary thyroid microcarcinomas are defined as papillary thyroid carcinomas (PTCs) of 1 cm or less in size. It has been reported that they are related to extremely low local or regional recurrence rates (2%-6%) and an even lower disease-specific mortality of less than 1%[9]. Since the majority of newly diagnosed thyroid carcinomas are microcarcinomas, there is growing pressure to stage the risk and minimize possible injury from the overtreatment of low-risk thyroid disease. To this effect, the American Thyroid Association indicates lobectomy as an alternative and less invasive approach in its new guidelines, as well as to minimize the major complications of total thyroidectomy, mainly hypocalcemia and recurrent laryngeal nerve palsy[8].

Lymph node metastases are common in papillary thyroid cancer, occurring in 20%–50% of patients, and they mostly occur in the central compartment of the neck (level VI). Lymph node metastases are also known to be an independent risk factor for local recurrence[10]. Lymph node dissection of the central, i.e., levels VI, VII with lateral compartments of the neck, i.e., Levels II to V will undoubtedly be recommended if there is a confirmed presence of lymph node metastases[8]. The necessity of prophylactic central neck lymph node dissection remains contested and in an ongoing controversy in the era of de-escalation.

This narrative review evaluates the role of prophylactic central neck lymph node dissection in well-differentiated thyroid carcinoma.

RESEARCH METHODS

The study was based on the data of an extensive literature review from PubMed until March 2023, focusing on the comparison of the efficacy and surgical safety of its prophylactic performance. Only full-text papers published in the English language were included. Since the aim of this review was to study the efficacy and oncological completeness of thyroidectomy with or without central neck lymph node dissection for well-differentiated thyroid carcinoma, studies for

nonmalignant thyroid pathologies were excluded.

CLINICAL ANATOMY AND EXTENT OF THOROUGH CENTRAL NECK DISSECTION

The central neck compartment is anatomically composed of level VI and the upper part of level VII. Level VI is bounded cranially by the hyoid bone, caudally by the upper margin of the sternum, and laterally by the left and right common carotid arteries. The anterior border of the superficial layer of the deep cervical fascia is the posterior margin of the sternothyroid muscle. The posterior border is the prevertebral fascia, the deep layer of the deep cervical fascia. Near the origin of the right brachiocephalic artery, which forms the lower edge, the caudal of level VI is extended to level VII. Four groups constitute the lymph nodes of the central neck compartment, *i.e.*, the prelaryngeal (Delphian), pretracheal, right paratracheal, and left paratracheal lymph nodes. Accurate central neck lymph node dissection requires imperatively meticulous complete removal of both the prelaryngeal and pretracheal regions and those of at least one paratracheal region, either left or right. In the case of the involvement of both paratracheal regions, central neck dissection should be bilateral[10].

Patients with clinical positivity for lymph node metastasis need to undergo therapeutic central neck dissection. Metastatic lymph node involvement is usually revealed either preoperatively, by ultrasound imaging, or intraoperatively by frozen section biopsy. Prophylactic central neck dissection means removal of all lymph nodes in both levels VI and VII, despite a negative preoperative diagnosis for suspected findings (cN0). The so-called berry picking, *i.e.*, the one by one excision of only the lymph nodes with the appearance of metastasis that are in the sites that are not apparently healthy, should not be considered as an option and should be avoided[11].

The resection of paratracheal lymph nodes constitutes one of the most challenging technical parts of central neck dissection because of the necessary preservation of the anatomical integrity of all crucial structures in this region, specifically the recurrent laryngeal nerve and parathyroid glands with their vascularity. The latter deals mainly with the inferior parathyroid gland, as almost 90% of cases receive blood supply from the inferior thyroid artery, which lies beneath the area, in which all contained lymph nodes are planned to be dissected. In contrast, preservation of the upper parathyroid gland is somewhat easier during paratracheal lymph node dissection, especially when its blood supply comes exclusively from the superior thyroid artery[8,10].

RISK STRATIFICATION: PREOPERATIVE EVALUATION

The last guidelines of the American Thyroid Association state that the relevant high-risk factors from the history, *i.e.*, rapid growth of nodules, sudden swallowing dysfunction, or dysphonia, must be investigated[8]. In addition, much relevant information can be obtained at the time of the patient's examination. There is agreement among authors that age equal to or more than 45 years, female sex, familial history of thyroid carcinoma, and previous neck irradiation are considered predisposing factors for developing thyroid carcinoma, as shown in Table 1[12-14].

Undoubtedly, a precise preoperative diagnosis is a necessary condition for successfully planning the operative strategy. It is imperative to examine all the central and lateral neck lymph nodes in patients with well-differentiated thyroid carcinoma preoperatively, as well as to examine the central compartment intraoperatively. The preoperative fundamental diagnostic tools include an ultrasound scan of high resolution and fine-needle aspiration cytology of all suspected nodes[15].

Determining the levels of thyroglobulin in the aspiration material from suspicious lymph nodes significantly increases the sensitivity of the whole diagnostic evaluation. The sensitivity and specificity to detect lateral lymph node metastasis are sometimes higher compared to the central compartment; the referred sensitivity of the lateral is 93.8%, which is in contrast to that of the central compartment, which is 30%[16]. This notable difference is attributed to the complex anatomy of the central compartment. The central lymph nodes are not only smaller in diameter than the lateral lymph nodes but are also located in a groove between the esophagus, trachea, and thyroid. The interpretation of ultrasound findings is undoubtedly operator dependent, and much expertise is needed. Moreover, the presence of lymphocytic thyroiditis (Hashimoto's disease) changes, which may be accompanied by inflammatory lymphadenopathy in majority of cases and may further interfere with the interpretation of the examination findings[17].

If a suspicion of extrathyroid spread exists that is accompanied by infiltration of a neighboring structure (larynx, esophagus, trachea and the main blood vessels in the neck) or if there is possible infiltration of the mediastinal and retropharyngeal lymph nodes, then a computed tomography (CT) scan of the head, neck and thorax needs to be performed. Magnetic resonance imaging of the head and neck could occasionally be a reliable alternative to CT scan; nonetheless, for the central compartment, it is less enlightening when compared to CT scan[15].

ROLE OF CENTRAL NECK NODAL STATUS

In patients with papillary thyroid cancer and clinical evidence of central with or without lateral lymph node metastases (cN1), the necessary initial treatment includes, central lymph node dissection with or without lateral neck dissection, in addition to total thyroidectomy. Level V lymph node dissection is mandatory in cases of central lymph node involvement. Additionally, it is necessary when ipsilateral with or without bilateral therapeutic lateral neck dissection,

Table 1 Papillary thyroid carcinoma, risk stratification and preoperative evaluation

No.	Parameter
1	Tumor size > 4 cm
2	Family history of thyroid carcinoma
3	Previous neck irradiation
4	Multifocality
5	Extrathyroid extension
6	Rapid growth of nodules
7	Sudden swallowing dysfunction or dysphonia
8	Age ≥ 45 yr
9	Female gender

including levels IIa, III, IV, and Vb, is required[8,10,18,19]. This is because of the pivotal role of the nodal status in patients with papillary thyroid cancer, which is mainly due to its prognostic contribution to the risk of recurrence[8]. The 2015 American Thyroid Association guidelines update, in contrast to those of 2009, more precisely determines the recommendations for therapeutic central and lateral lymph node dissection when they are clinically evident. Prophylactic central neck dissection can be considered in negative central lymph node (cN0) disease of large primary tumors (T3, T4), in clinical lateral lymph node disease (cN1b), or for staging purposes to define the plan of treatment strategy. This clarified aspect is important, as the 2009 guidelines recommended that routine level VI lymph node dissection "should be considered" in all patients undergoing total thyroidectomy for papillary thyroid cancer regardless of positive or negative nodal status[9]. This decisive statement has led to controversy, as many surgeons took it as an interpretation of the recommended surgery.

Well-differentiated thyroid carcinoma includes not only papillary but also follicular carcinomas. However, the latter has mainly hematogenous metastases and only occasional (less than 5% of cases) regional lymphatic metastases of the neck[20]. The most common locations for distant hematogenous metastases are the lungs and brain[21], with a metastasis rate that fluctuates between 6% and 20% of cases[22,23]. To this effect, there is no need for prophylactic central neck dissection in follicular cancers, except for those cases in which there are clinically evident central neck metastases.

Lymphatic metastases of papillary thyroid carcinoma are mainly located in the regional lymph nodes. They most often affect the lymph nodes of level VI (paratracheal) and, in distant time, those of the lateral neck compartment, specifically levels III and IV, and extremely rarely level I[24,25]. In the absence of central lymph node metastases, escaped lateral lymph node metastases have been reported with an overall incidence of 20%[26]. They are associated, in most cases, with carcinoma located in the superior thyroid third, which mainly has metastases in lymph node levels II and III[27]. Notably, approximately 83% of the cases with lateral lymph node involvement also have microscopic metastases of the ipsilateral central lymph nodes, and 4% of them cannot be revealed by any preoperative diagnostic tool. In this clinical scenario, a need exists for at least ipsilateral prophylactic central neck dissection regardless of the negative clinical status[28].

In 5%-10% of cases of papillary thyroid carcinoma, palpatory clinical evidence of regional metastatic disease (macroscopic disease) exists at the time of diagnosis. The use of more sophisticated diagnostic approaches, including high-resolution ultrasound with fine-needle aspiration biopsy, may increase the former incidence by up to 30%[29]. Hematoxylin and eosin staining, the classical histopathological tool, can reveal positive typical lymph nodes in 30% to 50% of patients with papillary thyroid carcinoma who underwent elective central with lateral lymph node dissection[30]. There are studies in which an additional immunohistochemical evaluation of the resection specimen revealed microscopic metastases in up to 90% of cases[31,32]. These reports sustain the aspect that papillary thyroid carcinoma in most cases is accompanied by microscopic dissemination of the disease at the time of diagnosis and does not usually exhibit clinical evidence.

ROLE OF PROPHYLACTIC CENTRAL NECK DISSECTION

The results from studies such as Tisell *et al*[33] and Barczyńsk *et al*[34] have suggested that prophylactic central neck dissection has a positive effect on patient survival, mainly by reducing the probability of locoregional recurrence. The effect of lymph node status in recurrence and survival in PTC is shown in Table 2. Such a recurrence is based on macroscopic metastases with infiltration beyond the thyroid caps, a larger number of either positive or negative nodes in the overall lymph nodes included in the performed dissection, as well as the existence of five or more nodes with metastasis in the initial specimen[35]. However, the recurrence ratio could be described as very low in the presence of microscopic metastases[36]. Although the incidence of nodal micrometastases in the central compartment ranges from 38% to 80%, the probability of local nodal recurrence is below 3.8%, and central neck dissection is either performed or not [37,38].

Table 2 Effect of lymph node status in recurrence and survival in papillary thyroid carcinoma

Ref.	Patients (Nu)	Trial	Survival
Tisell *et al*[33], 1996	195	Single center retrospective study	Increased in ≥ 45 yr; Unaffected in < 45 yr
Zaydfudim *et al*[20], 2008	30504	United States Registry, Surveillance	Increased in ≥ 45 yr; Unaffected in < 45 yr
Lundgren *et al*[38], 2006	5123	Swedish Registry Surveillance	Increased

In addition, the studies from Lundgren *et al*[38], including 5123 patients, and Zaydfudim *et al*[20], including 33088 patients, assessed the existence of metastases in the central and lateral compartments and documented a reduced survival rate. The recognized risk factors were age > 45 years in papillary cancer patients, male sex, metastases > 3 cm in size accompanied by spread beyond the thyroid caps, and the histopathological type of diffuse invasive follicular carcinoma [20,39,40]. According to the aforementioned, the selection of initial operative management has gained great importance; it should not be required to use only the tumor size as a criterion to determine the surgical plan.

Although there has been a worldwide agreement that lateral lymph node dissection should be preserved only in clinical N1b cases, the role of prophylactic central lymph node dissection in cN0 papillary thyroid carcinoma is still debated[40-45]. However, the preoperative evaluation of lymph nodes can be characterized as challenging. According to a meta-analysis from Liang *et al*[46], who included 23 "high-quality" studies, the proportion of central neck lymph node metastases fluctuates between 16.7% and 82.3% in those patients who underwent prophylactic central neck dissection.

Taking into account these broad ranges in the rate of central neck metastases, obtaining a high-quality evidence-based recommendation concerning prophylactic central neck dissection could be defined as demanding. A reason that could explain this heterogeneity in the literature's results could be the difference in the expertise of obtaining a preoperative assessment by ultrasound, the plan of surgical management, and the histopathological evaluation. The basic assertions that are in favor of prophylactic central lymph node dissection concern the better staging accuracy, a more precise allocation to radioiodine treatment and the more reduced levels of postoperative thyroglobulin, possibly contributing to a decrease in the recurrence risk[46,47].

Otherwise, the basic argument against the abovementioned is the increased potential for complications, mainly hypoparathyroidism and laryngeal nerve injury[40,48]. A more conservative approach, i.e., ipsilateral (IpsiCND) central neck dissection, provides a lower rate of complications and was proposed in patients with clinical unilateral papillary thyroid carcinoma. It includes removal of the prelaryngeal, pretracheal and paratracheal lymph nodes on the same side as the tumor[49].

Even if the preoperative evaluation of the central lymph node compartment has not confirmed nodal metastasis, it must not prevent the surgeon from sending any suspicious node for intraoperative frozen-section histopathological assessment, and the assessment should not be based only on an intraoperative clinical inspection and palpation. Depending on the outcome of the frozen-section biopsy, a decision on therapeutic central lymph node dissection can be made. Several authors have verified that the sensitivity and specificity of intraoperative frozen-section biopsy may reach 100%[50]. Nevertheless, even in experienced hands, only approximately 26% of the confirmed metastases of the lymph nodes could be revealed based only on the intraoperative clinical evaluation[51].

In consonance with the novel scientific evolution, there is no reason to perform ipsilateral prophylactic central neck dissection for small solitary (T1, T2) well-differentiated thyroid carcinomas. The incriminated factors for the development of locoregional metastases include the larger diameter thyroid carcinomas (T3, T4), multifocality, a tall cell, a diffuse sclerotic and insular tumor that represents an aggressive subtype[52-56] as well as positivity for *BRAF* gene mutations on genetic testing[57]. In such scenarios, ipsilateral prophylactic central neck dissection is recommended. Nonetheless, the majority of the aforementioned data concerning possible malignancy are available only postoperatively after a precise tumor histopathological evaluation. Unfortunately, that accurate information is not available in advance for determining the plan of the extent of operative resection, thus carrying out a prophylactic central neck dissection is ultimately required.

A reliable alternative could be a prophylactic ipsilateral neck dissection frozen section examination, as proposed by Raffaelli *et al*[58]. Taking into consideration the highly accurate rate of frozen section evaluation of the ipsilateral central lymph node compartment in assessing the nodal status of negative cases with papillary thyroid cancer (up to 90%), they hypothesized that the frozen section assessment of ipsilateral central lymph node dissection could be valuable to modulate the extension of surgical resection. Undoubtedly, if there is an occurrence of hidden ipsilateral central lymph node metastasis, then total thyroidectomy and therapeutic central compartment dissection will become mandatory. Currently, in a case control study (unpublished data), they adopted such operative tactics personalizing the extent of the attempted resection in patients with small (T1) papillary thyroid carcinoma, without both multifocality and central lymph node involvement. This evaluation included 60 patients with personalized management who were scheduled for initial lobectomy only. The results, as described, confirmed that frozen section evaluation of ipsilateral central lymph node dissection may be effective and accurate in identifying patients who could benefit from bilateral central neck dissection. Therefore, the advantages include the lower risk of recurrence and subsequently the reduced need for a second more complicated operation[58].

MORBIDITY IN ELECTIVE CENTRAL NECK DISSECTION

Another factor that supports the debate concerning prophylactic central neck dissection is the intraoperative and postoperative morbidity that accompany such a procedure. Actually, the question that arises is whether the benefits exceed the potential harm. It is well known and demonstrated that the percentage of complications, namely, recurrent laryngeal nerve injuries and hypocalcemia, is increased after total thyroidectomy in cases accompanied by central lymph node dissection[59-61].

Lee *et al*[62], including 103 patients, stated that ipsilateral central neck dissection is accompanied by fewer complications, especially temporary and permanent hypocalcemia, compared to bilateral dissection. On the other hand, a meta-analysis from Chisholm *et al*[63], including 1132 patients, supports that there is no statistically significant increase in the percentages of complications, especially when neck dissection is performed by an endocrine surgeon. Zhu *et al*[64] drew the same results after evaluating nine relevant studies including 2298 patients.

Over the last two decades, a notable increase in papillary thyroid cancer and multifocal lesions as well as the coexistence of Hashimoto's chronic thyroiditis was found. In addition, there was a gradual decrease in the papillary thyroid carcinoma sizes and subsequently an increase in micropapillary carcinoma[65]. The latter has led to controversy regarding the possible increase in lymph node metastasis reflecting central lymph node dissection. However, a recent study showed that multifocal lesions were not accompanied by a relevant increase in lymph node metastasis, but bilateral multifocality was associated with more aggressive clinical behavior and tumor histopathology. Thus, in this case, prophylactic central lymph node dissection is indicated, despite preoperative or intraoperative negative lymph node involvement[66].

The incidence of lymph node metastasis posterior to the right recurrent laryngeal nerve was estimated at 6%, making it necessary to thoroughly investigate this possibility in tumors of the lower pole that are greater than 0.5 cm in size[67]. Based on the recommendation by the American Thyroid Association for routine dissection of this lymph node[8], there is an increased risk of nerve injury and palsy in these tumors. Endoscopic thyroidectomy may offer an alternative safer approach[68].

A recent meta-analysis including 15 studies showed that total thyroidectomy with prophylactic lymph node dissection for papillary thyroid carcinoma was related to a lower local recurrence rate but a higher risk of permanent hypocalcemia and transient hypoparathyroidism than total thyroidectomy alone. There were no significant differences in transient hypocalcemia, permanent hypoparathyroidism, both temporary and permanent vocal cord paralysis, and recurrent laryngeal nerve injury[69]. The results of the above mentioned studies using routine prophylactic central neck lymph node dissection in PTC are shown in Table 3.

Hashimoto's thyroiditis may cause reactive hyperplasia of the central lymph nodes in patients with papillary thyroid cancer. Nevertheless, in this autoimmune thyroiditis, there are often false-positive findings on ultrasound, which lead to possible overtreatment and complications[70].

PRE/POSTOPERATIVE PREDICTION FACTORS-RECURRENCE

Several predisposing factors for potential central lymph node metastasis in T1-T2 papillary thyroid carcinoma have been recognized. Thus, predictive nomograms have been developed, and they can be useful in planning the extent of operative strategy[71-75].

They include age (less than 44 years), gender (male), race (white and other nonblack people), size of the tumor (larger than 10 mm), multiple focal lesions, and minimal extrathyroid extension[71].

The least absolute shrinkage and selection operator -based model includes age (equal to or more than 55 years), nodular goiter, mutations in the *BRAF* gene, and Hashimoto's thyroiditis as the most important factors[72].

The preoperative ultrasound suspicious findings (size of lymph node more than 5 mm, microcalcification, cystic degeneration, round shape, abnormal boundary, and cortical thickening) in addition to clinical data constitute another model[73]. Some statistical data of ultrasound signs are shown in Table 4[74].

Papillary thyroid carcinoma that is located in the isthmus exhibits aggressiveness and is related to poor prognosis. A nomogram including incriminated factors for metastatic lymph nodes and worse outcome (gender, age, size of malignant lesion, thyroid cap invasion, and Hashimoto's thyroiditis)[75], as for any other location of high-risk patients[76], predicts recurrence[77].

Hypervascularity in ultrasound is an independent risk factor for recurrence in papillary thyroid carcinoma[78].

The ratio of fibrinogen to neutrophile percentage has been proposed as another independent risk factor for recurrence in patients with the coexistence of papillary thyroid carcinoma and diabetes mellitus type 2[79].

Multifocality (presence of two or more foci) of papillary thyroid carcinoma was determined to be a risk factor for an increased rate of central lymph node metastasis (44.57%) and lateral lymph node metastasis (17.17%)[80].

A radiomics nomogram based on ultrasound features, sex, age, *BRAF* gene *V600E* mutation, and extrathyroid extension predicts lymph node metastasis in papillary thyroid carcinoma[81].

For stage pT1α papillary thyroid microcarcinoma, multivariate analyses have demonstrated that younger age, male sex, and subcapsular location of the lesion were predictive factors for central lymph node metastasis[82].

Based on the above mentioned studies, the main high-risk prediction factors of central lymph node recurrence in T1-T2 PTC are shown in Figure 1.

Small papillary thyroid carcinoma (equal to or less than 10 mm in diameter) was found to be a prediction factor for not detecting lymph node metastases, as shown in a recent study. Multivariate analyses have also showed that the values of

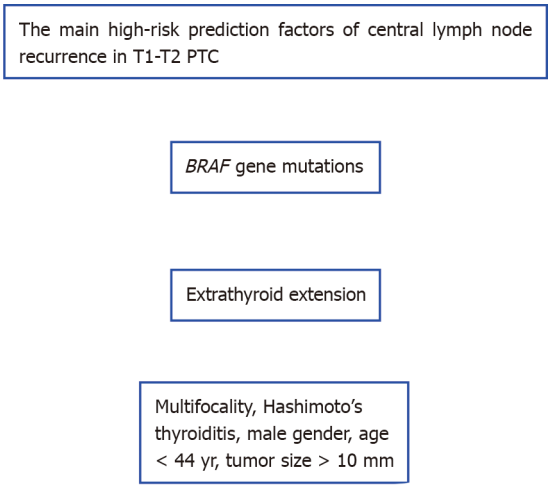
Table 3 Results of routine prophylactic central neck lymph node dissection in papillary thyroid carcinoma

Ref.	Patients (Nu)	Trial	Findings
Barczyński *et al*[34], 2013	640	Single center retrospective study	Bilateral pCND increases 10-yr disease-specific survival and locoregional control, No increased risk of permanent morbidity
Lee *et al*[62], 2007	103	Single center retrospective study	Increased transient hypocalcemia in bilateral than ipsilateral pCND
Chisholm *et al* [63], 2009	1132	Meta-analysis	No increased permanent morbidity
Zhu *et al*[64], 2013	2298	Meta-analysis	No more complications
Wang *et al* [69], 2023	2080	Meta-analysis	Reduced local recurrence; Higher risk of permanent hypocalcemia and transient hypopara-thyroidism; No significant differences in transient hypocalcemia, permanent hypopara-thyroidism, both temporary and permanent vocal cord paralysis, and recurrent laryngeal nerve injury

pCND: Prophylactic central neck lymph node dissection.

Table 4 Suspicious ultrasound findings of lymph nodes which predict malignant infiltration

Sign	Sensitivity, %	Specificity, %
Microcalcifications	5-69	93-100
Cystic degeneration	10-34	91-100
Vascularity peripheral	40-86	57-93
Hyperechogenicity	30-87	43-95
Shape round	37	70



DOI: 10.5306/wjco.v14.i7.247 **Copyright** ©The Author(s) 2023.

Figure 1 Scheme of high-risk prediction factors of central lymph node recurrence in T1-T2 papillary thyroid carcinoma. PTC: Papillary thyroid carcinoma.

stimulated thyroglobulin are related to shorter recurrence-free survival[83]. Thus, it must be considered a reliable prediction factor for recurrence.

Despite the presence of metastasis in the lateral neck lymph nodes, dissection of the central lymph nodes is not always necessary. A multivariate analysis showed that papillary thyroid carcinoma located in the center of the lobe and fewer than 4 positive lateral lymph nodes were protective factors against central lymph node involvement, which is subsequently a positive prognostic factor[84].

Patients with papillary thyroid carcinoma and a negative preoperative investigation for central lymph node involvement and who underwent total thyroidectomy alone without planned prophylactic lymph node dissection but with an incidentally removed lymph node positive for metastasis in the specimen biopsy had a worse course and high

rate of treatment failure. In such a case, as shown in a recent large and detailed trial, the cumulative disease-free survival (DFS) was significantly lower (61.8%) *vs* 93.9%, and the cumulative survival was 79% *vs* 96% within the following 60 mo in the patients without metastasis in their incidentally removed lymph nodes[85]. Thus, a positive incidental lymph node is considered a significant risk factor for a worse outcome.

Nevertheless, the utility of intraoperative ultrasound is important for the assessment of lymph node status. Small lymph nodes (2-3 mm in size) may be evaluated adequately for metastatic spread by high-resolution neck ultrasound. The recurrence rate and subsequent need for reoperation in patients with papillary thyroid cancer and negative central lymph node involvement has been limited by the intraoperative prediction of lateral lymph nodes *via* ultrasound and their prophylactic dissection[86].

It seems from all the above mentioned that preoperative evaluation is crucial for minimizing the possible risk of injury from overtreatment in the majority of patients who otherwise have a low risk of disease-specific mortality and morbidity, whereas properly treating and monitoring those patients at higher risk is important since in some cases, nodal metastases are found in the surgical specimen. Apparently, molecular genomic assessment of diagnostic cytology samples could be more informative when dealing with the aggressive behavior of well-differentiated thyroid carcinoma to reliably modulate the extent of the initial surgery. Ipsilateral central neck dissection frozen section examination could be a reliable intraoperative method to assess the nodal status.

CONCLUSION

Although there is a clear indication for therapeutic central neck dissection according to the current guidelines, the role of prophylactic treatment in cN0 patients with papillary thyroid carcinoma is still debated. In follicular thyroid carcinoma, which usually has hematogenous metastases, there is no need for prophylactic central lymph node dissection. In small solitary papillary carcinoma (T1, T2), prophylactic central neck dissection is not recommended, as it does not provide benefits regarding prolonged survival, while this simultaneously provides a significant increase in the postoperative complication risk concerning either temporary or permanent complications, such as recurrent laryngeal nerve palsy and hypoparathyroidism. Prophylactic central lymph node dissection has been recommended in large papillary thyroid carcinomas (T3 and T4 tumors) or small ones (T1 and T2 tumors) related to high-risk prediction factors of recurrence and diffuse nodal spread, such as in extrathyroid extension or when there is a mutation in the *BRAF* gene.

FOOTNOTES

Author contributions: Pavlidis TE designed research, contributed new analytic tools, analyzed data and review; Pavlidis ET performed research, analyzed data review and wrote the paper.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Greece

ORCID number: Efstathios T Pavlidis 0000-0002-7282-8101; Theodoros E Pavlidis 0000-0002-8141-1412.

S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **La Vecchia C**, Malvezzi M, Bosetti C, Garavello W, Bertuccio P, Levi F, Negri E. Thyroid cancer mortality and incidence: a global overview. *Int J Cancer* 2015; **136**: 2187-2195 [PMID: 25284703 DOI: 10.1002/ijc.29251]

3 **Vaccarella S**, Franceschi S, Bray F, Wild CP, Plummer M, Dal Maso L. Worldwide Thyroid-Cancer Epidemic? The Increasing Impact of Overdiagnosis. *N Engl J Med* 2016; **375**: 614-617 [PMID: 27532827 DOI: 10.1056/NEJMp1604412]

4 **Sentieri Working Group**. [Sentieri: mortality, cancer incidence and hospital discharges. Summary]. *Epidemiol Prev* 2014; **38**: 5-7 [PMID: 24986497]

5 **Davies L**, Welch HG. Current thyroid cancer trends in the United States. *JAMA Otolaryngol Head Neck Surg* 2014; **140**: 317-322 [PMID: 24557566 DOI: 10.1001/jamaoto.2014.1]

6 **Hoang JK**, Nguyen XV, Davies L. Overdiagnosis of thyroid cancer: answers to five key questions. *Acad Radiol* 2015; **22**: 1024-1029 [PMID: 26100186 DOI: 10.1016/j.acra.2015.01.019]

7 **Esserman LJ**, Thompson IM, Reid B, Nelson P, Ransohoff DF, Welch HG, Hwang S, Berry DA, Kinzler KW, Black WC, Bissell M, Parnes H, Srivastava S. Addressing overdiagnosis and overtreatment in cancer: a prescription for change. *Lancet Oncol* 2014; **15**: e234-e242 [PMID: 24807866 DOI: 10.1016/S1470-2045(13)70598-9]

8 **Haugen BR**, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* 2016; **26**: 1-133 [PMID: 26462967 DOI: 10.1089/thy.2015.0020]

9 **Kovatch KJ**, Hoban CW, Shuman AG. Thyroid cancer surgery guidelines in an era of de-escalation. *Eur J Surg Oncol* 2018; **44**: 297-306 [PMID: 28385370 DOI: 10.1016/j.ejso.2017.03.005]

10 **American Thyroid Association Surgery Working Group**; American Association of Endocrine Surgeons,; American Academy of Otolaryngology-Head and Neck Surgery; American Head and Neck Society, Carty SE, Cooper DS, Doherty GM, Duh QY, Kloos RT, Mandel SJ, Randolph GW, Stack BC Jr, Steward DL, Terris DJ, Thompson GB, Tufano RP, Tuttle RM, Udelsman R. Consensus statement on the terminology and classification of central neck dissection for thyroid cancer. *Thyroid* 2009; **19**: 1153-1158 [PMID: 19860578 DOI: 10.1089/thy.2009.0159]

11 **Musacchio MJ**, Kim AW, Vijungco JD, Prinz RA. Greater local recurrence occurs with "berry picking" than neck dissection in thyroid cancer. *Am Surg* 2003; **69**: 191-6; discussion 196 [PMID: 12678473]

12 **Matsuzu K**, Sugino K, Masudo K, Nagahama M, Kitagawa W, Shibuya H, Ohkuwa K, Uruno T, Suzuki A, Magoshi S, Akaishi J, Masaki C, Kawano M, Suganuma N, Rino Y, Masuda M, Kameyama K, Takami H, Ito K. Thyroid lobectomy for papillary thyroid cancer: long-term follow-up study of 1,088 cases. *World J Surg* 2014; **38**: 68-79 [PMID: 24081532 DOI: 10.1007/s00268-013-2224-1]

13 **Hay ID**, Thompson GB, Grant CS, Bergstralh EJ, Dvorak CE, Gorman CA, Maurer MS, McIver B, Mullan BP, Oberg AL, Powell CC, van Heerden JA, Goellner JR. Papillary thyroid carcinoma managed at the Mayo Clinic during six decades (1940-1999): temporal trends in initial therapy and long-term outcome in 2444 consecutively treated patients. *World J Surg* 2002; **26**: 879-885 [PMID: 12016468 DOI: 10.1007/s00268-002-6612-1]

14 **Ganly I**, Nixon IJ, Wang LY, Palmer FL, Migliacci JC, Aniss A, Sywak M, Eskander AE, Freeman JL, Campbell MJ, Shen WT, Vaisman F, Momesso D, Corbo R, Vaisman M, Shaha A, Tuttle RM, Shah JP, Patel SG. Survival from Differentiated Thyroid Cancer: What Has Age Got to Do with It? *Thyroid* 2015; **25**: 1106-1114 [PMID: 26148759 DOI: 10.1089/thy.2015.0104]

15 **Yeh MW**, Bauer AJ, Bernet VA, Ferris RL, Loevner LA, Mandel SJ, Orloff LA, Randolph GW, Steward DL; American Thyroid Association Surgical Affairs Committee Writing Task Force. American Thyroid Association statement on preoperative imaging for thyroid cancer surgery. *Thyroid* 2015; **25**: 3-14 [PMID: 25188202 DOI: 10.1089/thy.2014.0096]

16 **Hwang HS**, Orloff LA. Efficacy of preoperative neck ultrasound in the detection of cervical lymph node metastasis from thyroid cancer. *Laryngoscope* 2011; **121**: 487-491 [PMID: 21344423 DOI: 10.1002/lary.21227]

17 **Yoo YH**, Kim JA, Son EJ, Youk JH, Kwak JY, Kim EK, Park CS. Sonographic findings predictive of central lymph node metastasis in patients with papillary thyroid carcinoma: influence of associated chronic lymphocytic thyroiditis on the diagnostic performance of sonography. *J Ultrasound Med* 2013; **32**: 2145-2151 [PMID: 24277897 DOI: 10.7863/ultra.32.12.2145]

18 **Podnos YD**, Smith D, Wagman LD, Ellenhorn JD. The implication of lymph node metastasis on survival in patients with well-differentiated thyroid cancer. *Am Surg* 2005; **71**: 731-734 [PMID: 16468507 DOI: 10.1177/000313480507100907]

19 **Wang LY**, Ganly I. Nodal metastases in thyroid cancer: prognostic implications and management. *Future Oncol* 2016; **12**: 981-994 [PMID: 26948758 DOI: 10.2217/fon.16.10]

20 **Zaydfudim V**, Feurer ID, Griffin MR, Phay JE. The impact of lymph node involvement on survival in patients with papillary and follicular thyroid carcinoma. *Surgery* 2008; **144**: 1070-7; discussion 1077 [PMID: 19041020 DOI: 10.1016/j.surg.2008.08.034]

21 **Parameswaran R**, Shulin Hu J, Min En N, Tan WB, Yuan NK. Patterns of metastasis in follicular thyroid carcinoma and the difference between early and delayed presentation. *Ann R Coll Surg Engl* 2017; **99**: 151-154 [PMID: 27659362 DOI: 10.1308/rcsann.2016.0300]

22 **Lin JD**, Huang MJ, Juang JH, Chao TC, Huang BY, Chen KW, Chen JY, Li KL, Chen JF, Ho YS. Factors related to the survival of papillary and follicular thyroid carcinoma patients with distant metastases. *Thyroid* 1999; **9**: 1227-1235 [PMID: 10646663 DOI: 10.1089/thy.1999.9.1227]

23 **Schlumberger M**, Challeton C, De Vathaire F, Travagli JP, Gardet P, Lumbroso JD, Francese C, Fontaine F, Ricard M, Parmentier C. Radioactive iodine treatment and external radiotherapy for lung and bone metastases from thyroid carcinoma. *J Nucl Med* 1996; **37**: 598-605 [PMID: 8691248]

24 **Noguchi S**, Noguchi A, Murakami N. Papillary carcinoma of the thyroid. I. Developing pattern of metastasis. *Cancer* 1970; **26**: 1053-1060 [PMID: 5476786 DOI: 10.1002/1097-0142(197011)26:5<1053::aid-cncr2820260513>3.0.co;2-x]

25 **Caron NR**, Tan YY, Ogilvie JB, Triponez F, Reiff ES, Kebebew E, Duh QY, Clark OH. Selective modified radical neck dissection for papillary thyroid cancer-is level I, II and V dissection always necessary? *World J Surg* 2006; **30**: 833-840 [PMID: 16555024 DOI: 10.1007/s00268-005-0358-5]

26 **Park JH**, Lee YS, Kim BW, Chang HS, Park CS. Skip lateral neck node metastases in papillary thyroid carcinoma. *World J Surg* 2012; **36**: 743-747 [PMID: 22354485 DOI: 10.1007/s00268-012-1476-5]

27 **Bumber B**, Marjanovic Kavanagh M, Jakovcevic A, Sincic N, Prstacic R, Prgomet D. Role of matrix metalloproteinases and their inhibitors in the development of cervical metastases in papillary thyroid cancer. *Clin Otolaryngol* 2020; **45**: 55-62 [PMID: 31646745 DOI: 10.1111/coa.13466]

28 **Khafif A**, Ben-Yosef R, Abergel A, Kesler A, Landsberg R, Fliss DM. Elective paratracheal neck dissection for lateral metastases from papillary carcinoma of the thyroid: is it indicated? *Head Neck* 2008; **30**: 306-310 [PMID: 17615566 DOI: 10.1002/hed.20696]

29 **Stulak JM**, Grant CS, Farley DR, Thompson GB, van Heerden JA, Hay ID, Reading CC, Charboneau JW. Value of preoperative ultrasonography in the surgical management of initial and reoperative papillary thyroid cancer. *Arch Surg* 2006; **141**: 489-94; discussion 494 [PMID: 16702521 DOI: 10.1001/archsurg.141.5.489]

30 **Boi F**, Baghino G, Atzeni F, Lai ML, Faa G, Mariotti S. The diagnostic value for differentiated thyroid carcinoma metastases of thyroglobulin (Tg) measurement in washout fluid from fine-needle aspiration biopsy of neck lymph nodes is maintained in the presence of circulating anti-Tg antibodies. *J Clin Endocrinol Metab* 2006; **91**: 1364-1369 [PMID: 16434461 DOI: 10.1210/jc.2005-1705]

31 **Arturi F**, Russo D, Giuffrida D, Ippolito A, Perrotti N, Vigneri R, Filetti S. Early diagnosis by genetic analysis of differentiated thyroid cancer

metastases in small lymph nodes. *J Clin Endocrinol Metab* 1997; **82**: 1638-1641 [PMID: 9141564 DOI: 10.1210/jcem.82.5.4062]

32 **Qubain SW**, Nakano S, Baba M, Takao S, Aikou T. Distribution of lymph node micrometastasis in pN0 well-differentiated thyroid carcinoma. *Surgery* 2002; **131**: 249-256 [PMID: 11894028 DOI: 10.1067/msy.2002.120657]

33 **Tisell LE**, Nilsson B, Mölne J, Hansson G, Fjälling M, Jansson S, Wingren U. Improved survival of patients with papillary thyroid cancer after surgical microdissection. *World J Surg* 1996; **20**: 854-859 [PMID: 8678962 DOI: 10.1007/s002689900130]

34 **Barczyński M**, Konturek A, Stopa M, Nowak W. Prophylactic central neck dissection for papillary thyroid cancer. *Br J Surg* 2013; **100**: 410-418 [PMID: 23188784 DOI: 10.1002/bjs.8985]

35 **Schneider DF**, Mazeh H, Chen H, Sippel RS. Lymph node ratio predicts recurrence in papillary thyroid cancer. *Oncologist* 2013; **18**: 157-162 [PMID: 23345543 DOI: 10.1634/theoncologist.2012-0240]

36 **Bardet S**, Malville E, Rame JP, Babin E, Samama G, De Raucourt D, Michels JJ, Reznik Y, Henry-Amar M. Macroscopic lymph-node involvement and neck dissection predict lymph-node recurrence in papillary thyroid carcinoma. *Eur J Endocrinol* 2008; **158**: 551-560 [PMID: 18362303 DOI: 10.1530/EJE-07-0603]

37 **Gršić K**, Bumber B, Curić Radivojević R, Leović D. Prophylactic Central Neck Dissection in Well-differentiated Thyroid Cancer. Acta Clin Croat 2020; 59: 87-95

38 **Lundgren CI**, Hall P, Dickman PW, Zedenius J. Clinically significant prognostic factors for differentiated thyroid carcinoma: a population-based, nested case-control study. *Cancer* 2006; **106**: 524-531 [PMID: 16369995 DOI: 10.1002/cncr.21653]

39 **Sugitani I**, Kasai N, Fujimoto Y, Yanagisawa A. A novel classification system for patients with PTC: addition of the new variables of large (3 cm or greater) nodal metastases and reclassification during the follow-up period. *Surgery* 2004; **135**: 139-148 [PMID: 14739848 DOI: 10.1016/s0039-6060(03)00384-2]

40 **Bonnet S**, Hartl D, Leboulleux S, Baudin E, Lumbroso JD, Al Ghuzlan A, Chami L, Schlumberger M, Travagli JP. Prophylactic lymph node dissection for papillary thyroid cancer less than 2 cm: implications for radioiodine treatment. *J Clin Endocrinol Metab* 2009; **94**: 1162-1167 [PMID: 19116234 DOI: 10.1210/jc.2008-1931]

41 **Sancho JJ**, Lennard TW, Paunovic I, Triponez F, Sitges-Serra A. Prophylactic central neck disection in papillary thyroid cancer: a consensus report of the European Society of Endocrine Surgeons (ESES). *Langenbecks Arch Surg* 2014; **399**: 155-163 [PMID: 24352594 DOI: 10.1007/s00423-013-1152-8]

42 **Raffaelli M**, De Crea C, Sessa L, Giustacchini P, Revelli L, Bellantone C, Lombardi CP. Prospective evaluation of total thyroidectomy versus ipsilateral versus bilateral central neck dissection in patients with clinically node-negative papillary thyroid carcinoma. *Surgery* 2012; **152**: 957-964 [PMID: 23158170 DOI: 10.1016/j.surg.2012.08.053]

43 **Raffaelli M**, De Crea C, Sessa L, Giustacchini P, Bellantone R, Lombardi CP. Can intraoperative frozen section influence the extension of central neck dissection in cN0 papillary thyroid carcinoma? *Langenbecks Arch Surg* 2013; **398**: 383-388 [PMID: 23207498 DOI: 10.1007/s00423-012-1036-3]

44 **Raffaelli M**, De Crea C, Sessa L, Fadda G, Bellantone C, Lombardi CP. Ipsilateral Central Neck Dissection Plus Frozen Section Examination Versus Prophylactic Bilateral Central Neck Dissection in cN0 Papillary Thyroid Carcinoma. *Ann Surg Oncol* 2015; **22**: 2302-2308 [PMID: 25652046 DOI: 10.1245/s10434-015-4383-9]

45 **Sessa L**, Lombardi CP, De Crea C, Tempera SE, Bellantone R, Raffaelli M. Risk Factors for Central Neck Lymph Node Metastases in Micro- Versus Macro- Clinically Node Negative Papillary Thyroid Carcinoma. *World J Surg* 2018; **42**: 623-629 [PMID: 29238850 DOI: 10.1007/s00268-017-4390-z]

46 **Liang J**, Li Z, Fang F, Yu T, Li S. Is prophylactic central neck dissection necessary for cN0 differentiated thyroid cancer patients at initial treatment? A meta-analysis of the literature. *Acta Otorhinolaryngol Ital* 2017; **37**: 1-8 [PMID: 28374865 DOI: 10.14639/0392-100X-1195]

47 **Shaha AR**. Prophylactic central compartment dissection in thyroid cancer: a new avenue of debate. *Surgery* 2009; **146**: 1224-1227 [PMID: 19958952 DOI: 10.1016/j.surg.2009.10.020]

48 **Hughes DT**, White ML, Miller BS, Gauger PG, Burney RE, Doherty GM. Influence of prophylactic central lymph node dissection on postoperative thyroglobulin levels and radioiodine treatment in papillary thyroid cancer. *Surgery* 2010; **148**: 1100-6; discussion 1006 [PMID: 21134539 DOI: 10.1016/j.surg.2010.09.019]

49 **Giordano D**, Valcavi R, Thompson GB, Pedroni C, Renna L, Gradoni P, Barbieri V. Complications of central neck dissection in patients with papillary thyroid carcinoma: results of a study on 1087 patients and review of the literature. *Thyroid* 2012; **22**: 911-917 [PMID: 22827494 DOI: 10.1089/thy.2012.0011]

50 **Viola D**, Materazzi G, Valerio L, Molinaro E, Agate L, Faviana P, Seccia V, Sensi E, Romei C, Piaggi P, Torregrossa L, Sellari-Franceschini S, Basolo F, Vitti P, Elisei R, Miccoli P. Prophylactic central compartment lymph node dissection in papillary thyroid carcinoma: clinical implications derived from the first prospective randomized controlled single institution study. *J Clin Endocrinol Metab* 2015; **100**: 1316-1324 [PMID: 25590215 DOI: 10.1210/jc.2014-3825]

51 **Lee DH**, Yoon TM, Kim HK, Lee JK, Kang HC, Lim SC. Intraoperative Frozen Biopsy of Central Lymph Node in the Management of Papillary Thyroid Microcarcinoma. *Indian J Otolaryngol Head Neck Surg* 2016; **68**: 56-59 [PMID: 27066412 DOI: 10.1007/s12070-015-0900-1]

52 **Scherl S**, Mehra S, Clain J, Dos Reis LL, Persky M, Turk A, Wenig B, Husaini H, Urken ML. The effect of surgeon experience on the detection of metastatic lymph nodes in the central compartment and the pathologic features of clinically unapparent metastatic lymph nodes: what are we missing when we don't perform a prophylactic dissection of central compartment lymph nodes in papillary thyroid cancer? *Thyroid* 2014; **24**: 1282-1288 [PMID: 24787362 DOI: 10.1089/thy.2013.0600]

53 **Zhang L**, Wei WJ, Ji QH, Zhu YX, Wang ZY, Wang Y, Huang CP, Shen Q, Li DS, Wu Y. Risk factors for neck nodal metastasis in papillary thyroid microcarcinoma: a study of 1066 patients. *J Clin Endocrinol Metab* 2012; **97**: 1250-1257 [PMID: 22319042 DOI: 10.1210/jc.2011-1546]

54 **Koo BS**, Choi EC, Yoon YH, Kim DH, Kim EH, Lim YC. Predictive factors for ipsilateral or contralateral central lymph node metastasis in unilateral papillary thyroid carcinoma. *Ann Surg* 2009; **249**: 840-844 [PMID: 19387316 DOI: 10.1097/SLA.0b013e3181a40919]

55 **Roh JL**, Kim JM, Park CI. Central lymph node metastasis of unilateral papillary thyroid carcinoma: patterns and factors predictive of nodal metastasis, morbidity, and recurrence. *Ann Surg Oncol* 2011; **18**: 2245-2250 [PMID: 21327454 DOI: 10.1245/s10434-011-1600-z]

56 **Horvatic Herceg G**, Herceg D, Kralik M, Kulic A, Bence-Zigman Z, Tomic-Brzac H, Bracic I, Kusacic-Kuna S, Prgomet D. Urokinase plasminogen activator and its inhibitor type-1 as prognostic factors in differentiated thyroid carcinoma patients. *Otolaryngol Head Neck Surg* 2013; **149**: 533-540 [PMID: 23835563 DOI: 10.1177/0194599813496374]

57 **Howell GM**, Nikiforova MN, Carty SE, Armstrong MJ, Hodak SP, Stang MT, McCoy KL, Nikiforov YE, Yip L. BRAF V600E mutation

independently predicts central compartment lymph node metastasis in patients with papillary thyroid cancer. *Ann Surg Oncol* 2013; **20**: 47-52 [DOI: 10.1245/s10434-012-2611-0]

58 **Raffaelli M**, Tempera SE, Sessa L, Lombardi CP, De Crea C, Bellantone R. Total thyroidectomy versus thyroid lobectomy in the treatment of papillary carcinoma. *Gland Surg* 2020; **9**: S18-S27 [PMID: 32055495 DOI: 10.21037/gs.2019.11.09]

59 **Palestini N**, Borasi A, Cestino L, Freddi M, Odasso C, Robecchi A. Is central neck dissection a safe procedure in the treatment of papillary thyroid cancer? Our experience. *Langenbecks Arch Surg* 2008; **393**: 693-698 [PMID: 18592264 DOI: 10.1007/s00423-008-0360-0]

60 **Díez JJ**, Anda E, Sastre J, Pérez Corral B, Álvarez-Escolá C, Manjón L, Paja M, Sambo M, Santiago Fernández P, Blanco Carrera C, Galofré JC, Navarro E, Zafón C, Sanz E, Oleaga A, Bandrés O, Donnay S, Megía A, Picallo M, Sánchez Ragnarsson C, Baena-Nieto G, García JCF, Lecumberri B, de la Vega MS, Romero-Lluch AR, Iglesias P. Prevalence and risk factors for hypoparathyroidism following total thyroidectomy in Spain: a multicentric and nation-wide retrospective analysis. *Endocrine* 2019; **66**: 405-415 [PMID: 31317524 DOI: 10.1007/s12020-019-02014-8]

61 **Machens A**, Elwerr M, Thanh PN, Lorenz K, Schneider R, Dralle H. Impact of central node dissection on postoperative morbidity in pediatric patients with suspected or proven thyroid cancer. *Surgery* 2016; **160**: 484-492 [PMID: 27117577 DOI: 10.1016/j.surg.2016.03.007]

62 **Lee YS**, Kim SW, Kim SK, Kang HS, Lee ES, Chung KW. Extent of routine central lymph node dissection with small papillary thyroid carcinoma. *World J Surg* 2007; **31**: 1954-1959 [PMID: 17687598 DOI: 10.1007/s00268-007-9171-7]

63 **Chisholm EJ**, Kulinskaya E, Tolley NS. Systematic review and meta-analysis of the adverse effects of thyroidectomy combined with central neck dissection as compared with thyroidectomy alone. *Laryngoscope* 2009; **119**: 1135-1139 [PMID: 19358241 DOI: 10.1002/lary.20236]

64 **Zhu W**, Zhong M, Ai Z. Systematic evaluation of prophylactic neck dissection for the treatment of papillary thyroid carcinoma. *Jpn J Clin Oncol* 2013; **43**: 883-888 [PMID: 23858039 DOI: 10.1093/jjco/hyt087]

65 **Bakar B**, Taşar P, Kırdak T, Kılıçturgay S. What has changed in the last 20 years in the postoperative specimen findings of the papillary thyroid cancer cases? A retrospective analysis. *Turk J Surg* 2022; **38**: 345-352 [PMID: 36875266 DOI: 10.47717/turkjsurg.2022.5688]

66 **Ozdemir K**, Harmantepe AT, Gonullu E, Kocer B, Bayhan Z. Should multifocality be an indication for prophylactic central neck dissection in papillary thyroid cancer? *Updates Surg* 2023; **75**: 701-706 [PMID: 36871277 DOI: 10.1007/s13304-023-01479-7]

67 **Yang H**, Tao L. Lymph Node Posterior to the Right Recurrent Laryngeal Nerve Metastasis in Right Lobe T1a Papillary Thyroid Carcinoma: A Retrospective Cohort Study. *Cancer Control* 2023; **30**: 10732748221149819 [PMID: 36747345 DOI: 10.1177/10732748221149819]

68 **Si L**, Mei H, Wang Q, Wang F, Sha S, He Z, Ke J. Surgical outcomes of different approaches to dissection of lymph nodes posterior to right recurrent laryngeal nerve: a retrospective comparative cohort study of endoscopic thyroidectomy *via* the areolar approach and *via* the axillo-breast approach. *Gland Surg* 2022; **11**: 1936-1945 [PMID: 36654954 DOI: 10.21037/gs-22-661]

69 **Wang Y**, Xiao Y, Pan Y, Yang S, Li K, Zhao W, Hu X. The effectiveness and safety of prophylactic central neck dissection in clinically node-negative papillary thyroid carcinoma patients: A meta-analysis. *Front Endocrinol (Lausanne)* 2022; **13**: 1094012 [PMID: 36733809 DOI: 10.3389/fendo.2022.1094012]

70 **Tan HL**, Nyarko A, Duan SL, Zhao YX, Chen P, He Q, Zhang ZJ, Chang S, Huang P. Comprehensive analysis of the effect of Hashimoto's thyroiditis on the diagnostic efficacy of preoperative ultrasonography on cervical lymph node lesions in papillary thyroid cancer. *Front Endocrinol (Lausanne)* 2022; **13**: 987906 [PMID: 36714580 DOI: 10.3389/fendo.2022.987906]

71 **Sun Y**, Sun W, Xiang J, Zhang H. Nomogram for predicting central lymph node metastasis in T1-T2 papillary thyroid cancer with no lateral lymph node metastasis. *Front Endocrinol (Lausanne)* 2023; **14**: 1112506 [PMID: 36817601 DOI: 10.3389/fendo.2023.1112506]

72 **Zhao F**, Wang P, Yu C, Song X, Wang H, Fang J, Zhu C, Li Y. A LASSO-based model to predict central lymph node metastasis in preoperative patients with cN0 papillary thyroid cancer. *Front Oncol* 2023; **13**: 1034047 [PMID: 36761950 DOI: 10.3389/fonc.2023.1034047]

73 **Huang J**, Li Z, Zhong Q, Fang J, Chen X, Zhang Y, Huang Z. Developing and validating a multivariable machine learning model for the preoperative prediction of lateral lymph node metastasis of papillary thyroid cancer. *Gland Surg* 2023; **12**: 101-109 [PMID: 36761483 DOI: 10.21037/gs-22-741]

74 **Mizrachi A**, Shaha AR. Lymph Node Dissection for Differentiated Thyroid Cancer. *Mol Imaging Radionucl Ther* 2017; **26**: 10-15 [PMID: 28117285 DOI: 10.4274/2017.26.suppl.02]

75 **Zhao Y**, Shi W, Dong F, Wang X, Lu C, Liu C. Risk prediction for central lymph node metastasis in isolated isthmic papillary thyroid carcinoma by nomogram: A retrospective study from 2010 to 2021. *Front Endocrinol (Lausanne)* 2022; **13**: 1098204 [PMID: 36733797 DOI: 10.3389/fendo.2022.1098204]

76 **Li F**, Zhou FJ, Zhu TW, Qiu HL, Zhang XT, Ruan BW, Huang DY. Nomogram for predicting skip metastasis in cN0 papillary thyroid cancer patients at increased risk of lymph node metastasis. *Adv Clin Exp Med* 2023 [PMID: 36603142 DOI: 10.17219/acem/157240]

77 **Jang SW**, Park JH, Kim HR, Kwon HJ, Lee YM, Hong SJ, Yoon JH. Recurrence Risk Evaluation in Patients with Papillary Thyroid Carcinoma: Multicenter Machine Learning Evaluation of Lymph Node Variables. *Cancers (Basel)* 2023; **15** [PMID: 36672498 DOI: 10.3390/cancers15020550]

78 **Li W**, Li Y, Long M, Li J, Ma J, Luo Y. Vascularity depicted by contrast-enhanced ultrasound predicts recurrence of papillary thyroid cancer. *Eur J Radiol* 2023; **159**: 110667 [PMID: 36574742 DOI: 10.1016/j.ejrad.2022.110667]

79 **Zheng D**, Yang J, Qian J, Jin L, Huang G. Fibrinogen-to-Neutrophil Ratio as a New Predictor of Central Lymph Node Metastasis in Patients with Papillary Thyroid Cancer and Type 2 Diabetes Mellitus. *Cancer Manag Res* 2022; **14**: 3493-3505 [PMID: 36573167 DOI: 10.2147/CMAR.S366270]

80 **Zhang T**, He L, Wang Z, Dong W, Sun W, Zhang P, Zhang H. Risk factors of cervical lymph node metastasis in multifocal papillary thyroid cancer. *Front Oncol* 2022; **12**: 1003336 [PMID: 36568187 DOI: 10.3389/fonc.2022.1003336]

81 **Wen Q**, Wang Z, Traverso A, Liu Y, Xu R, Feng Y, Qian L. A radiomics nomogram for the ultrasound-based evaluation of central cervical lymph node metastasis in papillary thyroid carcinoma. *Front Endocrinol (Lausanne)* 2022; **13**: 1064434 [PMID: 36531493 DOI: 10.3389/fendo.2022.1064434]

82 **Tagliabue M**, Giugliano G, Mariani MC, Rubino M, Grosso E, Chu F, Calastri A, Maffini FA, Mauri G, De Fiori E, Manzoni MF, Ansarin M. Prevalence of Central Compartment Lymph Node Metastases in Papillary Thyroid Micro-Carcinoma: A Retrospective Evaluation of Predictive Preoperative Features. *Cancers (Basel)* 2021; **13** [PMID: 34885138 DOI: 10.3390/cancers13236028]

83 **Ryu YJ**, Kwon SY, Lim SY, Na YM, Park MH. Predictive Factors for Skip Lymph Node Metastasis and Their Implication on Recurrence in Papillary Thyroid Carcinoma. *Biomedicines* 2022; **10** [PMID: 35052858 DOI: 10.3390/biomedicines10010179]

84 **Graceffa G**, Orlando G, Cocorullo G, Mazzola S, Vitale I, Proclamà MP, Amato C, Saputo F, Rollo EM, Corigliano A, Melfa G, Cipolla C, Scerrino G. Predictors of Central Compartment Involvement in Patients with Positive Lateral Cervical Lymph Nodes According to Clinical and/or Ultrasound Evaluation. *J Clin Med* 2021; **10** [PMID: 34362189 DOI: 10.3390/jcm10153407]

85 **Pinheiro RA**, Leite AK, Cavalheiro BG, de Mello ES, Kowalski LP, Matos LL. Incidental Node Metastasis as an Independent Factor of Worse Disease-Free Survival in Patients with Papillary Thyroid Carcinoma. *Cancers (Basel)* 2023; **15** [PMID: 36765899 DOI: 10.3390/cancers15030943]

86 **Shen Y**, Li X, Tao L, Chen Y, Xie R. Clinical Efficacy of Intraoperative Ultrasound for Prophylactic Lymphadenectomy of the Lateral Cervical Neck in Stage CN0 Papillary Thyroid Cancer: A Prospective Study. *J Invest Surg* 2023; **36**: 2154416 [PMID: 36519315 DOI: 10.1080/08941939.2022.2154416]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 July 24; 14(7): 259-264

DOI: 10.5306/wjco.v14.i7.259

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

Relationship between anal cancer recurrence and cigarette smoking

Kevin R McMahon, Nicholas Gemma, McKenzie Clapp, Patricia Sanchez-Montejo, Joseph Dibello, Erica Laipply

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Lee PN, United Kingdom; Pietroletti R, Italy

Received: February 23, 2023
Peer-review started: February 23, 2023
First decision: March 28, 2023
Revised: May 10, 2023
Accepted: June 13, 2023
Article in press: June 13, 2023
Published online: July 24, 2023

Kevin R McMahon, Nicholas Gemma, McKenzie Clapp, Patricia Sanchez-Montejo, Joseph Dibello, Erica Laipply, Department of General Surgery, Summa Health System, Akron, OH 44304, United States

Corresponding author: Kevin R McMahon, MD, Doctor, Department of General Surgery, Summa Health System, 55 Arch Street, Akron, OH 44304, United States.
mcmahonkr@summahealth.org

Abstract

BACKGROUND

The incidence of anal cancer has been increasing in the United States. Smoking is a well-established risk factor; however, the impact of smoking on disease recurrence and outcome has not been well studied. The aim of this study was to assess the association between anal cancer recurrence and cigarette smoking.

AIM

To investigate the relationship between cigarette smoking status and anal cancer treatment outcome.

METHODS

The cancer registry from a single, community hospital was screened for patients with anal cancer between 2010 and 2021. The following characteristics were gathered from the database: Age; sex; cigarette smoking history; American Joint Committee on Cancer Clinical Stage Group; response to therapy; recurrence; time to recurrence; mortality; time to death; and length of follow-up. Patients were divided into the following groups: Current smokers; former smokers; and never smokers. SPSSv25.0 software (IBM Corp., Armonk, NY, United States) was used for statistical analysis.

RESULTS

A total of 95 patients from the database met the screening criteria. There were 37 never smokers, 22 former smokers, and 36 current smokers. There was no difference between groups in regards to race or sex. There was no difference in the American Joint Committee on Cancer Clinical Stage Group between groups. The former smokers were significantly older when compared to never smokers and current smokers (66.5 ± 13.17 *vs* 57.4 ± 7.82 *vs* 63.7 ± 13.80, P = 0.011). Former smokers and current smokers had a higher recurrence rate compared to never smokers (30.8% and 20.8% compared to zero, P = 0.009). There was not a significant difference in recurrence between former smokers and current smokers. There was no difference in the mortality, non-response rate, or time to death

between the groups.

CONCLUSION
Our data contributes evidence that cigarette smoking status is associated with increased recurrence for patients with anal cancer.

Key Words: Anal cancer; Smoking; Recurrence; Nigro protocol; Chemoradiation; Retrospective review

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: This retrospective review examined the impact of smoking on anal cancer treatment for 95 patients. Smoking status was associated with a significantly higher rate of anal cancer recurrence after standard treatment. There was not a significant association between smoking status and anal cancer treatment non-response or mortality. Further study is needed to determine if smoking cessation would alter the course of anal cancer or if adjunct therapy would be beneficial in patients with anal cancer and a smoking history.

Citation: McMahon KR, Gemma N, Clapp M, Sanchez-Montejo P, Dibello J, Laipply E. Relationship between anal cancer recurrence and cigarette smoking. *World J Clin Oncol* 2023; 14(7): 259-264
URL: https://www.wjgnet.com/2218-4333/full/v14/i7/259.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i7.259

INTRODUCTION

The incidence of anal cancer is increasing[1]. Several risk factors have been associated with anal cancer including human papilloma virus (HPV), HIV, age, immunosuppression, and smoking[1-3]. Although the association between anal cancer and smoking has been well-documented, the association between smoking status and recurrence is much less studied. A few prior studies have examined the impact of smoking status on anal cancer treatment, but these studies have been relatively small with the largest including 171 patients, while the other two included 64 and 68 patients[4-6]. Given smoking is a modifiable risk factor, studies examining its relationship with treatment success are important. This study aimed to contribute to the body of data examining treatments and outcomes for patients who smoke and have anal cancer.

MATERIALS AND METHODS

The study was conducted as a retrospective review of the cancer registry from a single health system. The registry was screened for patients with anal cancer between 2010 and 2020. All patients included in the registry were over the age of 18. The following characteristics were gathered from the database: Age; sex; cigarette smoking status; American Joint Committee on Cancer Clinical Stage Group; treatment pathway; response to therapy; recurrence; time to recurrence; mortality; time to death; and length of follow-up. Non-response was defined as persistent presence of disease despite completing standard chemoradiation. Recurrence was defined as the presence of disease after documentation that there was not any disease present. Unfortunately, HPV status and HIV status were not included in the database. Within the database, smoking status was divided into current smokers, never smokers, and former smokers. Smoking status was determined based on cigarette smoking alone. Current smokers were classified as any patient that reported cigarette smoking within 30 d of the time of diagnosis. Former smokers were classified as patients who had stopped smoking at least 30 d prior to diagnosis.

SPSSv25.0 software (IBM Corp., Armonk, NY, United States) was used for statistical analysis. Age and length of follow-up were analyzed between groups using single factor analysis of variance with Tukey post-hoc test. Patient race and sex were analyzed between groups using Fisher's exact test and Pearson's χ^2 test, respectively. *P* values were generated using an exact Mann-Whitney *U*-test to compare the mortality, non-response, recurrence, and time to recurrence between the current, former, and never smoker groups. In order to minimize type I error and given the small sample size, Bonferroni adjusted *z*-test was completed to compare the recurrence rate between groups. A subgroup analysis was completed to analyze time to death for the patients who did not respond using a single factor analysis of variance test.

RESULTS

A total of 95 patients were identified from the database. The patients were divided into three groups: Current smokers; former smokers; and never smokers. There was no significant difference in age, race, or sex between the groups (Table 1).

Table 1 Demographic data

Variable	Cigarette use			*P* value
	Never, *n* = 37	Former, *n* = 22	Current, *n* = 36	
Age in yr,				0.011
mean ± SD	63.70 ± 13.80	66.50 ± 13.17	57.40 ± 7.82	
Clinical stage group				0.066
1	5 (13.5)	4 (18.2)	8 (22.2)	
2	14 (37.8)	9 (40.9)	18 (50.0)	
3	14 (37.8)	9 (40.9)	8 (22.2)	
4	4 (10.8)	0	2 (5.6)	
Race				0.273
Black	2 (5.4)	0	4 (11.1)	
White	35 (94.6)	22 (100)	32 (88.9)	
Sex				0.078
Female	30 (81.1)	17 (77.3)	21 (58.3)	
Male	7 (18.9)	5 (22.7)	15 (41.7)	
Length of follow-up in d				0.759
mean ± SD	1195.10 ± 1135.96	1023.20 ± 855.35	1218.20 ± 986.20	

Data are *n* (%). SD: Standard deviation.

There was a difference in age between groups, with the former smokers being older than the smoker and never smoker groups (Table 1).

There was no significant difference in mortality or non-response between groups (Table 2). Former and current smokers did have a significantly higher recurrence rate compared to never smokers ($P = 0.009$). There was no difference in recurrence between the former and current smokers (Table 2).

Time to death was analyzed between the groups. On average, there was a shorter time to death in the current smoker arm, but this was not statistically significant (Table 3). The mortality rate between groups in the non-responder subset was also examined. Never smokers who did not respond to treatment were approximately twice as likely to die (43% *vs* 22%), but this did not achieve statistical significance (Table 3).

DISCUSSION

While three prior studies have examined the relationship between anal cancer treatment and smoking status, these studies have been small[4-6]. Our data contributes further evidence that smoking status is associated with a worse outcome and increased recurrence for patients with anal cancer. Additionally, it raises two interesting questions: (1) Should smokers and former smokers have more aggressive anal cancer treatment to reduce risk of recurrence?; and (2) Does smoking cessation result in an improvement in anal cancer outcomes? Lerman *et al*[4] raised the question of whether smokers would benefit from programmed cell death 1 inhibitors given the seemingly reduced efficacy of chemoradiation in smokers and a similar trend in non-small cell lung cancer. While our paper is limited in its evaluation, it does highlight the need for studies examining varying treatment options for smokers moving forward.

Interestingly, 14 of the never smoker patients did not respond to initial treatment. Of these patients, 43% died, with an average of 598 d after diagnosis (Table 3). This mortality rate was twice as high as the mortality rate for former and current smokers who did not respond. Although this did not achieve statistical significance due to the small numbers of this study, it is an interesting trend. One potential hypothesis is that never smokers who do not respond to initial therapy have a more aggressive tumor biology. Our data is not extensive enough to examine this further, but future research should examine this relationship. If confirmed, one could consider examining more aggressive treatment pathways for never smokers who do not respond to initial chemoradiation.

Unfortunately, the database used for this study did not include patient HPV status. This could be an important confounder that is not accounted for in this data. In similar cohorts of patients, 74%-88% of patients with anal cancer were HPV positive[1,3]. Although HPV is certainly linked to anal cancer, these same studies have shown that smoking status is an independent risk factor for anal cancer apart from HPV[1,3]. Additionally, the impact of HPV status on anal cancer outcome is not clear at this time as the two other largest studies examining anal cancer outcome and smoking status did not have HPV status collected for their cohorts either[4,5].

Table 2 Outcome data by group

Variable	Cigarette use			*P* value
	Never, *n* = 37	Former, *n* = 22	Current, *n* = 36	
Death	14 (37.8)	6 (27.3)	8 (22.2)	0.332
Non-response	14 (37.8)	9 (40.9)	14 (38.9)	0.973
Recurrence	0	4 (30.8)	5 (20.8)	0.009
Time to recurrence in d (median interquartile range)	NA	195.0 (159.0-351.0)	362.0 (214.5-1019.0)	0.413
Recurrence and non-response combined	14 (37.8)	13 (59.1)	18 (50.0)	0.264

NA: Not available.

Table 3 Mortality subgroup analysis

Feature	Cigarette use			*P* value
	Never	Former	Current	
Mortality* subgroup	*n* = 14	*n* = 6	*n* = 8	
Time to death in d, mean ± SD	598.30 ± 734.61	848.40 ± 756.83	393.80 ± 325.69	0.465
Non-responder subgroup	*n* = 14	*n* = 9	*n* = 14	
Deaths in non-responders subgroup, *n* (%)	6 (42.9)	2 (22.2)	3 (21.4)	0.285

*Death. SD: Standard deviation.

As noted before, this study is limited in its scope due to the retrospective nature and limitations of the collected data. A prospective study examining the impact of smoking cessation on anal cancer treatment would be valuable. Even without a prospective study, this study adds important data indicating an increased incidence of anal cancer recurrence in patients who smoke.

CONCLUSION

This paper highlighted the increased risk of anal cancer recurrence in patients who smoke. Although this study was small and limited in its scope, compared to current literature it is the second largest cohort of patients examining anal cancer, smoking, and recurrence. Further research is needed to examine the impact of smoking cessation on anal cancer treatment outcome and if adjuncts to standard therapy would be beneficial in patients who smoke.

ARTICLE HIGHLIGHTS

Research background

Despite the occurrence of approximately 50000 new cases of anal cancer per year and the clear link with smoking, very few studies have examined the relationship between smoking status and treatment outcome. It has already been shown that there is a link between anal cancer and smoking. This paper goes further and showed that there was an increased risk of recurrence in patients who smoke and have a history of smoking. This serves as a foundation for future research to examine modifications to the current treatment approach for patients with anal cancer.

Research motivation

Investigating the relationship between cigarette smoking status and anal cancer treatment outcome.

Research objectives

The main objective of this study was to examine the relationship between smoking status and outcomes for patients with anal cancer.

Research methods

A total of 95 patients were included in this data, making it the second largest study to examine the impact of smoking on anal cancer treatment outcomes. The patients were similar between the groups (never smokers, former smokers, and current smokers) in regards to important factors such as clinical stage group, race, and sex. Former and current smokers had a higher recurrence rate compared to never smokers. There was no difference in the mortality, non-response rate, or time to death between the groups. Unfortunately, data did not include human papilloma virus status, which would be an important area to include for future research.

Research results

There was an increased risk of anal cancer recurrence in patients who currently smoke and have a history of smoking.

Research conclusions

This study was the second largest study examining the relationship between treatment outcome and smoking status in patients with anal cancer. Although this data was limited in its scope, it contributed further to the limited body of evidence that smoking increases risk of recurrence of anal cancer.

Research perspectives

Future research should examine the impact of smoking cessation on treatment outcomes for patients with anal cancer as well as the role of adjuncts to standard chemoradiation in the treatment of anal cancer.

FOOTNOTES

Author contributions: McMahon KR designed and performed the research and wrote the paper; Gemma N helped write and revise the report; Dibello J assisted with data curation and editing of the report; Clapp M assisted with data curation and editing the report; Sanchez-Montejo P assisted with data analysis and editing the report; Laipply E designed the research and supervised the report.

Institutional review board statement: The study was reviewed and approved by the Summa Health System Institutional Review Board (Approval No. 21176).

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to inclusion in the cancer registry used in this review.

Conflict-of-interest statement: All the authors report having no relevant conflicts of interest for this article.

Data sharing statement: Statistical code and dataset available from the corresponding author at kevin.mcmahon88@outlook.com. Consent was not obtained, but the presented data are anonymized and risk of identification is low.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: United States

ORCID number: Kevin R McMahon 0000-0003-3881-0175.

S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Wang JJ

REFERENCES

1 **Daling JR**, Madeleine MM, Johnson LG, Schwartz SM, Shera KA, Wurscher MA, Carter JJ, Porter PL, Galloway DA, McDougall JK. Human papillomavirus, smoking, and sexual practices in the etiology of anal cancer. *Cancer* 2004; **101**: 270-280 [PMID: 15241823 DOI: 10.1002/cncr.20365]

2 **Holmes F**, Borek D, Owen-Kummer M, Hassanein R, Fishback J, Behbehani A, Baker A, Holmes G. Anal cancer in women. *Gastroenterology* 1988; **95**: 107-111 [PMID: 2836255 DOI: 10.1016/0016-5085(88)90297-1]

3 **Keller K**, Ramos-Cartagena JM, Guiot HM, Muñoz C, Rodríguez Y, Colón-López V, Deshmukh AA, Tirado-Gómez M, Ortiz AP. Association of smoking with anal high-risk HPV infection and histologically confirmed anal high-grade squamous intraepithelial lesions among a clinic-based population in Puerto Rico. *Cancer Treat Res Commun* 2021; **30**: 100503 [PMID: 34999478 DOI: 10.1016/j.ctarc.2021.100503]

4 **Lerman J**, Hennequin C, Etienney I, Abramowitz L, Goujon G, Gornet JM, Guillerm S, Aparicio T, Valverde A, Cattan P, Quéro L. Impact of tobacco smoking on the patient's outcome after (chemo)radiotherapy for anal cancer. *Eur J Cancer* 2020; **141**: 143-151 [PMID: 33137590 DOI: 10.1016/j.ejca.2020.09.039]

5 **Mai SK**, Welzel G, Haegele V, Wenz F. The influence of smoking and other risk factors on the outcome after radiochemotherapy for anal cancer. *Radiat Oncol* 2007; **2**: 30 [PMID: 17711570 DOI: 10.1186/1748-717X-2-30]

6 **Ramamoorthy S**, Luo L, Luo E, Carethers JM. Tobacco smoking and risk of recurrence for squamous cell cancer of the anus. *Cancer Detect Prev* 2008; **32**: 116-120 [PMID: 18639388 DOI: 10.1016/j.cdp.2008.04.004]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 July 24; 14(7): 265-284

DOI: 10.5306/wjco.v14.i7.265

ISSN 2218-4333 (online)

SYSTEMATIC REVIEWS

Cancer screening and management in the transgender population: Review of literature and special considerations for gender affirmation surgery

Juliet C Panichella, Sthefano Araya, Siddhartha Nannapaneni, Samuel G Robinson, Susan You, Sarah M Gubara, Maria T Gebreyesus, Theresa Webster, Sameer A Patel, Alireza Hamidian Jahromi

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ragosta S, United Kingdom; Withers R, United States

Received: December 28, 2022
Peer-review started: December 28, 2022
First decision: April 13, 2023
Revised: May 15, 2023
Accepted: June 27, 2023
Article in press: June 27, 2023
Published online: July 24, 2023

Juliet C Panichella, Samuel G Robinson, Susan You, Sarah M Gubara, Maria T Gebreyesus, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140, United States

Sthefano Araya, Siddhartha Nannapaneni, Sameer A Patel, Division of Plastic and Reconstructive Surgery, Fox Chase Cancer Center, Philadelphia, PA 19111, United States

Theresa Webster, Department of Plastic Surgery, Temple University, Philadelphia, PA 18045, United States

Alireza Hamidian Jahromi, Division of Plastic and Reconstructive Surgery, Temple University Hospitals, Philadelphia, PA 19140, United States

Corresponding author: Alireza Hamidian Jahromi, MD, MRCS, Division of Plastic and Reconstructive Surgery, Temple University Hospitals, 3401 Broad Street, Philadelphia, PA 19140, United States. alirezahamidian@yahoo.com

Abstract

BACKGROUND

Literature focused on cancer screening and management is lacking in the transgender population.

AIM

To action to increase contributions to the scientific literature that drives the creation of cancer screening and management protocols for transgender and gender nonconforming (TGNC) patients.

METHODS

We performed a systematic search of PubMed on January 5th, 2022, with the following terms: "TGNC", OR "transgender", OR "gender non-conforming", OR "gender nonbinary" AND "cancer screening", AND "breast cancer", AND "cervical cancer", AND "uterine cancer", AND "ovarian cancer", AND "prostate cancer", AND "testicular cancer", AND "surveillance", AND "follow-up", AND "management". 70 unique publications were used. The findings are discussed under "Screening" and "Management" categories.

RESULTS

Screening: Current cancer screening recommendations default to cis-gender protocols. However, long-term gender-affirming hormone therapy and loss to follow-up from the gender-specific specialties contribute to a higher risk for cancer development and possible delayed detection. The only known screening guidelines made specifically for this population are from the American College of Radiology for breast cancer. Management: Prior to undergoing Gender Affirmation Surgery (GAS), discussion should address cancer screening and management in the organs remaining in situ. Cancer treatment in this population requires consideration for chemotherapy, radiation, surgery and/or reconstruction. Modification of hormone therapy is decided on a case-by-case basis. The use of prophylactic *vs* aesthetic techniques in surgery is still debated.

CONCLUSION

When assessing transgender individuals for GAS, a discussion on the future oncologic risk of the sex-specific organs remaining in situ is essential. Cancer management in this population requires a multidisciplinary approach while the care should be highly individualized with considerations to social, medical, surgical and gender affirming surgery related specifications. Special considerations have to be made during planning for GAS as surgery will alter the anatomy and may render the organ difficult to sample for screening purposes. A discussion with the patient regarding the oncologic risk of remaining organs is imperative prior to GAS. Other special considerations to screening such as the conscious or unconscious will to unassociated with their remaining organs is also a key point to address. We currently lack high quality studies pertinent to the cancer topic in the gender affirmation literature. Further research is required to ensure more comprehensive and individualized care for this population.

Key Words: Gender affirmation surgery; Gender affirming surgery; Screening; Management; Transgender; Gender diverse

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Currently, a comprehensive guideline for cancer screening in the transgender and gender diverse (TGGD) population is lacking. Caring for the TGGD population undergoing Gender Affirmation Surgery is highly individualized and requires consideration of factors such as age at which individuals commenced hormonal therapy and the stage of transition. Once diagnosed with cancer, TGGD patients should receive care at institutions capable of providing a multi-disciplinary approach. This collective approach will ensure record upkeep and help delay any unnecessary delays in care.

Citation: Panichella JC, Araya S, Nannapaneni S, Robinson SG, You S, Gubara SM, Gebreyesus MT, Webster T, Patel SA, Hamidian Jahromi A. Cancer screening and management in the transgender population: Review of literature and special considerations for gender affirmation surgery. *World J Clin Oncol* 2023; 14(7): 265-284
URL: https://www.wjgnet.com/2218-4333/full/v14/i7/265.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i7.265

INTRODUCTION

The transgender and gender diverse (TGGD) population in the United States is estimated to be around 1.4 million, constituting 0.6% of the United States adult population[1]. There exists no census data to back this estimate and may be higher in the younger population. It is well known that cancer screening has led to a decrease in cancer mortality. Many organizations including American Cancer Society (ACS), United States Preventive Services Task Force (USPSTF) have clear recommendations for the early detection of cancer in cis-gender individuals. However, the TGGD population currently has no cancer screening recommendations specific to the TGGD population. The World Professional Association for Transgender Health, a non-profit, interdisciplinary professional and educational organization devoted to transgender health, states that due to a lack of prospective studies, there is not enough evidence for the recommendation of the appropriate type and frequency of screening in this population[2].

In addition to screening, no studies have commented on gender affirming surgery (GAS) and its impact on the screening, management, and surveillance of cancer in the TGGD population. Special considerations must be made during planning for GAS as surgery will alter the anatomy and may render the organ difficult to sample for screening purposes *i.e.*, prostate evaluation following the penile inversion vaginoplasty in the transgender woman. A discussion with the patient regarding the oncologic risk of remaining organs is imperative prior to GAS.

Of note, in this article, the distinction between sex and gender is made based on the former referring objectively to biology and the latter subjectively being psychosocially constructed. Overall, this article aims to review the current guidelines and practice patterns with regard to cancer screening and management in each sex-specific organ for the TGGD population.

MATERIALS AND METHODS

A systematic search of PubMed on January 5th, 2022, with the following terms: "TGNC", OR "transgender", OR "gender non-conforming", OR "gender nonbinary" AND "cancer screening", AND "breast cancer", AND "cervical cancer", AND "uterine cancer", AND "ovarian cancer", AND "prostate cancer", AND "testicular cancer", AND "surveillance", AND "follow-up", AND "management". After eliminating review articles, duplicates, abstracts, articles not relevant to the section topic or opinion pieces a total of 70 studies with original data were obtained (Figure 1). Articles relevant to the section topic, including the search terms were included in this systematic review. Search parameters were performed according to Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines. Two independent reviewers Araya S and Nannapaneni S carried out independent abstract revisions on January 11th, 2022, using systematic review software "Rayyan"[3] registered in Cambridge Massachusetts.

RESULTS

Breast

The PubMed Database was queried from April 1968 to January 2022 using the search text of "(gender nonbinary) OR (transgender and gender non conforming) OR (transsexual) AND (breast cancer)". This search produced 190 unique articles. Of these articles, 60 were assessed for eligibility and sub-classified based on the primary content of the paper as either screening or management relating to the breast. The term "transsexual" is outdated. However, as our search would span to the remote past, we used this term to be able to identify older publications.

Barriers to care

In addition to the physical limitations that GAS can impose on cancer screening, it is equally important to acknowledge the psychological health of each individual patient and the impact of gender dysphoria on their attitude towards the cancer screening process. The lack of protocols and education surrounding TGGD patients provided to healthcare workers has led to an environment where both providers and patients are uncomfortable with the quality healthcare currently being provided[4-10]. Finally, GAS adds to the technical complexity of oncologic screening protocols.

In different retrospective population studies, authors reported that while 92% of studied transgender men have retained their cervixes, they were 60% less likely to undergo cervical cancer screening, 70% less likely to have breast cancer screening, and 50% less likely to have colorectal cancer screening compared to cis-gender patients[9,11,12]. Of note, it is uncommon to remove the prostate during vaginoplasty in transgender women and these patients are also significantly less likely to receive prostate cancer screenings compared to their cis-gender counterparts[13].

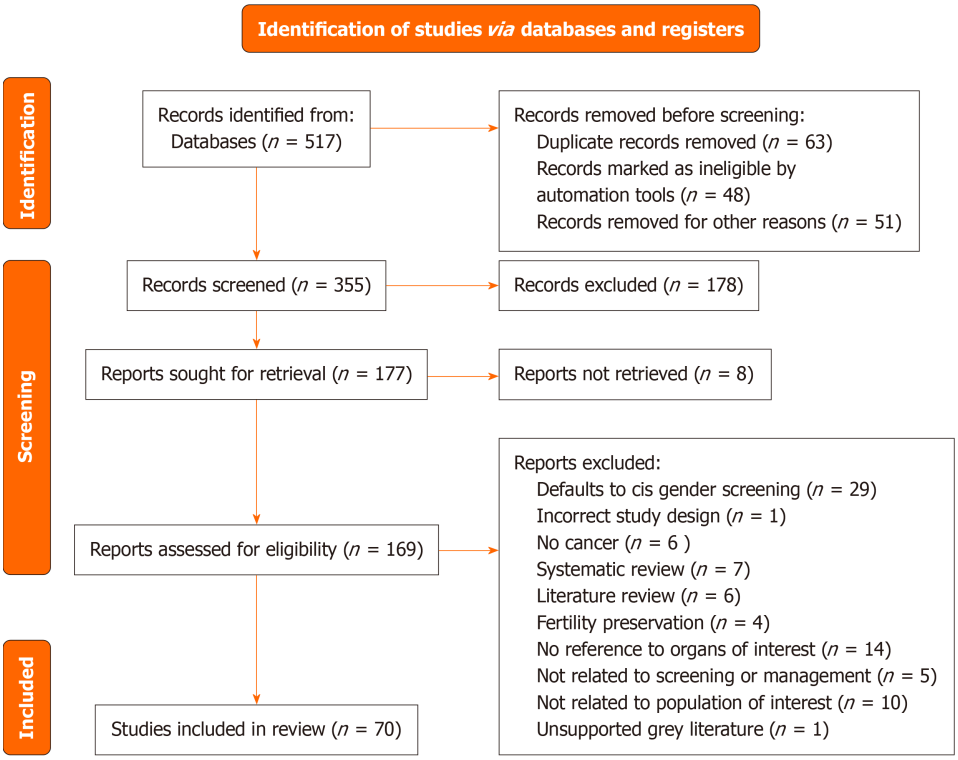
While some of these discrepancies can be attributed to differences in demographics as TGGD patients tend to be of a lower socioeconomic status, there are also hurdles these patients face within the healthcare system - including history of prior trauma, provider knowledge deficits, fear of mistreatment or mis-gendering, and lack of appropriate restrooms, gender affirming spaces or educational material[4-9]. There are also disparities of gender affirmation care, gender friendly facilities and services between different parts of the country.

As an example, the ACS recommendation for mammograms for women would miss screening of trans men or nonbinary people for whom the "chest" screening is relevant. Additionally, the lack of gender friendly language may create an additional barrier to care. Some TGGD individuals may want to mentally detach themselves from gender attributed organs *i.e.*, prostate in transgender women or breast in transgender men and attributed screening *i.e.*, a mammogram in the case of a transgender man as this may exacerbate their gender dysphoria. The mention of organs such as "breast" instead of "chest" or "vagina" instead of "current canal" can further promote gender dysphoria in TGGD individuals, and as a result, they are less likely to receive such life-saving screening[4].

Seventy percent of TGGD patients have reported some form of distrust with the healthcare system, and 33% of patients in this population have had negative experiences with healthcare providers that have ranged from incompetent providers and being refused care to harassment and assault[8,9,12]. During the time of the coronavirus disease 2019 pandemic, there has been an increase in anxiety, depression, and suicidal ideation among TGGD patients so providers should be mindful of the mental stress that these patients undergo in addition to the fear and mistrust they have experienced within the healthcare system[14]. Not only do providers need to be explicit in their welcoming of TGGD patients, but they need to invoke flexible methods of meeting the patients' needs, such as patient-collected HPV swabs, interviewing the patient prior to disrobing, creating a gender friendly environment *i.e.*, introducing themselves with their pronouns and the use of gender-inclusive language[4]. Providers also need to remain up to date on TGGD cancer screening recommendations as a study of gynecological providers found that only 35% felt comfortable providing gynecologic care to this community and even fewer (29%) felt equipped to do so[10]. The utilization of health navigators offers an additional form of support and knowledge for both patients and providers in accomplishing the best care of the patient[15].

Breast

Breast cancer is the most common form of cancer in cis-gender women and the second most common cause of cancer mortality in cis-gender women in the United States[16]. However, the reported lifetime risk for TGGD individuals is not reported due to insufficient data and research. Every year, more case studies are reported of TGGD individuals developing breast cancer. Studies have shown increased rates of breast cancer in TGGD women compared with cis-gender males, as well as a decreased risk of breast cancer in TGGD men compared to cis-gender females. For transgender men who have undergone chest surgery to remove the breasts, the decreased risk of breast cancer is an expected finding



DOI: 10.5306/wjco.v14.i7.265 **Copyright** ©The Author(s) 2023.

Figure 1 The preferred reporting items for systematic reviews and meta-analyses flowchart for overall cancer screening and management in the gender affirming surgery population.

and consistent with risk reducing mastectomy in the cis-gender female population[17]. However, there is a lack of data and recommendations on breast cancer screening and management of TGGD patients. This is compounded by the inherent risk of discrimination and poor access/barriers to healthcare in the TGGD population, leading to a high rate of disease progression before diagnosis[18,19]. This systematic review aims to elucidate the screening and management of breast cancer in TGGD individuals with a goal of improved care and treatment.

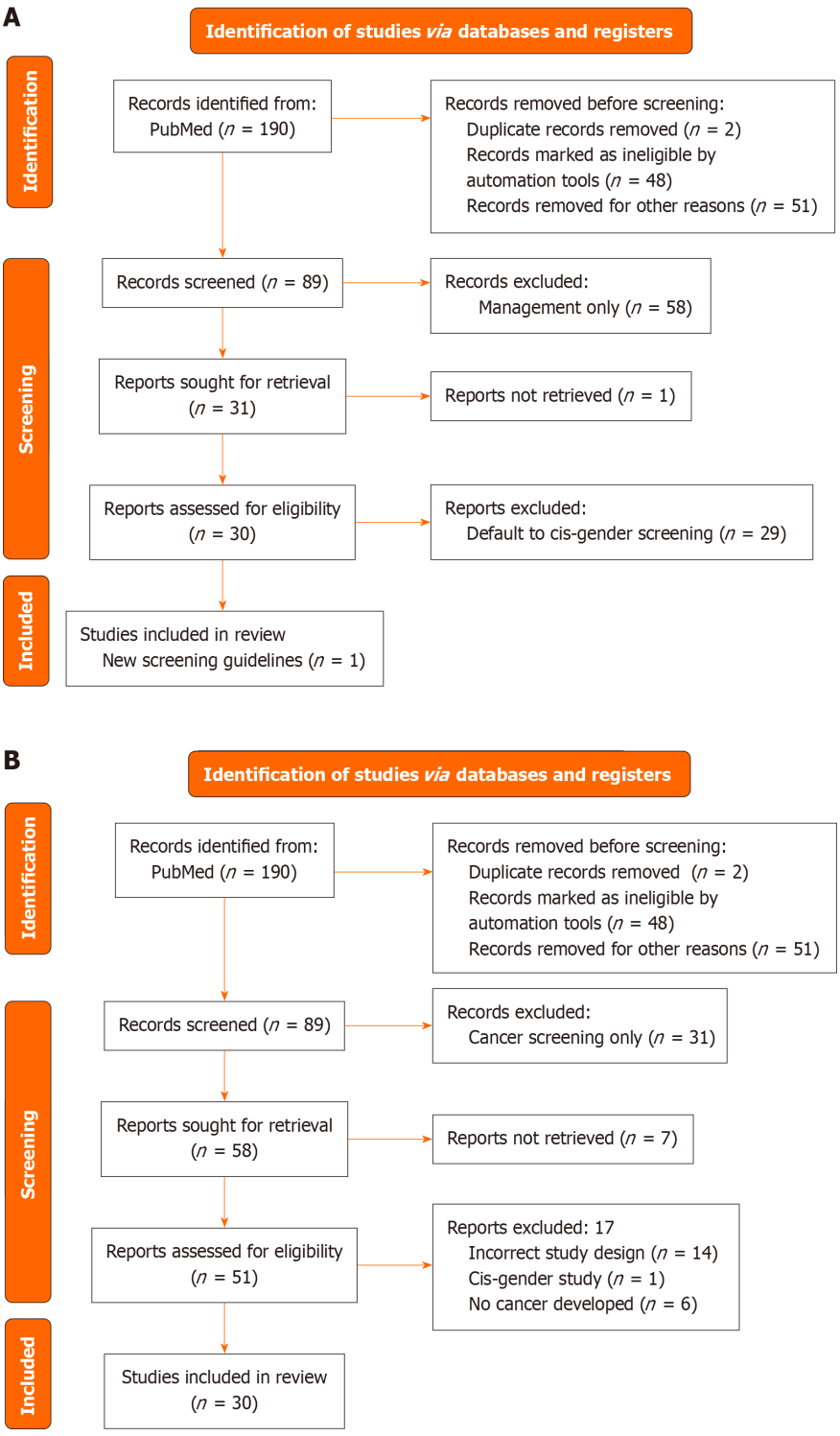
Screening

Of the 89 records screened, 30 records were sought for retrieval pertaining to screening for breast cancer in TGGD patients. The majority of these articles (*n* = 29) deferred management to cis-gender guidelines for TGGD patients or called for more studies on TGGD-specific screening recommendations (Figure 2A). Nevertheless, our review identified and included one article that was a comprehensively covered, evidence-based, breast cancer screening guideline for TGGD individuals provided by the American College of Radiology Appropriateness Criteria in 2021 (Figure 2A)[20]. These guidelines cover eight different variants of screening based on classification of gender affirming surgery, age, duration of exogenous hormone use, and risk category. Recommendations are graded for each variant by appropriateness categories including "Usually appropriate", "May be appropriate", and "Usually not appropriate". Each modality is also considered in relation to the amount of radiation involved. Screening modalities include digital breast tomosynthesis (DBT) screening, mammography screening, magnetic resonance imaging (MRI) breast with and without IV contrast, and ultrasound of breast. Overall, the higher the age, longer the length of use of hormones, and higher the risk category, the more appropriate the use of DBT and MRI becomes.

Management

Transgender women can undergo a variety of breast augmentation surgery procedures to create a feminine appearing chest. Included in this population are non binary individuals who may also undergo breast augmentation procedures. Breasts can be created through a variety of methods, including hormone therapy, fat grafting, saline implants or silicone implants, or autologous reconstruction. Chest masculinization, colloquially referred to as "top surgery", can be performed to create a more masculine appearing chest. Breast tissue is either reduced or completely removed *via* liposuction, mastopexy, or mastectomy to create a flat chest, while the nipples can be completely removed and/or resized and repositioned. The authors believe and practice with the gender spectrum concept and as such acknowledge the desired chest to be a spectrum.

Breast cancer in the cis-gender individuals is managed surgically with breast conserving surgery (lumpectomy and radiation), and/or mastectomy. Treatment may also include adjuvant or neoadjuvant chemotherapy and/or radiation pending nodal status along with hormonal therapy with anti-estrogen agents pending hormone receptor status. Currently, breast cancer in the TGGD individual is managed similarly. However, in TGGD patients, the timing of cancer presentation in relation to gender affirming surgery, as well as timing in relation to the use of hormone therapy are



DOI: 10.5306/wjco.v14.i7.265 **Copyright** ©The Author(s) 2023.

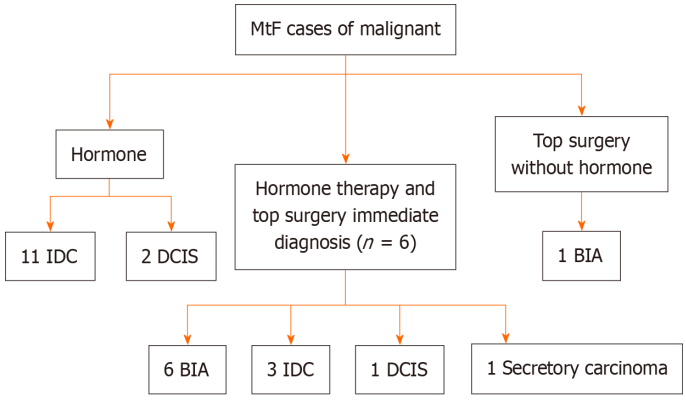
Figure 2 The preferred reporting items for systematic reviews and meta-analyses charts for the breast screening and management. A: The preferred reporting items for systematic reviews and meta-analyses (PRISMA) flowchart for articles about breast cancer screening; B: PRISMA flowchart for breast cancer management.

additional variables that will affect management.

Of the 89 records screened, 58 of them were sought for retrieval related specifically to management of breast cancer in TGGD patients. Of that 58, there were 30 case reports of breast cancer in TGGD patients (Figure 2B).

Chest Feminization Gender Affirming Surgery

There was a total of 25 male to female (MtF) gender affirming surgery cases among 18 case studies. Each group was further categorized according to hormonal status, gender affirming surgery, and the timing of detection (immediate or delayed) (Figure 3). Immediate detection describes patients whose breast cancer was discovered at the time of gender-



DOI: 10.5306/wjco.v14.i7.265 **Copyright** ©The Author(s) 2023.

Figure 3 Study design for male to female gender affirming surgery patients. MtF: Male to female; IDC: Invasive ductal carcinoma; DCIS: Ductal carcinoma in situ; BIA: Breast implant associated.

affirming breast augmentation. Delayed detection describes cases of breast cancer that were detected after breast augmentation. Patients that did not undergo surgery or hormone therapy were excluded as we were largely interested in understanding how these factors influenced breast cancer detection and management. Patients who were not diagnosed with breast cancer were excluded (Figure 2B).

Cancer Detection In Patients With Hormonal Therapy Only

Eleven papers identified 13 patients who were on estrogen hormone therapy regimens before gender affirming breast surgery. Of the 13 patient studies, two were diagnosed with ductal carcinoma in situ (DCIS) and 11 patients were diagnosed with Infiltrating Ductal Carcinoma (IDC). The average age of cancer diagnosis was 53.8 years old. The average time on hormone therapy prior to the surgery was 16.5 years. All patients mentioned were diagnosed with cancer.

Of the two patients who were diagnosed with DCIS, both had cancer that are Estrogen receptor (ER) positive. One patient demonstrated Progesterone receptor (PR) positive DCIS and hormone treatment was discontinued in the other patient who was PR negative. Additionally, the latter patient was further treated with lumpectomy and radiation with sentinel lymph node sampling, adjuvant chemotherapy, and aromatase inhibitor without any reported recurrence[21]. The patient with ER+/PR+ cancer reported family history of ovarian cancer and a mutation in Chek2 p.I157T, which confers a 1.4 increased risk of breast cancer development. Despite the higher risks, hormone therapy was not discontinued, and the patient was treated with breast conservation surgery and radiation without anti-estrogen therapy according to the patient's wishes. No follow-up recurrence was reported[22]. The difference in the treatment can be attributed to patient desires. Despite being aware of the higher risk, the patient opted to continue hormonal therapy and forgo anti-estrogen therapy.

In the group of 11 patients who were diagnosed with IDC, there was a variety of hormonal receptor status, treatments, and outcomes. Six patients had ER+/PR+ cancers. Of these six, two were positive for BRCA2 mutations. Both patients elected to discontinue hormone therapy[23,24]. The first patient declined tamoxifen and was just surgically treated with a simple unilateral mastectomy of the right side with sentinel lymph node biopsy. Local recurrence occurred 30 mo later and treatment with radiation therapy and adjuvant chemotherapy with aromatase inhibitors (epirubicin plus cyclophosphamide w/paclitaxel)[23]. The other patient was treated with bilateral mastectomy and sentinel lymph node dissection, neoadjuvant tamoxifen and adjuvant radiation (patient declined chemotherapy). No recurrence was reported[24]. This brings up the discussion on what treatment options should be for patients who are positive for BRCA2 mutations. Additionally, it is difficult to know whether ER positivity in these two patients is due to hormone therapy or the mutation itself[24]. This may require more research to determine the effect of BRCA2 mutations on ER+ cancer in the presence of gender affirming hormone (GAH) therapy.

The other four ER+ IDC patients were treated with tamoxifen. One of these patients did not stop hormone therapy and had good outcomes from treatment while two patients who did stop hormone therapy treatment did die from complications of metastatic breast cancer, 22 mo and 6.5 years after their diagnoses[25,26]. This further highlights the necessity to determine what the real impact of GAH therapy in on cancer. Further research is required to mitigate risk of gender-affirming care hormone therapy continuation.

Three patients were diagnosed with triple negative IDC, each of whom were taking hormone therapy for more than 10 years. One patient was only treated with tamoxifen after local wide excision and axillary clearance and did not discontinue their hormone therapy, Premarin. This patient reached remission and remained cancer free after 1 year of follow up[25]. The other two patients did discontinue hormone therapy treatment. Both of these patients were non-surgically treated with neoadjuvant chemotherapy and adjuvant radiation[27,28]. The first patient had no family history of breast cancer and genetic testing found no clinically significant mutations that would increase her risk. However, it should be noted that this patient had significant comorbidities including HIV that was well managed with medication, and depression that was managed through counseling. Additionally, though the patient was ER (-), her healthcare team decided to discontinue use of estrogen therapy to prevent the development of an ER+ tumor subset/second tumor. In addition to management of her breast cancer the patient attended counseling for management of psychological distress

attributed to the cessation of estrogen[27].

A second patient with comorbid severe depression on antipsychotic medications and possible secondary hyperprolactinemia attributed to the medications was managed with cessation of GAHs. The patient's cancer progressed and ultimately expired by intentional drug overdose[28]. Concerns of a patient's mental status due to aggravation of gender dysphoria and loss of feminine characteristics when halting hormone therapy and even creation of suicidal ideation *vs* the risk/benefit of hormone therapy on prolactin and cancer incidence is a debated issue in the current literature[28]. Authors describe prolactin screening for patients on long term estrogen given the possible tumor promoter actions in breast and prostate cancer[28].

Cancer Detection After Chest Feminization

Patients taking GAHs: This group received treatment with hormone therapy and underwent gender affirming breast augmentation surgery with implants prior to cancer diagnosis. There were 11 patients[6], who were diagnosed with Breast Implant-Associated Anaplastic Large Cell Lymphoma (BIA-ALCL), three who were diagnosed with IDC, one who was diagnosed with DCIS, and one patient who presented with a triple negative secretory carcinoma caused by a *ETV6-NTRK3* gene fusion mutation though no treatment was discussed[29]. The average age of cancer diagnosis was 45.3 years old. The average time on hormone therapy was 14.2 years.

The finding of six TGGD patients diagnosed with BIA-ALCL has implications on health care. For surgical treatment, all were treated with implant removal, capsulectomy, and tumor resection as per treatment in cis-gender women with ALCL [30-32]. This treatment has been shown to improve disease-free survival[32]. Complete surgical resection with *en-bloc* removal of the disease, implant, and capsule provides the best survival outcomes. However, for patients with extensive disease and regional lymph node involvement, adjuvant chemotherapy and/or radiation may be recommended[30].

According to the most recent National Comprehensive Cancer Network (NCCN) guidelines in the United States, adjuvant radiation therapy is indicated for patients with local residual disease with or without regional lymph node involvement or unresectable disease with chest wall invasion. Systemic chemotherapy is indicated for patients with Stage II–IV disease[32]. All six patients received textured implants, a possible risk factor for the formation of BIA-ALCL[33].

Three patients were diagnosed with IDC. One of the patients was advised to discontinue hormone therapy, however, decided to continue it against medical advice. No length of follow up or recurrence was reported[34]. However, the authors present an interesting debate as to what the acceptable balance of risk *vs* benefit is for cessation of hormone therapy in this group of patients given the often competing oncologic *vs* gender affirming interests[34].

Patients not taking GAHs: One paper identified a TGGD patient who underwent gender affirming breast augmentation surgery without prior hormone therapy treatment[32]. This patient was diagnosed with BIA-ALCL and subsequently treated with bilateral implant removal and capsulectomy of the affected side. The patient did not receive any radiation or chemotherapy and was tumor-free 10 mo post-operatively[32].

Chest Masculinizing Gender Affirming Surgery

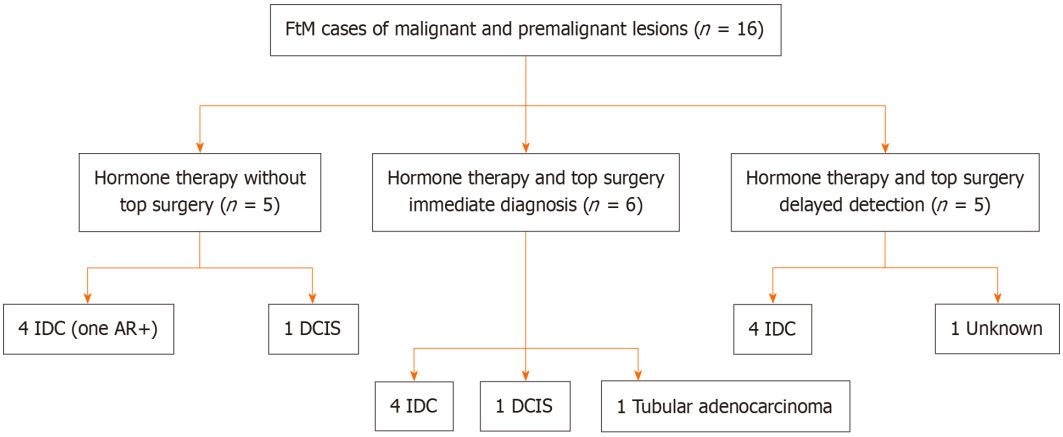
There was a total of 16 female to male gender affirming surgery patients among 12 case studies. Each group was further categorized according to hormonal status, gender affirming surgery, and the timing of detection (immediate or delayed) (Figure 4). Immediate detection describes patients whose breast cancer was discovered at the time of gender-affirming top surgery. Delayed detection describes cases of breast cancer that were detected after top surgery. Patients that did not undergo surgery or hormone therapy were excluded as we were largely interested in understanding how these factors influenced breast cancer detection and management. Patients who were not diagnosed with breast cancer were excluded (Figure 2B).

Cancer Detection Prior To Chest Masculinization

Four patients (out of 12 patients) among three papers were identified with intramuscular testosterone usage and development of breast cancer prior to top surgery (mastectomies)[26,35,36]. All four patients developed an IDC. There was a mix of hormone receptor positivity with no specific trend. The average time on intramuscular testosterone therapy was 4.7 years. The average age at diagnosis was 46.3 years old. Four patients among two papers were excluded due to no hormone therapy used[21,26].

One patient with ER+/PR+/HER2+/AR+ IDC was treated with bilateral mastectomies with right sentinel lymph node biopsy, nipple-areolar grafting, neoadjuvant chemotherapy, and continuation of testosterone therapy survived an unknown amount of time. A second patient diagnosed with ER+/PR-/HER2+ IDC was treated with unilateral mastectomy and adjuvant chemotherapy. Management with testosterone therapy was unknown. The patient expired within two years. A third patient with ER+/PR+ IDC was treated with lumpectomy, followed by bilateral nipple-sparing mastectomies 1 year later and unknown management of testosterone therapy after diagnosis was in remission at least 10 years. The fourth patient with ER-/PR+ IDC bilateral managed with nipple sparing mastectomy, adjuvant and neoadjuvant chemotherapy with permanent discontinuation of hormone therapy was in remission at least 5 years. Unfortunately, the sparse number of cases studied and incomplete patient history and follow up in these patients do not provide a good platform to draw conclusions for hormone continuation, surgical management, or survival.

One of the patients developed a clinically interesting finding of an androgen receptor (AR) positive IDC[37,38]. The authors of this paper emphasized the importance of testing for AR sensitivity in TGGD patients as some of the patients may be taking testosterone and stopping the hormone may impact their gender dysphoria. However, continuing GAH therapy could lead to progression or recurrence of the cancer after treatment given the cancer's responsiveness to the AR sensitivity.



DOI: 10.5306/wjco.v14.i7.265 **Copyright** ©The Author(s) 2023.

Figure 4 Study design for female to male gender affirming surgery patients. FtM: Female to male; IDC: Invasive ductal carcinoma; DCIS: Ductal carcinoma in situ; AR: Androgen receptor.

One patient developed DCIS, a premalignant lesion, in the setting of testosterone therapy. This is an interesting finding as even a premalignant lesion is a risk later down the line for these patients and begs the question of needing oncologic mastectomy to completely mitigate the risk. It is important to mention that the DCIS in the cis gender individual, not on androgen therapy, can undergo a risk reduction with hormone blockers and wide local excision and may not particularly necessitate a mastectomy. Had this pathology not been caught in the pre-operative setting, this patient could have been found to have a cancer or DCIS later during the top surgery or even in rare occasions in the post top surgery setting *i.e.*, in case the residual breast tissue will keep the burden of DCIS pathology. Therefore, this situation emphasizes the importance reevaluating GAH dosing or discussing discontinuing the hormone altogether.

Cancer Detection After Chest Masculinization

Immediate detection (surgical pathology): Five patients (out of 9 total patients) were found to have cancer based on surgical specimens sent for histologic evaluation during their top surgery. Four of these patients had invasive ductal carcinoma, one developed tubular adenocarcinoma[21,38,39]. In addition, one patient's pathology revealed a high grade DCIS[40]. The mean time on intramuscular testosterone therapy was 11.2 years. The average age at diagnosis was 45.4 years old. There were no patients that were found to have cancer during top surgery that did not take hormone therapy beforehand.

One patient with ER+/PR-/HER2- IDC was treated with bilateral mastectomies along with axillary lymph node dissection and chemotherapy. Later that patient presented with recurrence and underwent re-excision, radiotherapy, and tamoxifen treatment with unknown management of testosterone therapy after diagnosis with remission. Another patient with ER+/PR-/HER2+ IDC was treated after bilateral mastectomy with sentinel lymph node dissection plus chemotherapy. After a temporary discontinuation of testosterone therapy, the patient went into full remission. A third patient who was ER+/PR+/HER2+ IDC was treated with bilateral mastectomy with axillary lymph node dissection plus chemotherapy. After temporary discontinuation of testosterone, the patient had unknown survival. A fourth patient with ER+/PR+/HER2- IDC was treated after partial mastectomy breast reduction with full left mastectomy with sentinel node sampling with anastrozole plus radiation. After permanent discontinuation of testosterone, the patient had unknown survival. Finally, the last patient with ER+/PR+/HER2- tubular adenocarcinoma was treated with mastectomy and had a negative sentinel node biopsy. They did not discontinue testosterone, and received no further treatment, but had an unknown survival.

Overall, this section emphasizes the potential impact of having pathology sent for specimens at the time of surgery as earlier intervention on these patients could only improve the survival. All of these patients opted for full mastectomy (if not already done), whether unilateral or bilateral, for treatment of the cancer. Unfortunately, we are unable to draw clear conclusions from this subgroup for guidance on hormone discontinuation and survival outcome. However, one retrospective review comments on the increased risk of premalignant lesions and cancer found in surgical specimens of 193 bilateral mastectomies for TGGD patients both with and without hormones and reported an incidence of 8.8% of atypical lesions requiring further investigation[41]. Thus, even if no malignancy is anticipated in these patients, they would benefit from sending surgical specimens for pathologic evaluation.

Delayed detection (no surgical pathology): Four patients among five papers were found to have cancer based on screening post mastectomy. The mean amount of time post mastectomy for cancer diagnosis was 10 years. Four patients developed invasive ductal carcinoma, one patient's diagnosis was unknown[42-44]. The average time on intramuscular testosterone therapy was 7.7 years. The mean time after the first breast surgery was 10 years. The average age at diagnosis was 46.2 years old.

One patient with ER+/PR+/HER2 equivocal metastatic IDC discovered 20 years after bilateral mastectomy with free nipple grafts with unknown testosterone hormone management post diagnosis was treated with letrozole and had unknown survival. A second patient with ER+/PR+/AR+/HER2- IDC discovered 12 years post mastectomy, was treated

with breast partial resection, sentinel lymph node dissection, radiation therapy and aromatase inhibitors (patient refused tamoxifen due to feminization effects) with unknown testosterone hormone management post diagnosis and had unknown survival. A third patient who was diagnosed with triple negative IDC discovered seven years after bilateral mastectomy was treated with lumpectomy and adjuvant chemotherapy. This patient had unknown testosterone hormone management and had survived at least two years after treatment. A fourth patient with ER-/PR- metastatic IDC discovered one year after bilateral subcutaneous nipple sparing mastectomy was treated with neoadjuvant chemotherapy and radical mastectomy with axillary dissection. This patient had unknown testosterone hormone management post diagnosis and had unknown survival.

This subgroup of patients poses an interesting discussion of reduced risk of cancer from previous mastectomy, yet development of cancer in residual breast tissue shows such risk reduction not to be absolute. This would be due to the incomplete removal of breast tissue and pre-pectoral fascia in those that go for gender affirmation mastectomies *vs* oncologic mastectomies. The question as to whether we should offer a completion of (full oncologic) mastectomies (removing the pectoral fascia and the nipples) for such GAS patients remains uncertain. However, since the nature of these patients is higher loss to follow up and noncompliance with traditional screening, in addition to taking hormones, this population could be at higher risk than others for surreptitious development of cancer. Thus, they might benefit from a prophylactic oncologic mastectomy rather than a gender affirmation (subcutaneous) mastectomy. Clearly, this needs to be weighed against the cosmetic benefits of a subcutaneous mastectomy with nipple-areolar preservation and the quality-of-life implications that it affords.

BRCA

Of the 30 case studies, four patients were identified who were positive for BRCA2[23,24,33]. These four patients were transgender women. In cis-gender females with BRCA 1 or 2 mutation the lifetime risk of developing breast cancer is 55%-72%, while the lifetime risk in cis-females in 13%. In cis-gender men with a BRCA2 gene mutation, the lifetime risk of breast cancer is approximately 7 to 8 percent, while the lifetime risk of male breast cancer in the general population is approximately 0.1 percent[45,46].

In one case report, the patient, transgender woman, underwent bilateral skin-sparing mastectomy after confirming they were BRCA2 positive. A second patient, transgender woman, developed IDC two years after starting hormone therapy. She had bilateral mastectomies with immediate expander reconstruction and right sentinel lymph node sampling as well as adjuvant radiation therapy and then subsequently tested positive for BRCA2. A third patient, transgender woman, developed IDC after seven years on hormone therapy and underwent a right simple mastectomy with sentinel lymph node biopsy. There was recurrence 30 mo post-mastectomy, so radiation therapy and adjuvant chemotherapy were given as treatment. A fourth patient was identified as BRCA2 positive but had not developed cancer yet.

One of the most important points to be made about this subgroup analysis is that all six patients discontinued gender-affirming hormone therapy upon diagnosis with BRCA mutations. This seems to be the current standard of practice for management of these patients yet many patients choose not to discontinue hormone therapy. In fact, our review came across a few arguments against cessation, namely the history of treating advanced breast cancer with low dose estrogens and the deleterious effects of cessation on the mental well-being of TGGD patients[24,26,33]. More research is required to determine if there is a true therapeutic benefit to cessation of GAHs. Our systematic review also identified recommendations such as offering TGGD women who are BRCA1/2 positive risk-reducing mastectomies prior to breast augmentation rather than traditional aesthetic chest. Additionally, from oncology point of view TGGD men should be offered risk reducing mastectomies (gender affirmation subcutaneous mastectomies) over aesthetic chest surgeries[47]. It should be noted that there can be issues of coverage for certain procedures by insurance when the sex indicated on the patient's chart does not align with the sex-intended procedure especially if the insurance policies do not cover the gender affirmation as a separate group of procedure entities[47].

Additionally, this brings up an interesting discussion of whether we should routinely test these patients for BRCA before undergoing surgical intervention, or even prior to hormone initiation. This patient population would inherently benefit from more prophylactic interventions given the higher loss to follow-up and screening. Recommendations for the surgical management of the BRCA+ TGGD patients follow similar guidelines to the cis-gender individual for risk-reducing bilateral mastectomies over conservative, primarily aesthetic breast reductions. Overall, more studies need to be done to elucidate and strengthen further recommendations with regard to BRCA management in TGGD.

BIA-ALCL

Our search yielded seven cases of breast implant associated-anaplastic large cell lymphoma in transgender women[32,48]. There is a known increased risk of developing BIA-ALCL in cis-gender women with textured implants[32]. Thus, this risk is conferred in TGGD females as well. Loss of follow up and willing to seek medical attention may be further exacerbated by lack of provider knowledge on gender friendly language ultimately leading to delayed recognition and diagnosis[32]. Avoidance of gender specific language such as "breast" instead of "chest" as reference for anatomical parts may assist with patient willingness for follow-up and screening.

One patient underwent unilateral mastectomy (implant previously removed) with resection of pectoral muscle, and axillary node dissection and received chemotherapy. The second one underwent bilateral *en-bloc* resection of capsule and implant. The third one underwent *en-bloc* resection of implant, capsule, and mass (resection included part of pectoral muscle) plus chemotherapy. The fourth one underwent bilateral *en-bloc* resection of capsule and implant plus sentinel lymph node biopsy, excision of active lymph node and chemotherapy. The fifth patient underwent *en-bloc* resection of the capsule and implant. The sixth one underwent bilateral *en-bloc* resection of capsule and implant plus sentinel lymph node biopsy, along with chemotherapy and adjuvant radiation therapy. The seventh patient underwent bilateral *en-bloc*

resection of capsule, implant and tumor plus chemotherapy. The average time to diagnosis was 13.4 years, which is slightly more delayed yet comparable to cis-gender timeline of 9.75 years[49].

As BIA-ALCL is becoming more common in TGGD patients, surgeons should be aware of this and encourage follow up. Often patients experienced symptoms at least 2 years before going to their followed up, and with less frequency than cis-gender individuals[32]. Education of "signs and symptoms patients should look out for" may go a long way in improving rates of follow up as it makes patients aware of the dangers and gives them agency and involvement in their treatment.

Silicone Injections

Although it has been declared illegal since 1970s due to high number of complications, unfortunately free silicone injection has been and continues to be performed as a mode of breast augmentation in the TGGD individuals[50]. Secondary breast reconstruction after silicone injections is relevant to chest feminization. In one study, the incidence of prior silicone breast injections was 7.3%. In their cohort of 41 chest feminization surgery patients, there was only one patient with minor complications which healed without surgical intervention[51]. This study concluded that careful evaluation and planning can minimize the risk of complications in secondary breast reconstruction post silicone injections. Another study reported a case of TGGD patient with a false-positive axillary lymph nodes due to silicone adenitis from silicone leakage[52]. A final case reports two incidences TGGD patients with breast inflammation and necrosis as a result of silicone and paraffin injections[53].

A carefully performed history and physical exam are critical to planning reconstruction options. One point that was not discussed in these case reports is how silicone will affect breast imaging and routine cancer screening by obscuring the gland tissue. This has been addressed in the American College of Radiology (ACR) guidelines in more detail. Overall, successful reconstruction is possible as long as one familiarizes themselves with silicone usage and how it can mimic other pathologies. Patients with silicone may require further workup to ensure etiology of pathology before surgical planning can safely begin.

Uterine/Endometrial Cancer Screening

Figure 5 includes the PRISMA flow diagram regarding endometrial and cervical cancer studies. A lack of endometrial screening protocols for TGGD people on HRT, lead providers to follow the guidelines currently in place for cis-gender women. There is currently no evidence-based indication to perform prophylactic screening for endometrial cancer in cis-gender women. As such, diagnostic procedures like an endometrial biopsy or transvaginal ultrasound are not routinely recommended for transgender men regardless of hysterectomy status. Abnormal vaginal discharge and bleeding serve as signs to seek screening measures. The ACS recommends educating TGGD individuals with a vagina on the topic of unusual vaginal bleeding and to explore instances both pre and post hysterectomy. This may be difficult as TGGD individuals often avoid regular visits to their gynecologist, especially after undergoing a hysterectomy.

A uterine pathology study from Grimstad *et al*[54] reviewed 94 transgender men receiving testosterone therapy, reporting no case of endometrial cancer[55]. A similar pathologic analysis from Ralph *et al*[55] reported no evidence of malignant changes to the endometrium of transgender men in response to long-term testosterone treatment[55]. The uterine histological similarity to cis-gender women indicates regular endometrial screening is unlikely to be necessary for transgender men undergoing androgen therapy.

Uterine/Endometrial Cancer Treatment

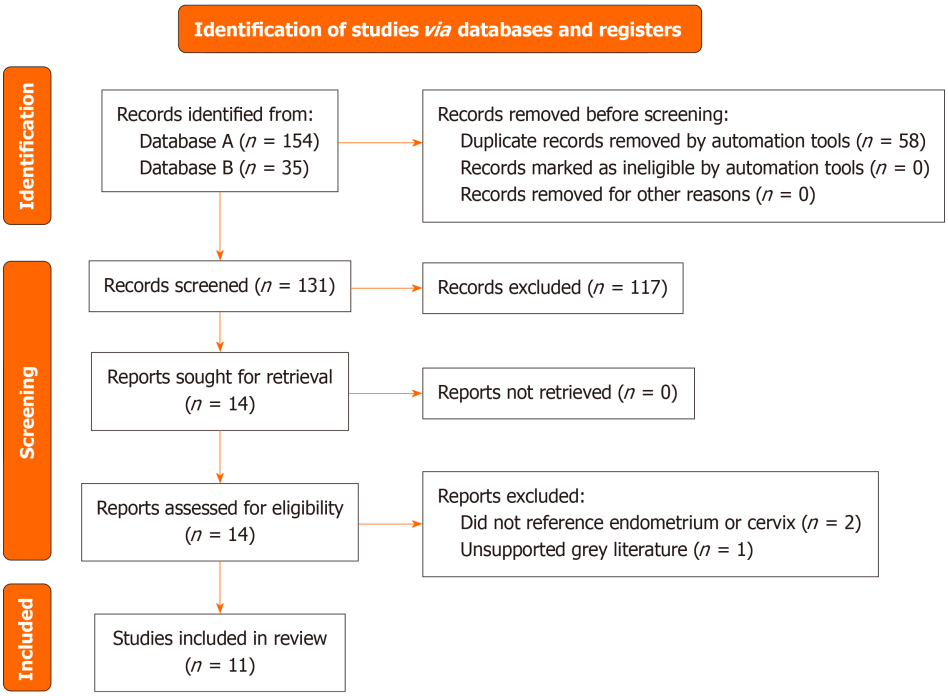
The literature documents one case of uterine cancer in a transgender man after he was found to have a mass noted during speculum examination for planned hysterectomy in preparation for GAS[56]. Post-operative pathology from a transmasculine person's radical hysterectomy revealed stage IIIC endometrioid adenocarcinoma of the uterus. The diagnosis included involvement of the parametrium and lymph nodes. The patient was treated with 6 cycles of chemotherapy (carboplatin and paclitaxel) before declining additional treatment. Two years later, the patient had evidence of recurrent disease and underwent additional chemotherapy. Follow up beyond this date is lost. The authors note the potential importance of evaluating the endometrium prior to undergoing a hysterectomy as the surgery could have been altered to more effectively treat the adenocarcinoma.

Cervical Cancer Screening

At present, there are no specific guidelines for transgender men regarding cervical cancer screening. As such, providers currently follow the guidelines created for cis-gender women when conducting screening on transgender men. The current recommendation indicates any cis-woman over 21 years old should have a Pap smear performed every 3 years or a human papillomavirus (HPV) test performed every 5 years. Screening may stop if the patient no longer has a cervix or if the patient is 65 years old and testing had been normal over the previous 10 years. A partial or supracervical hysterectomy preserves the cervix, indicating that not all transgender men with hysterectomies should stop receiving regular cervical cancer screenings.

In some cases, a routine postoperative histology workup may reveal cervical carcinoma in situ. Dysplasia of the cervix can spread to the vagina, which indicates the need for continued screening of the vaginal fornices even post-hysterectomy. It follows that convincing TGGD patients to continue regular cancer screenings after their hysterectomy poses a challenge, most importantly when partial cervix tissue remains[57].

A possible solution exists, such as increasing the availability of self-collected HPV DNA tests. Goldstein *et al*[58] reported a 2-fold increase in transgender men receiving HPV testing after introducing self-collected HPV swabbing options[58]. Additional research shows self-collected HPV tests have a 71.4% sensitivity when compared to provider-



DOI: 10.5306/wjco.v14.i7.265 **Copyright** ©The Author(s) 2023.

Figure 5 The preferred reporting items for systematic reviews and meta-analyses flow diagram indicated database search records and inclusion decision steps. The diagram includes records regarding endometrial and cervical cancer studies.

collected HPV tests[59]. The efficacy is consistent with the rates seen in the cis-gender women population choosing to use self-collected swabs.

Adding to the complexity of cancer screening is the inconsistent correlation between exogenous testosterone use and the risk of carcinoma in the female reproductive system. There is a report of TGGD patients on androgens having higher rates of unsatisfactory or abnormal Pap smear results when compared to cis-gender women[60]. Contradicting this study are two recent publications that showed no significant difference between rates of epithelial cell abnormalities between transgender and cis-gender women receiving Pap tests[61,62]. Further studies must be done to determine the extent to which exogenous testosterone treatment can influence cell growth in cervical tissue.

Cervical Cancer Treatment

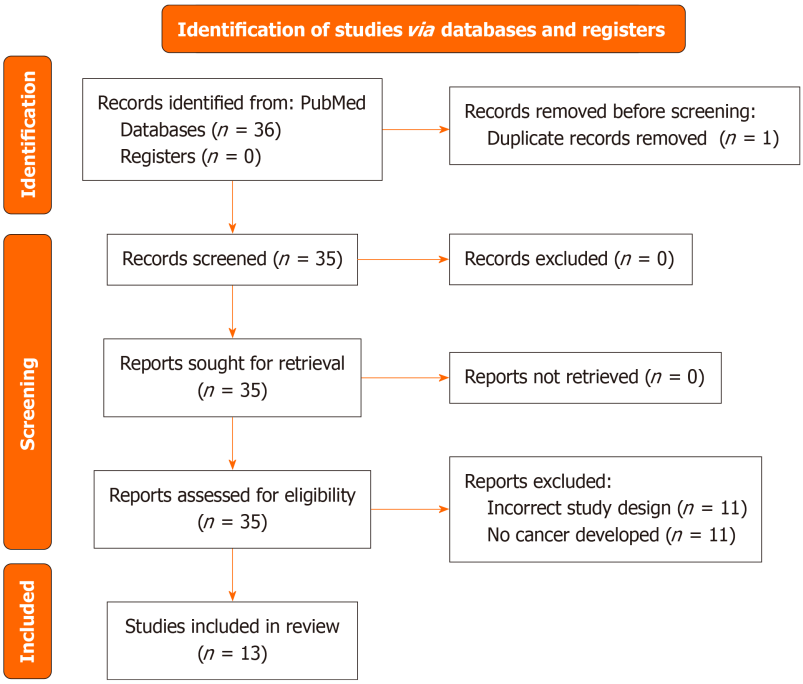
There are 3 reported cases of cervical cancer in transgender men documented in the literature. Driák and Šamudovský[63] report a case of localized squamous carcinoma, which was detected during a pathologic analysis of the cervix post-abdominal hysterectomy[63]. The patient had been on androgen therapy for the previous 4 years and did not need any oncological treatment beyond the hysterectomy. A case presented by Urban *et al*[56] follows a transgender man diagnosed with invasive stage IB adenoma malignum after receiving a laparoscopic total hysterectomy and bilateral salpingo-oophorectomy[56]. The patient reported vaginal bleeding for a 2-year period prior to the surgery, but contributed this to androgen therapy, which he had been on for the previous 7 years. He was subsequently treated with weekly cisplatin, external beam pelvic radiation, and intracavitary radiation to the upper vagina, which left him without evidence of disease. The most recent report of cervical carcinoma comes from Beswick *et al*[64], who present a transgender man diagnosed with stage IV A cervical cancer[64]. This 45-year-old patient had previously been on androgen therapy but stopped 18 mo prior to presenting with abnormal vaginal bleeding and subsequent squamous cell cervical carcinoma. The patient was treated with external beam radiation, weekly radiosensitizing cisplatin chemotherapy, and high-dose–rate intracavitary brachy-therapy. The patient showed no evidence of disease 6 mo after completing treatment. Ovaries see Figure 6.

Screening

Current screening guidelines state that there is no unique recommendation for TGGD individuals with ovaries. It is recommended that they follow the same guidelines established for cis-gender women: routine age-appropriate surveillance, a gynecological evaluation at least every 3 years (particularly for patients with a strong family history associated to ovarian cancer) with a pelvic examination, and routine ovarian cancer screening is not recommended[2,65].

Of the 13 articles included in our systematic review, seven described cases of ovarian cancer amongst TGGD and their respective management. There have been eight cases reported in the literature regarding cases of ovarian cancer amongst TGGD individuals, seven of whom had taken gender-affirming hormone therapy[66-72].

Cases in literature and their respective management: Figure 6 includes the PRISMA flow diagram regarding ovarian cancer studies. Hage *et al*[66] published the first journal article discussing two case reports about TGGD individuals who



DOI: 10.5306/wjco.v14.i7.265 **Copyright** ©The Author(s) 2023.

Figure 6 The preferred reporting items for systematic reviews and meta-analyses flow diagram indicated database search records and inclusion decision steps. The diagram includes records regarding ovarian cancer studies.

were diagnosed with ovarian cancer. Patient A was diagnosed with papillary cystadenocarcinoma and underwent a laparotomy, supracolic omentectomy, and left oophorectomy followed by adjuvant combination chemotherapy with taxol, epirubicin, cis-platinum. Patient B was diagnosed with papillary borderline tumor in the left ovary, which was discovered as the patient was admitted to undergo a hysterectomy and bilateral salpingo-oophorectomy. Patient B eventually underwent a laparotomy and resection of multicystic mass. No radiotherapy or chemotherapy was required. In both cases reported, Patient A and Patient B had a history of hormone therapy reported[66].

The next case was reported by Dizon *et al*[67], which described a 46-year-old transgender man who was diagnosed with endometrioid adenocarcinoma arising in the left ovary and fallopian tube. This patient underwent a total hysterectomy, bilateral salpingo-oophorectomy, omentectomy, pelvic and para-aortic node dissection, and peritoneal staging biopsies. Following surgery, chemotherapy was completed, consisting of carboplatin and paclitaxel. This case report also noted that the patient had used hormone therapy as part of their gender affirming surgery, but it was discontinued following surgery[67].

Another case report by Ferreira *et al*[68] described a 23-year-old transgender man with a history of testosterone therapy who was diagnosed with bilateral serous borderline ovarian tumor and underwent a total hysterectomy and bilateral salingo-oophorectomy. There was no discussion about subsequent chemotherapy[68].

Aubrey *et al*[69] published a similar case report about a 36-year-old transgender man diagnosed with stage IIA ovarian endometrium cancer who underwent a bilateral salpingo-oophorectomy followed by six cycles of chemotherapy. This patient also was using hormone therapy, which was discontinued after surgery[69].

Stevens and Abrahm[70] published another case report of a 67-year-old who was diagnosed with metastatic ovarian cancer and used exogenous testosterone. There was no mention about chemotherapy or surgery, and the patient remained in the hospital and received palliative care[70].

Bilash and Walker[71] published an article discussing Bilash's personal experiences as a transgender individual who was diagnosed with polycystic ovarian syndrome in his early 20s and underwent a bilateral oophorectomy and total hysterectomy at the age of 30 for stage III ovarian cancer. Bilash and Walker[71] began using testosterone therapy following surgery[71].

Millington *et al*[72] presented a case report about a 17-year-old transgender adolescent who was diagnosed with serous borderline ovarian tumor. The patient began subcutaneous testosterone cypionate 12 wk prior to the diagnosis. For treatment, the patient elected a right salpingo-oophorectomy. Post-operatively, testosterone was restarted two months following the procedure and surveillance of the remaining ovary was continued and eventually unremarkable over time [72].

Prevention and Management: As demonstrated by both the case reports and the current literature, there have been discussions about the possible relationship between using testosterone supplements and a potential increased risk of ovarian cancer. However, it has been repeatedly emphasized that there is currently a lack of reported cases and data in the TGGD community to prove the possible mitogenic effects of long-term exposure to exogenous androgens on ovaries [66,68,69,72,73].

This correlates to another topic that has been debated in the literature, which is the use of bilateral salpingo-oophorectomies as a possible preventative measure of ovarian cancer in the TGNC community. Currently, according to National Comprehensive Cancer Network guidelines for cis-gender women, TGGD who are carriers of the BRAA1 and BRCA2 mutation should be offered risk-reducing salpingo-oophorectomy. If patients chose to defer this procedure, serial monitoring is considered as an alternative[47]. Some articles explore the possibility of expanding preventative ovariectomies to TGGD patients who are eligible for gender-affirming surgery and are on hormone therapy (such as a simultaneous salpingo-oophorectomy for TGGD individuals who undergo hysterectomy)[66,74]. Other articles noted the lack of knowledge about the long-term effects of oophorectomy at the time of a hysterectomy and how oophorectomies affect the quality of life, gender dysphoria, and the risk reduction of ovarian cancer in the TGNC population[67,68,73,75]. Kwiatkowska *et al*[76] emphasizes the responsibility of the physician during hormone therapy, in which that gender-affirming surgeries must be beneficial for the overall well-being of the patient, which continues to remain a gray area due to the lack of research about the impact of hormone therapy on the risk of ovarian cancer and the benefits of prophylactic bilateral salpingo-oophorectomy in TGGD individuals using hormone therapy[76].

Overall current guidelines state TGGD individuals neither require routine ovarian cancer screening nor additional surveillance and prophylactic oophorectomies are not needed as TGGD individuals are not at an increased risk of ovarian cancer[2].

The healthcare needs of TGGD individuals are unique due to gender-affirming hormonal therapy and or surgical interventions. The most commonly used hormone therapies are antiandrogens combined with Estrogen. Subsequently, after 18–36 mo of hormone therapy[77], transgender women can undergo vaginoplasty, including orchiectomy. The Prostate usually is not removed during feminizing genital GAS (fgGAS) (vaginoplasty or vulvoplasty) due to potential significant complications such as incontinence. The permanence of the prostate after fgGAS poses a continued risk for prostate cancer.

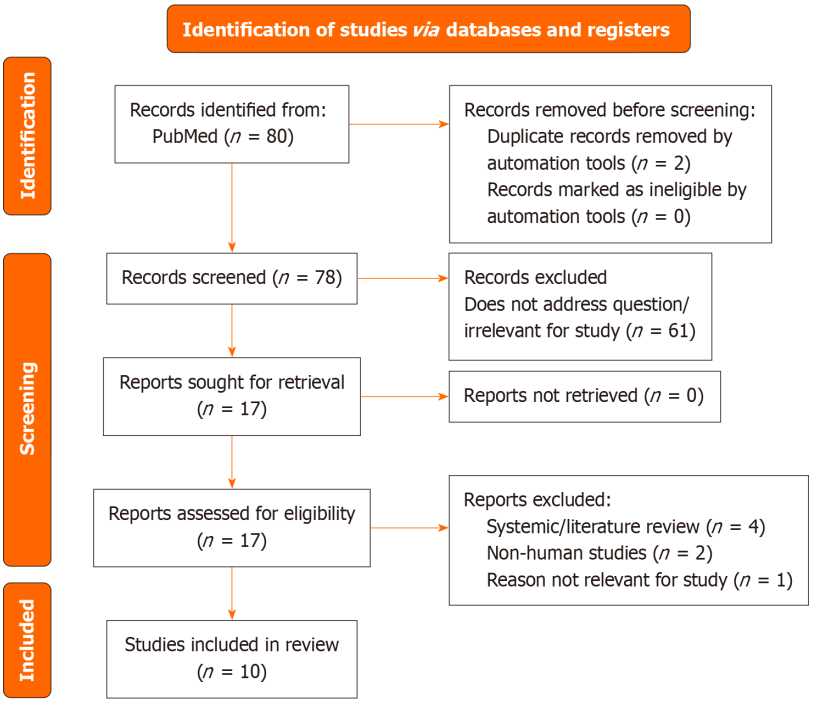
Antiandrogen and estrogen therapy with or without orchiectomy is theorized to have a lower incidence of prostate cancer in transgender women compared to cisgender men[78]. The main goal of hormone therapy is the regression of adult male sexual characteristics while inducing female sexual development in a transgender women with minimal long-term risk. While Estrogen has a short-term risk of thrombosis, the long-term risk of estrogen use is unclear[79]. Recent research has shown estrogen receptor–a, may have carcinogenic effects on the Prostate alone. A higher estradiol to dihydrotestosterone ratio may promote stromal cell growth in the prostate as well[79].

As part of antiandrogen treatment in male to female TGNC patients Prostate-specific antigen (PSA) and human glandular kallikrein (hK2) have been found to be elevated in plasma and urine after antiandrogen treatment in transgender women[80]. Both of these molecules are mainly produced by the Prostate, and androgens regulate their genes through the AR. Currently, screening guidelines for the TGGD population with prostates are the same as cis men. Transgender women 50 years and older should undergo annual prostate evaluation, consisting of digital rectal examination (DRE). Annual PSA evaluation might still have pertinence in prostate cancer screening and follow up. de Nie *et al*[81] performed a prostate biopsy in a transgender woman diagnosed with prostate cancer who had undergone orchiectomy with estrogen treatment[81]. The biopsy produced positive staining for prostate acid phosphatase (PAP) and prostate-specific antigen (PSA), showing that natural prostate activity persists in the castrated individuals and that this activity does not rely solely on androgens. As PSA is usually highly suppressed in these individuals following bilateral orchiectomy, any PSA value greater than 1.0 ng/mL should be regarded as concerning[82]. Further research on the adequate PSA monitoring threshold is required for this subset of patients.

A biopsy is a primary tool for diagnosing prostate cancer and determining a Gleason score for prognosis. Some studies have shown the difficulty of assigning a correct Gleason score due to morphologic changes to the Prostate induced by androgen deprivation adding a layer of complexity when interpreting results in the TGGD population[83]. For both cis men and transgender women diagnosed with prostate cancer multiple treatments are available. Amongst them include gonadotropin-releasing hormone (GnRH) agonists/antagonists, radiotherapy, chemotherapy, robotic-assisted laparoscopic prostatectomy, and cystoprostatectomy. New therapies such as abiraterone, enzalutamide, sipuleucel-T and cabazitaxel have been introduced to treat hormone-resistant prostate cancer[82].

In our review, we identified 14 TGGD individuals diagnosed with Prostate Cancer. Figure 7 includes the PRISMA flow diagram regarding prostate cancer studies. Of those 14, three underwent chemotherapy using estramustine, mitoxantrone, docetaxel, carboplatin, or prednisone. One patient underwent external beam radiotherapy, antiandrogen therapy, followed by mitoxantrone and prednisone, and passed away from a thromboembolic event[19]. Another patient underwent robotic-assisted laparoscopic prostatectomy and bilateral pelvic lymph node dissection[84]. One patient underwent cystoprostatectomy with resection of a right pelvic mass and lymph node dissection following chemotherapy with docetaxel and carboplatin[82]. Another patient was started with antiandrogen therapy using oral bicalutamide and oral dutasteride[82] one patient underwent radical radiotherapy and died after six months of therapy. Treatment for the other six patients was not discussed. There were only two reported deaths of the 14 reported Prostate cancer cases we identified.

The absence of TGGD-specific screening guidelines, unconfirmed effects of gender-affirming hormone therapy on prostate cancer, change of the pelvis anatomy following the surgery, and barriers of care by the healthcare providers and system can delay cancer diagnosis and treatment. The combination of factors may lead to poorer prognosis in this population[79]. Yet, although the incidence is lower in TGGD women, Jackson *et al*[79] have indicated that prostate cancer could be more aggressive amongst TGGD population with increased mortality amongst TGGD women. Incidence of prostate cancer after prolonged use of gender-affirming hormone therapy raises questions about the "protective" role of castrating status in cancer pathogenesis[85]. Further study regarding the effects of gender-affirming hormone therapy and orchiectomy is needed to shape the screening and treatment of Prostate cancer in TGGD women.



DOI: 10.5306/wjco.v14.i7.265 **Copyright** ©The Author(s) 2023.

Figure 7 The preferred reporting items for systematic reviews and meta-analyses flow diagram indicated database search records and inclusion decision steps. The diagram includes records regarding prostate cancer studies.

Currently, the USPSTF recommends against regular screening for Testicular Cancer in cis-gender men and has no recommendations for TGGD population. Figure 8 includes the PRISMA flow diagram regarding testicular cancer studies. Some societies recommend annual self-examinations. In TGGD population, hormonal therapy (primarily estradiol) is instituted with the goal to develop female secondary sex characteristics. Estrogen is thought to be a risk factor for development of testicular cancer although no large-scale studies have been done that show a link.

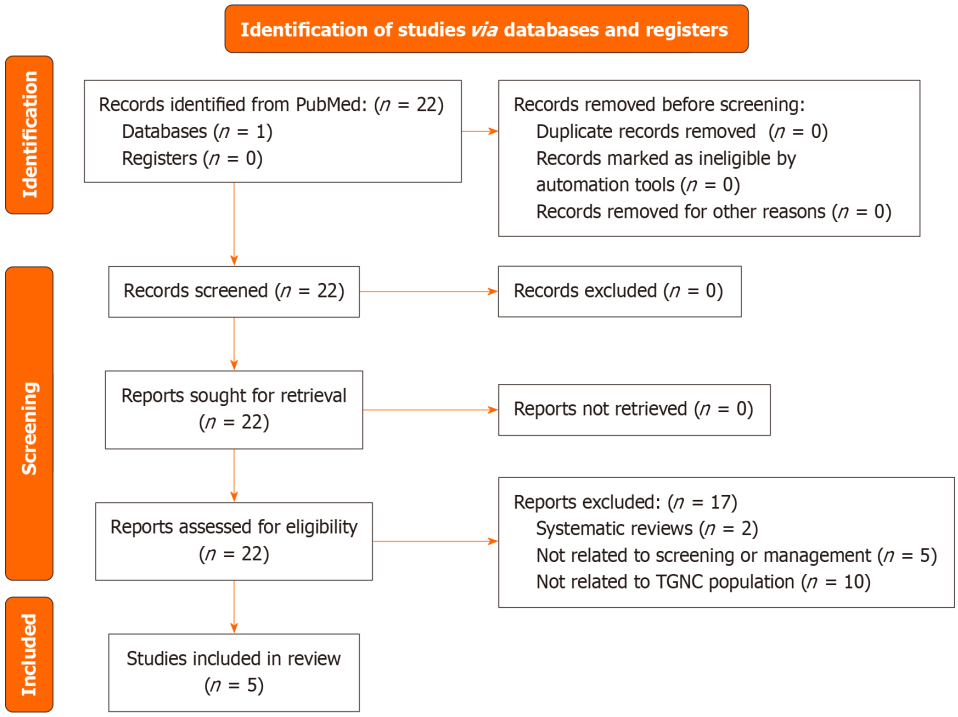
Standard management of testicular cancer involves tumor markers (β- human chorionic gonadotropin, Lactate Dehydrogenase (LDH) and alpha-fetoprotein), computed tomography scan of chest/abdomen/pelvis followed by radical orchiectomy. Tumor markers can help differentiate the type of cancer present, although standard of care involves a radical orchiectomy up front. Our review resulted in 5 cases of testicular cancer found in the TGGD population. Two cases were found when testosterone levels failed to suppress despite hormonal therapy. One case reported by Wolf-Gould and Wolf-Gould[86] was found to have an intratubular germ cells neoplasia (carcinoma in site), embryonal cell carcinoma[86]. Another case reported by Elshimy *et al*[87] was found to have a B-HCG secreting seminoma[87]. One case of seminoma reported by Kvach *et al*[88], was discovered incidentally after penile-inversion vaginoplasty[88]. A case by Chandhoke *et al*[89], reported a 38-year-old transgender woman with a testicular mass and a retroperitoneal tumor that was too morbid to resect[89]. The patient underwent radical orchiectomy followed by maintenance on chemotherapy and surveillance with serial imaging. An interesting case by Kobori *et al*[90] was revealed to have a mature testicular teratoma with positive estrogen receptor expression while undergoing hormonal therapy with estrogen and progesterone[90]. The authors note that although receptor expression does not necessarily imply causation, the contribution of estrogen cannot be ruled out. The patient elected to stop hormonal therapy in this case.

All patients underwent radical orchiectomy with chemotherapy reserved for patients who met criteria per cis-gender guidelines. Four patients elected to stop estrogen therapy; however, this was after an extensive discussion with the patient on the social and psychological effects of cessation.

DISCUSSION

Prior to breast cancer screening guidelines for the TGGD patient from the American College of Radiology in November 2021, no formal cancer screening guidelines were made for the TGGD population. In most instances, screening guidelines for the TGGD population default to cis-gender screening recommendations and management. Further, guidelines are needed to address non binary patients as existing literature in this select population is also lacking. Although screening suggestions based on this systematic review are alluded to in each organ section, the discussion of organ specific screening centers on a call to action for better research.

Discussion on cancer management is provided in each organ section in more detail. However, some overarching themes hold true for all cancer management. Provider education in the communication skills with the TGGD population in the form of gender friendly language is paramount to improve the existing barriers of care, improve healthcare access-



DOI: 10.5306/wjco.v14.i7.265 **Copyright** ©The Author(s) 2023.

Figure 8 The preferred reporting items for systematic reviews and meta-analyses flow diagram indicated database search records and inclusion decision steps. The diagram includes records regarding testicular cancer studies. TGNC: Transgender and gender nonconforming.

ibility and increase provider options for these patients. Addressing the limitations of care and actively participating in scientific research for this population will allow for earlier detection of cancer, improved treatment adherence, improved patient care accessibility and ultimately improved patient follow up and satisfaction.

CONCLUSION

Currently, a comprehensive guideline for cancer screening in the TGGD population is lacking. Prior to breast cancer screening guidelines for this population from the ACR in November 2021, no formal cancer screening guidelines were made for the TGGD population. In most instances, screening guidelines defaulted to cis gender screening recommendations and management. However, caring for the TGGD population undergoing gender affirming surgery is highly individualized and requires consideration of factors such as the age at which they commenced hormonal therapy, the stage of transition, and the disproportionate social determinants of health these patients are subject to. For all these reasons, these patients are at higher risk of developing cancer and or having their cancer detected at a later, more aggressive stage because they do not have access to the appropriate and comprehensive care they require.

This study performed systematic review of the current literature surrounding both the screening and management of cancer in the transgender and gender diverse population whom are considering gender affirming surgery. In addition to calling for better education and evidence based guidelines for physicians to follow, this paper is a call to action for physicians to openly address the limitations of care and to actively participating in scientific research for this population to allow for earlier detection of cancer, improved treatment adherence, improved patient care accessibility and ultimately improved patient satisfaction.

ARTICLE HIGHLIGHTS

Research background

Lack of screening and management guidelines in the transgender and gender diverse (TGGD) and non binary population.

Research motivation

A comprehensive guideline for cancer screening in the TGGD population is lacking. Caring for the TGGD population undergoing Gender Affirmation Surgery is highly individualized and requires consideration for the whole, integral patient including the physical and psychological realm. Communication and access to care should strive for inclusion and

avoid potential discrimination from misgendering. Once diagnosed with cancer, TGGD patients should receive care at institutions capable of providing a multi-disciplinary approach. This collective approach will ensure record upkeep and help delay any unnecessary delays in care. Resolving the lack of guidelines, improving inclusion, and diminishing the barriers of care will ultimately lead to more timely and efficient care for the TGGD population.

Research objectives

Literature is lacking regarding screening and management guidelines in the TGGD and non binary population. Barriers of care are present and need to be addressed to improve access and quality of care for this population.

Research methods

A systematic review utilizing the preferred reporting items for systematic reviews and meta-analyses guidelines was used. Rayyan software was used to organize and collaborate on articles for reviewers. A systematic search of PubMed on January 5th, 2022, with the following terms: "TGNC", OR "transgender", OR "gender non-conforming", OR "gender nonbinary" AND "cancer screening", AND "breast cancer", AND "cervical cancer", AND "uterine cancer", AND "ovarian cancer", AND "prostate cancer", AND "testicular cancer", AND "surveillance", AND "follow-up", AND "management". After eliminating review articles, duplicates, abstracts, articles not relevant to the section topic or opinion pieces a total of 70 studies with original data were obtained. Articles relevant to the section topic, including the search terms were included in this systematic review. Search parameters were performed according to Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines. Two independent reviewers Araya S and Nannapaneni S carried out independent abstract revisions on January 11th, 2022, using systematic review software "Rayyan" registered in Cambridge Massachusetts.

Research results

Literature is lacking regarding screening and management guidelines in the TGGD and non binary population. Barriers of care are present and need to be addressed to improve access and quality of care for this population.

Research conclusions

Caring for the TGGD and nonbinary patients is a complex process and requires understanding of three key points – care is highly individual, it depends on stage of gender affirming surgery, and it is centered on proper provider education and training. An understanding of the biopsychosocial model of health, where illness must be considered from not only the physical body, but also from the psychological and social aspects is required. Prior to breast cancer screening guidelines for the TGGD patient from the American College of Radiology in November 2021, no formal cancer screening guidelines were made for the TGGD population. In most instances, screening guidelines for the TGGD population default to cis gender screening recommendations and management. Further, guidelines are needed to address non binary patients as existing literature in this select population is also lacking. Although screening suggestions based on this systematic review are alluded to in each organ section, the discussion of organ specific screening centers on a call to action for better research. Discussion on cancer management is provided in each organ section in more detail. However, some overarching themes hold true for all cancer management. Provider education in the communication skills with the TGGD population in the form of gender friendly language is paramount to improve the existing barriers of care, improve healthcare accessibility and increase provider options for these patients. Addressing the limitations of care and actively participating in scientific research for this population will allow for earlier detection of cancer, improved treatment adherence, improved patient care accessibility and ultimately improved patient follow up and satisfaction.

Research perspectives

Creating specific cancer screening and management guidelines for the TGGD and non binary population while improving barriers to care.

FOOTNOTES

Author contributions: All authors contributed in a significant and meaningful way to the development of this article.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article. Accepted as poster presentation at Plastic Surgery the Meeting October 27th-30th 2022, Boston, Massachusetts.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: United States

ORCID number: Juliet C Panichella 0009-0008-8478-7694; Sthefano Araya 0000-0002-8245-8036; Alireza Hamidian Jahromi 0000-0002-7585-847X.

S-Editor: Li L
L-Editor: A
P-Editor: Xu ZH

REFERENCES

1 **Flores AR**, Herman JL, Gates GJ, Brown TNT. How Many Adults Identify as Transgender in the United States. The Williams Institute; 2016. [cited 1 June 2023]. Available from: https://williamsinstitute.law.ucla.edu/publications/trans-adults-united-states/
2 **Sterling J**, Garcia MM. Cancer screening in the transgender population: a review of current guidelines, best practices, and a proposed care model. *Transl Androl Urol* 2020; **9**: 2771-2785 [PMID: 33457249 DOI: 10.21037/tau-20-954]
3 **Ouzzani M**, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* 2016; **5**: 210 [PMID: 27919275 DOI: 10.1186/s13643-016-0384-4]
4 **Patel JM**, Dolitsky S, Bachman GA, Buckley de Meritens A. Gynecologic cancer screening in the transgender male population and its current challenges. *Maturitas* 2019; **129**: 40-44 [PMID: 31547911 DOI: 10.1016/j.maturitas.2019.08.009]
5 **Kiran T**, Davie S, Singh D, Hranilovic S, Pinto AD, Abramovich A, Lofters A. Cancer screening rates among transgender adults: Cross-sectional analysis of primary care data. *Can Fam Physician* 2019; **65**: e30-e37 [PMID: 30674526]
6 **Deebel NA**, Morin JP, Autorino R, Vince R, Grob B, Hampton LJ. Prostate Cancer in Transgender Women: Incidence, Etiopathogenesis, and Management Challenges. *Urology* 2017; **110**: 166-171 [PMID: 28882782 DOI: 10.1016/j.urology.2017.08.032]
7 **Nelson B**. A cancer screening crisis for transgender patients: Discrimination, patient unease, provider ignorance, and a highly gendered health care system are impeding cancer screening and risk assessment in the transgender population. In this article, the first of a 2-part series, we explore how clinicians can begin to address those barriers. *Cancer Cytopathol* 2019; **127**: 421-422 [PMID: 31291063 DOI: 10.1002/cncy.22159]
8 **Clarke CN**, Cortina CS, Fayanju OM, Dossett LA, Johnston FM, Wong SL. Breast Cancer Risk and Screening in Transgender Persons: A Call for Inclusive Care. *Ann Surg Oncol* 2022; **29**: 2176-2180 [PMID: 34097159 DOI: 10.1245/s10434-021-10217-5]
9 **Snow A**, Cerel J, Loeffler DN, Flaherty C. Barriers to Mental Health Care for Transgender and Gender-Nonconforming Adults: A Systematic Literature Review. *Health Soc Work* 2019; **44**: 149-155 [PMID: 31359065 DOI: 10.1093/hsw/hlz016]
10 **Unger CA**. Care of the transgender patient: a survey of gynecologists' current knowledge and practice. *J Womens Health (Larchmt)* 2015; **24**: 114-118 [PMID: 25525682 DOI: 10.1089/jwh.2014.4918]
11 **Gatos KC**. A Literature Review of Cervical Cancer Screening in Transgender Men. *Nurs Womens Health* 2018; **22**: 52-62 [PMID: 29433700 DOI: 10.1016/j.nwh.2017.12.008]
12 **Stenzel AE**, Moysich KB, Ferrando CA, Starbuck KD. Clinical needs for transgender men in the gynecologic oncology setting. *Gynecol Oncol* 2020; **159**: 899-905 [PMID: 33004214 DOI: 10.1016/j.ygyno.2020.09.038]
13 **James S**, Herman J, Rankin S, Keisling M, Mottet L, Anafi MA. The report of the 2015 US transgender survey. 2016. [cited 1 June 2023]. Available from: https://transequality.org/sites/default/files/docs/usts/USTS-Full-Report-Dec17.pdf
14 **Jarrett BA**, Peitzmeier SM, Restar A, Adamson T, Howell S, Baral S, Beckham SW. Gender-affirming care, mental health, and economic stability in the time of COVID-19: a global cross-sectional study of transgender and non-binary people. *medRxiv* 2020 [PMID: 33173876 DOI: 10.1101/2020.11.02.20224709]
15 **Loo S**, Almazan AN, Vedilago V, Stott B, Reisner SL, Keuroghlian AS. Understanding community member and health care professional perspectives on gender-affirming care-A qualitative study. *PLoS One* 2021; **16**: e0255568 [PMID: 34398877 DOI: 10.1371/journal.pone.0255568]
16 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin* 2021; **71**: 7-33 [PMID: 33433946 DOI: 10.3322/caac.21654]
17 **de Blok CJM**, Wiepjes CM, Nota NM, van Engelen K, Adank MA, Dreijerink KMA, Barbé E, Konings IRHM, den Heijer M. Breast cancer risk in transgender people receiving hormone treatment: nationwide cohort study in the Netherlands. *BMJ* 2019; **365**: l1652 [PMID: 31088823 DOI: 10.1136/bmj.l1652]
18 **Luehmann N**, Ascha M, Chwa E, Hackenberger P, Termanini K, Benning C, Sama D, Felt D, Beach LB, Gupta D, Kulkarni SA, Jordan SW. A Single-Center Study of Adherence to Breast Cancer Screening Mammography Guidelines by Transgender and Non-Binary Patients. *Ann Surg Oncol* 2022; **29**: 1707-1717 [PMID: 34704183 DOI: 10.1245/s10434-021-10932-z]
19 **Tabaac AR**, Sutter ME, Wall CSJ, Baker KE. Author Response to "Letter to the Editor Regarding 'Gender Identity Disparities in Cancer Screening Behaviors'". *Am J Prev Med* 2019; **56**: 162-166 [PMID: 30573145 DOI: 10.1016/j.amepre.2018.08.015]
20 **Brown A**, Lourenco AP, Niell BL, Cronin B, Dibble EH, DiNome ML, Goel MS, Hansen J, Heller SL, Jochelson MS, Karrington B, Klein KA, Mehta TS, Newell MS, Schechter L, Stuckey AR, Swain ME, Tseng J, Tuscano DS, Moy L; Expert Panel on Breast Imaging. ACR Appropriateness Criteria® Transgender Breast Cancer Screening. *J Am Coll Radiol* 2021; **18**: S502-S515 [PMID: 34794604 DOI: 10.1016/j.jacr.2021.09.005]
21 **Gooren L**, Bowers M, Lips P, Konings IR. Five new cases of breast cancer in transsexual persons. *Andrologia* 2015; **47**: 1202-1205 [PMID: 25611459 DOI: 10.1111/and.12399]
22 **Stafford A**, Shobieri A, Stamatakos M, Edmiston K. Ductal carcinoma in situ in the male-to-female transgender population. *Breast J* 2020; **26**: 2439-2440 [PMID: 33274801 DOI: 10.1111/tbj.14099]
23 **Corman V**, Potorac I, Manto F, Dassy S, Segers K, Thiry A, Bours V, Daly AF, Beckers A. Breast cancer in a male-to-female transsexual patient with a BRCA2 mutation. *Endocr Relat Cancer* 2016; **23**: 391-397 [PMID: 27000661 DOI: 10.1530/ERC-16-0057]
24 **Sieberg R**, Soriano K, Zuurbier R. A rare case of breast cancer in a transgender woman. *Radiol Case Rep* 2021; **16**: 3285-3288 [PMID: 34484532 DOI: 10.1016/j.radcr.2021.07.052]
25 **Ganly I**, Taylor EW. Breast cancer in a trans-sexual man receiving hormone replacement therapy. *Br J Surg* 1995; **82**: 341 [PMID: 7796003

DOI: 10.1002/bjs.1800820319]
26 **Brown GR**. Breast Cancer in Transgender Veterans: A Ten-Case Series. *LGBT Health* 2015; **2**: 77-80 [PMID: 26790021 DOI: 10.1089/lgbt.2014.0123]
27 **Nehlsen AD**, Bhardwaj A, Weltz C, Green S. Triple Negative Breast Cancer in a Male to Female Transgender Patient: A Case Report and Literature Review. *Adv Radiat Oncol* 2020; **5**: 1083-1089 [PMID: 33083671 DOI: 10.1016/j.adro.2020.06.026]
28 **Pattison ST**, McLaren BR. Triple negative breast cancer in a male-to-female transsexual. *Intern Med J* 2013; **43**: 203-205 [PMID: 23402485 DOI: 10.1111/imj.12047]
29 **Grabellus F**, Worm K, Willruth A, Schmitz KJ, Otterbach F, Baba HA, Kimmig R, Metz KA. ETV6-NTRK3 gene fusion in a secretory carcinoma of the breast of a male-to-female transsexual. *Breast* 2005; **14**: 71-74 [PMID: 15695086 DOI: 10.1016/j.breast.2004.04.005]
30 **Ali N**, Sindhu K, Bakst RL. A Rare Case of a Transgender Female With Breast Implant-Associated Anaplastic Large Cell Lymphoma Treated With Radiotherapy and a Review of the Literature. *J Investig Med High Impact Case Rep* 2019; **7**: 2324709619842192 [PMID: 31010324 DOI: 10.1177/2324709619842192]
31 **de Boer M**, van der Sluis WB, de Boer JP, Overbeek LIH, van Leeuwen FE, Rakhorst HA, van der Hulst RRWJ, Hijmering NJ, Bouman MB, de Jong D. Breast Implant-Associated Anaplastic Large-Cell Lymphoma in a Transgender Woman. *Aesthet Surg J* 2017; **37**: NP83-NP87 [PMID: 29036941 DOI: 10.1093/asj/sjx098]
32 **Zaveri S**, Yao A, Schmidt H. Breast Implant-Associated Anaplastic Large Cell Lymphoma Following Gender Reassignment Surgery: A Review of Presentation, Management, and Outcomes in the Transgender Patient Population. *Eur J Breast Health* 2020; **16**: 162-166 [PMID: 32656514 DOI: 10.5152/ejbh.2020.5480]
33 **Li JZ**, Tu HYV, Avram R, Pinthus J, Bordeleau L, Hodgson N. Cancer prevention and screening in a BRCA2-positive male to female transgender patient. *Breast J* 2018; **24**: 1112-1113 [PMID: 30036902 DOI: 10.1111/tbj.13096]
34 **Teoh ZH**, Archampong D, Gate T. Breast cancer in male-to-female (MtF) transgender patients: is hormone receptor negativity a feature? *BMJ Case Rep* 2015; **2015** [PMID: 25994431 DOI: 10.1136/bcr-2015-209396]
35 **Barghouthi N**, Turner J, Perini J. Breast Cancer Development in a Transgender Male Receiving Testosterone Therapy. *Case Rep Endocrinol* 2018; **2018**: 3652602 [PMID: 30693115 DOI: 10.1155/2018/3652602]
36 **Mingrino J**, Wang Y. Apocrine ductal carcinoma in situ associated with testosterone therapy in a transgender individual. *Breast J* 2021; **27**: 475-477 [PMID: 33547745 DOI: 10.1111/tbj.14187]
37 **Fehl A**, Ferrari S, Wecht Z, Rosenzweig M. Breast Cancer in the Transgender Population. *J Adv Pract Oncol* 2019; **10**: 387-394 [PMID: 33343986 DOI: 10.6004/jadpro.2019.10.4.6]
38 **Fundytus A**, Saad N, Logie N, Roldan Urgoiti G. Breast cancer in transgender female-to-male individuals: A case report of androgen receptor-positive breast cancer. *Breast J* 2020; **26**: 1007-1012 [PMID: 31749246 DOI: 10.1111/tbj.13655]
39 **Shao T**, Grossbard ML, Klein P. Breast cancer in female-to-male transsexuals: two cases with a review of physiology and management. *Clin Breast Cancer* 2011; **11**: 417-419 [PMID: 21831723 DOI: 10.1016/j.clbc.2011.06.006]
40 **Eismann J**, Heng YJ, Fleischmann-Rose K, Tobias AM, Phillips J, Wulf GM, Kansal KJ. Interdisciplinary Management of Transgender Individuals at Risk for Breast Cancer: Case Reports and Review of the Literature. *Clin Breast Cancer* 2019; **19**: e12-e19 [PMID: 30527351 DOI: 10.1016/j.clbc.2018.11.007]
41 **Jacoby A**, Rifkin W, Zhao LC, Bluebond-Langner R. Incidence of Cancer and Premalignant Lesions in Surgical Specimens of Transgender Patients. *Plast Reconstr Surg* 2021; **147**: 194-198 [PMID: 33370065 DOI: 10.1097/PRS.0000000000007452]
42 **Chotai N**, Tang S, Lim H, Lu S. Breast cancer in a female to male transgender patient 20 years post-mastectomy: Issues to consider. *Breast J* 2019; **25**: 1066-1070 [PMID: 31273889 DOI: 10.1111/tbj.13417]
43 **Katayama Y**, Motoki T, Watanabe S, Miho S, Kimata Y, Matsuoka J, Doihara H, Nanba Y. A very rare case of breast cancer in a female-to-male transsexual. *Breast Cancer* 2016; **23**: 939-944 [PMID: 26660332 DOI: 10.1007/s12282-015-0661-4]
44 **Nikolic DV**, Djordjevic ML, Granic M, Nikolic AT, Stanimirovic VV, Zdravkovic D, Jelic S. Importance of revealing a rare case of breast cancer in a female to male transsexual after bilateral mastectomy. *World J Surg Oncol* 2012; **10**: 280 [PMID: 23273269 DOI: 10.1186/1477-7819-10-280]
45 **Tai YC**, Domchek S, Parmigiani G, Chen S. Breast cancer risk among male BRCA1 and BRCA2 mutation carriers. *J Natl Cancer Inst* 2007; **99**: 1811-1814 [PMID: 18042939 DOI: 10.1093/jnci/djm203]
46 **Evans DG**, Susnerwala I, Dawson J, Woodward E, Maher ER, Lalloo F. Risk of breast cancer in male BRCA2 carriers. *J Med Genet* 2010; **47**: 710-711 [PMID: 20587410 DOI: 10.1136/jmg.2009.075176]
47 **Bedrick BS**, Fruhauf TF, Martin SJ, Ferriss JS. Creating Breast and Gynecologic Cancer Guidelines for Transgender Patients With BRCA Mutations. *Obstet Gynecol* 2021; **138**: 911-917 [PMID: 34735408 DOI: 10.1097/AOG.0000000000004597]
48 **Patzelt M**, Zarubova L, Klener P, Barta J, Benkova K, Brandejsova A, Trneny M, Gürlich R, Sukop A. Anaplastic Large-Cell Lymphoma Associated with Breast Implants: A Case Report of a Transgender Female. *Aesthetic Plast Surg* 2018; **42**: 451-455 [PMID: 29101436 DOI: 10.1007/s00266-017-1012-y]
49 **Clemens MW**, Jacobsen ED, Horwitz SM. 2019 NCCN Consensus Guidelines on the Diagnosis and Treatment of Breast Implant-Associated Anaplastic Large Cell Lymphoma (BIA-ALCL). *Aesthet Surg J* 2019; **39**: S3-S13 [PMID: 30715173 DOI: 10.1093/asj/sjy331]
50 **Murariu D**, Holland MC, Gampper TJ, Campbell CA. Illegal silicone injections create unique reconstructive challenges in transgender patients. *Plast Reconstr Surg* 2015; **135**: 932e-933e [PMID: 25700296 DOI: 10.1097/PRS.0000000000001192]
51 **Tirrell AR**, Abu El Hawa A, Bekeny JC, Del Corral G. Outcomes in chest feminization patients with a history of illicit hormone use and silicone injections. *Breast J* 2021; **27**: 352-358 [PMID: 33578450 DOI: 10.1111/tbj.14178]
52 **D'hulst L**, Nicolaij D, Beels L, Gheysens O, Alaerts H, Van de Wiele C, Maes A. False-Positive Axillary Lymph Nodes Due to Silicone Adenitis on (18)F-FDG PET/CT in an Oncological Setting. *J Thorac Oncol* 2016; **11**: e73-e75 [PMID: 26776866 DOI: 10.1016/j.jtho.2016.01.001]
53 **Bjerno T**, Basse PN, Siemssen PA, Møller TD. [Injection of high viscosity liquids. Acute or delayed excision?]. *Ugeskr Laeger* 1993; **155**: 1876-1878 [PMID: 8317048]
54 **Grimstad FW**, Fowler KG, New EP, Ferrando CA, Pollard RR, Chapman G, Gomez-Lobo V, Gray M. Uterine pathology in transmasculine persons on testosterone: a retrospective multicenter case series. *Am J Obstet Gynecol* 2019; **220**: 257.e1-257.e7 [PMID: 30579875 DOI: 10.1016/j.ajog.2018.12.021]
55 **Ralph O**, Shroff N, Christopher N, Ahmed A, Berner A, Barrett J, Sandison A, Ralph D. Mp59-16 response of endometrium to testosterone therapy in trans men and non-binary people undergoing hysterectomy. *J Urol* **201**: (Suppl 4) [DOI: 10.1097/01.JU.0000556728.56027.78]

56 **Urban RR**, Teng NN, Kapp DS. Gynecologic malignancies in female-to-male transgender patients: the need of original gender surveillance. *Am J Obstet Gynecol* 2011; **204**: e9-e12 [PMID: 21354550 DOI: 10.1016/j.ajog.2010.12.057]

57 **Peitzmeier SM**, Khullar K, Reisner SL, Potter J. Pap test use is lower among female-to-male patients than non-transgender women. *Am J Prev Med* 2014; **47**: 808-812 [PMID: 25455121 DOI: 10.1016/j.amepre.2014.07.031]

58 **Goldstein Z**, Martinson T, Ramachandran S, Lindner R, Safer JD. Improved Rates of Cervical Cancer Screening Among Transmasculine Patients Through Self-Collected Swabs for High-Risk Human Papillomavirus DNA Testing. *Transgend Health* 2020; **5**: 10-17 [PMID: 32322684 DOI: 10.1089/trgh.2019.0019]

59 **Reisner SL**, Deutsch MB, Peitzmeier SM, White Hughto JM, Cavanaugh TP, Pardee DJ, McLean SA, Panther LA, Gelman M, Mimiaga MJ, Potter JE. Test performance and acceptability of self- versus provider-collected swabs for high-risk HPV DNA testing in female-to-male trans masculine patients. *PLoS One* 2018; **13**: e0190172 [PMID: 29538411 DOI: 10.1371/journal.pone.0190172]

60 **Adkins BD**, Barlow AB, Jack A, Schultenover SJ, Desouki MM, Coogan AC, Weiss VL. Characteristic findings of cervical Papanicolaou tests from transgender patients on androgen therapy: Challenges in detecting dysplasia. *Cytopathology* 2018; **29**: 281-287 [PMID: 29488269 DOI: 10.1111/cyt.12525]

61 **Davis K**, Kwon R, Graham A, White M, Maleki Z, Rodriguez E. Comparison of Cervical Cancer Screen Results on Female-to-Male Transgender Patients With Female Patients. *Am J Clin Pathol* 2022; **157**: 540-545 [PMID: 34617991 DOI: 10.1093/ajcp/aqab158]

62 **Williams MPA**, Kukkar V, Stemmer MN, Khurana KK. Cytomorphologic findings of cervical Pap smears from female-to-male transgender patients on testosterone therapy. *Cancer Cytopathol* 2020; **128**: 491-498 [PMID: 32125771 DOI: 10.1002/cncy.22259]

63 **Driák D**, Samudovský M. Could a man be affected with carcinoma of cervix?--The first case of cervical carcinoma in trans-sexual person (FtM)--case report. *Acta Medica (Hradec Kralove)* 2005; **48**: 53-55 [PMID: 16080386]

64 **Beswick A**, Corkum M, D'Souza D. Locally advanced cervical cancer in a transgender man. *CMAJ* 2019; **191**: E76-E78 [PMID: 30665977 DOI: 10.1503/cmaj.181047]

65 **Beamer LC**. Hereditary Breast and Hereditary Ovarian Cancer: Implications for the Oncology Nurse. *Semin Oncol Nurs* 2019; **35**: 47-57 [PMID: 30711354 DOI: 10.1016/j.soncn.2018.12.001]

66 **Hage JJ**, Dekker JJ, Karim RB, Verheijen RH, Bloemena E. Ovarian cancer in female-to-male transsexuals: report of two cases. *Gynecol Oncol* 2000; **76**: 413-415 [PMID: 10684720 DOI: 10.1006/gyno.1999.5720]

67 **Dizon DS**, Tejada-Berges T, Koelliker S, Steinhoff M, Granai CO. Ovarian cancer associated with testosterone supplementation in a female-to-male transsexual patient. *Gynecol Obstet Invest* 2006; **62**: 226-228 [PMID: 16804313 DOI: 10.1159/000094097]

68 **Ferreira C**, Fraga J, Antunes C, Gonçalo M, Donato P. Serous Borderline Tumor in Transgender Female-to-Male Individuals: A Case Report of Androgen Receptor-Positive Ovarian Cancer. *Case Rep Radiol* 2021; **2021**: 8861692 [PMID: 34194862 DOI: 10.1155/2021/8861692]

69 **Aubrey C**, Saad N, Köbel M, Mattatall F, Nelson G, Glaze S. Implications for management of ovarian cancer in a transgender man: Impact of androgens and androgen receptor status. *Gynecol Oncol* 2021; **161**: 342-346 [PMID: 33663874 DOI: 10.1016/j.ygyno.2021.02.019]

70 **Stevens EE**, Abrahm JL. Adding Silver to the Rainbow: Palliative and End-of-Life Care for the Geriatric LGBTQ Patient. *J Palliat Med* 2019; **22**: 602-606 [PMID: 30513049 DOI: 10.1089/jpm.2018.0382]

71 **Bilash T**, Walker LM. Spare Parts: Navigating Ovarian Cancer as a Transgender Man. *J Clin Oncol* 2022; **40**: 1027-1029 [PMID: 35020447 DOI: 10.1200/JCO.21.01249]

72 **Millington K**, Hayes K, Pilcher S, Roberts S, Vargas SO, French A, Veneris J, O'Neill A. A serous borderline ovarian tumour in a transgender male adolescent. *Br J Cancer* 2021; **124**: 567-569 [PMID: 33106582 DOI: 10.1038/s41416-020-01129-4]

73 **Harris M**, Kondel L, Dorsen C. Pelvic pain in transgender men taking testosterone: Assessing the risk of ovarian cancer. *Nurse Pract* 2017; **42**: 1-5 [PMID: 28622261 DOI: 10.1097/01.NPR.0000520423.83910.e2]

74 **Mueller A**, Gooren L. Hormone-related tumors in transsexuals receiving treatment with cross-sex hormones. *Eur J Endocrinol* 2008; **159**: 197-202 [PMID: 18567667 DOI: 10.1530/EJE-08-0289]

75 **Cao CD**, Amero MA, Marcinkowski KA, Rosenblum NG, Chan JSY, Richard SD. Clinical Characteristics and Histologic Features of Hysterectomy Specimens From Transmasculine Individuals. *Obstet Gynecol* 2021; **138**: 51-57 [PMID: 34259463 DOI: 10.1097/AOG.0000000000004421]

76 **Kwiatkowska A**, Kułak K, Wertel I. Gender Dysphoria Disrupting the Course of Treatment of a Recurrent Juvenile Granulosa Cell Tumor in an Adolescent Female: A Case Report. *Case Rep Oncol* 2020; **13**: 1330-1336 [PMID: 33362514 DOI: 10.1159/000510810]

77 **Gooren L**, Morgentaler A. Prostate cancer incidence in orchidectomised male-to-female transsexual persons treated with oestrogens. *Andrologia* 2014; **46**: 1156-1160 [PMID: 24329588 DOI: 10.1111/and.12208]

78 **Randolph JF Jr**. Gender-Affirming Hormone Therapy for Transgender Females. *Clin Obstet Gynecol* 2018; **61**: 705-721 [PMID: 30256230 DOI: 10.1097/GRF.0000000000000396]

79 **Jackson SS**, Han X, Mao Z, Nogueira L, Suneja G, Jemal A, Shiels MS. Cancer Stage, Treatment, and Survival Among Transgender Patients in the United States. *J Natl Cancer Inst* 2021; **113**: 1221-1227 [PMID: 33704460 DOI: 10.1093/jnci/djab028]

80 **Obiezu CV**, Giltay EJ, Magklara A, Scorilas A, Gooren L, Yu H, Diamandis EP. Dramatic suppression of plasma and urinary prostate specific antigen and human glandular kallikrein by antiandrogens in male-to-female transsexuals. *J Urol* 2000; **163**: 802-805 [PMID: 10687981]

81 **de Nie I**, de Blok CJM, van der Sluis TM, Barbé E, Pigot GLS, Wiepjes CM, Nota NM, van Mello NM, Valkenburg NE, Huirne J, Gooren LJG, van Moorselaar RJA, Dreijerink KMA, den Heijer M. Prostate Cancer Incidence under Androgen Deprivation: Nationwide Cohort Study in Trans Women Receiving Hormone Treatment. *J Clin Endocrinol Metab* 2020; **105**: e3293-e3299 [PMID: 32594155 DOI: 10.1210/clinem/dgaa412]

82 **Ellent E**, Matrana MR. Metastatic Prostate Cancer 35 Years After Sex Reassignment Surgery. *Clin Genitourin Cancer* 2016; **14**: e207-e209 [PMID: 26707953 DOI: 10.1016/j.clgc.2015.11.007]

83 **Dorff TB**, Shazer RL, Nepomuceno EM, Tucker SJ. Successful treatment of metastatic androgen-independent prostate carcinoma in a transsexual patient. *Clin Genitourin Cancer* 2007; **5**: 344-346 [PMID: 17645834 DOI: 10.3816/CGC.2007.n.016]

84 **Sharif A**, Malhotra NR, Acosta AM, Kajdacsy-Balla AA, Bosland M, Guzman G, Prins GS, Abern MR. The Development of Prostate Adenocarcinoma in a Transgender Male to Female Patient: Could Estrogen Therapy Have Played a Role? *Prostate* 2017; **77**: 824-828 [PMID: 28191651 DOI: 10.1002/pros.23322]

85 **Turo R**, Jallad S, Prescott S, Cross WR. Metastatic prostate cancer in transsexual diagnosed after three decades of estrogen therapy. *Can Urol Assoc J* 2013; **7**: E544-E546 [PMID: 24032068 DOI: 10.5489/cuaj.175]

86 **Wolf-Gould CS**, Wolf-Gould CH. A Transgender Woman with Testicular Cancer: A New Twist on an Old Problem. *LGBT Health* 2016; **3**:

90-95 [PMID: 26698657 DOI: 10.1089/lgbt.2015.0057]

87 **Elshimy G**, Tran K, Harman SM, Correa R. Unmasked Testicular Seminoma During Use of Hormonal Transgender Woman Therapy: A Hidden hCG-Secreting Tumor. *J Endocr Soc* 2020; **4**: bvaa074 [PMID: 32666014 DOI: 10.1210/jendso/bvaa074]

88 **Kvach EJ**, Hyer JS, Carey JC, Bowers M. Testicular Seminoma in a Transgender Woman: A Case Report. *LGBT Health* 2019; **6**: 40-42 [PMID: 30650051 DOI: 10.1089/lgbt.2018.0173]

89 **Chandhoke G**, Shayegan B, Hotte SJ. Exogenous estrogen therapy, testicular cancer, and the male to female transgender population: a case report. *J Med Case Rep* 2018; **12**: 373 [PMID: 30563561 DOI: 10.1186/s13256-018-1894-6]

90 **Kobori Y**, Suzuki K, Iwahata T, Shin T, Sato R, Nishio K, Yagi H, Arai G, Soh S, Okada H. Mature Testicular Teratoma with Positive Estrogen Receptor Beta Expression in a Transgendered Individual on Cross-Sex Hormonal Therapy: A Case Report. *LGBT Health* 2015; **2**: 81-83 [PMID: 26790022 DOI: 10.1089/lgbt.2014.0061]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 August 24; 14(8): 285-323

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 8 August 24, 2023

MINIREVIEWS

285 Targeting *KRAS* in pancreatic adenocarcinoma: Progress in demystifying the holy grail

Elhariri A, Alhaj A, Ahn D, Sonbol MB, Bekaii-Saab T, Wu C, Rutenberg MS, Stauffer J, Starr J, Majeed U, Jones J, Borad M, Babiker H

ORIGINAL ARTICLE

Basic Study

297 Immune responses of six-transmembrane epithelial antigen of the prostate 4 functions as a novel biomarker in gastric cancer

Fang ZX, Hou YY, Wu Z, Wu BX, Deng Y, Wu HT, Liu J

Retrospective Study

311 Readmission rates and outcomes in adults with and without COVID-19 following inpatient chemotherapy admission: A nationwide analysis

Kanemo P, Musa KM, Deenadayalan V, Litvin R, Odeyemi OE, Shaka A, Baskaran N, Shaka H

ABOUT COVER

Peer Reviewer of *World Journal of Clinical Oncology*, Damian Kołat, BSc, MSc, Academic Research, Research Scientist, Senior Scientist, Teaching Assistant, Department of Biomedicine and Experimental Surgery, Medical University of Lodz, Lodz 90-136, lodzkie, Poland. damian.kolat@umed.lodz.pl

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJCO* as 2.8; IF without journal self cites: 2.8; 5-year IF: 3.0; Journal Citation Indicator: 0.36.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Xu Guo*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
August 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 August 24; 14(8): 285-296

DOI: 10.5306/wjco.v14.i8.285

ISSN 2218-4333 (online)

MINIREVIEWS

Targeting *KRAS* in pancreatic adenocarcinoma: Progress in demystifying the holy grail

Ahmed Elhariri, Ahmed Alhaj, Daniel Ahn, Mohamad Bassam Sonbol, Tanios Bekaii-Saab, Christina Wu, Michael Scott Rutenberg, John Stauffer, Jason Starr, Umair Majeed, Jeremy Jones, Mitesh Borad, Hani Babiker

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Gao W, China; Luchini C, Italy; Pan Y, China

Received: April 12, 2023
Peer-review started: April 12, 2023
First decision: June 21, 2023
Revised: July 5, 2023
Accepted: July 27, 2023
Article in press: July 27, 2023
Published online: August 24, 2023

Ahmed Elhariri, Ahmed Alhaj, Jason Starr, Umair Majeed, Jeremy Jones, Hani Babiker, Division of Hematology-Oncology, Department of Medicine, Mayo Clinic Florida, Mayo Clinic Cancer Center, Jacksonville, FL 32224, United States

Daniel Ahn, Mohamad Bassam Sonbol, Tanios Bekaii-Saab, Christina Wu, Mitesh Borad, Division of Hematology-Oncology, Department of Medicine, Mayo Clinic Arizona, Mayo Clinic Cancer Center, Phoenix, AZ 85054, United States

Michael Scott Rutenberg, Department of Radiation-Oncology, Mayo Clinic Florida, Mayo Clinic Cancer Center, Jacksonville, FL 32224, United States

John Stauffer, Department of Surgical Oncology, Hepatopancreatobiliary Surgery, Mayo Clinic Florida, Jacksonville, FL 32224, United States

Corresponding author: Ahmed Elhariri, MD, Research Fellow, Division of Hematology-Oncology, Department of Medicine, Mayo Clinic Florida, Mayo Clinic Cancer Center, 4500 San Pablo Rd, Jacksonville, FL 32224, United States. Ahmed.Elhariri@bcm.edu

Abstract

Pancreatic cancer (PC) remains one of the most challenging diseases, with a very poor 5-year overall survival of around 11.5%. Kirsten rat sarcoma virus *(KRAS)* mutation is seen in 90%-95% of PC patients and plays an important role in cancer cell proliferation, differentiation, metabolism, and survival, making it an essential mutation for targeted therapy. Despite extensive efforts in studying this oncogene, there has been little success in finding a drug to target this pathway, labelling it for decades as "undruggable". In this article we summarize some of the efforts made to target the KRAS pathway in PC, discuss the challenges, and shed light on promising clinical trials.

Key Words: Kirsten rat sarcoma virus; Targeted therapy; Pancreatic cancer; Drug resistance; Next generation sequencing; Clustered regularly interspaced short palindromic repeats

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Kirsten rat sarcoma virus (*KRAS*) mutation is the hallmark of pancreatic cancer (PC) and an important therapeutic target. Approaches to target this oncogene has been challenging. We herein discuss the role of *KRAS* in development of PC, efforts made to target this pathway, and ongoing clinical trials.

Citation: Elhariri A, Alhaj A, Ahn D, Sonbol MB, Bekaii-Saab T, Wu C, Rutenberg MS, Stauffer J, Starr J, Majeed U, Jones J, Borad M, Babiker H. Targeting *KRAS* in pancreatic adenocarcinoma: Progress in demystifying the holy grail. *World J Clin Oncol* 2023; 14(8): 285-296
URL: https://www.wjgnet.com/2218-4333/full/v14/i8/285.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i8.285

INTRODUCTION

In 2022, there was an estimated 62210 new pancreatic cancer (PC) cases and 49830 estimated deaths. PC is the fourth leading cause of cancer death in the United States[1]. PC is driven primarily by mutations in the Kirsten rat sarcoma virus (*KRAS*) gene, cyclin-dependent kinase inhibitor 2A, tumor protein 53, and mothers against decapentaplegic protein homolog 4. *KRAS* is one of the most frequently mutated oncogenes in human cancers. It is seen in more than 90% of PCs and more than 40% of colorectal and lung cancers[2]. 93% of all *KRAS* mutations occur at codon 12 (G12) with other common mutation sites at G13 and Q61. Missense mutation in glycine residues of G12 result in amino acid substitution, glycine substituted with aspartic acid (G12D), with valine (G12V), or with cysteine (G12C)[3]. The predominant mutation in PC is G12D followed by G12V (Figure 1)[4], but in lung cancer G12C is the most common. *KRAS* plays a major role in the development of PC and, as a result, there have been significant efforts to target the mutated *KRAS* pathway.

BACKGROUND

KRAS is a member of the rat sarcoma viral oncogene family (RAS), in addition to Neuroblastoma rat sarcoma virus and Harvey rat sarcoma virus. Identified in 1982, the *KRAS* is located on the short arm of chromosome 12[5,6]. It encodes two protein isoforms, KRAS-4B and KRAS-4A. Those are found in the inner side of the plasma membrane[7], and act as guanosine triphosphate (GTP)-binding proteins (G proteins), they bind guanine nucleotides that belong to the family of GTP-bound regulatory protein phosphatases (GTPase). An upstream signal *e.g.*, epidermal growth factor receptor (EGFR) stimulates the dissociation of guanosine diphosphate (GDP) from the GDP-bound G protein form, and allows the binding of GTP[8]. RAS functions as a binary switch, determined by two regulatory proteins called guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAP)[9]. KRAS binds to GDP in resting state due to its intrinsic GTPase activity. But with relevant stimuli, GEFs turn on signaling by catalyzing the exchange from a KRAS G-protein-bound GDP to GTP[10] (Figure 2). KRAS proteins can be activated by tyrosine kinase receptors, growth factors, chemokines, or calcium. This in turn activates multiple signaling pathways including the rapidly accelerated fibrosarcoma (RAF)-mitogen-activated protein kinase (MAPK)-extracellular regulated protein kinases (ERK) (MAPK/ERK; MEK) signaling pathway, the phosphoinositide 3-kinase (PI3K)-protein kinase (AKT)-mammalian target of rapamycin (mTOR) signaling pathway, and others. These pathways result in cell proliferation and DNA synthesis (Figure 3).

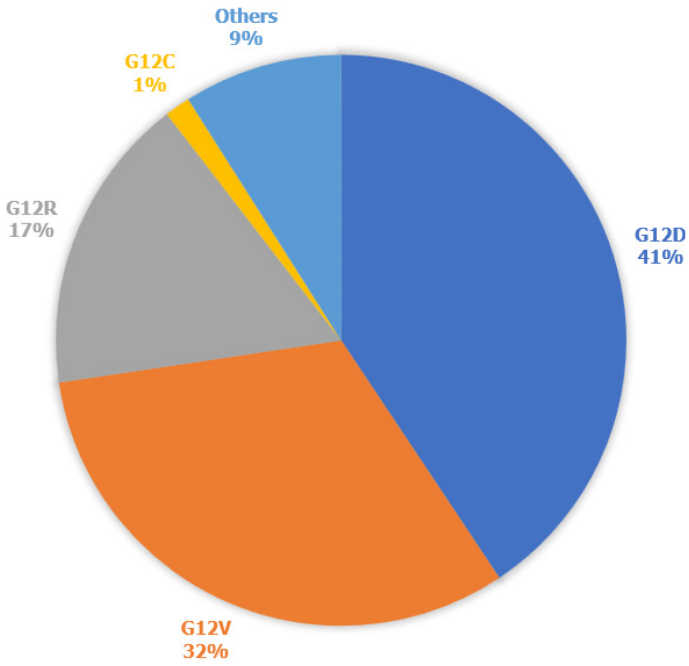
Precursor lesions of pancreatic ductal adenocarcinoma (PDAC) include pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm[11,12]. *KRAS* mutation was detected in 36% of PanIN-1A lesions and 87% of PanIN-2-3 lesions[13]. It was also found in 61% of patients with IPMN[14]. To study the role of *KRAS* in PC progression, scientists developed transgenic mice with inducible $KRAS^{G12D}$. Induction of oncogenic $KRAS^{G12D}$ altered normal epithelium and led to the development of precancerous lesions; on the other hand, inactivation of $KRAS^{G12D}$ in precursor lesions and during cancer progression led to disease regression[15]. These studies confirm the early role of *KRAS* mutation in the initiation and progression of precursor lesions into invasive PDAC as well as the correlation between frequency of *KRAS* mutation and degree of dysplasia.

KRAS mutation drives PC progression by resistance to apoptosis, induction of autophagy[16], immune evasion by downregulating major histocompatibility complex class I on tumor cells[17], and stimulating angiogenesis, resulting in cell survival and tumor progression.

TARGETED THERAPY

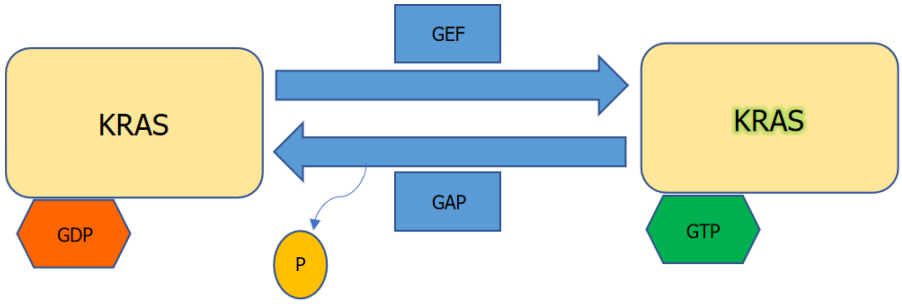
Upstream regulators

Some of the key regulators of KRAS include Son of Sevenless (SOS) and Src homology phosphatase 2 (SHP2). SOS is a GEF that activates KRAS, and SHP2 is a protein tyrosine phosphatase encoded by *PTPN11* that also promotes RAS activation, inhibiting either can delay tumor progression[18,19].



DOI: 10.5306/wjco.v14.i8.285 **Copyright** ©The Author(s) 2023.

Figure 1 Kirsten rat sarcoma virus mutations in pancreatic cancer. Types of Kirsten rat sarcoma virus (*KRAS*) mutations seen in pancreatic cancer, according to data publicly available on cBioPortal. 812 samples with altered *KRAS* collected from 5 pancreatic cancer studies. Others are A11T, A146T, A18V, G12A, G12I, G12L, G12S, G13C, G13D, G13H, G13P, G13R, L23V, Q61H, Q61K, Q61R.



DOI: 10.5306/wjco.v14.i8.285 **Copyright** ©The Author(s) 2023.

Figure 2 Kirsten rat sarcoma virus activation. Kirsten rat sarcoma virus is activated when guanine nucleotide exchange factor displaces guanosine diphosphate from nucleotide binding site allowing guanosine triphosphate (GTP) binding and inactivated upon GTP hydrolysis by intrinsic GTP-bound regulatory protein phosphatases (GTPase) activity enhanced by GTPase activating protein. GTP: Guanosine triphosphate; GAP: GTPase activating protein; GDP: Guanosine diphosphate; GEF: Guanine nucleotide exchange factor; KRAS: Kirsten rat sarcoma virus.

BI-3406 inhibits the interaction between KRAS and SOS1 which has been shown to cause tumor regression in KRAS-driven cancer cell models. Synergy was observed with SOS1/MEK inhibitors as this combination can counteract adaptive resistance to MEK inhibitors[20]. ERAS-601 is a small molecule allosteric inhibitor of SHP2 that stops KRAS from cycling into its GTP-active state, which inhibits cellular proliferation in multiple $KRAS^{G12C}$ mutated tumor cell models[21]. Recently the Food and Drug Administration (FDA) granted fast track designation to BBP-398 (SHP2 inhibitor) in combination with Sotorasib for $KRAS^{G12C}$-mutated metastatic non-small-cell lung carcinoma (NSCLC). There is an ongoing trial to evaluate the safety and efficacy of this combination [national clinical trial (NCT) 05480865]. Combination of $KRAS^{G12C}$ inhibitor (JAB-21822) and SHP2 inhibitor (JAB-3312) showed synergistic effect in $KRAS^{G12C}$-resistant tumor cells[22], currently in phase I/II trial for PDAC (NCT05288205).

MAPK/ERK pathway

The MAPK/ERK pathway was shown in Table 1.

KRAS

Direct inhibition of the KRAS protein remains a challenge, due to its small size of 21 kDa and the lack of hydrophobic pockets on its surface. Those pockets, if found, can then be blocked by small molecules and ultimately disrupt its interaction with other proteins[23]. Several attempts have been made to directly target KRAS, but results were non-

Table 1 Kirsten rat sarcoma virus-rapidly accelerated fibrosarcoma-mitogen-activated protein kinase/extracellular regulated protein kinases-extracellular regulated protein kinases pathway inhibitors

Agent	FDA approved[1]	Clinical trials[2]		
		Conditions (phase)	Combination	NCT number
SOS inhibitors				
BI-1701963	N/A	Advanced solid tumors (I); advanced solid tumors (I); metastatic colorectal cancer (I)	Trametinib; BI 3011441; irinotecan	NCT04111458; NCT04835714; NCT04627142
SHP2 inhibitors				
ERAS-601	N/A	Advanced/ metastatic solid tumors (I)	Cetuximab, pembrolizumab	NCT04670679
JAB-3312	N/A	Advanced solid tumors (I); advanced solid tumors (I/II)	N/A; binimetinib, pembrolizumab, sotorasib, osimertinib	NCT04045496; NCT04720976
BBP-398 (IACS-15509)	(+ sotorasib) fast track designation for metastatic NSCLC	Advanced solid tumor (I); advanced NSCLC (I); advanced solid tumors (I); advanced solid tumors (I)	N/A; nivolumab; N/A; sotorasib	NCT05621525; NCT05375084; NCT04528836; NCT05480865
RLY-1971	N/A	Advanced/metastatic solid tumors (I)	N/A	NCT04252339
TNO155	N/A	Advanced solid tumors (I); advanced solid tumors (I)	EGF816 (nazartinib); spartalizumab, ribociclib	NCT03114319; NCT04000529
RMC-4630	N/A	Relapsed/refractory solid tumors (I); NSCLC (II); metastatic KRAS mutant cancers (I); relapsed/refractory solid tumors, locally advanced/metastatic EGFR positive NSCLC (I/II)	N/A; sotorasib LY3214996; cobimetinib, osimertinib	NCT03634982; NCT05054725; NCT04916236; NCT03989115
Direct KRAS inhibitors				
G12C				
Sotorasib (AMG 510, Lumakras)	Advanced NSCLC	Colorectal cancer (III); advanced solid tumors (Ib/II)	Panitumumab; N/A	NCT05198934; NCT04185883
Adagrasib (MRTX849, Krazati)	Locally advanced or metastatic NSCLC	Metastatic PC (Ib); colorectal cancer (I); solid tumors (I/II); advanced solid tumors (I); advanced/metastatic cancers (I/II)	N/A; cetuximab and irinotecan; N/A; BI-1701963; TNO155	NCT05634525; NCT05722327; NCT05162443; NCT04975256; NCT04330664
JNJ-74699157	N/A	Advanced solid tumors (I)	N/A	NCT04006301
LY3499446	N/A	Advanced solid tumors (I/II)	Abemaciclib, cetuximab, erlotinib, docetaxel	NCT04165031
GDC 6036	N/A	Advanced/metastatic solid tumors (I)	Atezolizumab, cetuximab, bevacizumab, erlotinib, GDC-1971, inavolisib	NCT04449874
D-1553	N/A	Advanced/metastatic solid tumors (I/II); NSCLC (I/II)	N/A; N/A	NCT04585035; NCT05383898
G12D				
MRTX1133	N/A	Pancreatic, lung, and colorectal cancers (I/II)	N/A	Enters phase I in 2023
Tricomplex inhibitors				
RMC-6236	N/A	Advanced solid tumors (I)	N/A	NCT05379985
RMC-6291	N/A	Advanced solid tumors (I)	N/A	NCT05462717
RAF inhibitors				
Sorafenib (BAY43-9006, NEXAVAR)	Unresectable HCC; advanced RCC; thyroid cancer	PC that cannot be removed by surgery (II); unresectable PC (I); metastatic PC (II)	Erlotinib; gemcitabine, sorafenib and radiotherapy; alone or with gemcitabine	NCT00837876; NCT00375310; NCT00114244
Vemurafenib (PLX4032, RG7204,	BRAF V600E melanoma, ECD	PC (II)	Sorafenib	NCT05068752

RO5185426, ZELBORAF)				
Dabrafenib (GSK2118436, TAFINLAR)	(+ Trametinib) BRAF V600E or V600K melanoma, NSCLC, anaplastic thyroid cancer, solid tumors	Colorectal cancer (II); advanced/metastatic BRAF V600 colorectal cancer (I)	Trametinib + PDR001; trametinib, LTT462, LXH254, TNO155, spartalizumab, tislelizumab	NCT03668431; NCT04294160
Encorafenib (BRAFTOVI)	BRAF V600E metastatic colorectal cancer	Localized colon or upper rectum cancer with BRAF V600E mutation (II)	Cetuximab	NCT05706779
Regorafenib (BAY 73-4506, STIVARGA)	Metastatic colorectal cancer; advanced GIST	Solid tumors (II)	Nivolumab	NCT04704154
Lifirafeni (BGB-283)	N/A	Advanced or refractory solid tumors (I/II)	Mirdametinib	NCT03905148
Paradox breakers				
PLX7904/ PLX8394 (PB04)	N/A	Advanced cancers (I/IIa)	N/A	NCT02012231
Pan-RAF inhibitors				
LY3009120	N/A	Advanced cancer (I)	N/A	NCT02014116
MLN2480 (BIIB-024, TAK580, Tovorafenib)	N/A	Relapsed or refractory solid tumors followed by a dose expansion in participants with metastatic melanoma (I); advanced non-hematologic malignancies (I)	N/A; MLN0128 or alisertib, or paclitaxel, or cetuximab, or irinotecan	NCT01425008; NCT02327169
HM95573 (Belvarafenib)	N/A	Locally advanced or metastatic solid tumors (I)	Cobimetinib or cetuximab	NCT03284502
BMS-908662 (XL281)	N/A	Advanced or metastatic colorectal cancer (I/II); advanced solid tumors (I)	Alone or with cetuximab; N/A	NCT01086267; NCT00451880
MEK inhibitors				
Trametinib (GSK1120212, JTP-74057)	(+Dabrafenib) BRAF V600E or V600K melanoma, NSCLC, anaplastic thyroid cancer, solid tumors	Cancers with BRAF V600E mutations (II); solid tumors (I); PC (II); metastatic PC (II); biliary tract cancer (II)	Dabrafenib; gemcitabine; SBRT + pembrolizumab; gemcitabine; N/A	NCT04439292; NCT01428427; NCT02704156; NCT01231581; NCT01943864
Cobimetinib (XL-518, GDC-0973, RG7421, Cotellic)	Histiocytic neoplasms, melanoma	PC (I); locally advanced or metastatic PC (I)	N/A; calaspargase Pegol-mknl	NCT04005690; NCT05034627
Selumetinib (AZD6244, ARRY-142886, Koselugo)	Pediatric neurofibromatosis type 1	Advanced or metastatic PC who have failed first line gemcitabine (II); locally advanced or metastatic pancreatic cancer with KRAS G12R mutations (II); metastatic pancreatic cancer previously treated with chemotherapy (II); locally advanced or metastatic PC (II)	N/A; N/A; MK2206 (Akt inhibitor) or mFOLFOX; erlotinib hydrochloride	NCT00372944; NCT03040986; NCT01658943; NCT01222689
Binimetinib (ARRY-438162, ARRY-162, MEK162, MektoviI)	Unresectable or metastatic melanoma with a BRAF V600E mutation	Advanced BRAF mutant cancers (I/II); PC with somatic BRAF V600E mutation (II); advanced solid tumors harboring RAS or BRAFV60330E mutations (I)	Encorafenib; Encorafenib; RAF 265	NCT03843775; NCT04390243; NCT01352273
Pimasertib (AS703026, SAR24550, EMD1036239, MSC1936369B)	N/A	PC (I/II)	Gemcitabine	NCT01016483
Refametinib (RDEA119, BAY86-9766)	N/A	Advanced or metastatic cancer (I); RAS-mutant hepatocellular carcinoma (II); advanced cancer (Ib)	Regorafenib; N/A; copanlisib	NCT02168777; NCT01915589; NCT01392521
E6201 (ER 806201)	N/A	BRAF V600 mutated metastatic melanoma (I); advanced solid tumors (I)	Dabrafenib; N/A	NCT05388877; NCT00794781
PD-0325901 (Mirdametinib)	N/A	Advanced cancer (I)	PF-05212384 or Irinotecan	NCT01347866
AZD8330 (ARRY-424704, ARRY-704)	N/A	Advanced malignancies (I)	N/A	NCT00454090
GDC-0623 (RG7420, G-868)	N/A	Locally advanced or metastatic solid tumors (I)	N/A	NCT01106599

RO4987655 (CH4987655, RG7167)	N/A	Advanced solid tumors (I)	N/A	NCT00817518
RO5126766 (CH5126766, RG7304)	N/A	Advanced solid tumors (I)	N/A	NCT00773526
TAK733	N/A	Advanced nonhematologic malignancies (I)	N/A	NCT00948467
ERK inhibitors				
Ulixertinib (BVD-523)	N/A	Advanced pancreatic and other solid tumors (I); metastatic PC (I); advanced MAPK pathway-altered malignancies	Palbociclib; Nab-paclitaxel and gemcitabine; N/A	NCT03454035; NCT02608229; NCT04566393
GDC-0994 (RG7842)	N/A	Locally advanced or metastatic solid tumors (I)	N/A	NCT01875705
MK-8353 (SCH900353)	N/A	Advanced/metastatic solid tumors (I); advanced malignancies (I)	Selumetinib; pembrolizumab	NCT03745989; NCT02972034
JSI-1187	N/A	Advanced solid tumors with MAPK pathway mutations (I)	Alone or with dabrafenib	NCT04418167
ERAS-007	N/A	Advanced or metastatic solid tumors (I/II); advanced gastrointestinal malignancies (I/II)	ERAS-601; encorafenib, cetuximab, palbociclib	NCT04866134; NCT05039177
Menin inhibitor				
BMF-219	N/A	NSCLC, pancreatic, colorectal cancers (I)	N/A	NCT05631574

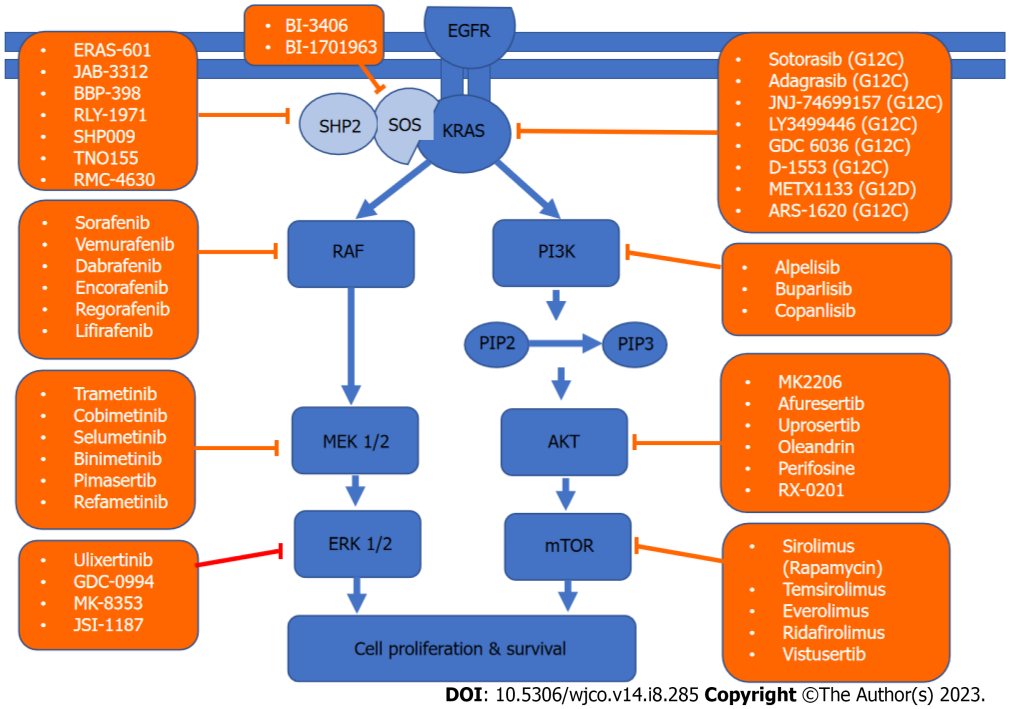
[1]www.fda.gov.
[2]clinicaltrials.gov.
FDA: Food and Drug Administration; SOS: Son of Sevenless; KRAS: Kirsten rat sarcoma virus; HCC: Hepatocellular carcinoma; RCC: Renal cell carcinoma; ECD: Erdheim-Chester disease; GIST: Gastrointestinal stroma tumors; PC: Pancreatic cancer; RAF: Rapidly accelerated fibrosarcoma; RAS: Rat sarcoma viral oncogene family; MAPK: Mitogen-activated protein kinases; NSCLC: Non-small-cell lung carcinoma; SHP2: Src homology-2 domain-containing protein tyrosine phosphatase-2; NCT: National clinical trial; MEK: Mitogen-activated protein kinase/extracellular regulated protein kinases; N/A: Not applicable.

satisfactory[24-26]. Only recently AMG 510 (sotorasib) was developed to target G12C mutation in NSCLC without inhibiting wild-type *KRAS*[27]. Adagrasib (MRTX849) which is also a $KRAS^{G12C}$ inhibitor is well tolerated, and preliminary results showed partial response in 50% of patients with PDAC harboring this mutation[28]. However, $KRAS^{G12C}$ only occurs in 1%-2% PC and attempts to target more common *KRAS* isoforms have failed. One promising compound is MRTX1133, a small molecule that selectively inhibits $KRAS^{G12D}$ by preventing SOS-catalyzed nucleotide exchange. Subsequently, it promotes tumor regression in immunocompetent PC models and alters the tumor microenvironment by increasing tumor associated macrophages (TAM) and tumor-infiltrating cytotoxic T-cells. MRTX1133 is expected to enter phase I trial in 2023[29,30]. Other agents inhibiting G12D in the pre-clinical phase include BI-KRASG12D, JAB-22000, and ERAS-4. A new category of drugs called tricomplex inhibitors has shown promising results in pre-clinical models of $KRAS^{G12V}$ mutant cancers[31] and in a phase I trial RMC-6236 in $KRAS^{G12}$-mutant advanced solid tumors excluding G12C (NCT05379985). A recent study was able to selectively target $KRAS^{G12R}$ using a small molecule electrophile[32]. Due to the challenging nature of direct KRAS inhibition focus was shifted on downstream signaling, knowing that some of the challenges include compensation by other pathways, and that inhibiting multiple pathways can result in toxicity[33].

Multiple mechanisms are implicated in the inevitable drug resistance seen with KRAS inhibitors, either by activation of wild-type KRAS which is mediated by receptor tyrosine kinase[34], synthesizing new $KRAS^{G12C}$ proteins in response to MAPK suppression[35], or developing secondary mutations in KRAS inhibitor binding pocket[36].

RAF

With regards to drugs targeting the MAPK pathway, sorafenib was the first RAF inhibitor to be FDA-approved, initially for advanced renal cell carcinoma, followed by unresectable/metastatic hepatocellular carcinoma and metastatic differentiated thyroid cancer[37]. In a phase II trial combining sorafenib and erlotinib, 12 of the first 15 patients required dose delays or reductions due to toxicity, and the study failed to reach its primary endpoint of 8-week progression-free survival (PFS)[38]. A second-generation of RAF inhibitors (*e.g.*, vemurafenib and dabrafenib) was proven to be effective in *BRAF V600E* mutant metastatic melanoma[39]. Dabrafenib in combination with trametinib received a tumor agnostic accelerated approval for treatment of unresectable/metastatic solid tumors with *BRAF V600E* mutation that progressed on prior treatment[40]. Unfortunately, vemurafenib and dabrafenib were not as effective in *KRAS*-mutant cancers, due to compensatory ERK activation that led to enhanced tumor growth[41,42]. A third generation of RAF inhibitors called "paradox breakers" (PLX7904 and PLX8394) also blocks MEK-ERK1/2, which can overcome this resistance mechanism [43], Unfortunately, a phase I/II trial to evaluate the safety of PLX8394 was terminated due to low accrual. Recently, another group called "pan-RAF inhibitors" (LY3009120, MLN2480, and HM95573) entered phase I trials. LY3009120 is a kinase inhibitor that showed efficacy in inhibiting mutated *KRAS* and *BRAF* in preclinical models of colorectal cancer



DOI: 10.5306/wjco.v14.i8.285 **Copyright** ©The Author(s) 2023.

Figure 3 Kirsten rat sarcoma virus signaling network and targeted therapy. A schematic of the two major Kirsten rat sarcoma virus pathways driving cell survival and drugs that target them. KRAS: Kirsten rat sarcoma virus; AKT: Protein kinase; EGFR: Epidermal growth factor receptor; PIP: Prolactin-induced protein; ERK: Extracellular regulated protein kinases; MEK: Mitogen-activated protein kinase/extracellular regulated protein kinases; mTOR: Mammalian target of rapamycin; PI3K: Phosphoinositide 3-kinase; RAF: Rapidly accelerated fibrosarcoma; SHP2: Src homology-2 domain-containing protein tyrosine phosphatase-2; SOS: Son of sevenless.

with minimal paradoxical MAPK activation[44,45], however, a phase I trial in advanced cancers was terminated early due to lack of sufficient clinical efficacy (NCT02014116). MLN2480 (tovorafenib) showed an acceptable safety profile[46], and HM95573 (belvarafenib) was well tolerated and showed anti-tumor activity in advanced solid tumors with RAS or RAF mutations[47]. The Yes-associated protein (YAP) is a transcription coregulator downstream from KRAS that promotes cell proliferation[48]. Combining LY3009120 and YAP-inhibitor (verteporfin) showed anti-tumor effect *in vivo* and *in vitro* by blocking compensatory activation of AKT pathway[49].

MEK

As mentioned above, trametinib is a MEK1/2 inhibitor FDA approved in combination with dabrafenib (RAF-inhibitor) as a tumor agnostic drug[50]. Trametinib was studied in combination with gemcitabine in a placebo controlled clinical trial for untreated metastatic PDAC. Unfortunately, it did not show improvement in overall survival (OS), PFS, or overall response rate (ORR)[51]. This is potentially due to a compensatory mechanism called autophagy, initiated through activation of the AKT pathway[52]. A Phase II trial of selumetinib (MEK1/2 kinase inhibitor) in PC did not show any significant difference in OS when compared to capecitabine[53], another phase II study of selumetinib targeting only PC patients with $KRAS^{G12R}$ mutation after at least two lines of prior systemic chemotherapy did not improve ORR, however, three patients had stable disease for ≥ 6 months[54]. A phase I/II trial studied the selective MEK1/2 inhibitor pimasertib in combination with gemcitabine *vs* gemcitabine alone in patients with metastatic PC. Despite the promising safety and efficacy of this combination, it did not improve PFS or OS[55]. Unfortunately, in whole there was no observed clinical benefit of MEK inhibitors in the multiple trials done in PC.

ERK

After resistance to BRAF and MEK inhibitors, the next downstream target is ERK. SCH772984[56] is a selective inhibitor of ERK1/2 that showed tumor regression in xenograft models refractory to BRAF and MEK inhibitors. Similar effects were seen with ulixertinib[57]. A phase Ib trial combining ERK1/2 inhibitor (GDC-0994) and MEK inhibitor (cobimetinib) in advanced solid tumors was terminated due to tolerability issues[58]. The ERK1/2 inhibitor JSI-1187-01 demonstrated pre-clinical efficacy in tumor models with MAPK pathway mutations, as well as synergy with BRAF inhibitors[59], and is being studied in a phase I trial (NCT04418167).

PI3K-AKT-mTOR-pathway

The PI3K-AKT-mTOR-pathway was shown in Table 2. One of the postulated reasons EGFR inhibitors and other targeted therapies develop resistance is the hyper-activation of PI3K-AKT-mTOR pathway, which can drive cancer progression and survival. PI3K is overexpressed in around 50% of patients with PC[60], and AKT2 is amplified in 10%-20% of PDAC [61]. TAM plays a role in the development of PC[62] by creating an immune-suppressive microenvironment, minimizing

Table 2 Phosphoinositide 3-kinase-protein kinase-mammalian target of rapamycin-pathway inhibitors

Agent	Combination	Phase	NCT number[1]
PI3K inhibitors (p110α) isoform			
Alpelisib (BYL719)	Gemcitabine and abraxane	I	NCT02155088
Buparlisib (BKM120)	mFOLFOX6; trametinib (MEKi)	I; I	NCT01571024; NCT01155453
Pan-PI3K inhibitors			
Copanlisib(BAY 80–6946)	N/A	I	NCT00962611
PI3K and mTOR inhibitors			
Voxtalisib (SAR245409, *XL765*)	N/A	I	NCT00485719
Dactolisib(NVP-BEZ235)	MEK162 (MEKi)	I	NCT01337765
Gedatolisib (PF-05212384, PKI-587)	Palbociclib (CDKi)	I	NCT03065062
Pan-Akt inhibitors			
MK2206	Monotherapy; dinaciclib (CDKi); selumitinib (MEKi) vs mFOLFOX6	I; I; II	NCT00848718; NCT01783171; NCT01658943
Afuresertib (GSK2110183)	Trametinib (MEKi); N/A	I; II	NCT01476137; NCT01531894
Uprosertib (GSK2141795)	Trametinib (MEKi)	I	NCT01138085
Oleandrin (PBI-05204)	N/A	II	NCT02329717
Perifosine	N/A	II; II	NCT00053924; NCT00059982
RX-0201	Gemcitabine	II	NCT01028495
Rapalogs (mTORC1 inhibitors)			
Sirolimus (rapamycin)	Sunitinib (RTKi); N/A; metformin; vismodegib (SMOi)	I; II; I/II; I	NCT00583063; NCT00499486; NCT02048384; NCT01537107
Temsirolimus (*CCI-779*, Torisel)	Lenalidomide; gemcitabine; nivolumab (PD-1i)	I; I; I/II	NCT01183663; NCT00593008; NCT02423954
Everolimus (RAD001)	Sorafenib (RTKi); trametinib (MEKi); gemcitabine; cetuximab (EGFRi) and capecitabine; N/A	I; I; I/II; I/II; II	NCT00981162; NCT00955773; NCT00560963; NCT01077986; NCT00409292
Ridafirolimus	Bevacizumab (VEGFRi)	I	NCT00781846
mTORC1/2 inhibitors			
Vistusertib (AZD2014)	N/A; selumitinib (MEKi); olaparib (PARPi)	I; II; II	NCT01026402; NCT02583542; NCT02576444

[1]clinicaltrials.gov

PI3K: Phosphoinositide 3-kinase; NCT: National clinical trial; MEKi: Mitogen-activated protein kinase/ extracellular regulated protein kinases inhibitor; CDKi: Cyclin-dependent kinase inhibitor; RTKi: Receptor tyrosine kinase inhibitor; SMOi: Smoothened inhibitor; PD-1i: Programmed cell death receptor-1 inhibitor; EGFRi: Epidermal growth factor receptor inhibitor; VEGFRi: Vascular endothelial growth factor receptor inhibitor; mTOR: mammalian target of rapamycin; PARPi: Poly (ADP-ribose) polymerase inhibitor; N/A: Not applicable.

the antitumor effect of T-cells[63]. PI3K helps drive this immune suppression, so its inhibition can restore immune response against cancer cells as well as potentiate the effect of chemotherapy[64]. Additionally, AKT mediates an anti-apoptotic effect and plays a role in chemoresistance[65]. Phosphatase and tensin homolog is a tumor suppressor of the AKT/mTOR pathway, its loss has been implicated in PC development, recurrence, and prognosis[66], as well as acceleration of *KRAS*^{G12D}-induced PDAC in mice[67]. An *in vivo* study tested PI3Kα-specific inhibitor (BYL) in combination with an EGFR inhibitor (erlotinib) and showed reduced tumor volume and apoptosis in PDAC cell lines[68]. Currently a clinical trial combining gedatolisib (PI3K/mTOR inhibitor) with palbociclib (CDK4/6 inhibitor) in advanced squamous cell cancers of the lung, pancreas, and solid tumors is recruiting (NCT03065062). A phase I/II trial studied the safety and efficacy of combining everolimus (mTOR inhibitor), cetuximab (EGFR inhibitor), and capecitabine, however, the combination resulted in significant epidermal and mucosal toxicities with minimal efficacy[69].

Small interfering RNA, MicroRNA, and clustered regularly interspaced short palindromic repeats

Pre-clinical studies show that small interfering RNAs (siRNAs) have potential in cancer treatment. To deliver siRNAs to target cancer cells, scientists devised two unique methods, one utilized nanoparticle[70] to target lung cancer cells and another study used a biodegradable polymeric matrix (LODER) to carry the anti *KRAS*^{G12D} siRNA. This resulted in the

decrease of KRAS levels and inhibited cell proliferation[71]. MicroRNAs (miRNA) regulate cell proliferation and contribute to PC development. Depending on their role they can act as tumour suppressor or oncogenic miRNAs[72,73]. MRX34 (miRNA-34 mimic) was used in a phase I clinical trial that utilized lipid-based vesicles (NOV40) as a delivery vector, for treating patients with advanced solid tumors. miRNA-96 directly targets *KRAS* oncogene decreasing PC cell invasion and slowing tumor growth both *in vivo* and *in vitro*[74]. Clustered regularly interspaced short palindromic repeat (CRISPR) is currently being studied in *KRAS*-mutated cancers. This technology is being harnessed to target inactivated tumor suppressor genes or overactive oncogenes. In a 2018 study CRISPR-Cas13a was developed to target $KRAS^{G12D}$ mRNA. Subsequently, it also suppressed downstream ERK and AKT proteins resulting in apoptosis and significant tumor suppression *in vivo* and *in vitro*[75]. Two phase I trials utilizing the CRISPR platform are currently ongoing in PC (NCT04426669 and NCT04842812).

CONCLUSION

KRAS mutation remains the hallmark genetic aberration leading to PC. Although several studies have demonstrated positive preclinical results, the resulting clinical trial results have been largely disappointing. As we continue to have a deeper understanding of the KRAS pathway, resistance mechanisms, and the role and function of the immune system; we get closer to developing effective therapies to outsmart the scourge that is PC. Ongoing clinical trials targeting more common *KRAS* mutations in PC will hopefully lead to more effective therapy and change the outcomes for the thousands of patients affected by this disease every year.

ACKNOWLEDGEMENTS

Dr. Babiker is a Paul Calabresi Scholar at the Mayo Clinic Cancer Center and acknowledges K-12 grant Program, K12CA090628.

FOOTNOTES

Author contributions: Elhariri A and Babiker H designed and wrote the manuscript; Alhaj A, Ahn D, Sonbol MB, Bekaii-Saab T, Wu C, Rutenberg MS, Stauffer J, Starr J, Majeed U, Jones J and Borad M critically reviewed and edited the manuscript; All authors approved the final version of the manuscript.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: United States

ORCID number: Ahmed Elhariri 0000-0002-3715-4842.

S-Editor: Lin C
L-Editor: A
P-Editor: Xu ZH

REFERENCES

1 **Siegel RL**, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin* 2022; **72**: 7-33 [PMID: 35020204 DOI: 10.3322/caac.21708]
2 **Huang L**, Guo Z, Wang F, Fu L. KRAS mutation: from undruggable to druggable in cancer. *Signal Transduct Target Ther* 2021; **6**: 386 [PMID: 34776511 DOI: 10.1038/s41392-021-00780-4]
3 **Veluswamy R**, Mack PC, Houldsworth J, Elkhouly E, Hirsch FR. KRAS G12C-Mutant Non-Small Cell Lung Cancer: Biology, Developmental Therapeutics, and Molecular Testing. *J Mol Diagn* 2021; **23**: 507-520 [PMID: 33618059 DOI: 10.1016/j.jmoldx.2021.02.002]
4 **Cerami E**, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C, Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012; **2**: 401-404 [PMID: 22588877 DOI: 10.1158/2159-8290.CD-12-0095]
5 **McBride OW**, Swan DC, Tronick SR, Gol R, Klimanis D, Moore DE, Aaronson SA. Regional chromosomal localization of N-ras, K-ras-1, K-ras-2 and myb oncogenes in human cells. *Nucleic Acids Res* 1983; **11**: 8221-8236 [PMID: 6672765 DOI: 10.1093/nar/11.23.8221]
6 **Shen X**, Niu N, Xue J. Oncogenic KRAS triggers metabolic reprogramming in pancreatic ductal adenocarcinoma. *J Transl Int Med* 2022; AOP [DOI: 10.2478/jtim-2022-0022]

7 **Santos E**, Nebreda AR. Structural and functional properties of ras proteins. *FASEB J* 1989; **3**: 2151-2163 [PMID: 2666231 DOI: 10.1096/fasebj.3.10.2666231]

8 **Takai Y**, Kaibuchi K, Kikuchi A, Kawata M. Small GTP-binding proteins. *Int Rev Cytol* 1992; **133**: 187-230 [PMID: 1577587 DOI: 10.1016/s0074-7696(08)61861-6]

9 **Drugan JK**, Rogers-Graham K, Gilmer T, Campbell S, Clark GJ. The Ras/p120 GTPase-activating protein (GAP) interaction is regulated by the p120 GAP pleckstrin homology domain. *J Biol Chem* 2000; **275**: 35021-35027 [PMID: 10954709 DOI: 10.1074/jbc.M004386200]

10 **Bos JL**, Rehmann H, Wittinghofer A. GEFs and GAPs: critical elements in the control of small G proteins. *Cell* 2007; **129**: 865-877 [PMID: 17540168 DOI: 10.1016/j.cell.2007.05.018]

11 **Singh M**, Maitra A. Precursor lesions of pancreatic cancer: molecular pathology and clinical implications. *Pancreatology* 2007; **7**: 9-19 [PMID: 17449961 DOI: 10.1159/000101873]

12 **Bian Y**, Jiang H, Zheng J, Shao C, Lu J. Basic Pancreatic Lesions: Radiologic-pathologic Correlation. *J Transl Int Med* 2022; **10**: 18-27 [PMID: 35702187 DOI: 10.2478/jtim-2022-0003]

13 **Löhr M**, Klöppel G, Maisonneuve P, Lowenfels AB, Lüttges J. Frequency of K-ras mutations in pancreatic intraductal neoplasias associated with pancreatic ductal adenocarcinoma and chronic pancreatitis: a meta-analysis. *Neoplasia* 2005; **7**: 17-23 [PMID: 15720814 DOI: 10.1593/neo.04445]

14 **Lee JH**, Kim Y, Choi JW, Kim YS. KRAS, GNAS, and RNF43 mutations in intraductal papillary mucinous neoplasm of the pancreas: a meta-analysis. *Springerplus* 2016; **5**: 1172 [PMID: 27512631 DOI: 10.1186/s40064-016-2847-4]

15 **Collins MA**, Bednar F, Zhang Y, Brisset JC, Galbán S, Galbán CJ, Rakshit S, Flannagan KS, Adsay NV, Pasca di Magliano M. Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. *J Clin Invest* 2012; **122**: 639-653 [PMID: 22232209 DOI: 10.1172/JCI59227]

16 **Ferreira A**, Pereira F, Reis C, Oliveira MJ, Sousa MJ, Preto A. Crucial Role of Oncogenic KRAS Mutations in Apoptosis and Autophagy Regulation: Therapeutic Implications. *Cells* 2022; **11** [PMID: 35883626 DOI: 10.3390/cells11142183]

17 **Dias Carvalho P**, Guimarães CF, Cardoso AP, Mendonça S, Costa ÂM, Oliveira MJ, Velho S. KRAS Oncogenic Signaling Extends beyond Cancer Cells to Orchestrate the Microenvironment. *Cancer Res* 2018; **78**: 7-14 [PMID: 29263151 DOI: 10.1158/0008-5472.CAN-17-2084]

18 **Ruess DA**, Heynen GJ, Ciecielski KJ, Ai J, Berninger A, Kabacaoglu D, Görgülü K, Dantes Z, Wörmann SM, Diakopoulos KN, Karpathaki AF, Kowalska M, Kaya-Aksoy E, Song L, van der Laan EAZ, López-Alberca MP, Nazaré M, Reichert M, Saur D, Erkan MM, Hopt UT, Sainz B Jr, Birchmeier W, Schmid RM, Lesina M, Algül H. Mutant KRAS-driven cancers depend on PTPN11/SHP2 phosphatase. *Nat Med* 2018; **24**: 954-960 [PMID: 29808009 DOI: 10.1038/s41591-018-0024-8]

19 **Kessler D**, Gerlach D, Kraut N, McConnell DB. Targeting Son of Sevenless 1: The pacemaker of KRAS. *Curr Opin Chem Biol* 2021; **62**: 109-118 [PMID: 33848766 DOI: 10.1016/j.cbpa.2021.02.014]

20 **Hofmann MH**, Gmachl M, Ramharter J, Savarese F, Gerlach D, Marszalek JR, Sanderson MP, Kessler D, Trapani F, Arnhof H, Rumpel K, Botesteanu DA, Ettmayer P, Gerstberger T, Kofink C, Wunberg T, Zoephel A, Fu SC, Teh JL, Böttcher J, Pototschnig N, Schachinger F, Schipany K, Lieb S, Vellano CP, O'Connell JC, Mendes RL, Moll J, Petronczki M, Heffernan TP, Pearson M, McConnell DB, Kraut N. BI-3406, a Potent and Selective SOS1-KRAS Interaction Inhibitor, Is Effective in KRAS-Driven Cancers through Combined MEK Inhibition. *Cancer Discov* 2021; **11**: 142-157 [PMID: 32816843 DOI: 10.1158/2159-8290.CD-20-0142]

21 **Martin L**, Patel R, Zhang J, Nevarez R, Congdon T, Brail L, Shoemaker B. Abstract 2670: ERAS-601, a potent allosteric inhibitor of SHP2, synergistically enhances the efficacy of sotorasib/adagrasib and cetuximab in NSCLC, CRC, and HNSCC tumor models. *Cancer Res* 2022; **82** Suppl 12: 2670 [DOI: 10.1158/1538-7445.Am2022-2670]

22 **Wang P**, Zheng Q, Kang D, Sun X, Zhu S, Wang Y, Long W, Lin Y. 30P Investigation of KRAS G12C inhibitor JAB-21822 as a single agent and in combination with SHP2 inhibitor JAB-3312 in preclinical cancer models. *Ann Oncol* 2022; **33** Suppl 9: S1441 [DOI: 10.1016/j.annonc.2022.10.040]

23 **Wang W**, Fang G, Rudolph J. Ras inhibition *via* direct Ras binding--is there a path forward? *Bioorg Med Chem Lett* 2012; **22**: 5766-5776 [PMID: 22902659 DOI: 10.1016/j.bmcl.2012.07.082]

24 **Taveras AG**, Remiszewski SW, Doll RJ, Cesarz D, Huang EC, Kirschmeier P, Pramanik BN, Snow ME, Wang YS, del Rosario JD, Vibulbhan B, Bauer BB, Brown JE, Carr D, Catino J, Evans CA, Girijavallabhan V, Heimark L, James L, Liberles S, Nash C, Perkins L, Senior MM, Tsarbopoulos A, Webber SE. Ras oncoprotein inhibitors: the discovery of potent, ras nucleotide exchange inhibitors and the structural determination of a drug-protein complex. *Bioorg Med Chem* 1997; **5**: 125-133 [PMID: 9043664 DOI: 10.1016/s0968-0896(96)00202-7]

25 **Peri F**, Airoldi C, Colombo S, Martegani E, van Neuren AS, Stein M, Marinzi C, Nicotra F. Design, synthesis and biological evaluation of sugar-derived Ras inhibitors. *Chembiochem* 2005; **6**: 1839-1848 [PMID: 16196015 DOI: 10.1002/cbic.200400420]

26 **Herrmann C**, Block C, Geisen C, Haas K, Weber C, Winde G, Möröy T, Müller O. Sulindac sulfide inhibits Ras signaling. *Oncogene* 1998; **17**: 1769-1776 [PMID: 9778042 DOI: 10.1038/sj.onc.1202085]

27 **Janes MR**, Zhang J, Li LS, Hansen R, Peters U, Guo X, Chen Y, Babbar A, Firdaus SJ, Darjania L, Feng J, Chen JH, Li S, Long YO, Thach C, Liu Y, Zarieh A, Ely T, Kucharski JM, Kessler LV, Wu T, Yu K, Wang Y, Yao Y, Deng X, Zarrinkar PP, Brehmer D, Dhanak D, Lorenzi MV, Hu-Lowe D, Patricelli MP, Ren P. Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor. *Cell* 2018; **172**: 578-589.e17 [PMID: 29373830 DOI: 10.1016/j.cell.2018.01.006]

28 **Ou SI**, Jänne PA, Leal TA, Rybkin II, Sabari JK, Barve MA, Bazhenova L, Johnson ML, Velastegui KL, Cilliers C, Christensen JG, Yan X, Chao RC, Papadopoulos KP. First-in-Human Phase I/IB Dose-Finding Study of Adagrasib (MRTX849) in Patients With Advanced KRAS(G12C) Solid Tumors (KRYSTAL-1). *J Clin Oncol* 2022; **40**: 2530-2538 [PMID: 35167329 DOI: 10.1200/JCO.21.02752]

29 **Wang X**, Allen S, Blake JF, Bowcut V, Briere DM, Calinisan A, Dahlke JR, Fell JB, Fischer JP, Gunn RJ, Hallin J, Laguer J, Lawson JD, Medwid J, Newhouse B, Nguyen P, O'Leary JM, Olson P, Pajk S, Rahbaek L, Rodriguez M, Smith CR, Tang TP, Thomas NC, Vanderpool D, Vigers GP, Christensen JG, Marx MA. Identification of MRTX1133, a Noncovalent, Potent, and Selective KRAS(G12D) Inhibitor. *J Med Chem* 2022; **65**: 3123-3133 [PMID: 34889605 DOI: 10.1021/acs.jmedchem.1c01688]

30 **Kemp SB**, Cheng N, Markosyan N, Sor R, Kim IK, Hallin J, Shoush J, Quinones L, Brown NV, Bassett JB, Joshi N, Yuan S, Smith M, Vostrejs WP, Perez-Vale KZ, Kahn B, Mo F, Donahue TR, Radu CG, Clendenin C, Christensen JG, Vonderheide RH, Stanger BZ. Efficacy of a Small-Molecule Inhibitor of KrasG12D in Immunocompetent Models of Pancreatic Cancer. *Cancer Discov* 2023; **13**: 298-311 [PMID: 36472553 DOI: 10.1158/2159-8290.CD-22-1066]

31 **Koltun E**, Cregg J, Rice MA, Whalen DM, Freilich R, Jiang J, Hansen R, Bermingham A, Knox, Dinglasan J, Seamon K, Blaj C, Chang SS, Liu Y, Huang J, Chou KJ, McDowell L, Lee BJ, Wildes D, Wang Z, Singh M, Gill AL, Smith JA. Abstract 1260: First-in-class, orally bioavailable KRASG12V(ON) tri-complex inhibitors, as single agents and in combinations, drive profound anti-tumor activity in preclinical

models of KRASG12V mutant cancers. *Cancer Res* 2021; **81** Suppl 13: 1260 [DOI: 10.1158/1538-7445.Am2021-1260]

32 **Zhang Z**, Morstein J, Ecker AK, Guiley KZ, Shokat KM. Chemoselective Covalent Modification of K-Ras(G12R) with a Small Molecule Electrophile. *J Am Chem Soc* 2022; **144**: 15916-15921 [PMID: 36001446 DOI: 10.1021/jacs.2c05377]

33 **Jørgensen F**, Hesse B, Grønbaek P, Fogh J, Haunsø S. Abnormal oesophageal function in patients with non-toxic goiter or enlarged left atrium, demonstrated by radionuclide transit measurements. *Scand J Gastroenterol* 1989; **24**: 1186-1192 [PMID: 2532392 DOI: 10.1038/nrd4389]

34 **Ryan MB**, Fece de la Cruz F, Phat S, Myers DT, Wong E, Shahzade HA, Hong CB, Corcoran RB. Vertical Pathway Inhibition Overcomes Adaptive Feedback Resistance to KRAS(G12C) Inhibition. *Clin Cancer Res* 2020; **26**: 1633-1643 [PMID: 31776128 DOI: 10.1158/1078-0432.CCR-19-3523]

35 **Xue JY**, Zhao Y, Aronowitz J, Mai TT, Vides A, Qeriqi B, Kim D, Li C, de Stanchina E, Mazutis L, Risso D, Lito P. Rapid non-uniform adaptation to conformation-specific KRAS(G12C) inhibition. *Nature* 2020; **577**: 421-425 [PMID: 31915379 DOI: 10.1038/s41586-019-1884-x]

36 **Awad MM**, Liu S, Rybkin II, Arbour KC, Dilly J, Zhu VW, Johnson ML, Heist RS, Patil T, Riely GJ, Jacobson JO, Yang X, Persky NS, Root DE, Lowder KE, Feng H, Zhang SS, Haigis KM, Hung YP, Sholl LM, Wolpin BM, Wiese J, Christiansen J, Lee J, Schrock AB, Lim LP, Garg K, Li M, Engstrom LD, Waters L, Lawson JD, Olson P, Lito P, Ou SI, Christensen JG, Jänne PA, Aguirre AJ. Acquired Resistance to KRAS(G12C) Inhibition in Cancer. *N Engl J Med* 2021; **384**: 2382-2393 [PMID: 34161704 DOI: 10.1056/NEJMoa2105281]

37 **Wilhelm S**, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R, Kelley S. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* 2006; **5**: 835-844 [PMID: 17016424 DOI: 10.1038/nrd2130]

38 **Cardin DB**, Goff L, Li CI, Shyr Y, Winkler C, DeVore R, Schlabach L, Holloway M, McClanahan P, Meyer K, Grigorieva J, Berlin J, Chan E. Phase II trial of sorafenib and erlotinib in advanced pancreatic cancer. *Cancer Med* 2014; **3**: 572-579 [PMID: 24574334 DOI: 10.1002/cam4.208]

39 **Flaherty KT**, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 2010; **363**: 809-819 [PMID: 20818844 DOI: 10.1056/NEJMoa1002011]

40 **Salama AKS**, Li S, Macrae ER, Park JI, Mitchell EP, Zwiebel JA, Chen HX, Gray RJ, McShane LM, Rubinstein LV, Patton D, Williams PM, Hamilton SR, Armstrong DK, Conley BA, Arteaga CL, Harris LN, O'Dwyer PJ, Chen AP, Flaherty KT. Dabrafenib and Trametinib in Patients With Tumors With BRAF(V600E) Mutations: Results of the NCI-MATCH Trial Subprotocol H. *J Clin Oncol* 2020; **38**: 3895-3904 [PMID: 32758030 DOI: 10.1200/JCO.20.00762]

41 **Callahan MK**, Rampal R, Harding JJ, Klimek VM, Chung YR, Merghoub T, Wolchok JD, Solit DB, Rosen N, Abdel-Wahab O, Levine RL, Chapman PB. Progression of RAS-mutant leukemia during RAF inhibitor treatment. *N Engl J Med* 2012; **367**: 2316-2321 [PMID: 23134356 DOI: 10.1056/NEJMoa1208958]

42 **Oberholzer PA**, Kee D, Dziunycz P, Sucker A, Kamsukom N, Jones R, Roden C, Chalk CJ, Ardlie K, Palescandolo E, Piris A, MacConaill LE, Robert C, Hofbauer GF, McArthur GA, Schadendorf D, Garraway LA. RAS mutations are associated with the development of cutaneous squamous cell tumors in patients treated with RAF inhibitors. *J Clin Oncol* 2012; **30**: 316-321 [PMID: 22067401 DOI: 10.1200/JCO.2011.36.7680]

43 **Basile KJ**, Le K, Hartsough EJ, Aplin AE. Inhibition of mutant BRAF splice variant signaling by next-generation, selective RAF inhibitors. *Pigment Cell Melanoma Res* 2014; **27**: 479-484 [PMID: 24422853 DOI: 10.1111/pcmr.12218]

44 **Peng SB**, Henry JR, Kaufman MD, Lu WP, Smith BD, Vogeti S, Rutkoski TJ, Wise S, Chun L, Zhang Y, Van Horn RD, Yin T, Zhang X, Yadav V, Chen SH, Gong X, Ma X, Webster Y, Buchanan S, Mochalkin I, Huber L, Kays L, Donoho GP, Walgren J, McCann D, Patel P, Conti I, Plowman GD, Starling JJ, Flynn DL. Inhibition of RAF Isoforms and Active Dimers by LY3009120 Leads to Anti-tumor Activities in RAS or BRAF Mutant Cancers. *Cancer Cell* 2015; **28**: 384-398 [PMID: 26343583 DOI: 10.1016/j.ccell.2015.08.002]

45 **Vakana E**, Pratt S, Blosser W, Dowless M, Simpson N, Yuan XJ, Jaken S, Manro J, Stephens J, Zhang Y, Huber L, Peng SB, Stancato LF. LY3009120, a panRAF inhibitor, has significant anti-tumor activity in BRAF and KRAS mutant preclinical models of colorectal cancer. *Oncotarget* 2017; **8**: 9251-9266 [PMID: 27999210 DOI: 10.18632/oncotarget.14002]

46 **Rasco DW**, Olszanski AJ, Patnaik A, Espino G, Neuwirth R, Faucette S, Bargfrede M, Gangolli EA, Walker RM, Kneissl M, Bozon V. MLN2480, an investigational oral pan-RAF kinase inhibitor, in patients (pts) with relapsed or refractory solid tumors: Phase I study. *J Clin Oncol* 2013; **31** Suppl 15: 2547 [DOI: 10.1200/jco.2013.31.15_suppl.2547]

47 **Kim TW**, Lee J, Shin SJ, Kim JS, Kim YJ, Han HS, Lee SJ, Lim HS, Hong YH, Noh YS, Kyoung Y, Han O, Yoon J, Lim JA, Kim SR. Belvarafenib, a novel pan-RAF inhibitor, in solid tumor patients harboring BRAF, KRAS, or NRAS mutations: Phase I study. *J Clin Oncol* 2019; **37** Suppl 15: 3000 [DOI: 10.1200/JCO.2019.37.15_suppl.3000]

48 **Zhang W**, Nandakumar N, Shi Y, Manzano M, Smith A, Graham G, Gupta S, Vietsch EE, Laughlin SZ, Wadhwa M, Chetram M, Joshi M, Wang F, Kallakury B, Toretsky J, Wellstein A, Yi C. Downstream of mutant KRAS, the transcription regulator YAP is essential for neoplastic progression to pancreatic ductal adenocarcinoma. *Sci Signal* 2014; **7**: ra42 [PMID: 24803537 DOI: 10.1126/scisignal.2005049]

49 **Zhao X**, Wang X, Fang L, Lan C, Zheng X, Wang Y, Zhang Y, Han X, Liu S, Cheng K, Zhao Y, Shi J, Guo J, Hao J, Ren H, Nie G. A combinatorial strategy using YAP and pan-RAF inhibitors for treating KRAS-mutant pancreatic cancer. *Cancer Lett* 2017; **402**: 61-70 [PMID: 28576749 DOI: 10.1016/j.canlet.2017.05.015]

50 **Vranic S**, Basu GD, Hall DW, Gatalica Z. Tumor-Type Agnostic, Targeted Therapies: BRAF Inhibitors Join the Group. *Acta Med Acad* 2022; **51**: 217-231 [PMID: 36799315 DOI: 10.5644/ama2006-124.392]

51 **Infante JR**, Somer BG, Park JO, Li CP, Scheulen ME, Kasubhai SM, Oh DY, Liu Y, Redhu S, Steplewski K, Le N. A randomised, double-blind, placebo-controlled trial of trametinib, an oral MEK inhibitor, in combination with gemcitabine for patients with untreated metastatic adenocarcinoma of the pancreas. *Eur J Cancer* 2014; **50**: 2072-2081 [PMID: 24915778 DOI: 10.1016/j.ejca.2014.04.024]

52 **Kinsey CG**, Camolotto SA, Boespflug AM, Guillen KP, Foth M, Truong A, Schuman SS, Shea JE, Seipp MT, Yap JT, Burrell LD, Lum DH, Whisenant JR, Gilcrease GW 3rd, Cavalieri CC, Rehbein KM, Cutler SL, Affolter KE, Welm AL, Welm BE, Scaife CL, Snyder EL, McMahon M. Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers. *Nat Med* 2019; **25**: 620-627 [PMID: 30833748 DOI: 10.1038/s41591-019-0367-9]

53 **Bodoky G**, Timcheva C, Spigel DR, La Stella PJ, Ciuleanu TE, Pover G, Tebbutt NC. A phase II open-label randomized study to assess the efficacy and safety of selumetinib (AZD6244 [ARRY-142886]) versus capecitabine in patients with advanced or metastatic pancreatic cancer who have failed first-line gemcitabine therapy. *Invest New Drugs* 2012; **30**: 1216-1223 [PMID: 21594619 DOI: 10.1007/s10637-011-9687-4]

54 **Kenney C**, Kunst T, Webb S, Christina D Jr, Arrowood C, Steinberg SM, Mettu NB, Kim EJ, Rudloff U. Phase II study of selumetinib, an orally active inhibitor of MEK1 and MEK2 kinases, in KRAS(G12R)-mutant pancreatic ductal adenocarcinoma. *Invest New Drugs* 2021; **39**:

821-828 [PMID: 33405090 DOI: 10.1007/s10637-020-01044-8]

55 **Van Cutsem E**, Hidalgo M, Canon JL, Macarulla T, Bazin I, Poddubskaya E, Manojlovic N, Radenkovic D, Verslype C, Raymond E, Cubillo A, Schueler A, Zhao C, Hammel P. Phase I/II trial of pimasertib plus gemcitabine in patients with metastatic pancreatic cancer. *Int J Cancer* 2018; **143**: 2053-2064 [PMID: 29756206 DOI: 10.1002/ijc.31603]

56 **Morris EJ**, Jha S, Restaino CR, Dayananth P, Zhu H, Cooper A, Carr D, Deng Y, Jin W, Black S, Long B, Liu J, Dinunzio E, Windsor W, Zhang R, Zhao S, Angagaw MH, Pinheiro EM, Desai J, Xiao L, Shipps G, Hruza A, Wang J, Kelly J, Paliwal S, Gao X, Babu BS, Zhu L, Daublain P, Zhang L, Lutterbach BA, Pelletier MR, Philippar U, Siliphaivanh P, Witter D, Kirschmeier P, Bishop WR, Hicklin D, Gilliland DG, Jayaraman L, Zawel L, Fawell S, Samatar AA. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. *Cancer Discov* 2013; **3**: 742-750 [PMID: 23614898 DOI: 10.1158/2159-8290.CD-13-0070]

57 **Germann UA**, Furey BF, Markland W, Hoover RR, Aronov AM, Roix JJ, Hale M, Boucher DM, Sorrell DA, Martinez-Botella G, Fitzgibbon M, Shapiro P, Wick MJ, Samadani R, Meshaw K, Groover A, DeCrescenzo G, Namchuk M, Emery CM, Saha S, Welsch DJ. Targeting the MAPK Signaling Pathway in Cancer: Promising Preclinical Activity with the Novel Selective ERK1/2 Inhibitor BVD-523 (Ulixertinib). *Mol Cancer Ther* 2017; **16**: 2351-2363 [PMID: 28939558 DOI: 10.1158/1535-7163.MCT-17-0456]

58 **Weekes C**, Lockhart A, LoRusso P, Murray E, Park E, Tagen M, Singh J, Sarkar I, Mueller L, Dokainish H, Shapiro G, Burris H. A Phase Ib Study to Evaluate the MEK Inhibitor Cobimetinib in Combination with the ERK1/2 Inhibitor GDC-0994 in Patients with Advanced Solid Tumors. *Oncologist* 2020; **25**: 833-e1438 [PMID: 32311798 DOI: 10.1634/theoncologist.2020-0292]

59 **Li A**, Jian S, Yuan X, Song F, Yang S, Du C, Tao Y, Wang L, Pan M, Dong P, Zhou J, Ge Z, Zhu Q, Hao W, Xu W, Zhang J, Li Q, Wang S. Abstract 4188: The ERK1/2 inhibitor, JSI-1187, demonstrates preclinical efficacy in tumor models with MAPK pathway mutations. *Cancer Research* 2020; **80** Suppl 16: 4188 [DOI: 10.1158/1538-7445.Am2020-4188]

60 **Baer R**, Cintas C, Therville N, Guillermet-Guibert J. Implication of PI3K/Akt pathway in pancreatic cancer: When PI3K isoforms matter? *Adv Biol Regul* 2015; **59**: 19-35 [PMID: 26166735 DOI: 10.1016/j.jbior.2015.05.001]

61 **Cheng JQ**, Ruggeri B, Klein WM, Sonoda G, Altomare DA, Watson DK, Testa JR. Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. *Proc Natl Acad Sci U S A* 1996; **93**: 3636-3641 [PMID: 8622988 DOI: 10.1073/pnas.93.8.3636]

62 **Liou GY**, Storz P. Inflammatory macrophages in pancreatic acinar cell metaplasia and initiation of pancreatic cancer. *Oncoscience* 2015; **2**: 247-251 [PMID: 25897428 DOI: 10.18632/oncoscience.151]

63 **Ruffell B**, Coussens LM. Macrophages and therapeutic resistance in cancer. *Cancer Cell* 2015; **27**: 462-472 [PMID: 25858805 DOI: 10.1016/j.ccell.2015.02.015]

64 **Kaneda MM**, Cappello P, Nguyen AV, Ralainirina N, Hardamon CR, Foubert P, Schmid MC, Sun P, Mose E, Bouvet M, Lowy AM, Valasek MA, Sasik R, Novelli F, Hirsch E, Varner JA. Macrophage PI3Kγ Drives Pancreatic Ductal Adenocarcinoma Progression. *Cancer Discov* 2016; **6**: 870-885 [PMID: 27179037 DOI: 10.1158/2159-8290.CD-15-1346]

65 **Schlieman MG**, Fahy BN, Ramsamooj R, Beckett L, Bold RJ. Incidence, mechanism and prognostic value of activated AKT in pancreas cancer. *Br J Cancer* 2003; **89**: 2110-2115 [PMID: 14647146 DOI: 10.1038/sj.bjc.6601396]

66 **Foo WC**, Rashid A, Wang H, Katz MH, Lee JE, Pisters PW, Wolff RA, Abbruzzese JL, Fleming JB. Loss of phosphatase and tensin homolog expression is associated with recurrence and poor prognosis in patients with pancreatic ductal adenocarcinoma. *Hum Pathol* 2013; **44**: 1024-1030 [PMID: 23260327 DOI: 10.1016/j.humpath.2012.09.001]

67 **Hill R**, Calvopina JH, Kim C, Wang Y, Dawson DW, Donahue TR, Dry S, Wu H. PTEN loss accelerates KrasG12D-induced pancreatic cancer development. *Cancer Res* 2010; **70**: 7114-7124 [PMID: 20807812 DOI: 10.1158/0008-5472.CAN-10-1649]

68 **Wong MH**, Xue A, Julovi SM, Pavlakis N, Samra JS, Hugh TJ, Gill AJ, Peters L, Baxter RC, Smith RC. Cotargeting of epidermal growth factor receptor and PI3K overcomes PI3K-Akt oncogenic dependence in pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2014; **20**: 4047-4058 [PMID: 24895459 DOI: 10.1158/1078-0432.CCR-13-3377]

69 **Kordes S**, Richel DJ, Klümpen HJ, Weterman MJ, Stevens AJ, Wilmink JW. A phase I/II, non-randomized, feasibility/safety and efficacy study of the combination of everolimus, cetuximab and capecitabine in patients with advanced pancreatic cancer. *Invest New Drugs* 2013; **31**: 85-91 [PMID: 22367239 DOI: 10.1007/s10637-012-9802-1]

70 **Xue W**, Dahlman JE, Tammela T, Khan OF, Sood S, Dave A, Cai W, Chirino LM, Yang GR, Bronson R, Crowley DG, Sahay G, Schroeder A, Langer R, Anderson DG, Jacks T. Small RNA combination therapy for lung cancer. *Proc Natl Acad Sci U S A* 2014; **111**: E3553-E3561 [PMID: 25114235 DOI: 10.1073/pnas.1412686111]

71 **Zorde Khvalevsky E**, Gabai R, Rachmut IH, Horwitz E, Brunschwig Z, Orbach A, Shemi A, Golan T, Domb AJ, Yavin E, Giladi H, Rivkin L, Simerzin A, Eliakim R, Khalaileh A, Hubert A, Lahav M, Kopelman Y, Goldin E, Dancour A, Hants Y, Arbel-Alon S, Abramovitch R, Galun E. Mutant KRAS is a druggable target for pancreatic cancer. *Proc Natl Acad Sci U S A* 2013; **110**: 20723-20728 [PMID: 24297898 DOI: 10.1073/pnas.1314307110]

72 **Zhang B**, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. *Dev Biol* 2007; **302**: 1-12 [PMID: 16989803 DOI: 10.1016/j.ydbio.2006.08.028]

73 **Wang H**, Peng R, Wang J, Qin Z, Xue L. Circulating microRNAs as potential cancer biomarkers: the advantage and disadvantage. *Clin Epigenetics* 2018; **10**: 59 [PMID: 29713393 DOI: 10.1186/s13148-018-0492-1]

74 **Yu S**, Lu Z, Liu C, Meng Y, Ma Y, Zhao W, Liu J, Yu J, Chen J. miRNA-96 suppresses KRAS and functions as a tumor suppressor gene in pancreatic cancer. *Cancer Res* 2010; **70**: 6015-6025 [PMID: 20610624 DOI: 10.1158/0008-5472.CAN-09-4531]

75 **Zhao X**, Liu L, Lang J, Cheng K, Wang Y, Li X, Shi J, Nie G. A CRISPR-Cas13a system for efficient and specific therapeutic targeting of mutant KRAS for pancreatic cancer treatment. *Cancer Lett* 2018; **431**: 171-181 [PMID: 29870774 DOI: 10.1016/j.canlet.2018.05.042]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 August 24; 14(8): 297-310

DOI: 10.5306/wjco.v14.i8.297

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

Immune responses of six-transmembrane epithelial antigen of the prostate 4 functions as a novel biomarker in gastric cancer

Ze-Xuan Fang, Yan-Yu Hou, Zheng Wu, Bing-Xuan Wu, Yu Deng, Hua-Tao Wu, Jing Liu

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C, C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Liu Q, China; Sato T, Japan; Senchukova M, Russia

Received: May 10, 2023
Peer-review started: May 10, 2023
First decision: June 7, 2023
Revised: June 19, 2023
Accepted: July 19, 2023
Article in press: July 19, 2023
Published online: August 24, 2023

Ze-Xuan Fang, Yan-Yu Hou, Zheng Wu, Jing Liu, Guangdong Provincial Key Laboratory for Diagnosis and Treatment of Breast Cancer, Cancer Hospital of Shantou University Medical College, Shantou 515041, Guangdong Province, China

Bing-Xuan Wu, Yu Deng, Hua-Tao Wu, Department of General Surgery, First Affiliated Hospital of Shantou University Medical College, Shantou 515041, Guangdong Province, China

Corresponding author: Jing Liu, MD, PhD, Academic Research, Associate Professor, Research Scientist, Senior Scientist, Guangdong Provincial Key Laboratory for Diagnosis and Treatment of Breast Cancer, Cancer Hospital of Shantou University Medical College, No. 22 Xinling Road, Shantou 515041, China. jliu12@stu.edu.cn

Abstract

BACKGROUND

Immune cells play an important role in regulating the behavior of tumor cells. According to emerging evidence, six-transmembrane epithelial antigen of the prostate 4 (STEAP4) performs a crucial part in tumor microenvironmental immune response and tumorigenesis, and serves as the potential target for cellular and antibody immunotherapy. However, the immunotherapeutic role of STEAP4 in gastric cancer (GC) remains unclear.

AIM

To investigate the expression of STEAP4 in GC and its relationship with immune infiltrating cells, and explore the potential value of STEAP4 as an immune prognostic indicator in GC.

METHODS

The expression level of STEAP4 was characterized by immunohistochemistry in tumors and adjacent non-cancerous samples in 96 GC patients. Tumor Immune Estimation Resource was used to study the correlation between STEAP4 and tumor immune infiltration level and immune infiltration gene signature. R package was used to analyze the relationship between STEAP4 expression and immune and stromal scores in GC (GSE62254) by the ESTIMATE algorithm, and Kaplan-Meier Plotter and Gene Expression Profiling Interactive Analysis were applied to analyze the effect of STEAP4 on clinical prognosis.

RESULTS

Immunohistochemistry analysis showed that STEAP4 expression was higher in

GC tissues than in adjacent tissues, and STEAP4 expression was positively correlated with the clinical stage of GC. In GC, the expression of STEAP4 was positively correlated with the infiltration levels of B cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells. The expression level of STEAP4 was strongly correlated with most of the immune markers. In addition, STEAP4 expression was inversely correlated with tumor purity, but correlated with stromal score ($r = 0.43$, $P < 0.001$), immune score ($r = 0.29$, $P < 0.001$) and estimate score ($r = 0.39$, $P < 0.001$). Moreover, stromal, immune, and estimate scores were higher in the STEAP4 high expression group, whereas tumor purity was higher in the STEAP4 Low expression group. The relationship between STEAP4 expression and prognosis of patients with GC was further investigated, and the results showed that high STEAP4 expression was associated with poor overall survival and disease-free survival. In addition, Kaplan-Meier Plotter showed that high expression of STEAP4 was significantly correlated with poor survival of patients with GC.

CONCLUSION

The current findings suggest an oncogenic role for STEAP4 in GC, with significantly high levels being associated with poor prognosis. Investigation of the GC tumor microenvironment suggests the potential function of STEAP4 is connected with the infiltration of diverse immune cells, which may contribute to the regulation of the tumor microenvironment. In conclusion, STEAP4 may serve as a potential therapeutic target for GC to improve the immune infiltration, as well as serve as a prognostic biomarker for judging the prognosis and immune infiltration status of GC.

Key Words: Six-transmembrane epithelial antigen of the prostate 4; Gastric cancer; Immune infiltration; Prognosis; Biomarker

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The present study analyzed the expression level of six-transmembrane epithelial antigen of the prostate 4 (STEAP4) in gastric cancer (GC) and found that high STEAP4 expression is significantly associated with poor survival of patients. STEAP4 is positively correlated with immune infiltration of different types of immune cells, and has strong correlations with most immune markers. STEAP4 may become a potential biomarker for predicting the prognosis of GC patients.

Citation: Fang ZX, Hou YY, Wu Z, Wu BX, Deng Y, Wu HT, Liu J. Immune responses of six-transmembrane epithelial antigen of the prostate 4 functions as a novel biomarker in gastric cancer. *World J Clin Oncol* 2023; 14(8): 297-310
URL: https://www.wjgnet.com/2218-4333/full/v14/i8/297.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i8.297

INTRODUCTION

Gastric cancer (GC), the fifth most common malignant tumor, is the second leading cause of cancer-related death worldwide[1,2]. Although the overall survival (OS) of GC patients has improved with standardized extended (D2) lymphadenectomy and the implementation of chemotherapy and targeted therapy, its survival rate is still less than 30% [3,4]. However, recent studies have shown that immune-involved mechanisms play a certain critical role in gastric tumors, and immunotherapy is considered a promising strategy for the therapeutics of gastric tumors[5]. In addition, Zhang *et al*[6] found that tumor-infiltrating lymphocytes can affect the prognosis and efficacy of chemotherapy and immunotherapy in GC patients. Therefore, there is an urgent need to elucidate the mechanism of tumor-immune interaction in GC, and to identify novel prognostic targets for immunotherapy.

Six-transmembrane epithelial antigen of the prostate 4 (STEAP4) consists of an N-terminal oxidoreductase domain and a six-helix transmembrane domain, serving as a transmembrane protein involved in metal reductase transport of copper and iron[7,8]. It is reported that high expression of STEAP4 is correlated with the pathogenesis of cancer and metabolic diseases[9-11]. STEAP4 is not only involved in the occurrence and development of breast cancer[12,13], but is also related to the inflammatory response of colon cancer[14]. It is also found that STEAP4 is highly expressed in prostate cancer tissues, serving as a promising prognostic indicator[15]. Nevertheless, the effect of STEAP4 in GC development and the mechanisms involved remain unclear.

In this study, the expression of STEAP4 and its correlation with the prognosis of GC patients are comprehensively analyzed. Moreover, the relevance between STEAP4 and different tumor-infiltrating immune cells and immune cell markers is also examined to clarify the essential role of STEAP4 in GC and provide a potential relationship and mechanism between STEAP4 and tumor-immune interactions.

MATERIALS AND METHODS

Patient information and ethics statement

Tissue array (XT17-037, OUTDO, China) recruited total 96 cases of GC, including 84 pairs of GC tissues and corresponding adjacent tissues, and 12 extra GC samples. This investigation of STEAP4 in GC was approved by the Ethics Committee of Shantou University Medical College.

Immunohistochemical staining

The protocol for immunohistochemical staining was conducted as described previously[16]. The primary antibody used was anti-STEAP4 antibody in 1:400 diluent (Proteintech 11944-AP). The sections were visualized and evaluated independently under a bright-field microscope (PerkinElmer Vectra, United States) by two investigators with no prior knowledge of the patient information. The evaluation of STEAP4 expression was based on the sum of the scores from the staining intensities (0-3 indicating colorless, light yellow, brown and dark) and the percentage of positive cells (0-4 for 0%, 1% to 25%, 26% to 50%, 51% to 74%, and 76% to 100%), and the patients were divided into two groups based on the sum score results[17].

STEAP4 mRNA expression in GC

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html), an interactive network from TCGA and GTEx projects was used to further analyze the expression level of STEAP4, in TCGA expression data, in different clinical stages of GC[18]. The survival information of GC patients was also evaluated based on STEAP4 expression in the GEPIA datasets.

Relationship between STEAP4 and infiltrating immune cells in GC

Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/) is an online dataset for systematic analysis of immune infiltration in various types of cancer[19]. The correlation between STEAP4 level and the abundance of infiltrating immune cells was analyzed using gene modules in the database. In addition, the correlation between STEAP4 level and biomarkers of tumor-infiltrating immune cells was also investigated, with scatterplots and Spearman's value for estimated statistical signifcance. Gene markers of tumor-infiltrating immune cells included CD8+ T cells, CD4+T cells, B cells, monocytes, TAMs, M1 macrophages, M2 macrophages, neutrophils, natural killer cells (NK), dendritic cells (DCs), T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, and follicle-helper T (Tfh) cells, T-helper 17 (Th17) cells, Tregs and exhausted T cells[20-22].

Expression of infiltrating immune cells in GC

The "ESTIMATE" algorithm of R package was used to calculate the immune score and stromal score of the GSE62254 dataset (n = 300), which was helpful for the evaluation of immune and stromal constitute in tumors. The immune and stromal scores were also calculated by STEAP4 expression in immune and stromal cells in GC.

The prognostic value of STEAP4 in GC

Kaplan-Meier Plotter (http://kmplot.com/analysis/) was applied to analyze the correlation between STEAP4 and survival rate of GC[23]. Hazard ratios (HRs) and log-rank P values for 95% confidence intervals were calculated simultaneously.

Statistical and survival analysis

SPSS software was used for χ^2 or Fisher's exact probability tests to analyze the relationship of STEAP4 level and clinic information of GC patients. To investigate the prognosis of GC patients, the Kaplan-Meier survival curve was conducted, along with log-rank test. Differences were achieved with $P < 0.05$.

RESULTS

STEAP4 is highly expressed in GC compared with adjacent normal tissues

To investigate the expression profiling of STEAP4 in GC tissues, cancerous tissues and adjacent normal tissues were obtained from GC patients. Representative images of STEAP4 expression are shown in Figure 1. Based on the quantitation of STEAP4 expression levels in GC, a significantly high level of STEAP4 in GC tissues was found, compared with corresponding adjacent normal tissues (P = 0.0056) (Table 1).

A high level of STEAP4 tends to contribute to GC progression

The expression level of STEAP4 in 96 GC patients was further analyzed with their clinicopathological parameters (Table 2). Although no statistical significance was found between the expression level of STEAP4 and the clinicopathologic parameters, including age of diagnosis, gender, lymph node status, vascular invasion and clinical stage ($P > 0.05$), the proportion of patients with high STEAP4 expression tended to increase with the progression of pathological stage, and high STEAP4 expression tended to be associated with lymph node metastasis and vascular invasion, indicating the potential contribution of STEAP4 to the progression of GC. The GEPIA database, regarding mRNA expression, was used

Table 1 Comparison of six-transmembrane epithelial antigen of the prostate 4 levels between gastric cancer and adjacent normal tissues

	Case (*n*)	STEAP4		χ^2	*P* value
		Low (%)	High (%)		
Tumor	84	20 (23.81)	64 (76.19)	7.674	0.0056
Normal	84	37 (44.05)	47 (55.95)		

STEAP4: Six-transmembrane epithelial antigen of the prostate 4.

Table 2 Correlation between six-transmembrane epithelial antigen of the prostate 4 expression and clinicopathological parameters in gastric cancer patients

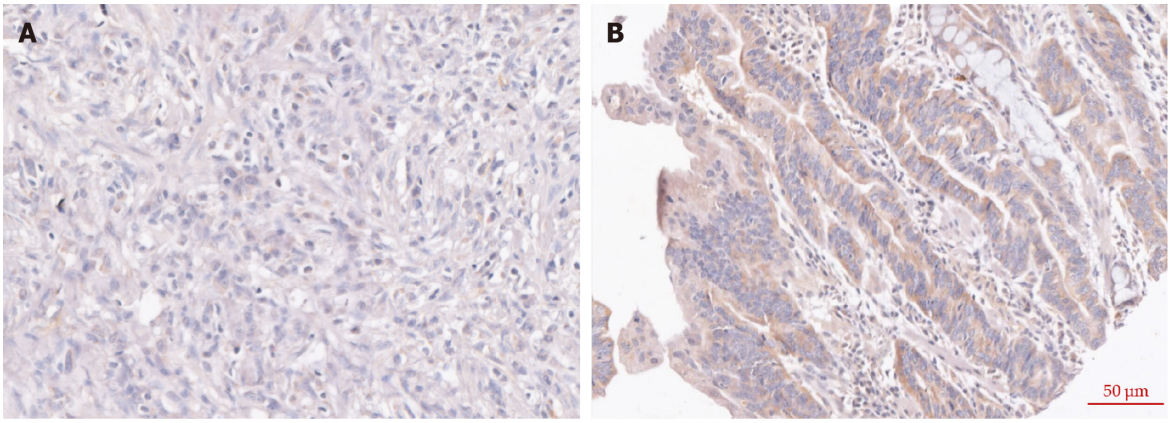
Clinical parameters	STEAP4		*P* value
	Low (%)	High (%)	
Age			
< 60	9 (25.0)	27 (75.0)	0.5662
≥ 60	12 (20.0)	48 (80.0)	
Gender			
Female	6 (19.4)	25 (80.6)	0.6800
Male	15 (23.1)	50 (76.9)	
T			
T1-3	15 (23.8)	48 (76.2)	0.5264
T4	6 (18.2)	27 (81.8)	
N			
N0	7 (35.0)	13 (65.0)	0.1105
N1-N3	14 (18.4)	62 (81.8)	
M			
M0	21 (22.3)	73 (77.7)	0.9999
M1	0 (0)	2 (100)	
Vascular invasion			
No	18 (26.1)	51 (73.9)	0.1105
Yes	3 (11.1)	24 (88.9)	
Clinical stage			
Phase 1	2 (25.0)	6 (75.0)	0.5900
Phase 2	8 (29.6)	19 (70.4)	
Phase 3	11 (18.6)	48 (81.4)	
Phase 4	0 (0)	2 (100)	

STEAP4: Six-transmembrane epithelial antigen of the prostate 4.

to verify the relationship of STEAP4 with clinical stage of GC. Interestingly, there was no significant difference in the expression of STEAP4 between 4 different clinical stages. However, an increased expression of STEAP4 was found in Stage III and Stage IV, compared with Stage I and Stage II, predicting the potential promoting role of STEAP4 in GC (Figure 2).

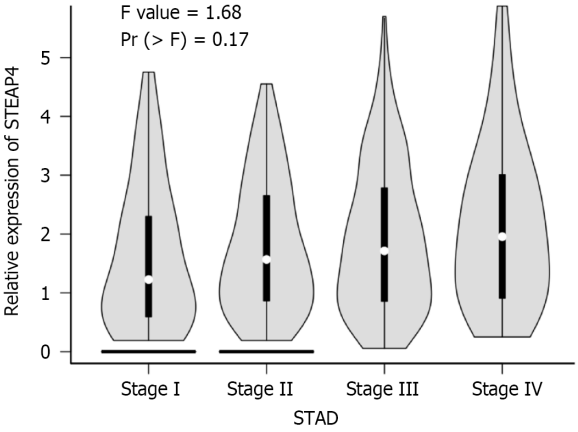
STEAP4 is positively correlated with the extent of immune infiltration in GC

Considering that tumor purity is an important factor affecting immune infiltration of clinical tumor samples analyzed by genomic approaches[24], it is of interest to investigate the tumor microenvironment-related immune infiltration with



DOI: 10.5306/wjco.v14.i8.297 **Copyright** ©The Author(s) 2023.

Figure 1 Representative images of six-transmembrane epithelial antigen of the prostate 4 expression in patients with gastric cancer. A: Low expression of six-transmembrane epithelial antigen of the prostate 4 (STEAP4); B: High expression of STEAP4.



DOI: 10.5306/wjco.v14.i8.297 **Copyright** ©The Author(s) 2023.

Figure 2 Relationship between six-transmembrane epithelial antigen of the prostate 4 level and clinical stage of gastric cancer patients. STAD: Stomach adenocarcinoma; STEAP4: Six-transmembrane epithelial antigen of the prostate 4.

STEAP4 levels. Interestingly, STEAP4 expression levels were found to be associated with higher immune infiltration in GC. The level of STEAP4 expression was positively associated with that of immune-infiltrating cells, including B cells, CD4+ T cells, neutrophils, macrophages and dendritic cells (Figure 3).

External verification confirms the positive correlation of STEAP4 with immune infiltration in GC

External verification was conducted on the GSE62254 dataset, with 300 GC samples, using the ESTIMATE algorithm in R software. Based on the features, stromal and immune scores were generated to reflect the proportion of stroma and immune cells, respectively, and single sample gene set enrichment analysis was used to combine the two to measure tumor purity. In Figure 4A-D, it is revealed that STEAP4 expression was inversely correlated with tumor purity and stromal score (r = 0.43, P < 0.001), immune score (r = 0.29, P < 0.001) and ESTIMATE score (r = 0.39, P < 0.001). In addition, stromal, immune, and estimate scores all increased with high STEAP4 expression (Figure 4F-H), whereas tumor purity was accompanied by low STEAP4 expression (Figure 4E).

Correlation analysis between STEAP4 expression and immunomarker sets

Due to the positive correlation between STEAP4 and immune infiltration was found in GC, further investigation was conducted to uncover the role of STEAP4 in the development of GC, and specify the subtype of immune cells associated with STEAP4. Diverse immunomarker sets were analyzed in TIMER database to verify the relationship of STEAP4 level with immune-infiltrating cells. After adjustment for purity, STEAP4 expression levels were significantly correlated with most of the immune marker sets of various immune cells and different T cells (Table 3).

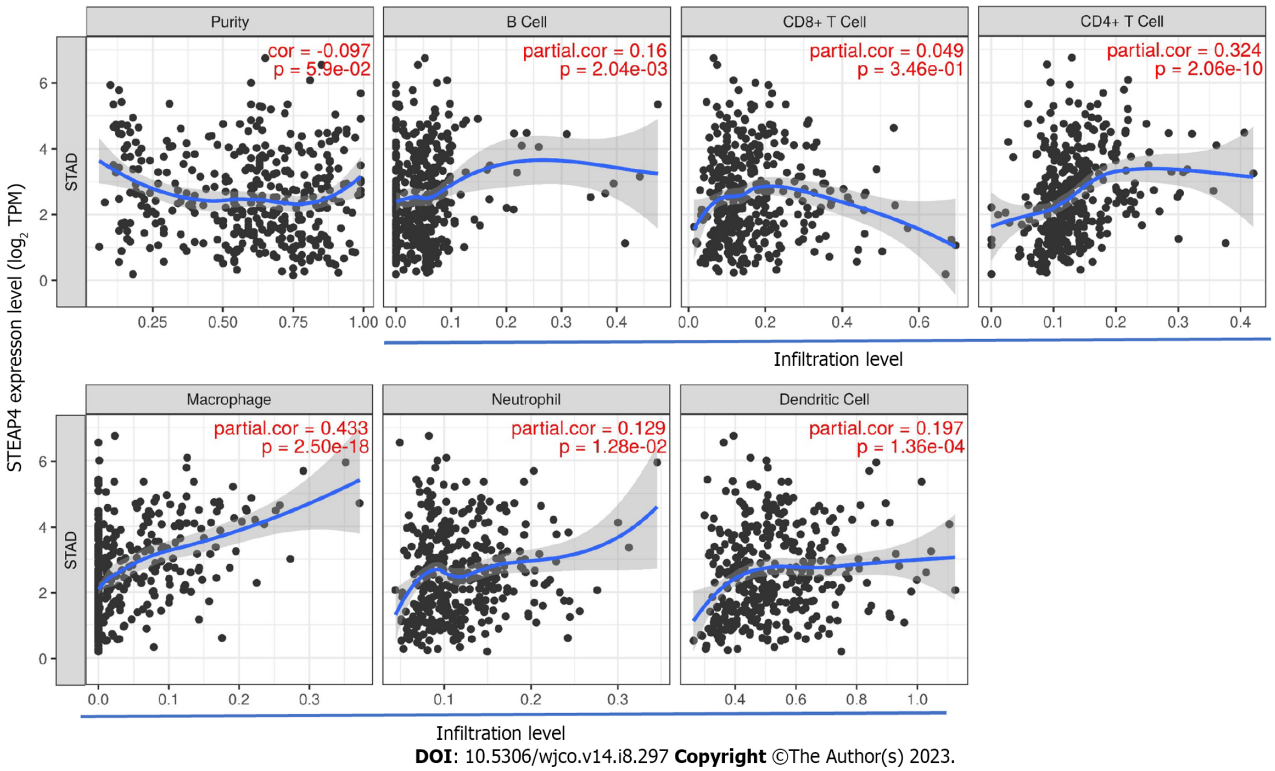
Interestingly, the expression levels of gene markers for B cells, monocytes, TAMs, M1 and M2 macrophages and other immune cells were correlated with the expression of STEAP4. Specifically, it was found that the expression level of CD19, B cell CD79A, CD86, monocyte CD115, TAM CCL2 and IL-10, M1 macrophage IRF5 and PTGS2, and M2 macrophage CD163, VSIG4, and MS4A4A were significantly correlated with STEAP4 expression (P < 0.01) (Table 3, Figure 5), suggesting a function of STEAP4 in regulating the infiltration of macrophages during the progression of GC.

Table 3 Correlation analysis between six-transmembrane epithelial antigen of the prostate 4 and the immunomarkers in gastric cancer

Immune cells	Gene markers	STAD			
		None		Tumor purity	
		Cor	*P* value	Cor	*P* value
CD8+ T cells	CD8A	0.083	0.0925	0.039	0.455
	CD8B	0.039	0.427	0.012	0.823
CD4+ T cells	CD3D	0.003	0.959	-0.059	0.254
	CD3E	0.043	0.382	-0.022	0.670
	CD2	0.048	0.325	-0.006	0.914
B cells	CD19	0.152	$P < 0.01$	0.127	$P < 0.05$
	CD79A	0.185	$P < 0.001$	0.148	$P < 0.01$
Monocytes	CD86	0.153	$P < 0.01$	0.109	$P < 0.05$
	CD115 (CSF1R)	0.298	$P < 0.001$	0.261	$P < 0.001$
TAMs	CCL2	0.334	$P < 0.001$	0.296	$P < 0.001$
	CD68	0.118	$P < 0.05$	0.085	0.0981
	IL10	0.250	$P < 0.001$	0.217	$P < 0.001$
M1 macrophages	INOS (NOS2)	-0.113	$P < 0.05$	-0.142	$P < 0.01$
	IRF5	0.220	$P < 0.001$	0.201	$P < 0.001$
	COX2 (PTGS2)	0.325	$P < 0.001$	0.312	$P < 0.001$
M2 macrophages	CD163	0.283	$P < 0.001$	0.248	$P < 0.001$
	VSIG4	0.232	$P < 0.001$	0.203	$P < 0.001$
	MS4A4A	0.479	$P < 0.001$	0.194	0.0999
Neutrophils	CD66b (CEACAM8)	-0.109	0.338	-0.089	0.452
	CD11b (ITGAM)	0.407	$P < 0.001$	0.118	0.320
	CCR7	0.384	$P < 0.001$	0.118	0.319
Natural killer cells	KIR2DL1	0.087	0.0768	0.078	0.129
	KIR2DL3	0.055	0.262	0.042	0.410
	KIR2DL4	-0.065	0.184	-0.088	0.087
	KIR3DL1	0.079	0.107	0.084	0.102
	KIR3DL2	0.073	0.138	0.063	0.217
	KIR3DL3	-0.071	0.148	-0.06	0.240
	KIR2DS4	0.019	0.693	0.012	0.819
Dendritic cells	HLA-DPB1	0.129	$P < 0.01$	0.08	0.120
	HLA-DQB1	0.025	0.609	-0.032	0.532
	HLA-DRA	0.086	0.0789	0.046	0.376
	HLA-DPA1	0.108	$P < 0.05$	0.066	0.199
	BDCA-1 (CD1C)	0.370	$P < 0.001$	0.351	$P < 0.001$
	BDCA-4 (NRP1)	0.533	$P < 0.001$	0.504	$P < 0.001$
	CD11c (ITGAX)	0.258	$P < 0.001$	0.217	$P < 0.001$
Th1	T-bet (TBX21)	0.050	0.307	0.008	0.881
	STAT4	0.204	$P < 0.001$	0.172	$P < 0.001$
	STAT1	-0.051	0.304	-0.070	0.174
	IFN-γ (IFNG)	-0.195	$P < 0.001$	-0.229	$P < 0.001$

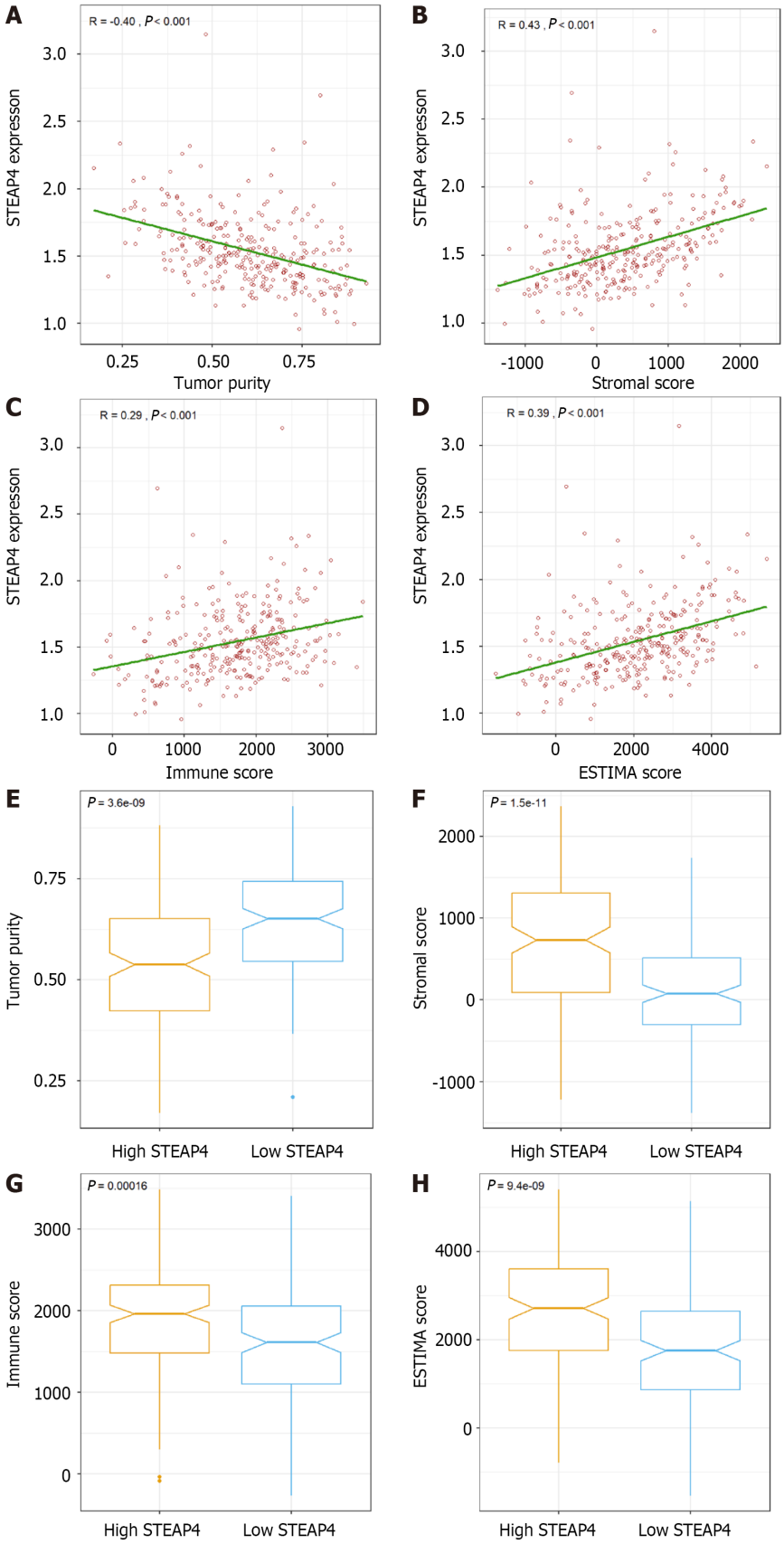
	IFN-α (TNF)	0.005	0.921	-0.045	0.387
Th2	GATA3	0.230	$P < 0.001$	0.205	$P < 0.001$
	STAT6	0.122	$P < 0.01$	0.119	$P < 0.05$
	STAT5A	0.220	$P < 0.001$	0.184	$P < 0.001$
	IL13	0.038	0.436	0.049	0.339
Tfh	BCL6	0.470	$P < 0.001$	0.451	$P < 0.001$
	IL21	-0.031	0.529	-0.055	0.285
Th17	STAT3	0.337	$P < 0.001$	0.310	$P < 0.001$
	IL17A	-0.268	$P < 0.001$	-0.278	$P < 0.001$
Treg	FOXP3	0.025	0.616	-0.028	0.589
	CCR8	0.158	$P < 0.01$	0.129	$P < 0.05$
	STAT5B	0.414	$P < 0.001$	0.383	$P < 0.001$
	TGFβ (TGFB1)	0.299	$P < 0.001$	0.266	$P < 0.001$
T cell exhaustion	PD-1 (PDCD1)	-0.060	0.221	-0.114	$P < 0.05$
	CTLA4	-0.072	0.141	-0.125	$P < 0.05$
	LAG3	-0.119	$P < 0.01$	-0.170	$P < 0.001$
	TIM-3 (HAVCR2)	0.124	$P < 0.05$	0.082	0.112
	GZMB	-0.121	$P < 0.01$	-0.169	$P < 0.001$

STAD: Stomach adenocarcinoma; TAM: Tumor-associated macrophage; Th: T helper cell; Tfh: Follicular helper T cell; Treg: Regulatory T cell; Cor: R value of Spearman's correlation; None: Correlation without adjustment. Tumor purity: Correlation adjusted by tumor purity.



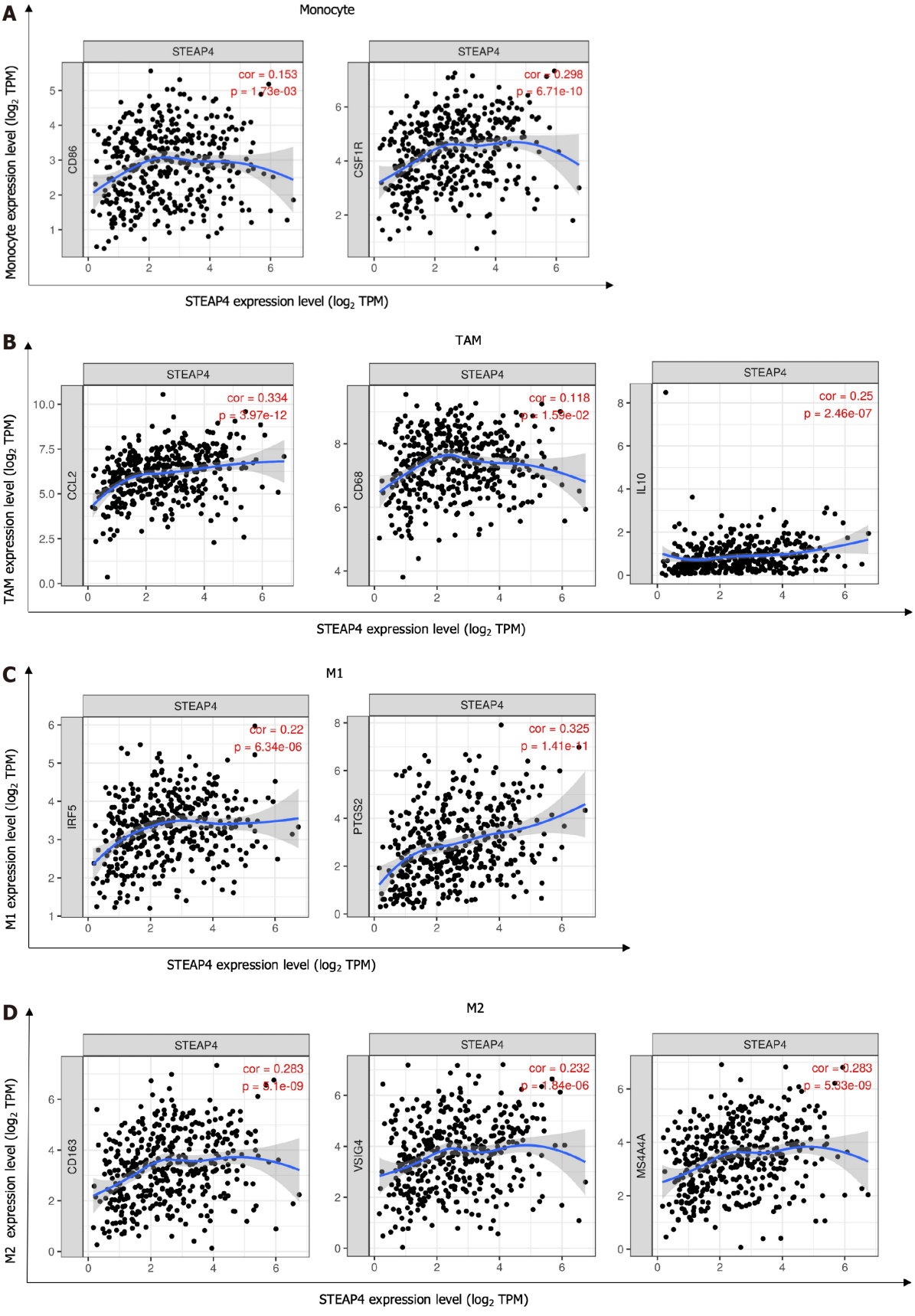
DOI: 10.5306/wjco.v14.i8.297 **Copyright** ©The Author(s) 2023.

Figure 3 Correlation of six-transmembrane epithelial antigen of the prostate 4 expression with immune infiltration level in gastric cancer.
STAD: Stomach adenocarcinoma.



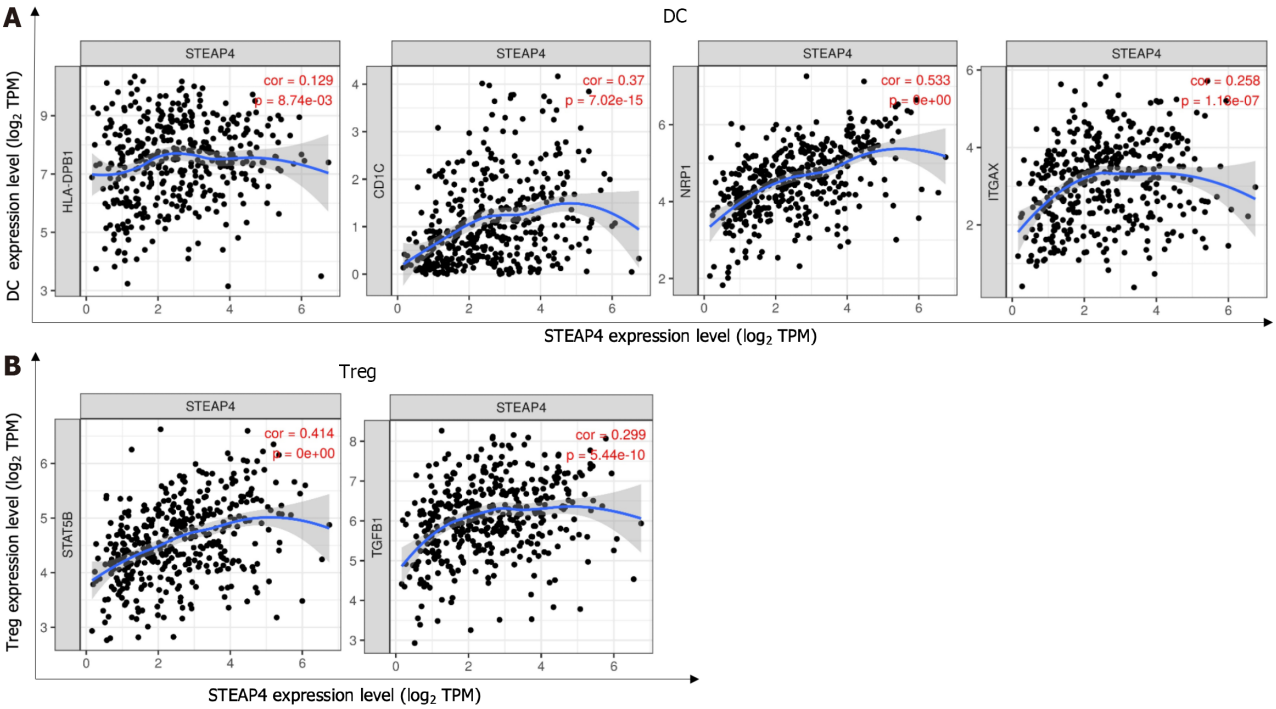
DOI: 10.5306/wjco.v14.i8.297 **Copyright** ©The Author(s) 2023.

Figure 4 Stromal and immune scores in relation to six-transmembrane epithelial antigen of the prostate 4 in gastric cancer. A: Tumor purity; B: Stromal score; C: Immune score; D: ESTIMATE score; E: Tumor purity was higher in the six-transmembrane epithelial antigen of the prostate 4 (STEAP4) low expression group; F-H: Stromal score, immune score and ESTIMATE score were higher in the STEAP4 high expression group. STEAP4: Six-transmembrane epithelial antigen of the prostate 4.



DOI: 10.5306/wjco.v14.i8.297 **Copyright** ©The Author(s) 2023.

Figure 5 Six-transmembrane epithelial antigen of the prostate 4 expression correlates with macrophage infiltration in stomach adenocarcinoma (*n* = 415). A-D: Scatter plots of associations between six-transmembrane epithelial antigen of the prostate 4 and gene markers, including CD86, CSF1R of monocytes (A); CCL2, CD68, and IL10 of TAMs (B); IRF5, PTGS2 of M1 macrophages (C); and CD163, VSIG4, and MS4A4A of M2 macrophages (D). STAD: Stomach adenocarcinoma; TAM: Tumor-associated macrophage; STEAP4: Six-transmembrane epithelial antigen of the prostate 4.



DOI: 10.5306/wjco.v14.i8.297 **Copyright** ©The Author(s) 2023.

Figure 6 The close relationship between six-transmembrane epithelial antigen of the prostate 4 and dendritic cell and Treg cell infiltration. Scatter plots of associations between six-transmembrane epithelial antigen of the prostate 4 and markers, including HLA-DPB1, CD1C, NRP1, and ITGAX of dendritic cells (A); and STAT5B, TGFB1 of Tregs (B). DC: Dendritic cell; Treg: Regulatory T cell; STEAP4: Six-transmembrane epithelial antigen of the prostate 4.

DCs promote tumor metastasis by increasing the activity of Treg cells and decreasing the activity of CD8+ T cells[25]. Here, high expression of STEAP4 was correlated with a high degree of DC infiltration, and DC markers such as HLA-DPB1, CD1C, NRP1 and ITGAX were also significantly correlated with STEAP4 expression ($P < 0.01$). In addition, STEAP4 was positively correlated with, that is STAT5B and TGFB1, biomarkers of Treg cells (Table 3, Figure 6), indicating a close relationship between STEAP4 and DC and Treg cell infiltration. However, whether STEAP4 can also mediate DC and tumor metastasis needs further research.

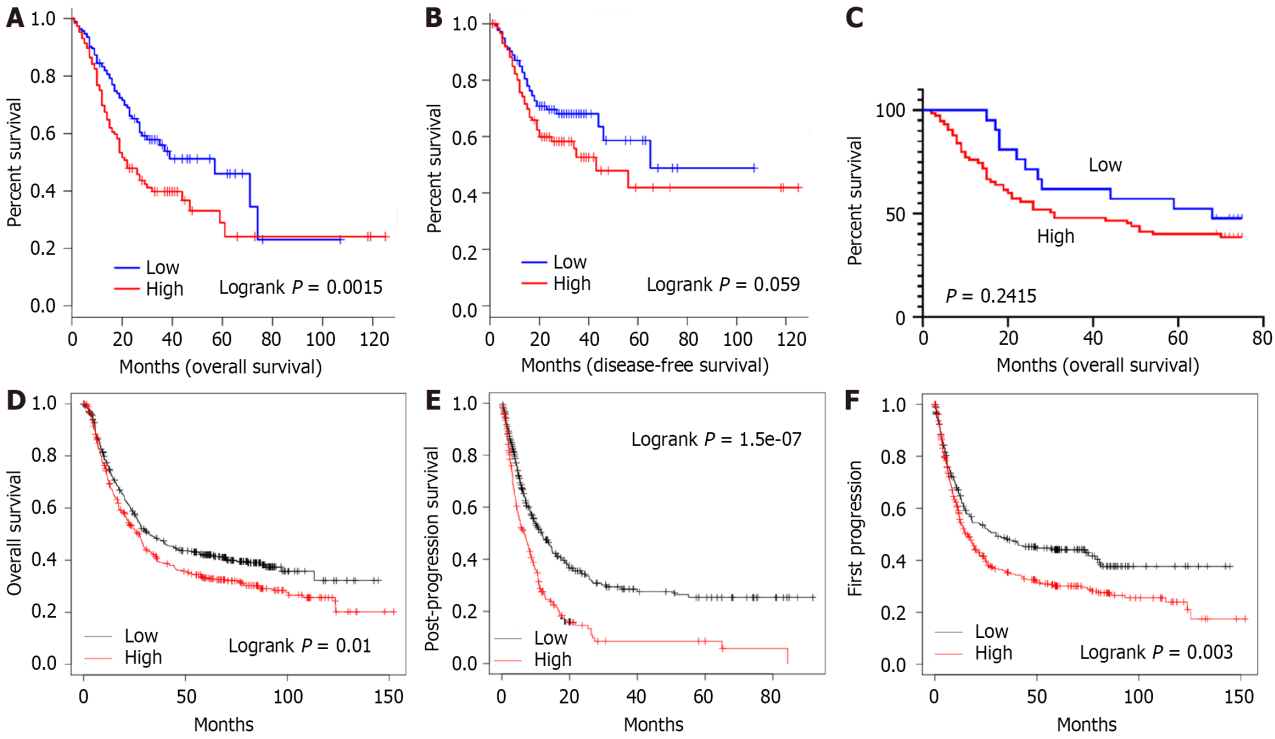
High expression of STEAP4 predicts poor prognosis in patients with GC

Based on the increased level of STEAP4 expression in GC, the prognostic value of STEAP4 was also evaluated on survival rate by using GEPIA database. It is worth noting that the expression of STEAP4 affects prognosis in all GC patients, and patients with high expression of STEAP4 have poor OS ($P = 0.0015$) and disease-free survival (DFS) ($P = 0.059$) (Figure 7A and B).

For immunohistochemical staining of STEAP4, it is showed that STEAP4 expression was not significantly correlated with OS. Although the difference did not meet statistical criteria ($P > 0.05$), high expression of STEAP4 tended to predict shorter OS in patients with GC, suggesting that STEAP4 protein levels could be used as a predictor of survival in patients with GC (Figure 7C). For further investigation, the Kaplan-Meier Plotter database was also applied to evaluate the prognostic signature of STEAP4. Interestingly, poor prognosis [OS: HR = 1.25, 95%CI: 1.05-1.48, $P = 0.01$; post-progression survival (PPS): HR = 1.8, 95%CI: 1.44-2.25, $P = 1.5e-07$; first progression (FP): HR = 1.38, 95%CI: 1.11-1.70, $P = 0.003$] was correlated with higher STEAP4 expression, suggesting that the level of STEAP4 influences the prognosis of GC patients (Figure 7D-F).

DISCUSSION

It is accepted that STEAP4 is an inflammatory metal reductase to catalyze the reduction of copper and iron, and the oxidation of NADPH. It has been shown that STEAP4 expression can promote the uptake of iron and copper, which can only be transported in reduced form through the cell membrane to exert their effects[9,14,26]. Liao *et al*[9] recently reported higher levels of cellular copper can enhance and maintain the activation of NF-κB, which leads to the production of inflammatory cytokines and chemokines, and Zhao *et al*[27] found STEAP4-mediated chemokine and cytokine induction enhances recruitment and activation of immune cells. As an important type of malignancy in gastrointestinal tract, GC is significantly associated with inflammatory and immune infiltration, both of which interact with the tumor microenvironment[28]. However, the regulatory factors in GC are not well characterized regarding inflammatory and immune infiltration.



DOI: 10.5306/wjco.v14.i8.297 **Copyright** ©The Author(s) 2023.

Figure 7 High expression of six-transmembrane epithelial antigen of the prostate 4 tends to be associated with poor prognosis in the patients of gastric cancer. A and B: Survival curves of overall survival (OS) and disease-free survival in the Gene Expression Profiling Interactive Analysis database (n = 384); C: Survival curves of OS in the tissue chip (n = 96); D-F: Survival curves of OS (D), post-progression survival (E), and first progression (F) in the Kaplan-Meier Plotter database (n = 875, n = 498, and n = 640). OS: Overall survival; DFS: Disease-free survival; PPS: Post-progression survival; FP: First progression.

Here, current research focused on STEAP4, a reductase related to oxidation, and its role in the progression of GC. We found that changes in STEAP4 expression levels are associated with the prognosis of GC, predicting poor prognosis of GC patients. Interestingly, high STEAP4 expression had a tendency to promote lymph node metastasis and vascular invasion, proposing STEAP4 as a predictor of tumor metastasis. In addition, we also show that in GC, the level of immune infiltration and multiple immune marker sets are correlated with STEAP4 expression level, and STEAP4 expression is positively correlated with stromal cells and immune cells of the tumor microenvironment. Thus, studies demonstrating the potential role of STEAP4 in tumor immunology and its use as a cancer biomarker provide insight.

In current investigation, we used a GC tissue microarray to determine the expression level of STEAP4 in GC and its adjacent tissues, and prognosis. Based on the immunohistochemical analysis, STEAP4 is highly expressed in GC compared with normal tissues, and is associated with poor prognosis. Although there was a significant correlation between STEAP4 expression and clinicopathological parameters, patients with high STEAP4 expression tended to have a higher pathological stage, lymph node metastasis and vascular invasion. Analysis of the GC cohort in TCGA showed that increased expression of STEP4 is associated with higher clinical stage. Furthermore, analysis of data from GEPIA and Kaplan-Meier Plotter revealed that high levels of STEAP4 expression are associated with high hazard ratios of OS, DFS, PPS, and FP. Together, these findings suggest that STEAP4 may be a prognostic biomarker in GC.

Another important aspect of this study is that STEAP4 expression correlates with different levels of immune infiltration in GC. Our results show a moderate to strong positive correlation between the infiltration levels of M1/M2 macrophages and DCs with STEAP4 expression levels in GC, implicating a potential regulatory function of STEAP4 in tumor-associated macrophage infiltration. Moreover, there is a significant correlation between STEAP4 expression and the regulation of several markers of helper T cells (Trf, Th17, and Treg), and it is known that the recruitment of regulatory T cells (Tregs) is another mechanism of immunosuppression[29]. Tumor cells secrete chemokines to attract Tregs and promote tumor angiogenesis[30], indicating that STEAP4 is a potential source for regulating T cell function in GC.

In addition, ESTIMATE algorithm analysis showed that high STEAP4 expression is positively correlated with stromal cells and immune cells. Interestingly, cancer develops in a complex tissue environment, and they rely on this environment for continuous growth, invasion and metastasis. Studies have shown that under the influence of carcinogenic factors, various cells in the tumor microenvironment undergo metabolic changes, which creates favorable conditions for the occurrence and development of tumors[31]. Not only immune cells, but also other stromal cells constituting the TME are also involved through metabolic reprogramming. Metabolites of stromal cells and immune cells not only serve as nutrient reservoirs to provide energy sources for tumor growth, but also act as messengers to transmit intercellular signals and participate in a variety of tumor-promoting signaling pathways[32]. This may be due to the recruitment of tumor-mediated immune cells by various chemokines secreted by tumor cells through activation of relevant signals in the TME[33]. Therefore, these results reveal that STEAP4 is specifically associated with immune-infiltrating cells, suggesting

that STEAP4 plays a role in immune escape in the microenvironment of GC.

CONCLUSION

The present study found that STEAP4 is a cancer-promoting factor in GC and can be used as a prognostic indicator in GC patients. GC patients with high expression of STEAP4 have a shorter survival time, and may play an important role in immune cell infiltration in GC patients, as well as serve as a prognostic biomarker.

ARTICLE HIGHLIGHTS

Research background

Six-transmembrane epithelial antigen of the prostate 4 (STEAP4), a transmembrane protein involved in metal reductase transport of copper and iron, has been reported as a potential target for cellular and antibody immunotherapy.

Research motivation

Few studies on STEAP4 in gastric cancer (GC), which may play a role in the immune response to the occurrence and development of GC.

Research objectives

The expression of STEAP4 in GC tissues and its correlation with the level of tumor immune infiltration were comprehensively analyzed and to explore the potential immune effect of STEAP4 in GC.

Research methods

The protein expression level, clinicopathological parameters and prognosis of STEAP4 in tumor and adjacent tissues of GC patients were detected by immunohistochemistry. An online database was used to study the correlation between STEAP4 and the level of tumor immunoinfiltration and the characteristics of immunoinfiltration genes. The relationship between STEAP4 expression and immune and stromal scores in the GC was analyzed by ESTIMATE algorithm.

Research results

Immunohistochemistry analysis showed that STEAP4 was highly expressed in GC and was positively correlated with the clinical stage of GC. The infiltration levels of immune cells such as B cells, CD4+ T cells, macrophages, neutrophils and dendritic cells were positively correlated with STEAP4. The expression level of STEAP4 was strongly correlated with most of the immune markers. In addition, the ESTIMATED algorithm analysis showed that the stromal, immune and estimated scores were higher in the group with high expression of STEAP4, while the tumor purity was higher in the STEAP4 Low expression group. The relationship between STEAP4 expression and prognosis of GC patients was further studied, and the results showed that high STEAP4 expression had shorter overall survival and disease-free survival. Moreover, Kaplan-Meier Plotter showed that high expression of STEAP4 was associated with poor survival in patients with GC.

Research conclusions

STEAP4 is indicated as a potential immune indicator of GC, targeting STEAP4 may provide a new therapeutic method for GC patients.

Research perspectives

The comprehensive analysis of STEAP4 function in GC still needs to explore the mechanism by which STEAP4 plays an immune role in GC.

FOOTNOTES

Author contributions: Liu J and Fang ZX designed the research study; Fang ZX performed the research; Fang ZX, Hou YY, Wu Z, Wu BX, Deng Y, and Wu HT analyzed the research and wrote the manuscript; Liu J revised the manuscript critically; and all authors have read and approved the final manuscript.

Supported by the National Natural Science Foundation of China, No. 82273457 and No. 81501539; Guangdong Basic and Applied Basic Research Foundation, No. 2023A1515012762 and No. 2021A1515012180; Special Grant for Key Area Programs of Guangdong Department of Education, No. 2021ZDZX2040; and Science and Technology Special Project of Guangdong Province, No. 210715216902829.

Institutional review board statement: The current study was reviewed and approved by the Ethics Committee of Shantou University Medical College (Approval No. SUMC-2022-075).

Informed consent statement: The informed consent was waived by the Ethics Committee because our experiment was conducted on commercial microarray.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Ze-Xuan Fang 0000-0002-6100-9012; Yan-Yu Hou 0000-0002-4249-8770; Zheng Wu 0000-0002-1393-7586; Bing-Xuan Wu 0000-0002-1212-7936; Yu Deng 0000-0002-0950-6169; Hua-Tao Wu 0000-0002-1640-6094; Jing Liu 0000-0002-7483-4572.

S-Editor: Chen YL
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
2 **Joshi SS**, Badgwell BD. Current treatment and recent progress in gastric cancer. *CA Cancer J Clin* 2021; **71**: 264-279 [PMID: 33592120 DOI: 10.3322/caac.21657]
3 **Songun I**, Putter H, Kranenbarg EM, Sasako M, van de Velde CJ. Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial. *Lancet Oncol* 2010; **11**: 439-449 [PMID: 20409751 DOI: 10.1016/S1470-2045(10)70070-X]
4 **Siegel RL**, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin* 2017; **67**: 7-30 [PMID: 28055103 DOI: 10.3322/caac.21387]
5 **Chen Y**, Jia K, Sun Y, Zhang C, Li Y, Zhang L, Chen Z, Zhang J, Hu Y, Yuan J, Zhao X, Gong J, Dong B, Zhang X, Li J, Shen L. Predicting response to immunotherapy in gastric cancer *via* multi-dimensional analyses of the tumour immune microenvironment. *Nat Commun* 2022; **13**: 4851 [PMID: 35982052 DOI: 10.1038/s41467-022-32570-z]
6 **Zhang H**, Liu H, Shen Z, Lin C, Wang X, Qin J, Qin X, Xu J, Sun Y. Tumor-infiltrating Neutrophils is Prognostic and Predictive for Postoperative Adjuvant Chemotherapy Benefit in Patients With Gastric Cancer. *Ann Surg* 2018; **267**: 311-318 [PMID: 27763900 DOI: 10.1097/SLA.0000000000002058]
7 **Ohgami RS**, Campagna DR, McDonald A, Fleming MD. The Steap proteins are metalloreductases. *Blood* 2006; **108**: 1388-1394 [PMID: 16609065 DOI: 10.1182/blood-2006-02-003681]
8 **Grunewald TG**, Bach H, Cossarizza A, Matsumoto I. The STEAP protein family: versatile oxidoreductases and targets for cancer immunotherapy with overlapping and distinct cellular functions. *Biol Cell* 2012; **104**: 641-657 [PMID: 22804687 DOI: 10.1111/boc.201200027]
9 **Liao Y**, Zhao J, Bulek K, Tang F, Chen X, Cai G, Jia S, Fox PL, Huang E, Pizarro TT, Kalady MF, Jackson MW, Bao S, Sen GC, Stark GR, Chang CJ, Li X. Inflammation mobilizes copper metabolism to promote colon tumorigenesis *via* an IL-17-STEAP4-XIAP axis. *Nat Commun* 2020; **11**: 900 [PMID: 32060280 DOI: 10.1038/s41467-020-14698-y]
10 **Cherner JA**, Cloud ML, Offen WW, Latz JE. Comparison of nizatidine and cimetidine as once-nightly treatment of acute duodenal ulcer. Nizatidine Multicenter Duodenal Ulcer Study Group. *Am J Gastroenterol* 1989; **84**: 769-774 [PMID: 2568086]
11 **Wellen KE**, Fucho R, Gregor MF, Furuhashi M, Morgan C, Lindstad T, Vaillancourt E, Gorgun CZ, Saatcioglu F, Hotamisligil GS. Coordinated regulation of nutrient and inflammatory responses by STAMP2 is essential for metabolic homeostasis. *Cell* 2007; **129**: 537-548 [PMID: 17482547 DOI: 10.1016/j.cell.2007.02.049]
12 **Wu HT**, Chen WJ, Xu Y, Shen JX, Chen WT, Liu J. The Tumor Suppressive Roles and Prognostic Values of STEAP Family Members in Breast Cancer. *Biomed Res Int* 2020; **2020**: 9578484 [PMID: 32802887 DOI: 10.1155/2020/9578484]
13 **Chen WJ**, Wu HT, Li CL, Lin YK, Fang ZX, Lin WT, Liu J. Regulatory Roles of Six-Transmembrane Epithelial Antigen of the Prostate Family Members in the Occurrence and Development of Malignant Tumors. *Front Cell Dev Biol* 2021; **9**: 752426 [PMID: 34778263 DOI: 10.3389/fcell.2021.752426]
14 **Xue X**, Bredell BX, Anderson ER, Martin A, Mays C, Nagao-Kitamoto H, Huang S, Győrffy B, Greenson JK, Hardiman K, Spence JR, Kamada N, Shah YM. Quantitative proteomics identifies STEAP4 as a critical regulator of mitochondrial dysfunction linking inflammation and colon cancer. *Proc Natl Acad Sci U S A* 2017; **114**: E9608-E9617 [PMID: 29078383 DOI: 10.1073/pnas.1712946114]
15 **Burnell SEA**, Spencer-Harty S, Howarth S, Bodger O, Kynaston H, Morgan C, Doak SH. Utilisation of the STEAP protein family in a diagnostic setting may provide a more comprehensive prognosis of prostate cancer. *PLoS One* 2019; **14**: e0220456 [PMID: 31393902 DOI: 10.1371/journal.pone.0220456]
16 **Liu J**, Wei XL, Huang WH, Chen CF, Bai JW, Zhang GJ. Cytoplasmic Skp2 expression is associated with p-Akt1 and predicts poor prognosis in human breast carcinomas. *PLoS One* 2012; **7**: e52675 [PMID: 23300741 DOI: 10.1371/journal.pone.0052675]
17 **Fang ZX**, Li CL, Chen WJ, Wu HT, Liu J. Potential of six-transmembrane epithelial antigen of the prostate 4 as a prognostic marker for colorectal cancer. *World J Gastrointest Oncol* 2022; **14**: 1675-1688 [PMID: 36187390 DOI: 10.4251/wjgo.v14.i9.1675]
18 **Tang Z**, Li C, Kang B, Gao G, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res* 2017; **45**: W98-W102 [PMID: 28407145 DOI: 10.1093/nar/gkx247]

19 **Chen D**, Sun Q, Zhang L, Zhou X, Cheng X, Zhou D, Ye F, Lin J, Wang W. The lncRNA HOXA11-AS functions as a competing endogenous RNA to regulate PADI2 expression by sponging miR-125a-5p in liver metastasis of colorectal cancer. *Oncotarget* 2017; **8**: 70642-70652 [PMID: 29050308 DOI: 10.18632/oncotarget.19956]

20 **Siemers NO**, Holloway JL, Chang H, Chasalow SD, Ross-MacDonald PB, Voliva CF, Szustakowski JD. Genome-wide association analysis identifies genetic correlates of immune infiltrates in solid tumors. *PLoS One* 2017; **12**: e0179726 [PMID: 28749946 DOI: 10.1371/journal.pone.0179726]

21 **Danaher P**, Warren S, Dennis L, D'Amico L, White A, Disis ML, Geller MA, Odunsi K, Beechem J, Fling SP. Gene expression markers of Tumor Infiltrating Leukocytes. *J Immunother Cancer* 2017; **5**: 18 [PMID: 28239471 DOI: 10.1186/s40425-017-0215-8]

22 **Sousa S**, Määttä J. The role of tumour-associated macrophages in bone metastasis. *J Bone Oncol* 2016; **5**: 135-138 [PMID: 27761375 DOI: 10.1016/j.jbo.2016.03.004]

23 **Lánczky A**, Nagy Á, Bottai G, Munkácsy G, Szabó A, Santarpia L, Győrffy B. miRpower: a web-tool to validate survival-associated miRNAs utilizing expression data from 2178 breast cancer patients. *Breast Cancer Res Treat* 2016; **160**: 439-446 [PMID: 27744485 DOI: 10.1007/s10549-016-4013-7]

24 **Yoshihara K**, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, Treviño V, Shen H, Laird PW, Levine DA, Carter SL, Getz G, Stemke-Hale K, Mills GB, Verhaak RG. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun* 2013; **4**: 2612 [PMID: 24113773 DOI: 10.1038/ncomms3612]

25 **Sawant A**, Hensel JA, Chanda D, Harris BA, Siegal GP, Maheshwari A, Ponnazhagan S. Depletion of plasmacytoid dendritic cells inhibits tumor growth and prevents bone metastasis of breast cancer cells. *J Immunol* 2012; **189**: 4258-4265 [PMID: 23018462 DOI: 10.4049/jimmunol.1101855]

26 **Jiang C**, Wu B, Xue M, Lin J, Hu Z, Nie X, Cai G. Inflammation accelerates copper-mediated cytotoxicity through induction of six-transmembrane epithelial antigens of prostate 4 expression. *Immunol Cell Biol* 2021; **99**: 392-402 [PMID: 33179273 DOI: 10.1111/imcb.12427]

27 **Zhao J**, Liao Y, Miller-Little W, Xiao J, Liu C, Li X, Kang Z. STEAP4 expression in CNS resident cells promotes Th17 cell-induced autoimmune encephalomyelitis. *J Neuroinflammation* 2021; **18**: 98 [PMID: 33879167 DOI: 10.1186/s12974-021-02146-7]

28 **Li J**, Xia Y, Sun B, Zheng N, Li Y, Pang X, Yang F, Zhao X, Ji Z, Yu H, Chen F, Zhang X, Zhao B, Jin J, Yang S, Cheng Z. Neutrophil extracellular traps induced by the hypoxic microenvironment in gastric cancer augment tumour growth. *Cell Commun Signal* 2023; **21**: 86 [PMID: 37127629 DOI: 10.1186/s12964-023-01112-5]

29 **Maharaj K**, Uriepero A, Sahakian E, Pinilla-Ibarz J. Regulatory T cells (Tregs) in lymphoid malignancies and the impact of novel therapies. *Front Immunol* 2022; **13**: 943354 [PMID: 35979372 DOI: 10.3389/fimmu.2022.943354]

30 **Facciabene A**, Peng X, Hagemann IS, Balint K, Barchetti A, Wang LP, Gimotty PA, Gilks CB, Lal P, Zhang L, Coukos G. Tumour hypoxia promotes tolerance and angiogenesis *via* CCL28 and T(reg) cells. *Nature* 2011; **475**: 226-230 [PMID: 21753853 DOI: 10.1038/nature10169]

31 **Hanahan D**, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; **144**: 646-674 [PMID: 21376230 DOI: 10.1016/j.cell.2011.02.013]

32 **Hanahan D**, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* 2012; **21**: 309-322 [PMID: 22439926 DOI: 10.1016/j.ccr.2012.02.022]

33 **Melero I**, Rouzaut A, Motz GT, Coukos G. T-cell and NK-cell infiltration into solid tumors: a key limiting factor for efficacious cancer immunotherapy. *Cancer Discov* 2014; **4**: 522-526 [PMID: 24795012 DOI: 10.1158/2159-8290.CD-13-0985]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 August 24; 14(8): 311-323

DOI: 10.5306/wjco.v14.i8.311

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

Readmission rates and outcomes in adults with and without COVID-19 following inpatient chemotherapy admission: A nationwide analysis

Philip Kanemo, Keffi Mubarak Musa, Vaishali Deenadayalan, Rafaella Litvin, Olubunmi Emmanuel Odeyemi, Abdultawab Shaka, Naveen Baskaran, Hafeez Shaka

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Haddadi S, Algeria; Wang LH, China

Received: May 7, 2023
Peer-review started: May 7, 2023
First decision: July 4, 2023
Revised: July 12, 2023
Accepted: August 8, 2023
Article in press: August 8, 2023
Published online: August 24, 2023

Philip Kanemo, Department of Internal Medicine, Rapides Regional Medical Center, Alexandria, LA 71301, United States

Keffi Mubarak Musa, Department of Medicine, Ahmadu Bello University Teaching Hospital, Zaria 88445, Kaduna, Nigeria

Vaishali Deenadayalan, Rafaella Litvin, Hafeez Shaka, Department of Internal Medicine, John H. Stroger, Jr. Hospital of Cook County, Chicago, IL 60623, United States

Olubunmi Emmanuel Odeyemi, Department of Internal Medicine, Ladoke Akintola University Teaching Hospital, Ogbomoso 210101, Oyo, Nigeria

Abdultawab Shaka, Department of Medicine, Windsor University School of Medicine, St. Kitts, Frankfort, IL 60423, United States

Naveen Baskaran, Department of Medicine, University of Florida, Gainesville, FL 32610, United States

Corresponding author: Keffi Mubarak Musa, MBBS, Doctor, Research Assistant, Department of Medicine, Ahmadu Bello University Teaching Hospital, Flat 8, street A, Transit Camp, Shika, Zaria 88445, Kaduna, Nigeria. keffimubarakmusa@gmail.com

Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic has received considerable attention in the scientific community due to its impact on healthcare systems and various diseases. However, little focus has been given to its effect on cancer treatment.

AIM

To determine the effect of COVID-19 pandemic on cancer patients' care.

METHODS

A retrospective review of a Nationwide Readmission Database (NRD) was conducted to analyze hospitalization patterns of patients receiving inpatient

chemotherapy (IPCT) during the COVID-19 pandemic in 2020. Two cohorts were defined based on readmission within 30 d and 90 d. Demographic information, readmission rates, hospital-specific variables, length of hospital stay (LOS), and treatment costs were analyzed. Comorbidities were assessed using the Elixhauser comorbidity index. Multivariate Cox regression analysis was performed to identify independent predictors of readmission. Statistical analysis was conducted using Stata® Version 16 software. As the NRD data is anonymous and cannot be used to identify patients, institutional review board approval was not required for this study.

RESULTS

A total of 87755 hospitalizations for IPCT were identified during the pandemic. Among the 30-day index admission cohort, 55005 patients were included, with 32903 readmissions observed, resulting in a readmission rate of 59.8%. For the 90-day index admission cohort, 33142 patients were included, with 24503 readmissions observed, leading to a readmission rate of 73.93%. The most common causes of readmission included encounters with chemotherapy (66.7%), neutropenia (4.36%), and sepsis (3.3%). Comorbidities were significantly higher among readmitted hospitalizations compared to index hospitalizations in both readmission cohorts. The total cost of readmission for both cohorts amounted to 1193000000.00 dollars. Major predictors of 30-day readmission included peripheral vascular disorders [Hazard ratio (HR) = 1.09, $P < 0.05$], paralysis (HR = 1.26, $P < 0.001$), and human immunodeficiency virus/acquired immuno-deficiency syndrome (HR = 1.14, $P = 0.03$). Predictors of 90-day readmission included lymphoma (HR = 1.14, $P < 0.01$), paralysis (HR = 1.21, $P = 0.02$), and peripheral vascular disorders (HR = 1.15, $P < 0.01$).

CONCLUSION

The COVID-19 pandemic has significantly impacted the management of patients undergoing IPCT. These findings highlight the urgent need for a more strategic approach to the care of patients receiving IPCT during pandemics.

Key Words: Chemotherapy; Coronavirus disease 2019 pandemic; Nationwide readmission database; Readmission rates; Cancer; Healthcare cost

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Our nationwide study explored care for patients undergoing inpatient chemotherapy during the coronavirus disease 2019 (COVID-19) pandemic. It is the first to analyze factors surrounding hospitalization for such patients. We found a higher readmission rate during the pandemic, with comorbidities posing a greater risk. Surprisingly, COVID-19 infection was not significantly linked to readmission. Hospitalization costs rose, averaging 22952.00 dollars. Our findings would interest the scientific community, hospital managers, and health policymakers. Understanding the pandemic's impact on cancer patients' care can lead to mitigating health policies.

Citation: Kanemo P, Musa KM, Deenadayalan V, Litvin R, Odeyemi OE, Shaka A, Baskaran N, Shaka H. Readmission rates and outcomes in adults with and without COVID-19 following inpatient chemotherapy admission: A nationwide analysis. *World J Clin Oncol* 2023; 14(8): 311-323

URL: https://www.wjgnet.com/2218-4333/full/v14/i8/311.htm

DOI: https://dx.doi.org/10.5306/wjco.v14.i8.311

INTRODUCTION

On the 11th of March 2020, the World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) a global pandemic following its discovery in December 2019 in Wuhan, China[1,2]. Since then, WHO has reported over 756 million cases and 6.8 million deaths worldwide[3], with the United States alone accounting for over 100 million cases and 1 million deaths[3]. COVID-19 is caused by a virus known as severe acute respiratory syndrome coronavirus 2[4], which results in a range of respiratory symptoms from mild to severe[4]. However, the introduction and widespread administration of COVID-19 vaccines have contributed to a decline in infection rates[5].

The COVID-19 pandemic has had a profound impact on various aspects of human life[6], with healthcare services and delivery being particularly affected[7,8]. The 30-day readmission rate serves as a crucial metric used by the Center for Medicare and Medicaid Services to evaluate hospitals and assess the quality of healthcare services[9,10]. In 2012, the Center for Medicare and Medicaid Services introduced the Hospital Readmission Reduction Program to enhance healthcare quality and reduce costs[11]. The annual cost associated with readmissions averages between 15 and 20 billion dollars[12]. Reducing the 30-day readmission rates can significantly decrease healthcare costs and alleviate the strain on healthcare facilities[13]. Assessing readmission rates becomes even more important for patients undergoing chemotherapy, as chemotherapy often entails extended periods of treatment and substantial healthcare expenses[13]. A systematic review conducted prior to the COVID-19 pandemic revealed readmission rates ranging from 3% to 34% for

patients undergoing chemotherapy[14]. Another study by Tennison *et al*[14] reported a 55% readmission rate for patients undergoing chemotherapy in United States hospitals. However, since the onset of the COVID-19 pandemic, there has been a scarcity of data regarding the hospitalization and care of patients receiving chemotherapy during this period.

This study aims to investigate the impact of the COVID-19 pandemic on 30-day and 90-day readmission rates among patients hospitalized for inpatient chemotherapy (IPCT). We also aim to identify common causes and independent predictors of readmission in this patient population. By conducting this study, we aim to gain a deeper understanding of the effects of COVID-19 on the management of cancer patients. Furthermore, the findings of this study can contribute to the development of strategies that improve the care of cancer patients. Finally, we believe that this study will pave the way for further research on the effects of pandemics on healthcare infrastructure and services.

MATERIALS AND METHODS

Study design and data source

We conducted a retrospective cross-sectional review of hospitalizations for IPCT across the United States during a one-year period in 2020. Hospitalization data for 2020 was retrieved from the Nationwide Readmission Database (NRD). The NRD is a national database that captures patients' hospitalization, readmissions, and other relevant discharge histories from over 31 different states in the United States. The NRD is a product of the Healthcare Cost and Utilization Project (HCUP), State Inpatient Databases, and the Agency for Healthcare Research and Quality[9]. The database records over 40 International Classification of Diseases-10 (ICD-10) recognized diagnoses and 25 procedures[9]. It covers approximately 62.2% of the United States population and 60.8% of total hospitalizations in the country[15]. It contains unique, verified de-identified patient linkage that enables tracking of individual hospitalizations and readmissions. Data within the NRD is available from January 1 to December 31 each year, and information outside of these dates cannot be accessed[16]. With over 18 million hospital stays recorded, the NRD provides ample and suitable data for our study.

Data Collection

We collected data on adult hospitalizations (age >18 years) for IPCT during the COVID-19 pandemic in 2020. Hospitalizations for conditions other than IPCT and those involving patients under 18 years of age were excluded from the study. Additionally, hospitalizations in December were excluded due to the lack of an adjoining 30-day period to determine 30-day readmission. The hospitalizations were divided into two groups: The 30-day readmission cohort (30DRC) and the 90-day readmission cohort (90DRC). Within each cohort, we identified and tagged each case that met our inclusion criteria as an index case on the first admission. Each index case was traced for readmission within 30 d of admission and tagged as a 30-day readmission in the 30DRC. Similarly, each index case was traced for readmission within 90 d of admission and tagged as a 90-day readmission in the 90DRC. Specific patient data, including demographics (age, sex, health insurance type, household income), mortality on readmission, LOS, and cost of admission, were collected. Hospital-specific variables, such as type of hospital, bed size, and hospital location, were also obtained. To account for the effects of comorbid conditions, we utilized the Elixhauser Comorbidity Index (ECI) to assess the level of comorbidities in the hospitalizations. The ECI is a software tool developed as part of HCUP, which identifies 38 different pre-existing comorbidities in hospital administrative data[15]. The ECI software has been refined for ICD-10 comorbidities and is available in nationwide HCUP databases for years 2019 onwards[15]. The ECI demonstrates a better prognostic value compared to the Charlson comorbidity index[16].

Outcome measures

The primary outcome of our study was the all-cause 30-day and 90-day readmission rates. Secondary outcomes included demographic characteristics, insurance type, mortality rate during readmission, average LOS, average cost of readmission, and independent predictors of readmission.

Analysis method

All analyses were performed using weighted samples for national estimates in accordance with HCUP regulations for using the NRD[17]. Data analysis was conducted using Stata® Version 16 software (StataCorp, Texas, United States). We examined demographic characteristics and calculated mean age, sex distribution, and mean household income. Additionally, we analyzed hospital-specific features, including hospital location, teaching status, and bed size. Comorbidities were calculated as proportions in our cohorts using the 31 ECI comorbidities, and the chi-square test was employed to compare characteristics between index hospitalizations and readmissions in 2020. A multivariate Cox regression analysis was performed to identify independent variables associated with readmission.

Ethical Consideration

As with other HCUP databases, the NRD data is anonymous and cannot be used to identify individual patients. Therefore, institutional review board approval was not required for our study.

RESULTS

We identified a total of 87756 hospitalizations for IPCT in the 2020 NRD database. In the 30DRC, we identified 55005 index hospitalizations during the study period. Among these, there were 32904 readmissions within 30 d, resulting in a 30-day readmission rate of 59.8%. Table 1 provides a comparison of the demographics of hospitalized patients between index hospitalizations and readmission cohort. Among the 90DRC, we identified 33636 index hospitalizations, of which 24503 patients were readmitted within 90 d of admission. The 90-day readmission rate was 73.93%. In both readmission cohorts, the proportion of male patients was higher than female patients. The majority of hospitalized patients in both cohorts were in their middle age. Private health insurance was the primary payer for hospital bills in most cases. A significant number of patients in both readmission cohorts belonged to the 26th-50th quantile of the national median household income. The rates of 30-day and 90-day readmission were higher in patients with Medicaid and private insurance, as well as those with a higher comorbidity burden (ECI score ≥ 4).

Comorbidities analyzed were significantly more prevalent in readmissions compared to index hospitalizations in both readmission cohorts. Detailed comparisons of comorbidities between index hospitalizations and readmissions in the 30DRCs and 90DRCs are listed in Tables 2 and 3, respectively. The majority of patients tested negative for COVID-19 in both index hospitalizations and readmissions, as depicted in Figure 1. Metropolitan teaching hospitals had the highest number of admissions in both cohorts. Table 4 summarizes the hospital characteristics among index hospitalizations and readmissions.

Common causes of readmission in both readmission cohorts included admissions for chemotherapy, neutropenia, non-specified sepsis, antineoplastic-induced pancytopenia, agranulocytosis secondary to chemotherapy, sepsis due to *Escherichia coli*, admissions for immunotherapy, acute myeloblastic leukemia, specified sepsis, and acute kidney failure. Figure 2 demonstrates the top causes of 30-day readmission during the COVID-19 pandemic. Mortality was higher among readmitted patients in both readmission cohorts. Figure 3 compares the mortality in index hospitalizations and 30DRCs and 90DRCs, respectively.

In both cohorts, readmissions had a shorter average LOS compared to index hospitalizations. The average LOS for readmitted patients was 5.60 d in the 30DRC, compared to 6.77 d for index cases ($P < 0.001$). In the 90DRC, the mean LOS for readmitted patients was 6.37 d, while index hospitalizations had a mean LOS of 7.51 d ($P < 0.001$). The total number of days lost due to hospitalization was higher in the 30DRC, totaling 184277 d compared to 156086 d in the 90-day cohort. The mean adjusted cost of hospitalization was higher in the 90-day cohort, with an average of 25646.4 dollars spent per index admission and 23477.0 dollars spent per readmission. In the 30DRC, the average cost per index admission was 22951.9 dollars, and 19220.8 dollars per readmission. The total cost incurred due to readmission across the country was 625 million dollars for the 30DRC and 568 million dollars for the 90DRC.

The results of the multivariable Cox regression analysis to identify independent predictors of 30-day and 90-day readmission are shown in Tables 5 and 6, respectively. Presence of comorbidities, including peripheral vascular disorder [Hazard ratio (HR) = 1.09, $P = 0.048$], paralysis (HR = 1.26, $P < 0.001$), human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) (HR = 1.14, $P = 0.03$), and lymphoma (HR = 1.23, $P < 0.001$), were associated with an increased risk of readmission for IPCT within 30 d of discharge during the COVID-19 pandemic. Being in the middle age group (HR = 0.83, $P < 0.001$), elderly age groups (HR = 0.78, $P < 0.001$), discharge against medical advice (HR = 0.69, $P = 0.031$), renal failure (HR = 0.89, $P = 0.004$), liver disease (HR = 0.9, $P = 0.02$), and coagulopathy (HR = 0.91, $P = 0.002$) were associated with a decreased risk of readmission within 30 d of discharge. Figure 4 shows a Kaplan-Meier readmission curve for 30-day readmissions by COVID-19 status, with a P-value < 0.01.

A similar profile of comorbidities increased the risk of 90-day readmission as observed in the 30DRC, except for HIV/AIDS (HR = 1.1, $P = 0.211$). Other variables analyzed for the risk of 90-day readmission followed the same trend as the 30DRC, except for coagulopathy (HR = 0.95, $P = 0.093$).

DISCUSSION

Our study provides a comprehensive nationwide view of the care received by patients undergoing IPCT during the COVID-19 pandemic. To the best of our knowledge, this is the first study that specifically focused on and analyzed factors related to hospitalization for patients receiving IPCT during the pandemic. We observed a 30-day readmission rate of 58.9% and a 90-day readmission rate of 73.93%, both of which are significantly higher than rates reported in previous similar studies[18,19]. This increase can be attributed to the strain imposed on the healthcare system by the pandemic. Similar findings were reported by Loo *et al*[20] and Matthews *et al*[21], who also observed an increase in readmission rates during the COVID-19 pandemic. The demographics of our patients were comparable and consistent with those reported in studies conducted before the pandemic[11,22].

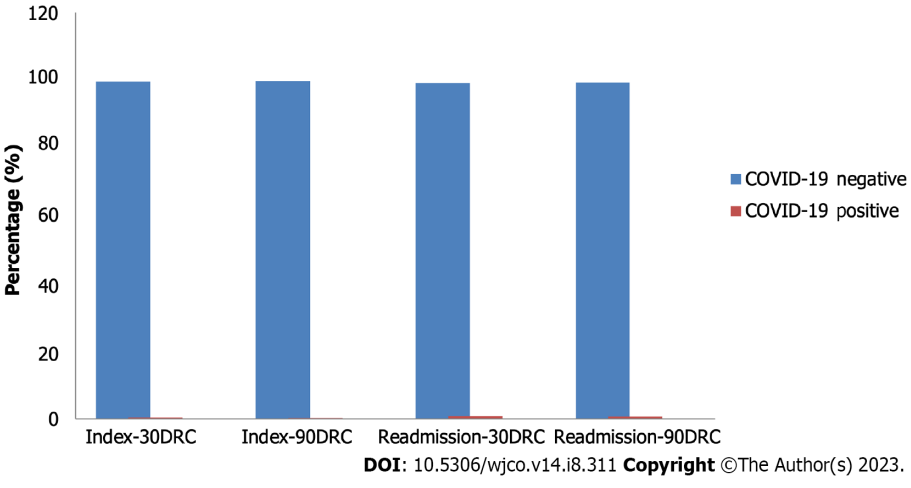
Several studies conducted during the pandemic have reported higher costs of hospitalization, and our study aligns with these findings[23,24]. With an average cost of re-hospitalization of 22952.0 dollars observed in our study, the cost was significantly higher than the average cost of 17035 dollars reported in similar hospitalizations before the pandemic [25]. However, contrary to the findings of higher readmission costs compared to index admissions reported by Kwei-Nsoro *et al*[9], our study revealed a higher cost of index admission.

The ECI scores were higher among readmitted hospitalizations compared to index hospitalizations due to the higher comorbidity burden among readmitted patients. Higher ECI scores are associated with higher mortality[26,27], which was also observed in our study, consistent with previous studies[28,29].

Table 1 Comparison of patients' demographics between inpatient chemotherapy and readmission for inpatient chemotherapy, Nationwide Readmission Database, 2020

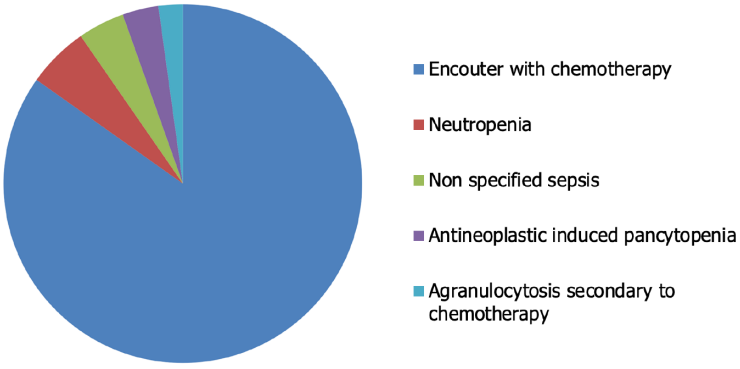
Variables (unit)	Index admission	30 d readmission	*P* value
Mean age ± SD (yr)	54.7 ± 17.71	53.53 ± 17.94	
Age range categories (%)			< 0.001
18-44 yr	26.86	29.5	
45-64 yr	40.06	39.62	
65 yr and above	33.08	30.89	
Sex (%)			0.182
Male	58.07	58.47	
Female	41.93	41.53	
Payer type (%)			< 0.001
Medicare	34.43	32.33	
Medicaid	17.06	18.14	
Private insurance	46.14	47.25	
Self pay	2.36	2.28	
Median household income (%)			0.617
0-25th quintile	22.28	21.96	
26th-50th quintile	27.91	28.02	
50th-75th quintile	25.61	25.85	
> 75th quintile	24.19	24.18	
ECI score (%)			< 0.001
0	4.88	4.14	
1	18.61	17.7	
2	23.75	23.35	
3	20.61	20.53	
≥ 4	32.15	34.28	

ECI: Elixhauser comorbidity index.



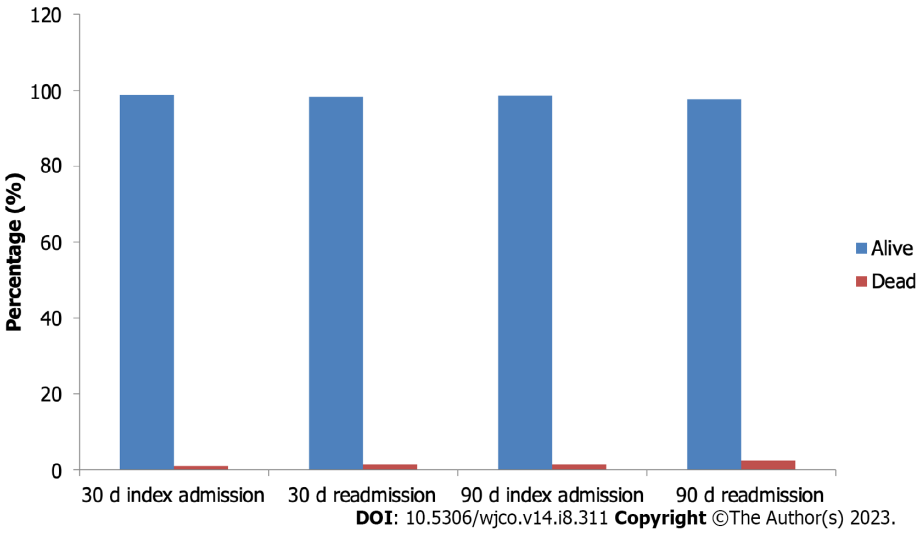
DOI: 10.5306/wjco.v14.i8.311 **Copyright** ©The Author(s) 2023.

Figure 1 Coronavirus disease 2019 status of index and readmitted case for 30-days and 90-days readmission cohort, 2020. COVID-19: Coronavirus disease 2019; DRC: Day readmission cohort.



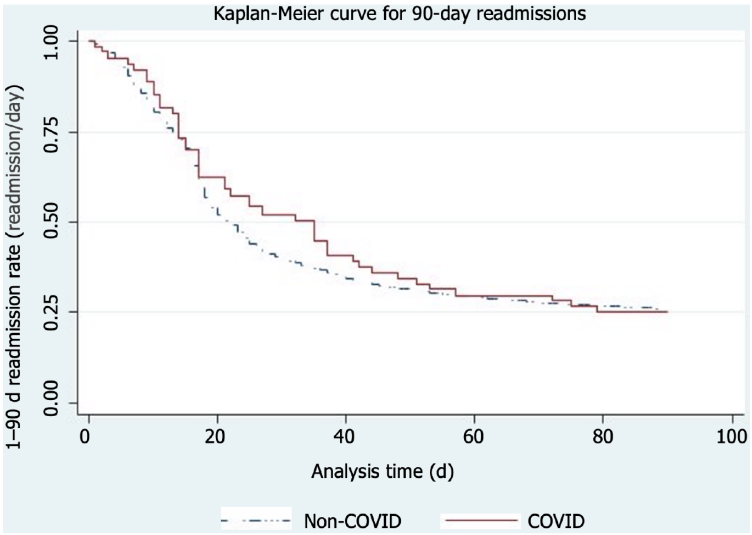
DOI: 10.5306/wjco.v14.i8.311 **Copyright** ©The Author(s) 2023.

Figure 2 Top five causes of 30-days readmission for inpatient chemotherapy during coronavirus disease 2019 pandemic, Nationwide Readmission Database, 2020.



DOI: 10.5306/wjco.v14.i8.311 **Copyright** ©The Author(s) 2023.

Figure 3 Comparison of Mortality Between Index Admission and Readmission in 30-days and 90-days readmission cohort, Nationwide Readmission Database, 2020.



DOI: 10.5306/wjco.v14.i8.311 **Copyright** ©The Author(s) 2023.

Figure 4 Kaplan-Meier curve for 90-readmision, Nationwide Readmission Database, 2023. COVID: Coronavirus disease.

Table 2 Comparing the 31 elixhauser comorbidities between index admission and 30-days readmission for inpatient chemotherapy, Nationwide Readmission Database, 2020

Variables (%)	Index	Readmission	*P* value
Congestive heart failure	5.45	5.83	0.02
Cardiac arrhythmias	12.14	13.13	< 0.001
Valvular diseases	2.48	2.12	0.001
Pulmonary circulation disorders	2.1	2.47	0.001
Peripheral vascular disorders	4.81	5.1	0.141
Hypertension, uncomplicated	34.12	32.88	0.001
Paralysis	1.06	1.17	0.181
Other neurologic disorders	4.72	5.72	< 0.001
Chronic pulmonary disease	11.07	10.5	0.012
Diabetics, uncomplicated	8.44	8.5	0.757
Diabetics, complicated	8.63	8.28	0.105
Hypothyroidism	9.88	9.61	0.134
Renal failure	7.75	6.89	< 0.001
Liver disease	4.06	4.09	0.854
Peptic ulcer disease	0.34	0.33	0.859
HIV/AIDS	1.26	1.44	0.003
Lymphoma	45.89	48.77	< 0.001
Metastatic cancer	14.93	14.78	0.637
Solid tumor without metastasis	24.4	24.84	0.255
RA/collagen vascular disease	2.03	1.9	0.226
Coagulopathy	12.64	14.41	< 0.001
Obesity	10.33	9.81	0.159
Weight loss	10.76	11.12	0.207
Fluid and electrolyte disorders	23.17	28.16	< 0.001
Blood loss anemia	0.39	0.35	0.497
Deficiency anemia	3.09	2.63	0.001
Alcohol abuse	1.13	0.98	0.103
Drug abuse	2.32	2.35	0.832
Psychosis	0.68	0.76	0.089
Depression	12.39	12.59	0.429
Hypertension, complicated	8.04	7.66	0.063

HIV/AIDS: Human immunodeficiency virus/acquired immuno-deficiency syndrome; RA: Rheumatoid arthritis.

The most common cause of readmission was admission for chemotherapy. The Kaplan-Meier curve (Figure 4) demonstrated a shorter time to 50% readmission in the non-COVID-19 group (20 d) compared to the COVID-19 group (36 d). This could be explained by the fact that COVID-19 positivity delayed the admission for chemotherapy, which was the most common cause of readmissions. Other causes of readmissions included neutropenia, sepsis, and acute kidney injury, in line with previous studies[13,30].

We observed a significant number of patients undergoing IPCT being managed in medium-sized metropolitan teaching hospitals. However, we did not observe any significant difference in the type of treatment center between index hospitalizations and readmissions. Middle-aged and elderly patients had a decreased risk of readmission, likely due to the higher prevalence of comorbidities in these age groups. Our results showed that comorbidities were associated with an increased risk of readmission, consistent with findings in other studies[31,32].

Table 3 Comparison of the 31 elixhauser comorbidities between index admission and 90-days readmission for inpatient chemotherapy, Nationwide Readmission Database, 2020

Variables (%)	Index	Readmission	*P* value
Congestive heart failure	5.88	6.59	0.002
Cardiac arrhythmias	13.16	14.17	0.001
Valvular diseases	2.69	2.19	0.001
Pulmonary circulation disorders	2.28	2.57	0.052
Peripheral vascular disorders	4.72	5.19	0.024
Hypertension, uncomplicated	34.58	33.12	< 0.001
Paralysis	1.09	1.13	0.645
Other neurologic disorders	4.81	5.91	< 0.001
Chronic pulmonary disease	11.68	11.09	0.026
Diabetics, uncomplicated	8.54	8.51	0.908
Diabetics, complicated	8.86	8.7	0.539
Hypothyroidism	10.31	9.77	0.003
Renal failure	8.38	7.88	0.033
Liver disease	4.36	4.42	0.814
Peptic ulcer disease	0.29	0.37	0.196
HIV/AIDS	1.09	1.12	0.667
Lymphoma	43.95	44.89	0.012
Metastatic cancer	15.5	14.48	< 0.001
Solid tumor without metastasis	24.39	23.94	0.181
RA/collagen vascular disease	2.13	1.91	0.077
Coagulopathy	13.27	15.73	< 0.001
Obesity	10.41	9.77	0.166
Weight loss	11.8	12.44	0.055
Fluid and electrolyte disorders	24.87	30.26	< 0.001
Blood loss anemia	0.44	0.42	0.876
Deficiency anemia	3.16	2.51	< 0.001
Alcohol abuse	1.29	1.13	0.144
Drug abuse	2.33	2.31	0.922
Psychosis	0.71	0.77	0.269
Depression	12.23	12.95	0.015
Hypertension, complicated	8.81	8.75	0.785

HIV/AIDS: Human immunodeficiency virus/acquired immuno-deficiency syndrome; RA: Rheumatoid arthritis.

Previous studies have indicated that discharge against medical advice increases the risk of readmission, but our results were contrary to this[16,32]. This could be explained by the possibility that patients who left the hospital against medical advice had limited access to the healthcare system, which was heavily impacted by the pandemic[8,33]. However, further research is needed to explore this area. We found weight loss to be an independent predictor of 90-day readmission, which is consistent with a survey of approximately 10000 general medicine discharges where weight loss was identified as a significant predictor of 30-day readmissions, aligning with our findings in the 90DRC[33]. However, we did not find weight loss to be an independent predictor of 30-day readmission, and the reason for this remains unclear. Additionally, contrary to our expectations and findings in similar studies[21,34], COVID-19 was not identified as an independent predictor of readmission. This could be due to the smaller percentage of COVID-19-infected patients in our study population or could be an area for further investigation.

Table 4 Comparison of hospital specific characteristics of index admissions and readmissions for inpatient chemotherapy, Nationwide Readmission Database, 2020

Variables	Index	Readmission	*P* value
Hospital bed size (%)			0.002
Small	7.51	8.49	
Medium	15.96	16.01	
Large	76.53	75.5	
Teaching status of hospital (%)			0.002
Metropolitan, non teaching	5.51	6.09	
Metropolitan teaching	93.48	92.27	
Non-metropolitan	1.01	1.64	

Finally, we acknowledge some limitations in our study. The readmission rates may vary across different states, but the NRD does not provide state-specific data, preventing us from drawing conclusions at the state level. Our study excluded elective hospitalizations in December, potentially leading to a missed number of readmissions during that month.

CONCLUSION

The COVID-19 pandemic has significantly impacted the management of patients receiving IPCT. There is a need for a more strategic approach in the care of patients undergoing IPCT during pandemics.

Table 5 Independent predictors of 30 d readmission for inpatient chemotherapy, Nationwide Readmission Database, 2020

Variables	Hazard ratio	Confidence interval	*P* value
Age category			
45-64 years	0.83	0.79-0.87	< 0.001
65 years and above	0.78	0.72-0.85	< 0.001
Discharge AMA	0.69	0.49-0.97	0.031
Payer type			
Medicaid	1.04	0.97-1.12	0.299
Private insurance	1.02	0.99-1.09	0.564
Self pay	1.05	0.90-1.23	0.522
Median household income			
25th-50th quantile	1.04	0.97-1.12	0.137
50th-75th quantile	1.04	0.96-1.09	0.103
> 75th quantile	1.03	0.90-1.09	0.263
COVID-19	0.91	0.68-1.22	0.543
Comorbidities			
Congestive heart failure	0.92	0.85-1.00	0.059
Cardiac arrhythmias	0.96	0.91-1.01	0.147
Peripheral vascular disorders	1.09	1.00-1.19	0.048
Hypertension, uncomplicated	1.03	0.99-1.07	0.111
Paralysis	1.26	1.08-1.47	0.003
Chronic pulmonary disease	0.96	0.91-1.02	0.233
Diabetics, uncomplicated	0.94	0.89-1.00	0.058

Renal failure	0.89	0.82-0.96	0.004
Liver disease	0.9	0.82-0.98	0.023
HIV/AIDS	1.14	1.01-1.30	0.038
Lymphoma	1.23	1.17-1.28	< 0.001
Coagulopathy	0.91	0.86-0.97	0.002
Weight loss	0.97	0.92-1.04	0.457
Hypertension, complicated	1.05	0.96-1.15	0.28

AMA: Against medical advice; HIV/AIDS: Human immunodeficiency virus/acquired immuno-deficiency syndrome.

Table 6 Independent predictors of 90 d readmission for inpatient chemotherapy, Nationwide Readmission Database, 2020

Variables	Hazard ratio	Confidence interval	*P* value
Age category			
45-64 yr	0.84	0.80-0.89	< 0.001
65 yr and above	0.74	0.68-0.80	< 0.001
Discharge AMA	0.65	0.47-0.90	0.01
Payer type			
Medicaid	1.02	0.95-1.09	0.633
Private insurance	1.02	0.95-1.08	0.631
Self pay	1.15	0.99-1.33	0.056
Median household income			
25th-50th quintile	1.03	0.97-1.09	0.329
50th-75th quintile	1.04	0.98-1.11	0.166
> 75th quintile	1.03	0.96-1.10	0.471
COVID-19	0.88	0.66-1.18	0.396
Comorbidities			
Congestive heart failure	0.93	0.85-1.01	0.086
Cardiac arrhythmias	0.97	0.92-1.02	0.198
Peripheral vascular disorders	1.15	1.04-1.26	0.004
Hypertension, uncomplicated	1.02	0.98-1.07	0.296
Paralysis	1.21	1.02-1.43	0.027
Chronic pulmonary disease	0.97	0.92-1.03	0.386
Diabetics, uncomplicated	0.92	0.87-0.99	0.01
Renal failure	0.91	0.83-0.99	0.046
Liver disease	0.89	0.82-0.98	0.017
HIV/AIDS	1.1	0.94-1.29	0.211
Lymphoma	1.14	1.09-1.20	< 0.001
Coagulopathy	0.95	0.90-1.00	0.093
Weight loss	0.93	0.88-0.99	0.019
Hypertension, complicated	1.03	0.93-1.14	0.604

AMA: Against medical advice; HIV/AIDS: Human immunodeficiency virus/acquired immuno-deficiency syndrome.

ARTICLE HIGHLIGHTS

Research background

The coronavirus disease 2019 (COVID-19) pandemic has had a profound impact on healthcare services and has resulted in modifications to the management of various diseases.

Research motivation

The treatment of cancer has undergone significant changes during the COVID-19 pandemic. Understanding the effects of these changes can provide valuable insights to better prepare for future pandemics.

Research objectives

This study aims to provide insights into the outcomes of hospitalization for in hospital chemotherapy during the COVID-19 pandemic.

Research methods

We conducted a retrospective review of a Nationwide Readmission Database for patients undergoing inpatient chemotherapy (IPCT) during the COVID-19 pandemic. We analyzed data on readmission rates, causes of readmission, and predictors of readmission.

Research results

We found a 90-day readmission rate of 59.8% and a 30-day readmission rate of 73.93%. The most common cause of readmission was chemotherapy encounters (66.7%). Predictors of readmission included peripheral vascular disorders [Hazard ratio (HR) = 1.09, P = 0.04] and paralysis (HR = 1.26, P < 0.001). The total cost incurred due to readmission during the pandemic was 1193000000.00 dollars.

Research conclusions

The COVID-19 pandemic has had a significant impact on the management of cancer patients. There is a need for a more strategic approach to the care of patients undergoing IPCT during pandemics.

Research perspectives

This study opens the door for further investigation into the effects of pandemics on disease management.

FOOTNOTES

Author contributions: Kanemo P and Shaka H conceived of the presented idea and designed and proposed the study protocol; Deenadayalan V and Litvin R extracted data from the nationwide readmission database; Shaka A and Baskaran N provided tools for analysis and conducted the analysis; Musa KM, Shaka H and Odeyemi OE interpreted the analysis results and wrote the manuscript; Shaka H and Kanemo P supervised the findings of this work; all authors discussed the results and contributed to the final manuscript.

Institutional review board statement: As the nationwide readmission database data is anonymous and cannot be used to identify patients, institutional review board approval was not required for this study.

Informed consent statement: As the nationwide readmission database data is anonymous and cannot be used to identify patients, informed consent statement was not required for this study.

Conflict-of-interest statement: The authors hereby declare no conflict of interest.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: United States

ORCID number: Keffi Mubarak Musa 0000-0003-0946-156X; Olubunmi Emmanuel Odeyemi 0000-0003-1386-8936; Hafeez Shaka 0000-0002-9456-4581.

S-Editor: Lin C
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **World Health Organization**. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. Mar 11, 2020. [cited Feb 23, 2023]. Available from: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020

2 **Umakanthan S**, Sahu P, Ranade AV, Bukelo MM, Rao JS, Abrahao-Machado LF, Dahal S, Kumar H, Kv D. Origin, transmission, diagnosis and management of coronavirus disease 2019 (COVID-19). *Postgrad Med J* 2020; **96**: 753-758 [PMID: 32563999 DOI: 10.1136/postgradmedj-2020-138234]

3 **World Health Organization**; WHO Coronavirus (COVID-19) Dashboard. Feb 23, 2022. [cited Feb 25, 2022]. Available from: https://covid19.who.int

4 **Sharma A**, Ahmad Farouk I, Lal SK. COVID-19: A Review on the Novel Coronavirus Disease Evolution, Transmission, Detection, Control and Prevention. *Viruses* 2021; **13** [PMID: 33572857 DOI: 10.3390/v13020202]

5 **da Silva SJR**, do Nascimento JCF, Germano Mendes RP, Guarines KM, Targino Alves da Silva C, da Silva PG, de Magalhães JJF, Vigar JRJ, Silva-Júnior A, Kohl A, Pardee K, Pena L. Two Years into the COVID-19 Pandemic: Lessons Learned. *ACS Infect Dis* 2022; **8**: 1758-1814 [PMID: 35940589 DOI: 10.1021/acsinfecdis.2c00204]

6 **Haleem A**, Javaid M, Vaishya R. Effects of COVID-19 pandemic in daily life. *Curr Med Res Pract* 2020; **10**: 78-79 [PMID: 32292804 DOI: 10.1016/j.cmrp.2020.03.011]

7 **Kirchberg TN**, Costantini TW, Santorelli J, Doucet JJ, Godat LN. Predictors of Readmission Following Treatment for Traumatic Hemothorax. *J Surg Res* 2022; **277**: 365-371 [PMID: 35569214 DOI: 10.1016/j.jss.2022.04.031]

8 **Global Health Research Group on Children's Non-Communicable Diseases Collaborative**. Impact of the COVID-19 pandemic on patients with paediatric cancer in low-income, middle-income and high-income countries: a multicentre, international, observational cohort study. *BMJ Open* 2022; **12**: e054690 [PMID: 35410925 DOI: 10.1136/bmjopen-2021-054690]

9 **Kwei-Nsoro R**, Ojemolon P, Laswi H, Ebhohon E, Shaka A, Mir WA, Siddiqui AH, Philipose J, Shaka H. Rates, Reasons, and Independent Predictors of Readmissions in Portal Venous Thrombosis Hospitalizations in the USA. *Gastroenterology Res* 2022; **15**: 253-262 [PMID: 36407807 DOI: 10.14740/gr1561]

10 **Kocher RP**, Adashi EY. Hospital readmissions and the Affordable Care Act: paying for coordinated quality care. *JAMA* 2011; **306**: 1794-1795 [PMID: 22028355 DOI: 10.1001/jama.2011.1561]

11 **Wang S**, Zhu X. Nationwide hospital admission data statistics and disease-specific 30-day readmission prediction. *Health Inf Sci Syst* 2022; **10**: 25 [PMID: 36065327 DOI: 10.1007/s13755-022-00195-7]

12 **Brooks GA**, Li L, Sharma DB, Weeks JC, Hassett MJ, Yabroff KR, Schrag D. Regional variation in spending and survival for older adults with advanced cancer. *J Natl Cancer Inst* 2013; **105**: 634-642 [PMID: 23482657 DOI: 10.1093/jnci/djt025]

13 **Bell JF**, Whitney RL, Reed SC, Poghosyan H, Lash RS, Kim KK, Davis A, Bold RJ, Joseph JG. Systematic Review of Hospital Readmissions Among Patients With Cancer in the United States. *Oncol Nurs Forum* 2017; **44**: 176-191 [PMID: 28222076 DOI: 10.1011/17.ONF.176-191]

14 **Tennison JM**, Rianon NJ, Manzano JG, Munsell MF, George MC, Bruera E. Thirty-day hospital readmission rate, reasons, and risk factors after acute inpatient cancer rehabilitation. *Cancer Med* 2021; **10**: 6199-6206 [PMID: 34313031 DOI: 10.1002/cam4.4154]

15 **Agency for Healthcare Research and Quality**. HCUP Nationwide Readmission Database (NRD). 2018. Healthcare Cost and Utilization Project (HCUP). [cited 3 August 2022]. Available from: https://www.hcup-us.ahrq.gov/nrdoverview.jsp.

16 **Khan MS**, Sreenivasan J, Lateef N, Abougergi MS, Greene SJ, Ahmad T, Anker SD, Fonarow GC, Butler J. Trends in 30- and 90-Day Readmission Rates for Heart Failure. *Circ Heart Fail* 2021; **14**: e008335 [PMID: 33866827 DOI: 10.1161/CIRCHEARTFAILURE.121.008335]

17 **Agency for Healthcare Research and Quality**. Methodological Issues when Studying Readmissions and Revisits Using Hospital Administrative Data. 2011. HCUP Methods Series Report # 2011-01. Mar 9, 2011. [cited 3 August 2022]. Available from: http://www.hcupus.ahrq.gov/reports/methods/methods.jsp

18 **Shivani Handa**, MBBS, Kamesh Gupta, Jasdeep Singh Sidhu, Giulia Petrone, MD MBBS, Sridevi Rajeeve, MD, Karan Jatwani. Etiologies and Predictors of 30-Day Readmission in Patients Undergoing Induction Chemotherapy for Acute Myeloid Leukemia. *Blood* 2019; **134** Suppl 1: 4759 [DOI: 10.1182/blood-2019-132180]

19 **Martin AS**, Abbott DE, Hanseman D, Sussman JE, Kenkel A, Greiwe P, Saeed N, Ahmad SH, Sussman JJ, Ahmad SA. Factors Associated with Readmission After Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for Peritoneal Carcinomatosis. *Ann Surg Oncol* 2016; **23**: 1941-1947 [PMID: 26842489 DOI: 10.1245/s10434-016-5109-3]

20 **Loo WK**, Hasikin K, Suhaimi A, Yee PL, Teo K, Xia K, Qian P, Jiang Y, Zhang Y, Dhanalakshmi S, Azizan MM, Lai KW. Systematic Review on COVID-19 Readmission and Risk Factors: Future of Machine Learning in COVID-19 Readmission Studies. *Front Public Health* 2022; **10**: 898254 [PMID: 35677770 DOI: 10.3389/fpubh.2022.898254]

21 **Matthews A**, Kramer RA, Peterson CM, Mitan L. Higher admission and rapid readmission rates among medically hospitalized youth with anorexia nervosa/atypical anorexia nervosa during COVID-19. *Eat Behavh* 2021; **43**: 101573 [PMID: 34619464 DOI: 10.1016/j.eatbeh.2021.101573]

22 **Shen Y**, Tang S, Xu J, Xie X, Chen Z. Modified Intraperitoneal Chemotherapy Without Bevacizumab as a First-Line Therapy for Newly Diagnosed Advanced Epithelial Ovarian Cancer-Two Centers Experiences. *Front Med (Lausanne)* 2022; **9**: 846352 [PMID: 35372416 DOI: 10.3389/fmed.2022.846352]

23 **Graves JA**, Baig K, Buntin M. The Financial Effects and Consequences of COVID-19: A Gathering Storm. *JAMA* 2021; **326**: 1909-1910 [PMID: 34714325 DOI: 10.1001/jama.2021.18863]

24 **Iwashyna TJ**, Kamphuis LA, Gundel SJ, Hope AA, Jolley S, Admon AJ, Caldwell E, Monahan ML, Hauschildt K, Thompson BT, Hough CL; NHLBI Prevention and Early Treatment of Acute Lung Injury (PETAL) Network. Continuing Cardiopulmonary Symptoms, Disability, and Financial Toxicity 1 Month After Hospitalization for Third-Wave COVID-19: Early Results From a US Nationwide Cohort. *J Hosp Med* 2021 [PMID: 34424190 DOI: 10.12788/jhm.3660]

25 **J I**. PCN41 - Hospital Cancer BurdenIn Argentina: Costs, Mortality And Readmissions. *Value Health* 2015; **18**: A196 [DOI: 10.1016/j.jval.2015.03.1138]

26 **Kumar A**, Karmarkar A, Downer B, Vashist A, Adhikari D, Al Snih S, Ottenbacher K. Current Risk Adjustment and Comorbidity Index Underperformance in Predicting Post-Acute Utilization and Hospital Readmissions After Joint Replacements: Implications for Comprehensive Care for Joint Replacement Model. *Arthritis Care Res (Hoboken)* 2017; **69**: 1668-1675 [PMID: 28118530 DOI: 10.1002/acr.23195]

27 **Menendez ME**, Neuhaus V, van Dijk CN, Ring D. The Elixhauser comorbidity method outperforms the Charlson index in predicting inpatient death after orthopaedic surgery. *Clin Orthop Relat Res* 2014; **472**: 2878-2886 [PMID: 24867450 DOI: 10.1007/s11999-014-3686-7]

28 **Shaw JA**, Stiliannoudakis S, Qaiser R, Layman E, Sima A, Ali A. Thirty-Day Hospital Readmissions: A Predictor of Higher All-cause Mortality for Up to Two Years. *Cureus* 2020; **12**: e9308 [PMID: 32839677 DOI: 10.7759/cureus.9308]

29 **Krumholz HM**, Lin Z, Keenan PS, Chen J, Ross JS, Drye EE, Bernheim SM, Wang Y, Bradley EH, Han LF, Normand SL. Relationship between hospital readmission and mortality rates for patients hospitalized with acute myocardial infarction, heart failure, or pneumonia. *JAMA* 2013; **309**: 587-593 [PMID: 23403683 DOI: 10.1001/jama.2013.333]

30 **Jairam V**, Lee V, Park HS, Thomas CR Jr, Melnick ER, Gross CP, Presley CJ, Adelson KB, Yu JB. Treatment-Related Complications of Systemic Therapy and Radiotherapy. *JAMA Oncol* 2019; **5**: 1028-1035 [PMID: 30946433 DOI: 10.1001/jamaoncol.2019.0086]

31 **Chakrabarti B**, Lane S, Jenks T, Higgins J, Kanwar E, Allen M, Wotton D. Predictors of 30-day readmission following hospitalisation with community-acquired pneumonia. *BMJ Open Respir Res* 2021; **8** [PMID: 33771814 DOI: 10.1136/bmjresp-2021-000883]

32 **Yong TY**, Fok JS, Hakendorf P, Ben-Tovim D, Thompson CH, Li JY. Characteristics and outcomes of discharges against medical advice among hospitalised patients. *Intern Med J* 2013; **43**: 798-802 [PMID: 23461391 DOI: 10.1111/imj.12109]

33 **Tessema GA**, Kinfu Y, Dachew BA, Tesema AG, Assefa Y, Alene KA, Aregay AF, Ayalew MB, Bezabhe WM, Bali AG, Dadi AF, Duko B, Erku D, Gebrekidan K, Gebremariam KT, Gebremichael LG, Gebreyohannes EA, Gelaw YA, Gesesew HA, Kibret GD, Leshargie CT, Meazew MW, Mekonnen A, Mirkuzie AH, Mohammed H, Tekle DY, Tesfay FH. The COVID-19 pandemic and healthcare systems in Africa: a scoping review of preparedness, impact and response. *BMJ Glob Health* 2021; **6** [PMID: 34853031 DOI: 10.1136/bmjgh-2021-007179]

34 **Moynihan R**, Sanders S, Michaleff ZA, Scott AM, Clark J, To EJ, Jones M, Kitchener E, Fox M, Johansson M, Lang E, Duggan A, Scott L, Albarqouni L. Impact of COVID-19 pandemic on utilisation of healthcare services: a systematic review. *BMJ Open* 2021; **11**: e045343 [PMID: 33727273 DOI: 10.1136/bmjopen-2020-045343]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 September 24; 14(9): 324-356

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 9 September 24, 2023

MINIREVIEWS

324 Progress in the research of cuproptosis and possible targets for cancer therapy

Wang J, Luo LZ, Liang DM, Guo C, Huang ZH, Sun GY, Wen J

335 Advances in drug resistance of triple negative breast cancer caused by pregnane X receptor

Rao ZZ, Tang ZW, Wen J

343 Effectiveness and safety of COVID-19 vaccines in patients with oncological diseases: State-of-the-art

Ivanov N, Krastev B, Miteva DG, Batselova H, Alexandrova R, Velikova T

ABOUT COVER

Peer Reviewer of *World Journal of Clinical Oncology*, Mohammad-Salar Hosseini, MD, MPH, Doctor, Research Associate, Research Center for Evidence-Based Medicine, Tabriz University of Medical Sciences, Golgasht St., Tabriz, East Azerbaijan 51666, Iran. hosseini.msalar@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJCO* as 2.8; IF without journal self cites: 2.8; 5-year IF: 3.0; Journal Citation Indicator: 0.36.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Xu Guo*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
September 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 September 24; 14(9): 324-334

DOI: 10.5306/wjco.v14.i9.324

ISSN 2218-4333 (online)

MINIREVIEWS

Progress in the research of cuproptosis and possible targets for cancer therapy

Jiang Wang, Lan-Zhu Luo, Dao-Miao Liang, Chao Guo, Zhi-Hong Huang, Guo-Ying Sun, Jie Wen

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): E

P-Reviewer: Amin A, United Arab Emirates; El-Arabey AA, Egypt; Suvvari TK, India

Received: April 28, 2023
Peer-review started: April 28, 2023
First decision: July 28, 2023
Revised: August 5, 2023
Accepted: September 4, 2023
Article in press: September 4, 2023
Published online: September 24, 2023

Jiang Wang, Lan-Zhu Luo, Zhi-Hong Huang, Children Medical Center, Hunan Provincial People's Hospital, the First Affiliated Hospital of Hunan Normal University, Changsha 410013, Hunan Province, China

Dao-Miao Liang, Chao Guo, Department of Hepatobiliary Surgery, Hunan Provincial People's Hospital, the First Affiliated Hospital of Hunan Normal University, Changsha 410013, Hunan Province, China

Guo-Ying Sun, Department of Histology and Embryology, Hunan Normal University School of Medicine, Changsha 410013, Hunan Province, China

Jie Wen, Department of Pediatric Orthopedics, Hunan Provincial People's Hospital, the First Affiliated Hospital of Hunan Normal University, Changsha 410013, Hunan Province, China

Corresponding author: Jie Wen, PhD, Associate Professor, Department of Pediatric Orthopedics, Hunan Provincial People's Hospital, the First Affiliated Hospital of Hunan Normal University, No. 61 West Jiefang Rd, Changsha 410013, Hunan Province, China. cashwj@qq.com

Abstract

Developing novel cancer therapies that exploit programmed cell death pathways holds promise for advancing cancer treatment. According to a recently published study in Science, copper death (cuproptosis) occurs when intracellular copper is overloaded, triggering aggregation of lipidated mitochondrial proteins and Fe–S cluster proteins. This intriguing phenomenon is triggered by the instability of copper ions. Understanding the molecular mechanisms behind cuproptosis and its associated genes, as identified by Tsvetkov, including ferredoxin 1, lipoic acid synthase, lipoyltransferase 1, dihydrolipid amide dehydrogenase, dihydrolipoamide transacetylase, pyruvate dehydrogenase α1, pyruvate dehydrogenase β, metallothionein, glutaminase, and cyclin-dependent kinase inhibitor 2A, may open new avenues for cancer therapy. Here, we provide a new understanding of the role of copper death and related genes in cancer.

Key Words: Cuproptosis; Cuproptosis-related genes; Cancer; Targeted therapy

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Developing novel cancer therapies that exploit programmed cell death pathways holds promise for advancing cancer treatment. Cuproptosis-related genes were identified by Tsvetkov, including ferredoxin 1, lipoic acid synthase, lipoyltransferase 1, dihydrolipid amide dehydrogenase, dihydrolipoamide transacetylase, pyruvate dehydrogenase α1, pyruvate dehydrogenase β, metallothionein, glutaminase, and cyclin-dependent kinase inhibitor 2A. Here, we provide a new understanding of the role of copper death and related genes in cancer.

Citation: Wang J, Luo LZ, Liang DM, Guo C, Huang ZH, Sun GY, Wen J. Progress in the research of cuproptosis and possible targets for cancer therapy. *World J Clin Oncol* 2023; 14(9): 324-334
URL: https://www.wjgnet.com/2218-4333/full/v14/i9/324.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i9.324

INTRODUCTION

Tsvetkov *et al*[1] have proposed an intriguing new form of programmed cell death related to the mitochondrial tricarboxylic acid (TCA) cycle, resulting in proteotoxic stress and copper-induced death, referred to as cuproptosis. These forms of oxidative-stress-induced cell death are characterized by mitochondrial stress, including the accumulation of fatty acylated mitochondrial enzymes and the loss of Fe–S cluster proteins[1]. The dysregulation of copper homeostasis promotes cancer growth and causes irreversible cellular damage. A variety of mechanisms have been suggested for the ability of copper to induce cell death, such as oxidative stress, proteasome inhibition, and antiangiogenesis[2].

The exact molecular mechanism underlying cuproptosis remains unclear, but recent studies have shed light on potential contributors. For instance, knockout of the ferredoxin (FDX) 1 gene attenuates copper ionophore-induced cell death. Additionally, genes associated with the loss of lipidated mitochondrial enzymes and Fe-S cluster proteins loss, such as lipoic acid synthase (LIAS), lipoyltransferase (LIPT) 1, and dihydrolipoamide transacetylase (DLAT), may contribute to cuproptosis[1,3].

Although the precise correlation between cuproptosis and cancer is yet to be fully understood, imbalances in copper homeostasis have been implicated in cancer growth and cause irreversible cellular damage. Copper metabolism *in vivo* and cancer therapy has been extensively studied[4,5]. Certain genes involved in the cuproptosis pathway, such as FDX1, may also play a role in cancer development, serving as a key regulator of proptosis and associated with poor prognoses in specific cancer types[6]. Here, we review the progress of copper ions in cancer therapy, the function of cuproptosis-related genes in cancer, and the possible target in cuproptosis.

COPPER IONS AND CANCER THERAPY

Recent studies have revealed three distinct mechanisms through which copper ions may induce cancer cell death. (1) Oxidative stress induction: Anticancer drug elesclomol has been found to exert its therapeutic effects through the transfer of copper ions to mitochondria, leading to oxidative stress[7]. Liu *et al*[8] demonstrated that flavonoids can induce mitochondrial apoptosis through modification of the redox cycle of copper ions; (2) inhibition of proteasomes: Chen *et al*[9] synthesized copper diethyldithiocarbamate [Cu(DDC)(2)] nanoparticles (NPs) that improved the resistance of prostate cancer to treatment. Copper-ion-mediated endoplasmic reticulum (ER) stress is induced by proteasome inhibition and accumulation of ubiquitinated proteins. Proteasome inhibitors like bortezomib and carfilzomib have been explored for their potential as cancer treatment options in the form of various complexes, such as clioquinol and dithiocarbamates[10]; and (3) reduce angiogenesis: Copper ions play a significant role in endothelial cell migration, proliferation, and fibronectin synthesis, crucial steps in angiogenesis[11,12]. However, copper depletion can act as an antiangiogenic switch, blocking the growth of endothelial cells and preventing their proliferation. By inhibiting copper transporters or chaperones like human antioxidant protein 1 and consolidation tumor ratio-1, in addition to direct capture of intracellular copper, copper imbalance can be induced, leading to antiangiogenic effects[13,14]. Combining this approach with vascular targeting techniques, such as immunotherapy, can enhance the cancer-killing effects[15]. The tumor microenvironment (TME) is a complex ecosystem where various immune cells interact and influence tumor growth and progression [16,17]. In the early stage of tumor growth, neutrophils promote inflammation and tumor cell apoptosis by releasing cytokines. However, in the middle and late stages of tumor formation, neutrophils contribute to angiogenesis, accelerating tumor progression and local infiltration. Different T cell populations are involved in TME, among which $CD8^+$ T cells can target and destroy tumor cells, secrete interferon, and inhibit angiogenesis. $CD4^+$ T cells coordinate immune responses, with Th1 cells promoting cancer and T regulatory cells promoting tumor formation and survival, by secreting auxin and cytokines, which then interacts with fibroblasts and epithelial cells. Although less prevalent than T cells, tumor-infiltrating B cells have antitumor effects, including antigen presentation to T cells, production of antitumor antibodies, and secretion of cytokines that promote cytotoxic immune responses. Regulatory B cells, in contrast, promote tumors by producing cytokines that promote the immunosuppressive phenotype in macrophages, neutrophils, and cytotoxic T cells. Tumor-associated macrophages (TAMs) are the predominant immune cells in the TME. They are involved in coordinating cancer-related inflammation and can release macrophage colony-stimulating factor to recruit TAMs, which have been implicated in cancer development. Moreover, TAMs can release epidermal growth factor,

modify cancer cells, and accelerate cell migration and metastasis. Medullary suppressive cells promote tumor invasion by weakening innate and adaptive antitumor responses.

In light of the mechanisms described above for copper ions in cancer treatment, copper complexes have been extensively studied for their potential in anticancer therapy (Figure 1). For instance, copper-amino acid sulfhydryl NPs can reduce Cu^{2+} to Cu^{+} when reacting with localized glutathione. The generated Cu^{+} then reacts with hydrogen peroxide, resulting in an increase in reactive oxygen species (ROS) levels. Excessive ROS can induce apoptosis of cancer cells[18]. A copper-containing complex known as Cu-tuberous sclerosis complex (TSC) is another widely used complex to enhance cytotoxicity of TSC and ROS production[19]. Chronic inflammation in the body can induce carcinogenesis and facilitate cancer spread. Copper complexes containing nonsteroidal anti-inflammatory drugs (NSAIDs) are used to treat inflammation and prevent cancer development (Table 1). In breast cancer stem-cell-like cells, Boodram *et al*[20] demonstrated that Cu-NSAID complexes could induce ROS accumulation, DNA damage, and cyclooxygenase-2 inhibition. Copper complexes with subcellular targeting properties can deliver more precise attacks on cancer cells. Kaur *et al*[21] reported that copper complexes containing polypyridine ligands could enter the ER in situ, leading to increased ROS levels and ER-stress-induced immunogenic cell death in cancer cells[22]. Although copper-complex-related therapies hold promise as a new anticancer strategy, their biocompatibility and application safety are critical challenges. Researchers have shown that copper complexes are cancer-killing, but long-term stability and biosafety tests remain to be conducted before these therapies can be translated into clinical applications.

THE ROLE OF CUPROPTOSIS-RELATED GENES IN CANCER

Cuproptosis remains an area of active exploration in its relationship with cancer. However, significant research has been conducted to understand the mechanisms through which cuproptosis-related gene molecules contribute to cancer development (Table 2). Figure 2 illustrates how these genes induce cuproptosis.

FDX1

FDX1 is a FDX protein primarily found in mitochondria, with diverse physiological functions, including the conversion of cytochromes during steroid hormone synthesis and vitamin D metabolism[23]. Shi *et al*[24] demonstrated that FDX1 is critical for Fe–S cluster biogenesis. Recent research has identified FDX1 as a key gene in the regulation of cuproptosis[25]. Zhang *et al*[26] study found that FDX1 expression did not significantly differ across clinical stages in most cancers. Although the reduction in FDX1 expression may not directly impact the growth, apoptosis, or cell cycle distribution of LUAD cells, it could affect their metabolism, as FDX1 knockout has been shown to promote glycolysis and fatty acid oxidation. Further investigations into the mechanisms of FDX1 in cancer pathogenesis revealed significant positive correlations between FDX1 expression and immune cells in most cancers. FDX1 has been associated with major histocompatibility complex, immune activation, immune suppression, chemokines, and chemotaxis[27]. Additionally, the products of factor receptors were positively coexpressed with FDX1, except for 1-aminocyclopropane-1-carboxylic acid and tetrahydrocannabinolic acid. This indicates that FDX1 expression is closely related to the immune response of cancer cells, which has implications for prognosis and represents a potential target for immunosuppressants[28,29]. Given the crucial role of copper ions in cuproptosis, the significance of FDX1 as a key gene in this process makes it an intriguing target for cancer therapy. Studies exploring its role may offer valuable insights as it directly influences the protein fatty acylation cycle, leading to the aggregation of these proteins and interference with respiratory chain iron-sulfur cluster proteins.

LIAS

LIAS encodes a protein belonging to the biotin and LIAS families. Located in the mitochondria, this Fe–S enzyme contributes to lipoic acid biosynthesis, serving as the final step in the process. Diseases like diabetes, atherosclerosis, and neonatal epilepsy are associated with a lack of LIAS expression. Current studies on the association between the LIAS gene and cancer have predominantly focused on lung cancer[29].

Using in situ hybridization and real-time quantitative PCR, Mabeta *et al*[30] investigated the differential expression of the LIAS gene in normal lung tissue and lung cancer samples. Their findings suggest that alteration in LIAS expression levels can promote lung cancer development, making LIAS an attractive target for novel therapies[29]. However further studies are warranted to confirm its therapeutic effectiveness.

LIPT1

As a member of the fatty acyltransferase family, LIPT1 encodes an enzyme that catalyzes the transfer of fatty acyl groups from fatty acyl-AMPs to specific lysine residues in fatty-acid-dependent enzymes. LIPT1-related disorders include fatty acyltransferase 1 deficiency and leukodystrophy[31]. While there have been relatively few studies on LIPT1 in cancer, Chen *et al*[32] conducted a systematic investigation of genes related to prognosis in bladder cancer using the pathological

Table 1 Copper-related compounds and their antitumor mechanism

Compounds	Mechanism	Ref.
Elesclomol	Transferring copper ions to mitochondria and increasing ROS level	Nagai *et al*[7]
Flavonoid drugs	Interfering with copper ion redox and inducing mitochondrial apoptosis	Liu *et al*[8]
(Cu(DDC)2)	Inhibiting proteasome and leading to ER stress activation	Chen *et al*[9]
Copper ion chelating agent	Inhibiting endothelial cell proliferation and angiogenesis	Zhou *et al*[15]
Copper ion transporter inhibitor	Inhibit endothelial cell proliferation and angiogenesis	Yee *et al*[13], Karginova *et al*[14]
NPs(Cu-CysNPs)	Reacting with glutathione to increase ROS level	Ma *et al*[18]
Cu-TSC	Inducing ROS accumulation	Sîrbu *et al*[19]
Cu-NSAID compound	Inducing ROS accumulation, DNA damage and COX-2 activity inhibition	Boodram *et al*[20]
Copper complexes containing polypyridine ligands	Increasing ROS level and inducing ER stress	Kaur *et al*[21]

ROS: Reactive oxygen species; TSC: Tuberous sclerosis complex; COX-2: Cyclooxygenase-2; NSAID: Non-steroidal anti-inflammatory drugs; (Cu(DDC)2): Copper diethyldithiocarbamate; ER: Endoplasmic reticulum.

Table 2 Functions of cuproptosis-related genes in cancer

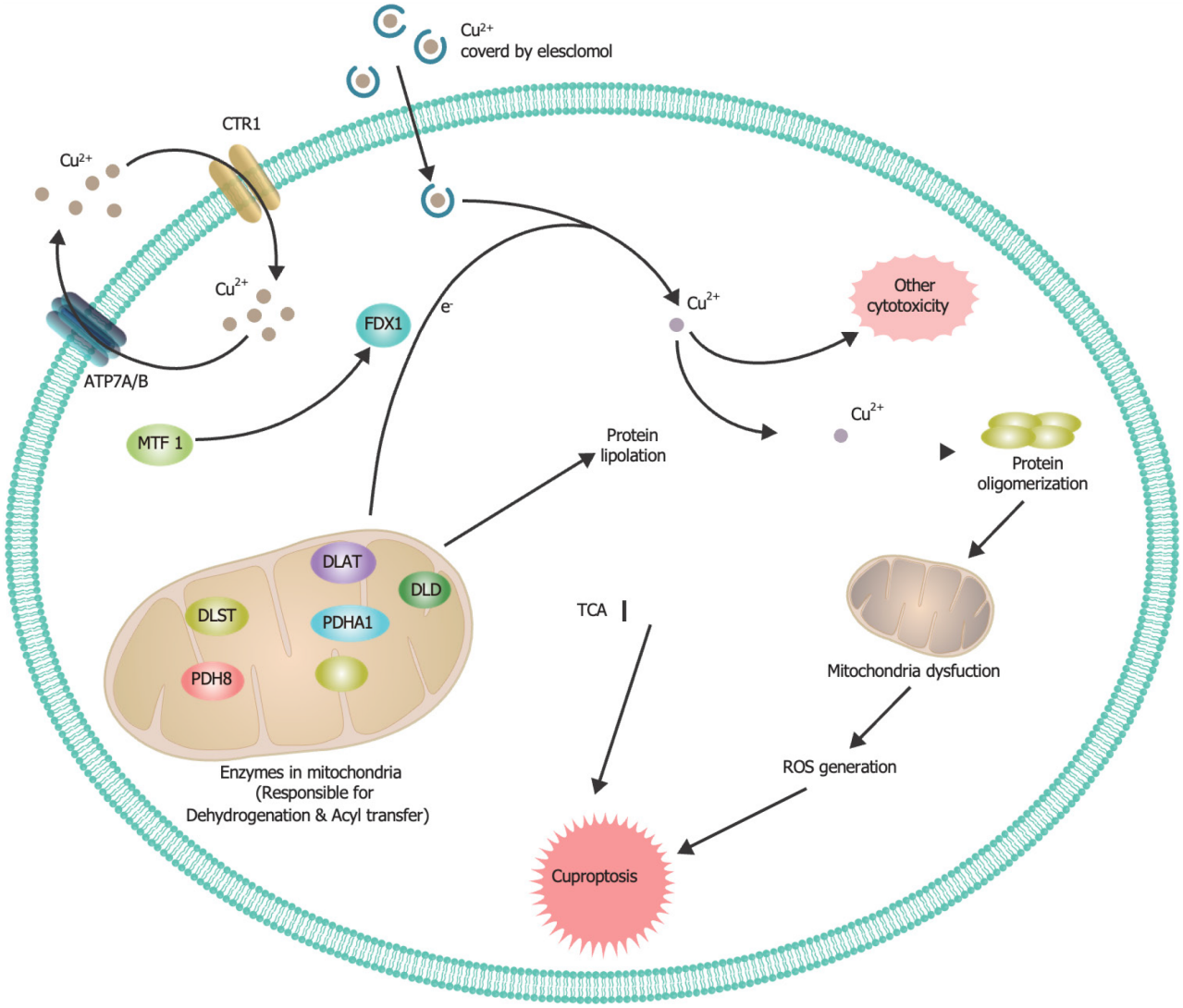
Genes	Mechanism	Ref.
FDX1	FDX1 knockout promotes glycolysis and fatty acid oxidation and alters amino acid metabolism	Zhang *et al*[26]
LIAS	Involved in lipoic acid biosynthesis. Abnormally elevated transcript levels of LIAS contribute to the development of lung cancer	Burr *et al*[29]
LIPT1	Participating in the tricarboxylic acid cycle and is related to the prognosis of bladder cancer	Solmonson *et al*[31], Chen *et al*[32]
DLD		Wang *et al*[33]
DLAT	Converting pyruvate to acetyl-COA Promoting cancer cell growth by activating pentose phosphate pathway	Shan *et al*[40]
PDHA1	Inhibition of PDHA1 expression promotes glycolysis and cell proliferation	Zhuang *et al*[43]
	PDHA1 promotes mitochondrial lipid synthesis	Chen *et al*[45]
PDHB	Overexpression of PDHB inhibits the proliferation and invasiveness	Zhu *et al*[46]
MTF1	Induced co-expression of metallothionein with other genes involved in metal homeostasis contributes to tumor biogenesis and development	Günther *et al*[51]
GLS	Encoding K-type mitochondrial glutaminase and is dysregulated in many tumors	Choi and Park[52], Momcilovic *et al*[53]
CDKN2A	A cyclin with mutations and aberrant methylation in a variety of tumors	Zhao *et al*[56], Tam *et al*[60]

FDX1: Ferredoxin 1; LIAS: Lipoic acid synthase; LIPT1: Lipoyltransferase 1; DLD: Dihydrolipoamide dehydrogenase; DLAT: Dihydrolipoamide transacetylase; PDHA1: Pyruvate dehydrogenase alpha 1; PDHB: Pyruvate dehydrogenase beta; MTF1: Metallothionein; GLS: Glutaminase; CDKN2A: Cyclin dependent kinase inhibitor 2A.

atlas of the Cancer Genome Atlas. Their findings revealed a correlation between LIPT1 expression and bladder cancer prognosis[32]. However, further research is needed to elucidate the role of LIPT1 in other cancer types.

DIHYDROLIPOAMIDE DEHYDROGENASE (DLD)

DLD, encoded by the DLD gene, is an essential enzyme that significantly impacts cell metabolism, particularly pyruvate metabolism and the TCA cycle[33]. There is evidence that DLD could be used as a cancer-targeted therapy. In head and neck squamous cell carcinoma, DLD has been shown to be closely related to cystine deprivation and glutaminolysis. The biological function of DLD enhances mitochondrial KDH, MMP, and glutaminase activity. Increasing mitochondrial iron



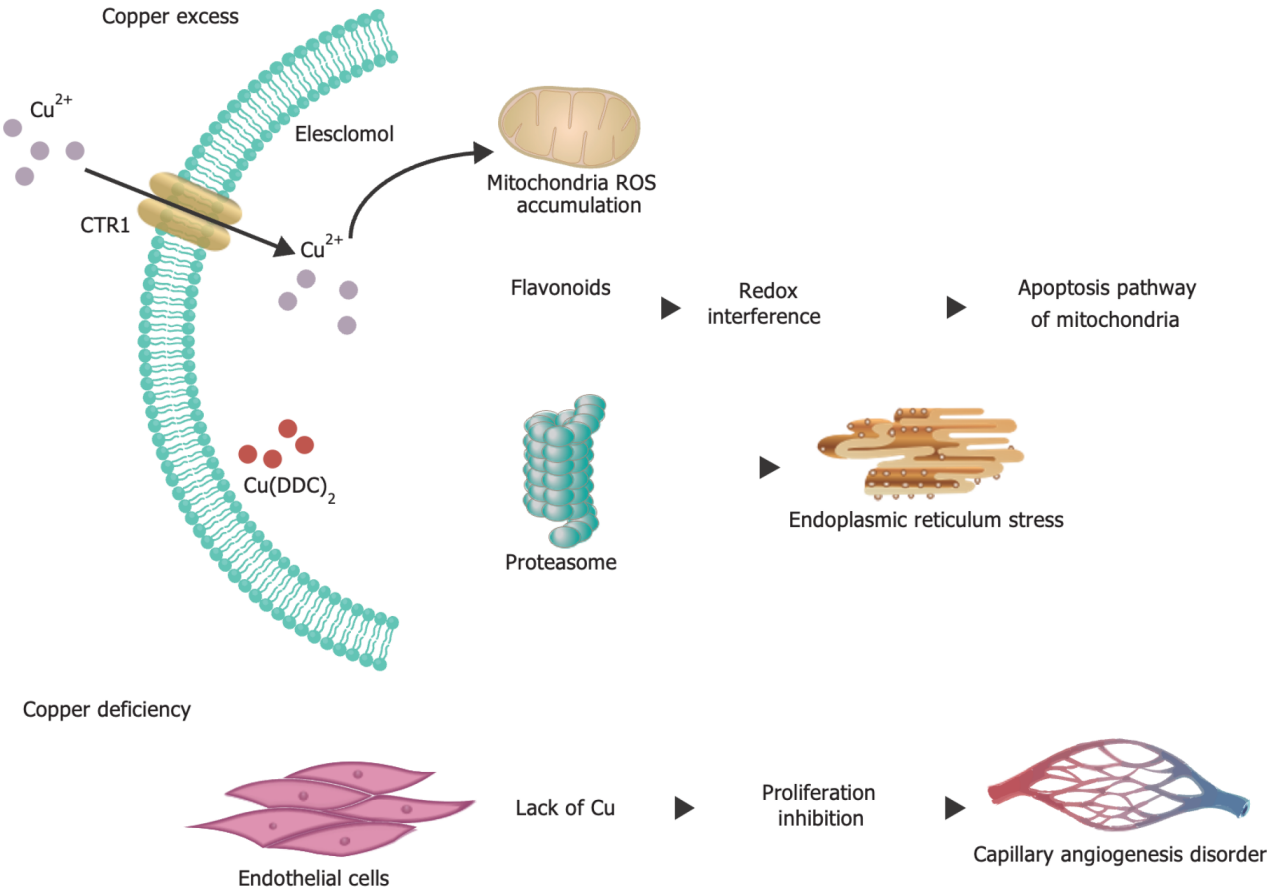
DOI: 10.5306/wjco.v14.i9.324 **Copyright** ©The Author(s) 2023.

Figure 1 Effects of excess copper and copper deficiency in cancer. Four copper-related pathways with cancer inhibition effects are described. Elesclomol mediates the entry of Cu^{2+} into the mitochondria and causes reactive oxygen species accumulation. Flavonoids interfere with copper ion oxidation and reduction, inducing mitochondrial apoptosis pathway activation. Copper diethyldithiocarbamate can inhibit proteasome and result in endoplasmic reticulum stress. Copper deficiency can suppress the proliferation and migration of endothelial cells and the formation of connexin, bridling tumor angiogenesis. TCA: Tricarboxylic acid; ROS: Reactive oxygen species; FDX1: Ferredoxin 1; MTF1: Metallothionein; CTR1: Consolidation tumor ratio-1.

levels can facilitate mitochondrial lipid peroxidation, or silencing DLD, which effectively reduces the proportion of cells undergoing death from cystine deprivation and reduces ROS levels in cystine-deprived cells. These processes have been closely related to cancer-programmed death[34]. Patients with endometrial cancer have exhibited abnormal levels of IgA and non-DLD IgG autoantibodies in their sera, indicating a correlation with mitochondrial DLD protein[35]. Comparing DLD protein expression levels between breast cancer and normal tissues revealed significant differences, highlighting the potential of DLD as a diagnostic and therapeutic target in breast cancer[36]. Using DLDH-based exogenous ROS to target skin cancer cells, Avraham *et al*[37] developed a method for targeting cancer cells, which could be a potential approach for melanoma treatment in the future.

DLAT

DLAT is an essential component of the pyruvate dehydrogenase complex, along with DLD and pyruvate dehydrogenase. This enzyme complex plays a crucial role in the synthesis of pyruvate acetyl-CoA. As the sole enzyme capable of converting citric acid into acetyl-CoA, DLAT can control the citric acid cycle–oxidative phosphorylation pathway, thus affecting the energy supply of cancer cells[38]. In gastric cancer cells, DLAT expression was significantly upregulated[39], making it a potential therapeutic target. DLAT promotes the growth of cancer cells by activating the pentose phosphate pathway[40]. Alternol, a compound that binds to multiple Krebs cycle enzymes, inhibits mitochondrial respiration and ATP production. This discovery offers a novel therapeutic strategy for treating prostate cancer[41].



DOI: 10.5306/wjco.v14.i9.324 **Copyright** ©The Author(s) 2023.

Figure 2 General molecular biological process of cuproptosis. Copper can be transported into cells through the action of consolidation tumor ratio-1 and elesclomol encapsulation. When Cu^{2+} encapsulated by elesclomol enter the mitochondria, it gains an electron from ferrodoxin 1 (FDX1) (FDX1 expression can be promoted by metallothionein) and converts into Cu^{+}. Concurrently, proteins responsible for dehydrogenation and acyl transfer (dihydrolipoamide transacetylase, dihydrolipoamide S-succinyltransferase, dihydrolipoamide dehydrogenase, pyruvate dehydrogenase α1, and pyruvate dehydrogenase β) undergo electron loss and are liporated by lipoic acid synthase. Subsequently, Cu^{+} promotes the oligomerization of liporated proteins. This cascade of events leads to a series of phenomena, including reactive oxygen species accumulation, mitochondrial dysfunction, and tricarboxylic acid inhibition, ultimately culminating in cuproptosis. CTR1: Consolidation tumor ratio-1; (Cu (DDC)2): Copper diethyldithiocarbamate; FDX1: Ferrodoxin 1.

PYRUVATE DEHYDROGENASE α1 (PDHA1) AND PYRUVATE DEHYDROGENASE β (PDHB)

PDHA1 and PDHB encode subunits of the pyruvate dehydrogenase complex, an essential enzyme complex within the mitochondria responsible for catalyzing pyruvate oxidation to acetyl-CoA, connecting glycolysis and the TCA cycle.

PDHA1 inhibition can increase proliferation, glycolysis, and Warburg effect in certain cancer cells. Gastric cancer has been shown to downregulate PDHA1, and elevated expression of PDHA1 correlates with poor prognosis[42]. Downregulation of PDHA1 promotes the growth of gastric cancer. Exosomal miR-21-5p suppresses PDHA1 expression, thereby promoting glycolysis and cell proliferation in gastric cancer cells. PDHA1 expression in gastric cancer samples is negatively correlated with miR-21-5p levels[42]. Additionally, miR-21-5p/PDHA may influence ovarian cancer drug resistance through exosomal miR-21-5p-mediated regulation of PDHA1 expression[43]. The knockout strains had increased glycolysis, glucose intake, and glutamine consumption, while oxidative phosphorylation was inhibited, indicating enhanced Warburg effect and PDHA1. The proliferative capacity, angiogenic capacity, and drug resistance of the knockout esophageal cancer cells were significantly improved[44]. PDHA1 is closely associated with prostate cancer growth, where it is involved in mitochondrial lipid synthesis. Therefore, PDHA1 may be useful as a therapeutic target for prostate cancer[45].

PDHB also acts as a cancer suppressor gene. PDHB overexpression inhibits colon cancer cell proliferation, invasiveness, and glycolysis as it targets miR-146b-5p at the 3'-UTR end of the gene, promoting cancer cell growth[46]. Gastric cancer cells overexpressing PDHB exhibit reduced proliferation and migration[47]. PDHB inhibitors have also been shown to suppress cancer growth in various studies. For instance, reduced PDHB expression in non-small cell lung cancer indicates poor prognosis for patients[48], while PDHB may serve as a biomarker for breast cancer[49]. Thus, the progress made in the research on PDHA1 and PDHB in cancer highlights the broad potential applications of therapeutic drugs targeting these molecular targets.

METALLOTHIONEIN (MTF1)

MTF1 plays a crucial role in the treatment resistance of malignant cancers[50]. Cells stimulated with heavy metals, such as copper, trigger the production of products encoded by MTF1, leading to the induction of metal sulfur production. During tumor biogenesis and progression, coexpression of proteins and other genes involved in metal homeostasis is implicated. Notably, MTF1 is highly expressed in ovarian cancer tissues, and its high expression is associated with poor patient survival and disease recurrence[51]. MTF1 knockout can inhibit the epithelial–mesenchymal transition process of ovarian cancer cells, thereby suppressing their proliferation, migration, and invasion, indicating that MTF1 may serve as a novel biomarker and therapeutic target for ovarian cancer[50]. Given the multiple aspects of MTF-1 activities, monitoring changes in its expression and activity during cellular stress and cancer may prove valuable for cancer screening and prognosis studies.

GLUTAMINASE (GLS)

GLS encodes mitochondrial glutaminase K, which is dysregulated in many cancers. GLS can modulate promoter methylation modification and influence the clinical prognosis. In both in vitro and in vivo studies, GLS-targeted therapy has demonstrated its potential to inhibit cancer growth[52,53]. Similarly, GLS has been detected in clinical samples from breast cancer, esophageal cancer, head and neck cancer, and leukemia. The expression of GLS is associated with poor prognosis in statistical analysis. Therefore, GLS can be considered a prognostic biomarker for certain types of cancer[54]. However, its use as a prognostic biomarker remains controversial and further research is necessary to clarify its role and potential clinical applications[55].

CYCLIN-DEPENDENT KINASE INHIBITOR 2A (CDKN2A)

During cancer development, aberrant gene silencing is highly associated with cell cycle regulation. Dysregulation of CDKN2A, which encodes the p16INK4a protein, has been causally linked to the pathogenesis of various cancer types, contributing to cancer recurrence, poor prognosis, cancer genesis, and metastasis[56]. CDKN2A mutations are responsible for 20%-40% of familial cancers and 2%-3% of sporadic melanomas[57]. Nonsynonymous mutations of CDKN2A were found in approximately 16% (9/56) of cutaneous melanoma metastases[58]. Activation of CDKN2A has been reported in 95% of pancreatic adenocarcinoma cases due to promoter hypermethylation[59]. In lung cancer, CDKN2A inactivation has been observed in 75% of cases (30/40), including 16 homozygous deletions, 10 methylations, and four mutations[60]. CDKN2A gene mutations and abnormal methylation have also been reported in ovarian, gastric, and colorectal cancers, among others[56]. Reactivating CDKN2A genetically and epigenetically could offer promising approaches for cancer prevention and treatment.

DISCUSSION

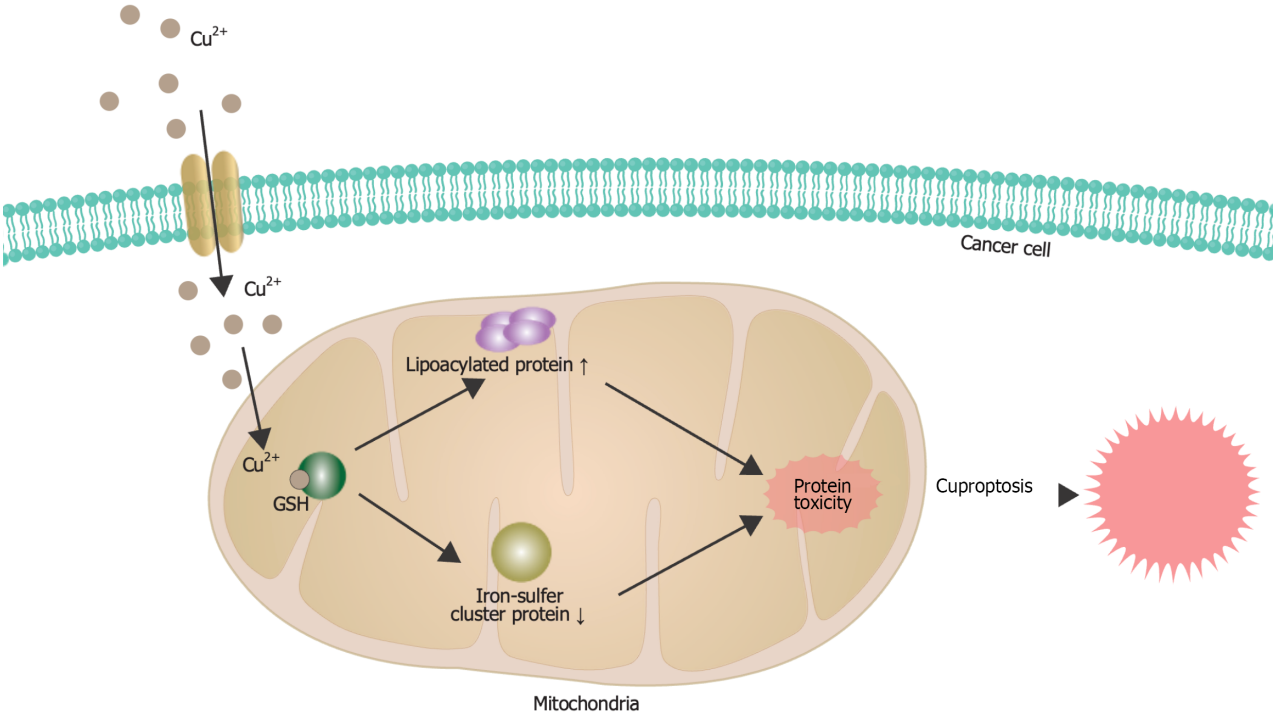
Copper ion concentration in the human body is tightly regulated by a homeostatic mechanism to maintain trace levels, as excess copper becomes toxic and leads to cell death. However, the mechanism underlying copper-induced cytotoxicity is still unclear [61,62]. Recently, a novel form of cell death, cuproptosis, was discovered, which operates independently of known cell death mechanisms[1]. Cuproptosis-related genes were identified using CRISPR-Cas9 loss-of-function screens, which revealed seven positively regulated and three negatively regulated genes.

So far, the identified copper-ionophore-induced death genes include DLD, fatty acylated protein targets PDH complex including DLAT, PDHA, and PDHB. While studies on these genes in cancer have been more extensive[3], other components of the lipoic acid pathway, such as fatty acyl synthase LIAS and FDX1, remain relatively understudied in cancer, and further experiments are needed to verify their roles in different cancer types[1,3]. High cuproptosis activity status has been found to be a good prognostic indicator.

While some progress has been made in utilizing other types of programmed cell death for cancer treatment, there are still limitations in their application. Cuproptosis, being a novel form of programmed cell death, offers new perspectives on the correlation between its related genes and cancer prognosis. The combination of cuproptosis-targeted molecular drugs with existing therapies might open up new avenues for cancer treatment.

Currently, cuproptosis research is still in its infancy, and the existence of other signaling pathways for cell cuproptosis is not yet clear. Additionally, existing copper agents have poor targeting specificity and can cause serious side effects in patients undergoing treatment. These limitations and deficiencies impede the development and clinical implementation of cancer treatment strategies based on cuproptosis mechanisms.

In the future, researchers should focus on improving our understanding of the mechanism of cuproptosis in cancer cells and conducting thorough investigations into relevant mechanisms. Additionally, efforts should be directed towards developing copper-related formulations with high targeting and specificity (such as targeted nano-drug delivery systems) to maximize the targeting of cancer treatment while reducing toxic side effects. Lastly, it is necessary to develop and improve copper treatment plans in clinical practice in order to conduct relevant clinical trials and treatments for patients with cancer.



DOI: 10.5306/wjco.v14.i9.324 **Copyright** ©The Author(s) 2023.

Figure 3 The mechanisms underlying cuproptosis in cancer cells. GSH: Glutathione.

CONCLUSION

Cuproptosis is triggered by the direct interaction of copper ions with the fatty acylated components in the citric acid cycle of mitochondrial respiration. This interaction results in the aggregation of fatty acylated proteins and subsequent down regulation of Fe–S cluster proteins, leading to protein toxic stress and, ultimately leading to cell death (Figure 3). The elucidation of this mechanism provides a clear understanding of how previous copper ion drugs exert their antitumor effects. This provides potential possibilities for the clinical application of these drugs in antitumor therapy and also broadens the path for the development of new drugs targeting copper in the future.

FOOTNOTES

Author contributions: Wang J and Luo LZ contributed equally to this study, and share joint first authorship; Wang J wrote the paper; Luo LZ and Liang DM did the literature review; Guo C and Huang ZH did the data analysis; Luo LZ conceived and coordinated the study; Sun GY and Wen J contributed equally to this study, and are joint corresponding authors; All authors reviewed the results and approved the final version of the manuscript.

Supported by Scientific Research Project of Hunan Education Department, No. 21A0054.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Jiang Wang 0000-0002-2036-1263; Jie Wen 0000-0002-5734-4678.

S-Editor: Qu XL
L-Editor: Kerr C
P-Editor: Zhang XD

REFERENCES

1 **Tsvetkov P**, Coy S, Petrova B, Dreishpoon M, Verma A, Abdusamad M, Rossen J, Joesch-Cohen L, Humeidi R, Spangler RD, Eaton JK, Frenkel E, Kocak M, Corsello SM, Lutsenko S, Kanarek N, Santagata S, Golub TR. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science* 2022; **375**: 1254-1261 [PMID: 35298263 DOI: 10.1126/science.abf0529]

2 **Oliveri V**. Selective Targeting of Cancer Cells by Copper Ionophores: An Overview. *Front Mol Biosci* 2022; **9**: 841814 [PMID: 35309510 DOI: 10.3389/fmolb.2022.841814]

3 **Tang D**, Chen X, Kroemer G. Cuproptosis: a copper-triggered modality of mitochondrial cell death. *Cell Res* 2022; **32**: 417-418 [PMID: 35354936 DOI: 10.1038/s41422-022-00653-7]

4 **Allensworth JL**, Evans MK, Bertucci F, Aldrich AJ, Festa RA, Finetti P, Ueno NT, Safi R, McDonnell DP, Thiele DJ, Van Laere S, Devi GR. Disulfiram (DSF) acts as a copper ionophore to induce copper-dependent oxidative stress and mediate anti-tumor efficacy in inflammatory breast cancer. *Mol Oncol* 2015; **9**: 1155-1168 [PMID: 25769405 DOI: 10.1016/j.molonc.2015.02.007]

5 **Babak MV**, Ahn D. Modulation of Intracellular Copper Levels as the Mechanism of Action of Anticancer Copper Complexes: Clinical Relevance. *Biomedicines* 2021; **9** [PMID: 34440056 DOI: 10.3390/biomedicines9080852]

6 **Zhang C**, Zeng Y, Guo X, Shen H, Zhang J, Wang K, Ji M, Huang S. Pan-cancer analyses confirmed the cuproptosis-related gene FDX1 as an immunotherapy predictor and prognostic biomarker. *Front Genet* 2022; **13**: 923737 [PMID: 35991547 DOI: 10.3389/fgene.2022.923737]

7 **Nagai M**, Vo NH, Shin Ogawa L, Chimmanamada D, Inoue T, Chu J, Beaudette-Zlatanova BC, Lu R, Blackman RK, Barsoum J, Koya K, Wada Y. The oncology drug elesclomol selectively transports copper to the mitochondria to induce oxidative stress in cancer cells. *Free Radic Biol Med* 2012; **52**: 2142-2150 [PMID: 22542443 DOI: 10.1016/j.freeradbiomed.2012.03.017]

8 **Liu ZH**, Yang CX, Zhang L, Yang CY, Xu XQ. Baicalein, as a Prooxidant, Triggers Mitochondrial Apoptosis in MCF-7 Human Breast Cancer Cells Through Mobilization of Intracellular Copper and Reactive Oxygen Species Generation. *Onco Targets Ther* 2019; **12**: 10749-10761 [PMID: 31849483 DOI: 10.2147/OTT.S222819]

9 **Chen W**, Yang W, Chen P, Huang Y, Li F. Disulfiram Copper Nanoparticles Prepared with a Stabilized Metal Ion Ligand Complex Method for Treating Drug-Resistant Prostate Cancers. *ACS Appl Mater Interfaces* 2018; **10**: 41118-41128 [PMID: 30444340 DOI: 10.1021/acsami.8b14940]

10 **Zhang Z**, Wang H, Yan M, Zhang C. Novel copper complexes as potential proteasome inhibitors for cancer treatment (Review). *Mol Med Rep* 2017; **15**: 3-11 [PMID: 27959411 DOI: 10.3892/mmr.2016.6022]

11 **Park KC**, Fouani L, Jansson PJ, Wooi D, Sahni S, Lane DJ, Palanimuthu D, Lok HC, Kovačević Z, Huang ML, Kalinowski DS, Richardson DR. Copper and conquer: copper complexes of di-2-pyridylketone thiosemicarbazones as novel anti-cancer therapeutics. *Metallomics* 2016; **8**: 874-886 [PMID: 27334916 DOI: 10.1039/c6mt00105j]

12 **Baldari S**, Di Rocco G, Toietta G. Current Biomedical Use of Copper Chelation Therapy. *Int J Mol Sci* 2020; **21** [PMID: 32041110 DOI: 10.3390/ijms21031069]

13 **Yee EMH**, Brandl MB, Pasquier E, Cirillo G, Kimpton K, Kavallaris M, Kumar N, Vittorio O. Dextran-Catechin inhibits angiogenesis by disrupting copper homeostasis in endothelial cells. *Sci Rep* 2017; **7**: 7638 [PMID: 28794411 DOI: 10.1038/s41598-017-07452-w]

14 **Karginova O**, Weekley CM, Raoul A, Alsayed A, Wu T, Lee SS, He C, Olopade OI. Inhibition of Copper Transport Induces Apoptosis in Triple-Negative Breast Cancer Cells and Suppresses Tumor Angiogenesis. *Mol Cancer Ther* 2019; **18**: 873-885 [PMID: 30824611 DOI: 10.1158/1535-7163.MCT-18-0667]

15 **Zhou P**, Qin J, Zhou C, Wan G, Liu Y, Zhang M, Yang X, Zhang N, Wang Y. Multifunctional nanoparticles based on a polymeric copper chelator for combination treatment of metastatic breast cancer. *Biomaterials* 2019; **195**: 86-99 [PMID: 30623789 DOI: 10.1016/j.biomaterials.2019.01.007]

16 **El-Arabey AA**, Abdalla M, Abd-Allah AR. SnapShot: TP53 status and macrophages infiltration in TCGA-analyzed tumors. *Int Immunopharmacol* 2020; **86**: 106758 [PMID: 32663767 DOI: 10.1016/j.intimp.2020.106758]

17 **Labani-Motlagh A**, Ashja-Mahdavi M, Loskog A. The Tumor Microenvironment: A Milieu Hindering and Obstructing Antitumor Immune Responses. *Front Immunol* 2020; **11**: 940 [PMID: 32499786 DOI: 10.3389/fimmu.2020.00940]

18 **Ma B**, Wang S, Liu F, Zhang S, Duan J, Li Z, Kong Y, Sang Y, Liu H, Bu W, Li L. Self-Assembled Copper-Amino Acid Nanoparticles for in Situ Glutathione "AND" H(2)O(2) Sequentially Triggered Chemodynamic Therapy. *J Am Chem Soc* 2019; **141**: 849-857 [PMID: 30541274 DOI: 10.1021/jacs.8b08714]

19 **Sîrbu A**, Palamarciuc O, Babak MV, Lim JM, Ohui K, Enyedy EA, Shova S, Darvasiová D, Rapta P, Ang WH, Arion VB. Copper(ii) thiosemicarbazone complexes induce marked ROS accumulation and promote nrf2-mediated antioxidant response in highly resistant breast cancer cells. *Dalton Trans* 2017; **46**: 3833-3847 [PMID: 28271099 DOI: 10.1039/c7dt00283a]

20 **Boodram JN**, Mcgregor IJ, Bruno PM, Cressey PB, Hemann MT, Suntharalingam K. Breast Cancer Stem Cell Potent Copper(II)-Non-Steroidal Anti-Inflammatory Drug Complexes. *Angew Chem Int Ed Engl* 2016; **55**: 2845-2850 [PMID: 26806362 DOI: 10.1002/anie.201510443]

21 **Kaur P**, Johnson A, Northcote-Smith J, Lu C, Suntharalingam K. Immunogenic Cell Death of Breast Cancer Stem Cells Induced by an Endoplasmic Reticulum-Targeting Copper(II) Complex. *Chembiochem* 2020; **21**: 3618-3624 [PMID: 32776422 DOI: 10.1002/cbic.202000553]

22 **Zhang L**, Wan SS, Li CX, Xu L, Cheng H, Zhang XZ. An Adenosine Triphosphate-Responsive Autocatalytic Fenton Nanoparticle for Tumor Ablation with Self-Supplied H(2)O(2) and Acceleration of Fe(III)/Fe(II) Conversion. *Nano Lett* 2018; **18**: 7609-7618 [PMID: 30383966 DOI: 10.1021/acs.nanolett.8b03178]

23 **Ewen KM**, Ringle M, Bernhardt R. Adrenodoxin--a versatile ferredoxin. *IUBMB Life* 2012; **64**: 506-512 [PMID: 22556163 DOI: 10.1002/iub.1029]

24 **Shi Y**, Ghosh M, Kovtunovych G, Crooks DR, Rouault TA. Both human ferredoxins 1 and 2 and ferredoxin reductase are important for iron-sulfur cluster biogenesis. *Biochim Biophys Acta* 2012; **1823**: 484-492 [PMID: 22101253 DOI: 10.1016/j.bbamcr.2011.11.002]

25 **Kahlson MA**, Dixon SJ. Copper-induced cell death. *Science* 2022; **375**: 1231-1232 [PMID: 35298241 DOI: 10.1126/science.abo3959]

26 **Zhang Z**, Ma Y, Guo X, Du Y, Zhu Q, Wang X, Duan C. FDX1 can Impact the Prognosis and Mediate the Metabolism of Lung Adenocarcinoma. *Front Pharmacol* 2021; **12**: 749134 [PMID: 34690780 DOI: 10.3389/fphar.2021.749134]

27 **Bian Z**, Fan R, Xie L. A Novel Cuproptosis-Related Prognostic Gene Signature and Validation of Differential Expression in Clear Cell Renal Cell Carcinoma. *Genes (Basel)* 2022; **13** [PMID: 35627236 DOI: 10.3390/genes13050851]

28 **Ohtani H**. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. *Cancer Immun* 2007; **7**: 4 [PMID: 17311363]

29 **Burr SP**, Costa AS, Grice GL, Timms RT, Lobb IT, Freisinger P, Dodd RB, Dougan G, Lehner PJ, Frezza C, Nathan JA. Mitochondrial Protein Lipoylation and the 2-Oxoglutarate Dehydrogenase Complex Controls HIF1α Stability in Aerobic Conditions. *Cell Metab* 2016; **24**: 740-752 [PMID: 27923773 DOI: 10.1016/j.cmet.2016.09.015]

30 **Mabeta P**, Hull R, Dlamini Z. LncRNAs and the Angiogenic Switch in Cancer: Clinical Significance and Therapeutic Opportunities. *Genes (Basel)* 2022; **13** [PMID: 35052495 DOI: 10.3390/genes13010152]

31 **Solmonson A**, Faubert B, Gu W, Rao A, Cowdin MA, Menendez-Montes I, Kelekar S, Rogers TJ, Pan C, Guevara G, Tarangelo A, Zacharias LG, Martin-Sandoval MS, Do D, Pachnis P, Dumesnil D, Mathews TP, Tasdogan A, Pham A, Cai L, Zhao Z, Ni M, Cleaver O, Sadek HA, Morrison SJ, DeBerardinis RJ. Compartmentalized metabolism supports midgestation mammalian development. *Nature* 2022; **604**: 349-353 [PMID: 35388219 DOI: 10.1038/s41586-022-04557-9]

32 **Chen Y**, Xu T, Xie F, Wang L, Liang Z, Li D, Liang Y, Zhao K, Qi X, Yang X, Jiao W. Evaluating the biological functions of the prognostic genes identified by the Pathology Atlas in bladder cancer. *Oncol Rep* 2021; **45**: 191-201 [PMID: 33200223 DOI: 10.3892/or.2020.7853]

33 **Wang Y**, Guo YR, Liu K, Yin Z, Liu R, Xia Y, Tan L, Yang P, Lee JH, Li XJ, Hawke D, Zheng Y, Qian X, Lyu J, He J, Xing D, Tao YJ, Lu Z. KAT2A coupled with the α-KGDH complex acts as a histone H3 succinyltransferase. *Nature* 2017; **552**: 273-277 [PMID: 29211711 DOI: 10.1038/nature25003]

34 **Shin D**, Lee J, You JH, Kim D, Roh JL. Dihydrolipoamide dehydrogenase regulates cystine deprivation-induced ferroptosis in head and neck cancer. *Redox Biol* 2020; **30**: 101418 [PMID: 31931284 DOI: 10.1016/j.redox.2019.101418]

35 **Yoneyama K**, Shibata R, Igarashi A, Kojima S, Kodani Y, Nagata K, Kurose K, Kawase R, Takeshita T, Hattori S. Proteomic identification of dihydrolipoamide dehydrogenase as a target of autoantibodies in patients with endometrial cancer. *Anticancer Res* 2014; **34**: 5021-5027 [PMID: 25202086 DOI: 10.1158/0008-5472.can-05-3365]

36 **Abdullah Al-Dhabi N**, Srigopalram S, Ilavenil S, Kim YO, Agastian P, Baaru R, Balamurugan K, Choi KC, Valan Arasu M. Proteomic Analysis of Stage-II Breast Cancer from Formalin-Fixed Paraffin-Embedded Tissues. *Biomed Res Int* 2016; **2016**: 3071013 [PMID: 27110560 DOI: 10.1155/2016/3071013]

37 **Avraham H**, Avraham S, Taniguchi Y. Receptor protein tyrosine phosphatases in hematopoietic cells. *J Hematother Stem Cell Res* 2000; **9**: 425-432 [PMID: 10982240 DOI: 10.1089/152581600419080]

38 **Patel MS**, Nemeria NS, Furey W, Jordan F. The pyruvate dehydrogenase complexes: structure-based function and regulation. *J Biol Chem* 2014; **289**: 16615-16623 [PMID: 24798336 DOI: 10.1074/jbc.R114.563148]

39 **Goh WQ**, Ow GS, Kuznetsov VA, Chong S, Lim YP. DLAT subunit of the pyruvate dehydrogenase complex is upregulated in gastric cancer-implications in cancer therapy. *Am J Transl Res* 2015; **7**: 1140-1151 [PMID: 26279757 DOI: 10.5772/48582]

40 **Shan C**, Elf S, Ji Q, Kang HB, Zhou L, Hitosugi T, Jin L, Lin R, Zhang L, Seo JH, Xie J, Tucker M, Gu TL, Sudderth J, Jiang L, DeBerardinis RJ, Wu S, Li Y, Mao H, Chen PR, Wang D, Chen GZ, Lonial S, Arellano ML, Khoury HJ, Khuri FR, Lee BH, Brat DJ, Ye K, Boggon TJ, He C, Kang S, Fan J, Chen J. Lysine acetylation activates 6-phosphogluconate dehydrogenase to promote tumor growth. *Mol Cell* 2014; **55**: 552-565 [PMID: 25042803 DOI: 10.1016/j.molcel.2014.06.020]

41 **Li C**, He C, Xu Y, Xu H, Tang Y, Chavan H, Duan S, Artigues A, Forrest ML, Krishnamurthy P, Han S, Holzbeierlein JM, Li B. Alternol eliminates excessive ATP production by disturbing Krebs cycle in prostate cancer. *Prostate* 2019; **79**: 628-639 [PMID: 30663084 DOI: 10.1002/pros.23767]

42 **Liu Z**, Yu M, Fei B, Fang X, Ma T, Wang D. miR215p targets PDHA1 to regulate glycolysis and cancer progression in gastric cancer. *Oncol Rep* 2018; **40**: 2955-2963 [PMID: 30226598 DOI: 10.3892/or.2018.6695]

43 **Zhuang L**, Zhang B, Liu X, Lin L, Wang L, Hong Z, Chen J. Exosomal miR-21-5p derived from cisplatin-resistant SKOV3 ovarian cancer cells promotes glycolysis and inhibits chemosensitivity of its progenitor SKOV3 cells by targeting PDHA1. *Cell Biol Int* 2021; **45**: 2140-2149 [PMID: 34288231 DOI: 10.1002/cbin.11671]

44 **Liu L**, Cao J, Zhao J, Li X, Suo Z, Li H. PDHA1 Gene Knockout In Human Esophageal Squamous Cancer Cells Resulted In Greater Warburg Effect And Aggressive Features In Vitro And In Vivo. *Onco Targets Ther* 2019; **12**: 9899-9913 [PMID: 31819487 DOI: 10.2147/OTT.S226851]

45 **Chen J**, Guccini I, Di Mitri D, Brina D, Revandkar A, Sarti M, Pasquini E, Alajati A, Pinton S, Losa M, Civenni G, Catapano CV, Sgrignani J, Cavalli A, D'Antuono R, Asara JM, Morandi A, Chiarugi P, Crotti S, Agostini M, Montopoli M, Masgras I, Rasola A, Garcia-Escudero R, Delaleu N, Rinaldi A, Bertoni F, Bono J, Carracedo A, Alimonti A. Compartmentalized activities of the pyruvate dehydrogenase complex sustain lipogenesis in prostate cancer. *Nat Genet* 2018; **50**: 219-228 [PMID: 29335542 DOI: 10.1038/s41588-017-0026-3]

46 **Zhu Y**, Wu G, Yan W, Zhan H, Sun P. miR-146b-5p regulates cell growth, invasion, and metabolism by targeting PDHB in colorectal cancer. *Am J Cancer Res* 2017; **7**: 1136-1150 [PMID: 28560062 DOI: 10.11569/wcjd.v31.i10.397]

47 **Cai Z**, Zhao JS, Li JJ, Peng DN, Wang XY, Chen TL, Qiu YP, Chen PP, Li WJ, Xu LY, Li EM, Tam JP, Qi RZ, Jia W, Xie D. A combined proteomics and metabolomics profiling of gastric cardia cancer reveals characteristic dysregulations in glucose metabolism. *Mol Cell Proteomics* 2010; **9**: 2617-2628 [PMID: 20699381 DOI: 10.1074/mcp.M110.000661]

48 **Giannos P**, Kechagias KS, Gal A. Identification of Prognostic Gene Biomarkers in Non-Small Cell Lung Cancer Progression by Integrated Bioinformatics Analysis. *Biology (Basel)* 2021; **10** [PMID: 34827193 DOI: 10.3390/biology10111200]

49 **Carlini MJ**, Recouvreux MS, Simian M, Nagai MA. Gene expression profile and cancer-associated pathways linked to progesterone receptor isoform a (PRA) predominance in transgenic mouse mammary glands. *BMC Cancer* 2018; **18**: 682 [PMID: 29940887 DOI: 10.1186/s12885-018-4550-z]

50 **Ji L**, Zhao G, Zhang P, Huo W, Dong P, Watari H, Jia L, Pfeffer LM, Yue J, Zheng J. Knockout of MTF1 Inhibits the Epithelial to Mesenchymal Transition in Ovarian Cancer Cells. *J Cancer* 2018; **9**: 4578-4585 [PMID: 30588241 DOI: 10.7150/jca.28040]

51 **Günther V**, Lindert U, Schaffner W. The taste of heavy metals: gene regulation by MTF-1. *Biochim Biophys Acta* 2012; **1823**: 1416-1425 [PMID: 22289350 DOI: 10.1016/j.bbamcr.2012.01.005]

52 **Choi YK**, Park KG. Targeting Glutamine Metabolism for Cancer Treatment. *Biomol Ther (Seoul)* 2018; **26**: 19-28 [PMID: 29212303 DOI: 10.4062/biomolther.2017.178]

53 **Momcilovic M**, Bailey ST, Lee JT, Fishbein MC, Magyar C, Braas D, Graeber T, Jackson NJ, Czernin J, Emberley E, Gross M, Janes J, Mackinnon A, Pan A, Rodriguez M, Works M, Zhang W, Parlati F, Demo S, Garon E, Krysan K, Walser TC, Dubinett SM, Sadeghi S, Christofk HR, Shackelford DB. Targeted Inhibition of EGFR and Glutaminase Induces Metabolic Crisis in EGFR Mutant Lung Cancer. *Cell Rep* 2017; **18**: 601-610 [PMID: 28099841 DOI: 10.1016/j.celrep.2016.12.061]

54 **Sheikh TN**, Patwardhan PP, Cremers S, Schwartz GK. Targeted inhibition of glutaminase as a potential new approach for the treatment of NF1 associated soft tissue malignancies. *Oncotarget* 2017; **8**: 94054-94068 [PMID: 29212209 DOI: 10.18632/oncotarget.21573]

55 **Saha SK**, Islam SMR, Abdullah-Al-Wadud M, Islam S, Ali F, Park KS. Multiomics Analysis Reveals that GLS and GLS2 Differentially Modulate the Clinical Outcomes of Cancer. *J Clin Med* 2019; **8** [PMID: 30871151 DOI: 10.3390/jcm8030355]

56 **Zhao R**, Choi BY, Lee MH, Bode AM, Dong Z. Implications of Genetic and Epigenetic Alterations of CDKN2A (p16(INK4a)) in Cancer. *EBioMedicine* 2016; **8**: 30-39 [PMID: 27428416 DOI: 10.1016/j.ebiom.2016.04.017]

57 **Kostaki M**, Manona AD, Stavraka I, Korkolopoulou P, Levidou G, Trigka EA, Christofidou E, Champsas G, Stratigos AJ, Katsambas A, Papadopoulos O, Piperi C, Papavassiliou AG. High-frequency p16(INK) (4A) promoter methylation is associated with histone methyltransferase SETDB1 expression in sporadic cutaneous melanoma. *Exp Dermatol* 2014; **23**: 332-338 [PMID: 24673285 DOI: 10.1111/exd.12398]

58 **Jonsson A**, Tuominen R, Grafström E, Hansson J, Egyhazi S. High frequency of p16(INK4A) promoter methylation in NRAS-mutated cutaneous melanoma. *J Invest Dermatol* 2010; **130**: 2809-2817 [PMID: 20703244 DOI: 10.1038/jid.2010.216]

59 **Jiao L**, Zhu J, Hassan MM, Evans DB, Abbruzzese JL, Li D. K-ras mutation and p16 and preproenkephalin promoter hypermethylation in plasma DNA of pancreatic cancer patients: in relation to cigarette smoking. *Pancreas* 2007; **34**: 55-62 [PMID: 17198183 DOI: 10.1097/01.mpa.0000246665.68869.d4]

60 **Tam KW**, Zhang W, Soh J, Stastny V, Chen M, Sun H, Thu K, Rios JJ, Yang C, Marconett CN, Selamat SA, Laird-Offringa IA, Taguchi A, Hanash S, Shames D, Ma X, Zhang MQ, Lam WL, Gazdar A. CDKN2A/p16 inactivation mechanisms and their relationship to smoke exposure and molecular features in non-small-cell lung cancer. *J Thorac Oncol* 2013; **8**: 1378-1388 [PMID: 24077454 DOI: 10.1097/JTO.0b013e3182a46c0c]

61 **Tan MS**, Tan L, Jiang T, Zhu XC, Wang HF, Jia CD, Yu JT. Amyloid-β induces NLRP1-dependent neuronal pyroptosis in models of Alzheimer's disease. *Cell Death Dis* 2014; **5**: e1382 [PMID: 25144717 DOI: 10.1038/cddis.2014.348]

62 **Ghobrial IM**, Witzig TE, Adjei AA. Targeting apoptosis pathways in cancer therapy. *CA Cancer J Clin* 2005; **55**: 178-194 [PMID: 15890640 DOI: 10.3322/canjclin.55.3.178]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 September 24; 14(9): 335-342

DOI: 10.5306/wjco.v14.i9.335

ISSN 2218-4333 (online)

MINIREVIEWS

Advances in drug resistance of triple negative breast cancer caused by pregnane X receptor

Zhou-Zhou Rao, Zhong-Wen Tang, Jie Wen

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kukongviriyapan V, Thailand; PhD CKP, India

Received: June 21, 2023
Peer-review started: June 21, 2023
First decision: August 10, 2023
Revised: August 17, 2023
Accepted: August 29, 2023
Article in press: August 29, 2023
Published online: September 24, 2023

Zhou-Zhou Rao, Department of Physiology, Hunan Normal University School of Medicine, Changsha 410003, Hunan Province, China

Zhong-Wen Tang, Jie Wen, Department of Pediatric Orthopedics, Hunan Provincial People's Hospital, the First Affiliated Hospital of Hunan Normal University, Changsha 410013, Hunan Province, China

Corresponding author: Jie Wen, PhD, Assistant Professor, Department of Pediatric Orthopedics, Hunan Provincial People's Hospital, the First Affiliated Hospital of Hunan Normal University, No. 61 West Jiefang Rd, Changsha 410013, Hunan Province, China. cashwj@qq.com

Abstract

Breast cancer is the most common malignancy in women worldwide. Triple-negative breast cancer (TNBC), refers breast cancer negative for estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2, characterized by high drug resistance, high metastasis and high recurrence, treatment of which is a difficult problem in the clinical treatment of breast cancer. In order to better treat TNBC clinically, it is a very urgent task to explore the mechanism of TNBC resistance in basic breast cancer research. Pregnane X receptor (PXR) is a nuclear receptor whose main biological function is to participate in the metabolism, transport and clearance of allobiological agents in PXR. PXR plays an important role in drug metabolism and clearance, and PXR is highly expressed in tumor tissues of TNBC patients, which is related to the prognosis of breast cancer patients. This reviews synthesized the important role of PXR in the process of high drug resistance to TNBC chemotherapeutic drugs and related research progress.

Key Words: Triple-negative breast cancer; Pregnane X receptor; Drug resistance; Cytochrome P450; Uridinediphosphate glucuronyl transferases; Glutathione transferases; ATP-binding cassette transporter

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Treatment of triple-negative breast cancer (TNBC) is a difficult problem in the clinical treatment of breast cancer. It is a very urgent task to explore the mechanism of TNBC resistance in basic breast cancer research. Pregnane X receptor (PXR) is a nuclear receptor whose main biological function is to participate in the metabolism, transport and clearance of allobiological agents in PXR. This reviews synthesized the important role of PXR in the process of high drug resistance to TNBC chemotherapeutic drugs and related research progress.

Citation: Rao ZZ, Tang ZW, Wen J. Advances in drug resistance of triple negative breast cancer caused by pregnane X receptor. *World J Clin Oncol* 2023; 14(9): 335-342
URL: https://www.wjgnet.com/2218-4333/full/v14/i9/335.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i9.335

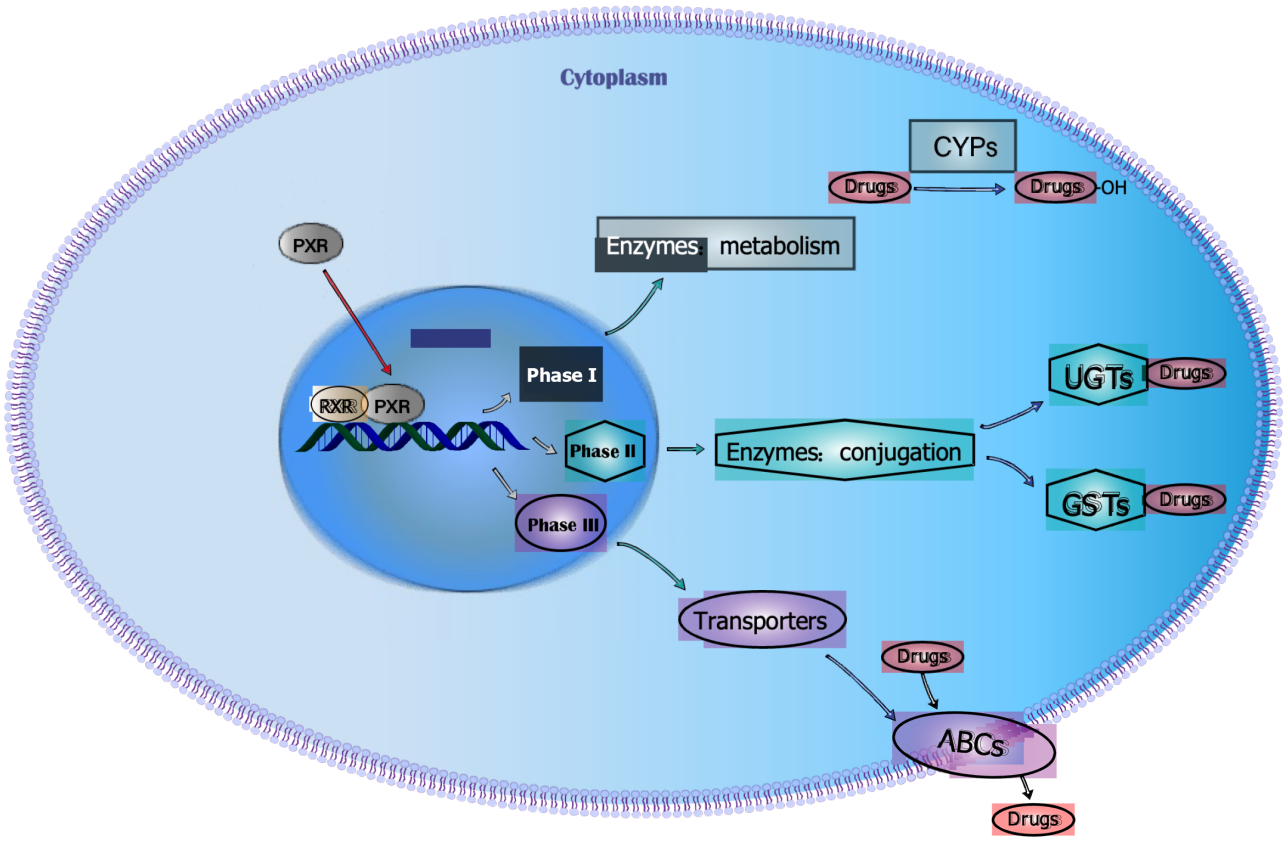
INTRODUCTION

Cancer and cardiovascular disease are the two leading causes of death in the world, which seriously endanger people's physical and mental health[1]. In recent years, the incidence of cancer has been showing an upward trend worldwide, and the growth rate and mortality rate of breast cancer in women are grim[2]. According to the overall cancer data in the world in 2020[3], breast cancer has exceeded lung cancer to become the number one malignant tumor in the world, accounting for 11.7% of all different types of cancer. The incidence and mortality of breast cancer rank the first in most countries in the world. Literature reports that in 2020, the number of new breast cancer cases in the world was more than 2.26 million, and the number of deaths reached 685000, among which Chinese patients accounted for 18.4% of all cases in the world[4]. Therefore breast cancer has become the most threatening malignant tumor that endangers women's health.

According to the different express of estrogen receptor (ER), human epidermal growth factor receptor 2 (HER-2), progesterone receptor (PR), and insufficient expression of proliferating cell nuclear antigen-67, breast cancer have been classified into several subtypes, these include: Luminal A, HER-2 overexpression, Luminal B and triple negative[5]. In all kinds of breast cancer, the type of breast cancer which is negative for PR, ER, and HER-2 is called triple-negative breast cancer (TNBC). It accounts for 10% to 20% of all types of breast cancer[6] and occurs mostly in young women[7]. TNBC mainly metastasize to the lung and brain, and its own biological characteristics make it have poor response to general local treatment and poor prognosis[8]. Although there have been great breakthroughs in the treatment of breast cancer recently, the treatment of advanced metastatic breast cancer (especially TNBC) is still a great clinical challenge. Although there are so many different subtypes in breast cancer, TNBC is the most clinically complex subtype to treat. Because the lackness of effective molecular targets, theraputic attempts for non-TNBC, such as endocrine therapy and HER2-targeted therapy, cannot benefit TNBC patients[9]. Poly (ADP-ribose) polymerase inhibitors and immune checkpoint-based immunotherapy have made important progress in preclinical and clinical research[10]. However, although these treatment strategies can benefit some patients, the overall benefit of all TNBC patients is still very limited. At present, chemotherapy is still an important treatment for TNBC[11]. However, TNBC is not all sensitive to chemotherapy, and the main reason for the failure of chemotherapy is the resistance of TNBC to chemotherapy[12]. In summary, this type of breast cancer is characterized by high degree of deterioration, high recurrence rate, high metastasis rate and low survival rate. It is particularly important to study the mechanism of chemotherapy resistance[13].

In 1998, when Kliewer *et al*[14] searched the mouse liver HHMI EST database, they found a sequence with high homology to the known nuclear receptor, and the protein encoded by this sequence can be activated by a series of natural or synthetic pregnane hormones, so they named it pregnane X receptor (PXR). Human PXR is expressed by the nuclear receptor subfamily 1 group I member 2 gene, located on chromosome 3q13-21, and consists of 10 exons and 9 introns, with a gene size of approximately 40 kb. In contrast to other nucleoid receptors, PXR possesses a large and somewhat flexible spherical ligand-binding domain, allowing it to bind a large number of compounds of different sizes and structures. Phosphorylation of residues at positions T248, Y249, and T422 of PXR is required for its ligand-activated function[15]. When PXR binds to its ligand, its conformation changes and activates the PXR pathway, which causes PXR to translocate from the cytoplasm to the nucleus and bind to the retinal X receptor to form a heterodimer, which in turn combine with the DNA response elements in the target gene's specific promoter region to regulate their transcription[16]. The main biological function of PXR is to participate in the metabolism, transport and clearance of xenobiotics including chemotherapeutic drugs[17]. There are three phases involved in the metabolic process of PXR: Phase I, metabolizing enzymes; Phase II, conjugating enzymes; phase III, transporter[18] (Figure 1).

Although PXR is mainly expressed in liver, intestinal and colon tissues, it has been found that it is also expressed in normal breast tissues, and its expression level is even higher in breast cancer tissues[19]. PXR can affect the expression of drug resistance-related genes, thereby enhancing the metabolism and clearance function of chemotherapy drugs in cancer cells[20], and then plays an important role in breast cancer[21]. Studies have shown that the expression of PXR increased in docetaxel-resistant TNBC cells and tumor xenograft mice[22]. This article reviews the role of PXR in the drug resistance mechanism of TNBC.



DOI: 10.5306/wjco.v14.i9.335 **Copyright** ©The Author(s) 2023.

Figure 1 Three phases in the chemotherapy drug resistance mechanism caused by pregnane X receptor in triple-negative breast cancer. RXR: Retinal X receptor; PXR: Pregnane X receptor; ABC: Adenosine triphosphate binding cassette; CYPs: Cytochrome P450s; UGTs: Uridine diphosphate glucuronosyltransferase; GSTs: Glutathione transferase.

PXR AND METABOLIZING ENZYMES IN PHASE I OF DRUG METABOLISM

Drug metabolizing enzymes refers a special kind of enzymes, which responsible for the metabolism function of a variety of substances such as exogenous chemicals and endogenous biological small molecules. Cytochrome P450 (CYP) is an important enzyme system involved in the metabolism of xenobiotics in cells. CYP was first discovered in rat liver microsomes in 1958[23]. CYP is named for its typical absorption peak at 450 nm wave length[24]. The rules for CYP nomenclature include: Different numbers after the family represent different families, different letters after the family represent different subfamilies, and different numbers after the subfamily represent different peptides[25]. There are 18 CYP families in human body, including 26 subfamilies and more than 50 different isoforms with catalytic functions[26]. Three families, CYP1, CYP2 and CYP3, account for nearly 70% of the human CYP family and response for most drugs' metabolism progress. It is the dominant superfamily enzyme system not only involved in the drug metabolism phase I, but also affected drug oxidation, reduction or hydrolysis[27]. For patients with liver cancer, clarifying the expression information of CYP, strengthening the monitoring of medication, adjusting the dose and frequency of drugs, and reducing drug resistance and side effects are of great significance for the precise treatment of anticancer drugs[28].

It is demonstrated by Murray *et al*[29] that CYP2S1, CYP4V2, CYP3A4, and CYP26A1 were connected to the final survive rate of breast cancer patients, which also indicated the potential of CYP as a marker for the clinical results of breast cancer patients. A large number of studies have shown that CYP enzymes are related to breast cancer drug metabolism. Among them, CYP enzymes have been experimentally confirmed to be: CYP3A4, CYP3A5, CYP2C8, CYP2C9, CYP2J2, CYP1A1, CYP1B1, CYP17A1, CYP2B6, CYP2D6, CYP2C19, *etc*[30-33]. Alexanian *et al*[34] reported the lower expressions of CYP4A11 and CYP4A22 in normal breast tissues than those in TNBC tissues. Overexpression of CYP3A4 can promote the metabolism of docetaxel in triple negative breast cancer stem cells and further induce reduced accumulation of chemotherapy drugs in cancer cells, leading to cell drug resistance[22]. Two major metabolic enzymes of paclitaxel (CYP2C8, CYP3A4) and other genes involved in taxane heterogenic metabolism (*e.g.*, CYP1B1) are associated with drug resistance in TNBC[35]. Numerous experiments have shown that CYP enzymes are significantly upregulated in TNBC patients [22,29,35]. Therefore, the association between CYP enzymes and tumor resistance in TNBC has attracted increasing attention.

It has been reported that activated PXR can transcriptically up-regulate the expression of CYP450 family members such as CYP3A4, CYP3A23, CYP2B6, CYP2B9, CYP2C55, CYP2C9 and CYP1A[36,37]. In experimental studies related to TNBC drug resistance, it has been confirmed that PXR can regulate the expression of CYP3A4, resulting in increased drug metabolism in TNBC, which is obviously related to TNBC chemotherapy resistance[22].

PXR AND CONJUGATIVE ENZYMES IN PHASE II OF DRUG METABOLISM

Conjugation enzymes in phase II of drug metabolism are mainly various transferases, such as glutathione transferase (GST) and uridine diphosphate glucuronosyltransferase (UGT)[30]. GST, as an important part of the detoxification system of the body, is responsible for catalyzing the combination of glutathione and drugs, and expelling the conjugate from the body under the action of multidrug resistant-related proteins, all of above made GST plays a detoxification role[38]. UGT is the most important enzyme involved in human phase II of drug metabolism, and about 40%-70% of drugs and traditional Chinese medicine are metabolized by UGT[39]. UGT and GST can make exogenous harmful substances into water-soluble harmless small molecular substances, and then excreted in the form of bile and urine.

In 1978, Lawrence *et al*[40] found that there was a glutathione peroxidase without selenium in the liver tissue of mice, named GST. The GST family plays a crucial role in cellular defense by catalyzing the coupling reaction of carcinogens to glutathione, thereby preventing cell damage. Any mutation in the gene that expresses this enzyme may alter the catalytic process, which in turn can alter drug bioavailability and may amplify or reduce drug efficacy and toxicity[41]. Multidrug resistance (MDR) mediated by the overexpression of GST is the main cause of chemotherapy failure in breast cancer[42]. Compared with non-TNBC cells, GSTP1 expression is higher in TNBC, and GSTP1 plays a crucial role in the chemoresistance of TNBC cells[43]. In GSTA1-overexpressing cancer cells, an unexpected lack of chemotherapeutic agents leads to enhanced cytotoxicity[44]. Overexpression of GSTA2 protects cancer cells from apoptosis can also induced by chemotherapeutic agents[45]. Upregulation of GSTA2 is associated with doxorubicin resistance[46]. A case-control study, which investigated children suffered acute lymphoblastic leukemia treated with different anticancer agents (vincristine, daunorubicin, cytarabine, *etc.*), showed that GSTM1 deficiency reduced the risk of recurrence by 18 times[47]. In addition, low survival rate was observed in patients with high GSTM1 expression who received high-dose cyclophosphamide, carmustine and cisplatin as initial chemotherapy for breast cancer[48]. Clearly, GST family is associated with drug resistance of breast cancer, and it also involved in the drug resistance of TNBC.

UGTs are a superfamily, so named because they mainly utilize uridine diphosphate glucuronic acid as a glycosyl donor. UGT catalyzes the binding of the substrate to the uridine diphosphate glucuronate group, making it more hydrophilic and conducive to elimination from the body. The human UGT superfamily is divided into two families based on nucleotide sequence similarity: UGT1A and UGT2[49]. The UGT1A gene cluster, encoded by a gene cluster located at 2q37, contains a total of 17 exons. UGT1A enzymes, especially UGT1A1, have been shown to be overexpressed in tumor tissues and play a role in anticancer drug resistance[50], as well as in TNBC[51]. Overexpression of UGT1A6 counteracts the cytotoxicity caused by the breast cancer chemotherapy drug methotrexate[52]. UGT2B7 can induce epirubicin resistance in breast cancer cells[53]. To sum up that UGT, as a conjugation enzyme in phase II of drug metabolism, plays a important role in breast cancer resistance. Although there are few reports on UGT family in TNBC, the only reports can also illustrate the role of UGT in tumor resistance.

Among the conjugated enzymes in phase II of drug metabolism, the target genes of PXR have been found to include UGT1A1, UGT1A6, UGT1A3, UGT1A4 and GSTA1, GSTA2, GSTA3, GSTM1, GSTM2, GSTM3, GSTM4[30]. The mechanism of which PXR regulates UGT and GST, further lead to drug resistance in TNBC may be one of the drug resistance mechanisms, but due to the lack of relevant reports, more experiments are needed to prove it.

PXR AND TRANSPORTERS IN PHASE III OF DRUG METABOLISM

The transporters in phase III of drug metabolism are mainly adenosine triphosphate binding cassette (ABC) membrane transporters, including MDR protein, multidrug resistation-associated protein (MRP) and breast cancer resistance protein (BCRP), which are mainly involved in drug transport and clearance[54].

ABC membrane transporters affect the therapeutic effect of drugs on malignant tumors by affecting the absorption and metabolism of drugs in cells. ABC transporters use adenosine triphosphate to efflux various compounds, including chemotherapeutic drugs of different structures and properties. A variety of ABC transporters are closely related to chemotherapy resistance of solid tumors including breast cancer, and increased drug efflux mediated by ABC transporters is the most common mechanism of MDR caused by drug efflux[55]. The ABC family of membrane transporters includes seven isoforms (ABCA-ABCG), among which the MDR protein 1 (MDR1/P-gp) gene is a membrane transporter encoded by the ABCB1 gene, with a relative molecular weight of 170 KDa, composed of 1280 amino acids, and located on the cell membrane. The energy released by ATP hydrolysis can be used to transport the hydrophobic and lipophilic drugs outside the cell, when MDR1/P-gp is overexpressed, drug efflux is increased through the role of efflux pump, thereby reducing the accumulation of drugs in cells and the effect of drugs on cells, thus causing drug resistance in tumor cells[56]. Overexpression of MDR has become an important mechanism of drug resistance mediated by TNBC, which is associated with poor outcome, reduced survival rate and chemoresistance of patients[57]. The MRP gene is a membrane transporter encoded by the ABCC gene, whereas BCRP is a membrane transporter encoded by the ABCG gene. In breast cancer related studies, ABCC1, ABCC3, ABCB1 and ABCG2 are associated with drug resistance [22,30,33]. Compared with other breast cancer subtypes, tmultidrug resistance protein-1 (ABCC1/MRP1), MDR protein-8 (ABCC11/MRP8) and BCRP (ABCG2/BCRP) is significantly overexpressed in TNBC[58,59], which is closely related to chemotherapy resistance[60].

PXR regulates a variety of proteins, including MDR protein (ABCB1, ABCB2), MDR associated protein (ABCC2, ABCC3, ABCC4, ABCC5) and so on. These enzymes are mainly bile acid transporters, which mediate the metabolism and excretion of bile acids, as well as the transmembrane transport and clearance of chemotherapeutic drugs[61]. Overexpression of PXR leads to increased cellular levels of resistance proteins such as ABCC1 and ABCG2[62,63]. Studies have

Table 1 Role of pregnane X receptor in the mechanism of drug resistance in breast cancer (including triple-negative breast cancer)

Phase	Resistance-associated proteins associated with PXR	Resistance-associated proteins associated with breast cancer	Resistance associated proteins associated with TNBC	Resistance related proteins known to be regulated by PXR in TNBC	Possible regulatory targets of PXR in TNBC (unconfirmed)
Phase I	CYP3A4, CYP3A23	CYP3A4, CYP3A5	CYP3A4	CYP3A4	CYP2C8
Enzymes metabolism	CYP3A11, CYP2B6	CYP2C8, CYP2C9	CYP4A11		
CYPs	CYP2C8, CYP2C9	CYP2J2, CYP1A1	CYP4A22		
	CYP2C19, CYP1A	CYP1B1, CYP17A1	CYP2C8		
	CYP2B9, CYP2C55	CYP2B6, CYP2D6	CYP1B1		
		CYP2C19, CYP2S1			
		CYP4V2, CYP26A1			
		CYP4A11, CYP4A22			
Phase II	GSTA1, GSTA2	GSTM1, GSTP1	GSTP1		
Enzymes conjugation	GSTA3, GSTM1	GSTA1, GSTA2			
GSTs	GSTM2, GSTM3				
	GSTM4				
UGTs	UGT1A1, UGT1A6	UGT1A, UGT2B7	UGT1A1		UGT1A1
	UGT1A3, UGT1A4				
Phase III	ABCB1, ABCB2	ABCC1, ABCC3	ABCC1	ABCC1	
Ttansporters	ABCC1, ABCC2	ABCB1, ABCG2	ABCG2	ABCG2	
ABCs	ABCC3, ABCC4	ABCC11	ABCC11		
	ABCC5, ABCG2				

PXR: Pregnane X receptor; TNBC: Triple-negative breast cancer; ABC: Adenosine triphosphate binding cassette; CYPs: Cytochrome P450s; UGTs: Uridine diphosphate glucuronosyltransferase; GSTs: Glutathione transferase.

also shown that PXR-mediated induction of ABCC2 seems to be involved in chemotherapy resistance in tamoxifen-resistant breast cancer [64,65]. PXR has been confirmed to regulate two membrane transporters ABCB1 and ABCG2 in TNBC[66]. Clearly, PXR-mediated upregulation of ABC membrane transporter family expression in TNBC cancer patients is one of the mechanisms of chemotherapy resistance in TNBC.

CONCLUSION

In conclusion, although PXR is mainly expressed in liver, intestinal and colon tissues, it is also expressed in normal breast tissues, and its expression level is even higher in breast cancer tissues[67-70]. PXR is associated with the phenotype of TNBC and is a powerful and independent poor prognostic factor[71]. PXR can accelerate the metabolism and clearance of chemotherapy drugs in TNBC through the regulation of three phases of the metabolism of chemotherapy drugs: phase I drug metabolism enzymes CYPs, phase II drug binding enzymes GSTs and UGTs, and phase III drug transporter ABCs, thus resulting in drug resistance (Table 1). Among them, experiments have confirmed that PXR can regulate the expression of CYP3A4, ABCC1, and ABCG2 in TNBC, resulting in TNBC drug resistance. In the future, researchers should focus on improving our understanding of the mechanism of PXR in TNBC drug resistance, including regulation of PXR and function of PXR independence of drug metabolism.

FOOTNOTES

Author contributions: Rao ZZ did the literature search and wrote the paper; Tang ZW revised the paper; Wen J conceived and coordinated the study, designed; Tang ZW and Wen J contribute equally to this study, they share co-corresponding author.

Supported by Science project of Hunan Provincial Health Commission, No. B202304089304.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Zhong-Wen Tang 0000-0001-6201-4625; Jie Wen 0000-0002-5734-4678.

S-Editor: Qu XL
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Bray F**, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. *Cancer* 2021; **127**: 3029-3030 [PMID: 34086348 DOI: 10.1002/cncr.33587]
2 **Chong FF**, Yin LY, Liu J, Guo J, Fan Y, Zhang ML, Zhang L, He MY, Zhang HM. Malnutrition increases therisk of mortality in hospitalized lung cancer patients. *J NutrOncol* 2022; 7: 49-57
3 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]
4 **Qiu H**, Cao S, Xu R. Cancer incidence, mortality, and burden in China: a time-trend analysis and comparison with the United States and United Kingdom based on the global epidemiological data released in 2020. *Cancer Commun (Lond)* 2021; **41**: 1037-1048 [PMID: 34288593 DOI: 10.1002/cac2.12197]
5 **Burstein HJ**, Curigliano G, Thürlimann B, Weber WP, Poortmans P, Regan MM, Senn HJ, Winer EP, Gnant M; Panelists of the St Gallen Consensus Conference. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. *Ann Oncol* 2021; **32**: 1216-1235 [PMID: 34242744 DOI: 10.1016/j.annonc.2021.06.023]
6 **Coughlin SS**. Epidemiology of Breast Cancer in Women. *Adv Exp Med Biol* 2019; **1152**: 9-29 [PMID: 31456177 DOI: 10.1007/978-3-030-20301-6_2]
7 **Yu KD**, Ye FG, He M, Fan L, Ma D, Mo M, Wu J, Liu GY, Di GH, Zeng XH, He PQ, Wu KJ, Hou YF, Wang J, Wang C, Zhuang ZG, Song CG, Lin XY, Toss A, Ricci F, Shen ZZ, Shao ZM. Effect of Adjuvant Paclitaxel and Carboplatin on Survival in Women With Triple-Negative Breast Cancer: A Phase 3 Randomized Clinical Trial. *JAMA Oncol* 2020; **6**: 1390-1396 [PMID: 32789480 DOI: 10.1001/jamaoncol.2020.2965]
8 **Zhou YD**, Li J, Du L, Mahdi F, Le TP, Chen WL, Swanson SM, Watabe K, Nagle DG. Biochemical and Anti-Triple Negative Metastatic Breast Tumor Cell Properties of Psammaplins. *Mar Drugs* 2018; **16** [PMID: 30423844 DOI: 10.3390/md16110442]
9 **Yin L**, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Res* 2020; **22**: 61 [PMID: 32517735 DOI: 10.1186/s13058-020-01296-5]
10 **Lee A**, Djamgoz MBA. Triple negative breast cancer: Emerging therapeutic modalities and novel combination therapies. *Cancer Treat Rev* 2018; **62**: 110-122 [PMID: 29202431 DOI: 10.1016/j.ctrv.2017.11.003]
11 **Bianchini G**, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. *Nat Rev Clin Oncol* 2016; **13**: 674-690 [PMID: 27184417 DOI: 10.1038/nrclinonc.2016.66]
12 **Nedeljković M**, Damjanović A. Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer-How We Can Rise to the Challenge. *Cells* 2019; **8** [PMID: 31443516 DOI: 10.3390/cells8090957]
13 **Singh DD**, Yadav DK. TNBC: Potential Targeting of Multiple Receptors for a Therapeutic Breakthrough, Nanomedicine, and Immunotherapy. *Biomedicines* 2021; **9** [PMID: 34440080 DOI: 10.3390/biomedicines9080876]
14 **Kliewer SA**, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterström RH, Perlmann T, Lehmann JM. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* 1998; **92**: 73-82 [PMID: 9489701 DOI: 10.1016/s0092-8674(00)80900-9]
15 **Doricakova A**, Novotna A, Vrzal R, Pavek P, Dvorak Z. The role of residues T248, Y249 and T422 in the function of human pregnane X receptor. *Arch Toxicol* 2013; **87**: 291-301 [PMID: 22976785 DOI: 10.1007/s00204-012-0937-9]
16 **Chen Y**, Tang Y, Guo C, Wang J, Boral D, Nie D. Nuclear receptors in the multidrug resistance through the regulation of drug-metabolizing enzymes and drug transporters. *Biochem Pharmacol* 2012; **83**: 1112-1126 [PMID: 22326308 DOI: 10.1016/j.bcp.2012.01.030]
17 **Xing Y**, Yan J, Niu Y. PXR: a center of transcriptional regulation in cancer. *Acta Pharm Sin B* 2020; **10**: 197-206 [PMID: 32082968 DOI: 10.1016/j.apsb.2019.06.012]
18 **Baldwin WS**. Phase 0 of the Xenobiotic Response: Nuclear Receptors and Other Transcription Factors as a First Step in Protection from Xenobiotics. *Nucl Receptor Res* 2019; **6** [PMID: 31815118 DOI: 10.32527/2019/101447]
19 **Verma S**, Tabb MM, Blumberg B. Activation of the steroid and xenobiotic receptor, SXR, induces apoptosis in breast cancer cells. *BMC Cancer* 2009; **9**: 3 [PMID: 19123943 DOI: 10.1186/1471-2407-9-3]
20 **Shao Z**, Li Y, Dai W, Jia H, Zhang Y, Jiang Q, Chai Y, Li X, Sun H, Yang R, Cao Y, Feng F, Guo Y. ETS-1 induces Sorafenib-resistance in hepatocellular carcinoma cells *via* regulating transcription factor activity of PXR. *Pharmacol Res* 2018; **135**: 188-200 [PMID: 30114438 DOI: 10.1016/j.phrs.2018.08.003]

21 **Feng F**, Jiang Q, Cao S, Cao Y, Li R, Shen L, Zhu H, Wang T, Sun L, Liang E, Sun H, Chai Y, Li X, Liu G, Yang R, Yang Z, Yang Y, Xin S, Li BA. Pregnane X receptor mediates sorafenib resistance in advanced hepatocellular carcinoma. *Biochim Biophys Acta Gen Subj* 2018; **1862**: 1017-1030 [PMID: 29369785 DOI: 10.1016/j.bbagen.2018.01.011]

22 **Qiao EQ**, Yang HJ, Yu XF, Gong LJ, Zhang XP, Chen DB. Curcuma zedoaria petroleum ether extract reverses the resistance of triple-negative breast cancer to docetaxel *via* pregnane X receptor. *Ann Transl Med* 2021; **9**: 1389 [PMID: 34733941 DOI: 10.21037/atm-21-4199]

23 **Gajjar K**, Martin-Hirsch PL, Martin FL. CYP1B1 and hormone-induced cancer. *Cancer Lett* 2012; **324**: 13-30 [PMID: 22561558 DOI: 10.1016/j.canlet.2012.04.021]

24 **Luthra A**, Denisov IG, Sligar SG. Spectroscopic features of cytochrome P450 reaction intermediates. *Arch Biochem Biophys* 2011; **507**: 26-35 [PMID: 21167809 DOI: 10.1016/j.abb.2010.12.008]

25 **Nelson DR**. Cytochrome P450 nomenclature. *Methods Mol Biol* 1998; **107**: 15-24 [PMID: 14577209 DOI: 10.1385/0-89603-519-0:15]

26 **Pelkonen O**, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J, Raunio H. Inhibition and induction of human cytochrome P450 enzymes: current status. *Arch Toxicol* 2008; **82**: 667-715 [PMID: 18618097 DOI: 10.1007/s00204-008-0332-8]

27 **Guengerich FP**. Mechanisms of cytochrome P450 substrate oxidation: MiniReview. *J Biochem Mol Toxicol* 2007; **21**: 163-168 [PMID: 17936929 DOI: 10.1002/jbt.20174]

28 **Ul-Islam S**, Ahmed MB, Shehzad A, Ul-Islam M, Lee YS. Failure of Chemotherapy in Hepatocellular Carcinoma Due to Impaired and Dysregulated Primary Liver Drug Metabolizing Enzymes and Drug Transport Proteins: What to Do? *Curr Drug Metab* 2018; **19**: 819-829 [PMID: 29807513 DOI: 10.2174/1389200219666180529113818]

29 **Murray GI**, Patimalla S, Stewart KN, Miller ID, Heys SD. Profiling the expression of cytochrome P450 in breast cancer. *Histopathology* 2010; **57**: 202-211 [PMID: 20716162 DOI: 10.1111/j.1365-2559.2010.03606.x]

30 **Ihunnah CA**, Jiang M, Xie W. Nuclear receptor PXR, transcriptional circuits and metabolic relevance. *Biochim Biophys Acta* 2011; **1812**: 956-963 [PMID: 21295138 DOI: 10.1016/j.bbadis.2011.01.014]

31 **Panigrahy D**, Kaipainen A, Greene ER, Huang S. Cytochrome P450-derived eicosanoids: the neglected pathway in cancer. *Cancer Metastasis Rev* 2010; **29**: 723-735 [PMID: 20941528 DOI: 10.1007/s10555-010-9264-x]

32 **Sneha S**, Baker SC, Green A, Storr S, Aiyappa R, Martin S, Pors K. Intratumoural Cytochrome P450 Expression in Breast Cancer: Impact on Standard of Care Treatment and New Efforts to Develop Tumour-Selective Therapies. *Biomedicines* 2021; **9** [PMID: 33809117 DOI: 10.3390/biomedicines9030290]

33 **van Eijk M**, Boosman RJ, Schinkel AH, Huitema ADR, Beijnen JH. Cytochrome P450 3A4, 3A5, and 2C8 expression in breast, prostate, lung, endometrial, and ovarian tumors: relevance for resistance to taxanes. *Cancer Chemother Pharmacol* 2019; **84**: 487-499 [PMID: 31309254 DOI: 10.1007/s00280-019-03905-3]

34 **Alexanian A**, Miller B, Roman RJ, Sorokin A. 20-HETE-producing enzymes are up-regulated in human cancers. *Cancer Genomics Proteomics* 2012; **9**: 163-169 [PMID: 22798501]

35 **Stewart DA**, Winnike JH, McRitchie SL, Clark RF, Pathmasiri WW, Sumner SJ. Metabolomics Analysis of Hormone-Responsive and Triple-Negative Breast Cancer Cell Responses to Paclitaxel Identify Key Metabolic Differences. *J Proteome Res* 2016; **15**: 3225-3240 [PMID: 27447733 DOI: 10.1021/acs.jproteome.6b00430]

36 **Ding X**, Staudinger JL. Induction of drug metabolism by forskolin: the role of the pregnane X receptor and the protein kinase a signal transduction pathway. *J Pharmacol Exp Ther* 2005; **312**: 849-856 [PMID: 15459237 DOI: 10.1124/jpet.104.076331]

37 **Miki Y**, Suzuki T, Tazawa C, Blumberg B, Sasano H. Steroid and xenobiotic receptor (SXR), cytochrome P450 3A4 and multidrug resistance gene 1 in human adult and fetal tissues. *Mol Cell Endocrinol* 2005; **231**: 75-85 [PMID: 15713537 DOI: 10.1016/j.mce.2004.12.005]

38 **Leake CD**. Annual review of pharmacology and toxicology: review of reviews. *Annu Rev Pharmacol Toxicol* 1978; **18**: 581-588 [PMID: 348070 DOI: 10.1146/annurev.pa.18.040178.003053]

39 **Mano ECC**, Scott AL, Honorio KM. UDP-glucuronosyltransferases: Structure, Function and Drug Design Studies. *Curr Med Chem* 2018; **25**: 3247-3255 [PMID: 29484974 DOI: 10.2174/0929867325666180226111311]

40 **Lawrence RA**, Parkhill LK, Burk RF. Hepatic cytosolic non selenium-dependent glutathione peroxidase activity: its nature and the effect of selenium deficiency. *J Nutr* 1978; **108**: 981-987 [PMID: 650300 DOI: 10.1093/jn/108.6.981]

41 **Zhang BL**, Sun T, Zhang BN, Zheng S, Lü N, Xu BH, Wang X, Chen GJ, Yu DK, Lin DX. Polymorphisms of GSTP1 is associated with differences of chemotherapy response and toxicity in breast cancer. *Chin Med J (Engl)* 2011; **124**: 199-204 [PMID: 21362365 DOI: 10.3760/cma.j.issn.0366-6999.2011.02.008]

42 **Gangemi R**, Paleari L, Orengo AM, Cesario A, Chessa L, Ferrini S, Russo P. Cancer stem cells: a new paradigm for understanding tumor growth and progression and drug resistance. *Curr Med Chem* 2009; **16**: 1688-1703 [PMID: 19442140 DOI: 10.2174/092986709788186147]

43 **Yang M**, Li Y, Shen X, Ruan Y, Lu Y, Jin X, Song P, Guo Y, Zhang X, Qu H, Shao Y, Quan C. CLDN6 promotes chemoresistance through GSTP1 in human breast cancer. *J Exp Clin Cancer Res* 2017; **36**: 157 [PMID: 29116019 DOI: 10.1186/s13046-017-0627-9]

44 **van Gisbergen MW**, Cebula M, Zhang J, Ottosson-Wadlund A, Dubois L, Lambin P, Tew KD, Townsend DM, Haenen GR, Drittij-Reijnders MJ, Saneyoshi H, Araki M, Shishido Y, Ito Y, Arnér ES, Abe H, Morgenstern R, Johansson K. Chemical Reactivity Window Determines Prodrug Efficiency toward Glutathione Transferase Overexpressing Cancer Cells. *Mol Pharm* 2016; **13**: 2010-2025 [PMID: 27093577 DOI: 10.1021/acs.molpharmaceut.6b00140]

45 **Xie J**, Shults K, Flye L, Jiang F, Head DR, Briggs RC. Overexpression of GSTA2 protects against cell cycle arrest and apoptosis induced by the DNA inter-strand crosslinking nitrogen mustard, mechlorethamine. *J Cell Biochem* 2005; **95**: 339-351 [PMID: 15778998 DOI: 10.1002/jcb.20440]

46 **Lee JY**, Han CY, Yang JW, Smith C, Kim SK, Lee EY, Kim SG, Kang KW. Induction of glutathione transferase in insulin-like growth factor type I receptor-overexpressed hepatoma cells. *Mol Pharmacol* 2007; **72**: 1082-1093 [PMID: 17615245 DOI: 10.1124/mol.107.038174]

47 **Rocha JC**, Cheng C, Liu W, Kishi S, Das S, Cook EH, Sandlund JT, Rubnitz J, Ribeiro R, Campana D, Pui CH, Evans WE, Relling MV. Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. *Blood* 2005; **105**: 4752-4758 [PMID: 15713801 DOI: 10.1182/blood-2004-11-4544]

48 **Salinas AE**, Wong MG. Glutathione S-transferases--a review. *Curr Med Chem* 1999; **6**: 279-309 [PMID: 10101214 DOI: 10.2174/0929867306666220208213032]

49 **Mackenzie PI**, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners JO, Owens IS, Nebert DW. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genomics* 2005; **15**: 677-685 [PMID: 16141793 DOI: 10.1097/01.fpc.0000173483.13689.56]

50 **Pathania S**, Bhatia R, Baldi A, Singh R, Rawal RK. Drug metabolizing enzymes and their inhibitors' role in cancer resistance. *Biomed*

Pharmacother 2018; **105**: 53-65 [PMID: 29843045 DOI: 10.1016/j.biopha.2018.05.117]

51 **Li Y**, Zhou Y, Mao F, Shen S, Zhao B, Xu Y, Lin Y, Zhang X, Cao X, Chen C, Zhang J, Sun Q. miR-452 Reverses Abnormal Glycosylation Modification of ERα and Estrogen Resistance in TNBC (Triple-Negative Breast Cancer) Through Targeting UGT1A1. *Front Oncol* 2020; **10**: 1509 [PMID: 32983995 DOI: 10.3389/fonc.2020.01509]

52 **de Almagro MC**, Selga E, Thibaut R, Porte C, Noé V, Ciudad CJ. UDP-glucuronosyltransferase 1A6 overexpression in breast cancer cells resistant to methotrexate. *Biochem Pharmacol* 2011; **81**: 60-70 [PMID: 20854796 DOI: 10.1016/j.bcp.2010.09.008]

53 **Parmar S**, Stingl JC, Huber-Wechselberger A, Kainz A, Renner W, Langsenlehner U, Krippl P, Brockmöller J, Haschke-Becher E. Impact of UGT2B7 His268Tyr polymorphism on the outcome of adjuvant epirubicin treatment in breast cancer. *Breast Cancer Res* 2011; **13**: R57 [PMID: 21658222 DOI: 10.1186/bcr2894]

54 **van den Heuvel-Eibrink MM**, Sonneveld P, Pieters R. The prognostic significance of membrane transport-associated multidrug resistance (MDR) proteins in leukemia. *Int J Clin Pharmacol Ther* 2000; **38**: 94-110 [PMID: 10739113 DOI: 10.5414/cpp38094]

55 **Szakács G**, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. *Nat Rev Drug Discov* 2006; **5**: 219-234 [PMID: 16518375 DOI: 10.1038/nrd1984]

56 **Abolhoda A**, Wilson AE, Ross H, Danenberg PV, Burt M, Scotto KW. Rapid activation of MDR1 gene expression in human metastatic sarcoma after in vivo exposure to doxorubicin. *Clin Cancer Res* 1999; **5**: 3352-3356 [PMID: 10589744]

57 **Abd El-Aziz YS**, Spillane AJ, Jansson PJ, Sahni S. Role of ABCB1 in mediating chemoresistance of triple-negative breast cancers. *Biosci Rep* 2021; **41** [PMID: 33543229 DOI: 10.1042/BSR20204092]

58 **Yamada A**, Ishikawa T, Ota I, Kimura M, Shimizu D, Tanabe M, Chishima T, Sasaki T, Ichikawa Y, Morita S, Yoshiura K, Takabe K, Endo I. High expression of ATP-binding cassette transporter ABCC11 in breast tumors is associated with aggressive subtypes and low disease-free survival. *Breast Cancer Res Treat* 2013; **137**: 773-782 [PMID: 23288347 DOI: 10.1007/s10549-012-2398-5]

59 **Kumar H**, Gupta NV, Jain R, Madhunapantula SV, Babu CS, Kesharwani SS, Dey S, Jain V. A review of biological targets and therapeutic approaches in the management of triple-negative breast cancer. *J Adv Res* 2023 [PMID: 36791960 DOI: 10.1016/j.jare.2023.02.005]

60 **Sharom FJ**. ABC multidrug transporters: structure, function and role in chemoresistance. *Pharmacogenomics* 2008; **9**: 105-127 [PMID: 18154452 DOI: 10.2217/14622416.9.1.105]

61 **Kliewer SA**, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev* 2002; **23**: 687-702 [PMID: 12372848 DOI: 10.1210/er.2001-0038]

62 **Revathidevi S**, Sudesh R, Vaishnavi V, Kaliyanasundaram M, MaryHelen KG, Sukanya G, Munirajan AK. Screening for the 3'UTR Polymorphism of the PXR Gene in South Indian Breast Cancer Patients and its Potential Role in Pharmacogenomics. *Asian Pac J Cancer Prev* 2016; **17**: 3971-3977 [PMID: 27644647]

63 **Nabekura T**, Kawasaki T, Jimura M, Mizuno K, Uwai Y. Microtubule-targeting anticancer drug eribulin induces drug efflux transporter P-glycoprotein. *Biochem Biophys Rep* 2020; **21**: 100727 [PMID: 31993509 DOI: 10.1016/j.bbrep.2020.100727]

64 **Bhagyaraj E**, Ahuja N, Kumar S, Tiwari D, Gupta S, Nanduri R, Gupta P. TGF-β induced chemoresistance in liver cancer is modulated by xenobiotic nuclear receptor PXR. *Cell Cycle* 2019; **18**: 3589-3602 [PMID: 31739702 DOI: 10.1080/15384101.2019.1693120]

65 **Kodama S**, Negishi M. Pregnane X receptor PXR activates the GADD45beta gene, eliciting the p38 MAPK signal and cell migration. *J Biol Chem* 2011; **286**: 3570-3578 [PMID: 21127053 DOI: 10.1074/jbc.M110.179812]

66 **Yang H**, Ren L, Wang Y, Bi X, Li X, Wen M, Zhang Q, Yang Y, Jia Y, Li Y, Zang A, Wei Y, Dai G. FBI-1 enhanced the resistance of triple-negative breast cancer cells to chemotherapeutic agents *via* the miR-30c/PXR axis. *Cell Death Dis* 2020; **11**: 851 [PMID: 33051436 DOI: 10.1038/s41419-020-03053-0]

67 **Chen Y**, Tang Y, Chen S, Nie D. Regulation of drug resistance by human pregnane X receptor in breast cancer. *Cancer Biol Ther* 2009; **8**: 1265-1272 [PMID: 19746521 DOI: 10.4161/cbt.8.13.8696]

68 **Dotzlaw H**, Leygue E, Watson P, Murphy LC. The human orphan receptor PXR messenger RNA is expressed in both normal and neoplastic breast tissue. *Clin Cancer Res* 1999; **5**: 2103-2107 [PMID: 10473093]

69 **Meyer zu Schwabedissen HE**, Tirona RG, Yip CS, Ho RH, Kim RB. Interplay between the nuclear receptor pregnane X receptor and the uptake transporter organic anion transporter polypeptide 1A2 selectively enhances estrogen effects in breast cancer. *Cancer Res* 2008; **68**: 9338-9347 [PMID: 19010908 DOI: 10.1158/0008-5472.CAN-08-0265]

70 **Miki Y**, Suzuki T, Kitada K, Yabuki N, Shibuya R, Moriya T, Ishida T, Ohuchi N, Blumberg B, Sasano H. Expression of the steroid and xenobiotic receptor and its possible target gene, organic anion transporting polypeptide-A, in human breast carcinoma. *Cancer Res* 2006; **66**: 535-542 [PMID: 16397270 DOI: 10.1158/0008-5472.CAN-05-1070]

71 **Theocharis S**, Giaginis C, Gourzi S, Alexandrou P, Tsourouflis G, Sarantis P, Danas E, Michail A, Tsoukalas N, Pergaris A, Politis PK, Nakopoulou L. High Pregnane X Receptor (PXR) Expression Is Correlated with Poor Prognosis in Invasive Breast Carcinoma. *Diagnostics (Basel)* 2021; **11** [PMID: 34829293 DOI: 10.3390/diagnostics11111946]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 September 24; 14(9): 343-356

DOI: 10.5306/wjco.v14.i9.343

ISSN 2218-4333 (online)

MINIREVIEWS

Effectiveness and safety of COVID-19 vaccines in patients with oncological diseases: State-of-the-art

Nedelcho Ivanov, Boris Krastev, Dimitrina Georgieva Miteva, Hristiana Batselova, Radostina Alexandrova, Tsvetelina Velikova

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Chen LJ, China; Emran TB, Bangladesh

Received: June 28, 2023
Peer-review started: June 28, 2023
First decision: July 6, 2023
Revised: August 6, 2023
Accepted: September 12, 2023
Article in press: September 12, 2023
Published online: September 24, 2023

Nedelcho Ivanov, Department of Clinical Immunology with Stem Cell Bank, University Hospital Alexanrovska, Sofia 1431, Bulgaria

Boris Krastev, Medical Center Nadezhda, Medical Center Nadezhda, Sofia 1407, Bulgaria

Dimitrina Georgieva Miteva, Department of Genetics, Faculty of Biology, Sofia University St. Kliment Ohridski, Sofia 1164, Bulgaria

Hristiana Batselova, Department of Epidemiology and Disaster Medicine, Medical University, Plovdiv, University Hospital St. George, Plovdiv 6000, Bulgaria

Radostina Alexandrova, Department of Pathology, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia 1000, Bulgaria

Tsvetelina Velikova, Medical Faculty, Sofia University St. Kliment Ohridski, Sofia 1407, Bulgaria

Corresponding author: Nedelcho Ivanov, FRCS (Hon), MD, PhD, Assistant Professor, Department of Clinical Immunology with Stem Cell Bank, University Hospital Alexanrovska, St. Georfi Sofiiski 1 str, Sofia 1431, Bulgaria. nedelcho.ivanov93@gmail.com

Abstract

Although the coronavirus disease 2019 (COVID-19) pandemic was declared to be no longer "a public health emergency of international concern" with its wide range of clinical manifestations and late complications, severe acute respiratory syndrome coronavirus 2 infection proved to be a serious threat, especially to the elderly and patients with comorbidities. Patients with oncologic diseases are vulnerable to severe infection and death. Indeed, patients with oncohematological diseases have a higher risk of severe COVID-19 and impaired post-vaccination immunity. Unfortunately, cancer patients are usually excluded from vaccine trials and investigations of post-vaccinal immune responses and the effectiveness of the vaccines. We aimed to elucidate to what extent patients with cancer are at increased risk of developing severe COVID-19 and what is their overall case fatality rate. We also present the current concept and evidence on the effectiveness and safety of COVID-19 vaccines, including boosters, in oncology patients. In conclusion, despite the considerably higher mortality in the cancer patient group than the general population, countries with high vaccination rates have demon-

strated trends toward improved survival of cancer patients early and late in the pandemic.

Key Words: COVID-19; COVID-19 vaccines; RNA vaccines; Cancer; Oncological; Safety; Efficacy; Immunogenicity

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The coronavirus disease 2019 (COVID-19) pandemic has greatly impacted the lives of cancer patients. Their medical care has been challenging, given the competing risks of death from cancer and serious complications from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Cancer patients are at high risk of severe complications and death from COVID-19. Protective SARS-CoV-2 antibodies and cellular immune response are induced after infection or/and COVID-19 vaccination. Vaccines decrease the risk of hospitalization and death from COVID-19. Therefore, vaccination of specific vulnerable groups, such as oncological patients, and all people in general, will slow the virus spread and save lives.

Citation: Ivanov N, Krastev B, Miteva DG, Batselova H, Alexandrova R, Velikova T. Effectiveness and safety of COVID-19 vaccines in patients with oncological diseases: State-of-the-art. *World J Clin Oncol* 2023; 14(9): 343-356
URL: https://www.wjgnet.com/2218-4333/full/v14/i9/343.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i9.343

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has considerably impacted the lives of cancer patients. Their medical care has been challenging because of the competing risks of death from cancer or serious complications from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the likely higher lethality in immunocompromised hosts[1,2]. Furthermore, patients diagnosed with malignancies are at higher risk of developing severe COVID-19[3] and fatal outcomes due to the disease. Studies have demonstrated variable mortality rates among subjects with hematological cancers and solid tumors, with some reporting fatality cases of as much as 40% of the infected subjects[4]. Despite this considerably higher mortality than the one observed in the general population, trends towards improved survival during the evolution of the pandemic have already been demonstrated in Europe, and much of this could be a direct result of the rigorous COVID-19 vaccination in this region[5].

Since the beginning of the pandemic, hundreds of different therapeutic options have been studied, including those well-known in the treatment of other diseases, such as reoriented drugs. Amongst them are remdesivir (initially developed for hepatitis C treatment, tocilizumab-rheumatoid arthritis, hydroxychloroquine-malaria, lupus, *etc*), corticosteroids, plasma from donors who have recovered from COVID-19, monoclonal antibodies (casirivimab + imdevimab, bamlanivimab, sotrovimab, cilgavimab + tixagevimab, *etc*), Janus kinase inhibitors (baricitinib), and even mesenchymal stem cells[6,7]. Targeting both the virus itself and the host's immune response with variable effectiveness during the different stages of the disease. However, prevention in the form of COVID vaccines remains the most desirable option for the general population both in long-term health-related and financial terms. Cancer patients are no exception in this regard. But exactly how effective are vaccines in cancer patients compared to the general population? This is the question we will try to answer.

In this review, we elucidated to what extent patients with cancer are at increased risk of developing severe COVID-19 and what is their overall case fatality rate. We also present the current concept and evidence on the effectiveness and safety of COVID-19 vaccines, including boosters, in oncology patients.

SEARCH STRATEGY

We performed a modified form of a biomedical narrative review according to recent recommendations for writing[8]. First, we thoroughly searched the scientific bibliographic databases Medline (PubMed) and Scopus. We used relevant free-text and Medical Subject Headings terms, as follows: ("COVID-19" OR "SARS-CoV-2") AND ("cancer patients" OR "oncological patients") AND ("COVID-19 vaccine" OR "mRNA vaccine"). We confined the search from January 1, 2020 to June 20, 2023. Then we identified additional papers using the search engine Google Scholar. Information from advisory committee meetings was also added.

COVID-19 AND PATIENTS WITH ONCOLOGICAL DISEASES

Patients with oncologic diseases are affected by SARS-CoV-2 in many different ways. Similar to many other infections, COVID-19 poses an additional risk of a fatal outcome for cancer patients. However, it is challenging to say to what extent patients with malignancies are threatened by complications of severe infections. As oncological diseases and treatment

protocols are extremely diverse, it can be expected that the course of SARS-CoV2 infection would also be quite different [9-11].

The stage of disease, type of malignancy, and the sort and phase of the applied treatment modalities (surgery, chemotherapy, radiation therapy, and immunotherapy) introduce even more variables and more superimposing confounding factors, making this group of patients even more heterogeneous and difficult for overall risk assessment. Cancer patients who have recently undergone surgery or chemotherapy (especially during the induction phase with high-dose intensive regimens) are at a dramatically increased risk of death from COVID[12,13]. Side effects of chemotherapy, such as secondary immunodeficiency due to severe leukopenia and specific tissue toxicity due to some chemo- and immunotherapeutics, can significantly alter the course of COVID-19 infection, from worsening the patient's overall condition and increasing the risk of complications and death to masking or mimicking the radiological pulmonary signs (*e.g.*, immune checkpoint related pneumonitis)[13]. Finally, another confounding factor is the various therapeutic regimens used to treat infection in hospitals and intensive care units worldwide. Cancer patients are treated as high risk by default, which carries a risk (polypharmacy, drug interactions, adverse drug effects, acute kidney or liver failure, *etc*) [14].

Below, we present data from several studies that attempt to measure and objectify this risk. The first large-scale meta-analysis by 2020 done by Zhang *et al*[11] of 15 studies involving a total of 3019 patients from Europe, the United Kingdom, the United States, Canada, and Asia detected 22.4% circulating free RNAs (CFRs) in cancer patients with COVID-19, compared to 5.9% in noncancer patients. As in other patients, risk factors influencing the course and mortality are: Being over 65 years old, male sex, and having comorbidities (especially hypertension and diabetes). No significant difference in mortality was found between different continents. The study found that mortality in patients with lung cancer and hematological malignancies was highest, although the incidence of complications did not differ[11].

A study by Yang *et al*[15] involving 1575 patients, of whom 52 with various cancers (lung, colorectal, breast, cervical, thyroid, *etc*) showed that oncologic patients are at higher risk of presenting as severe/critical cases and are more likely to develop acute respiratory distress syndrome. Also, other life-threatening complications such as myocardial infarction and shock are significantly increased in frequency. Lower lymphocyte count, as well as higher concentrations of C-reactive protein, D-dimer, procalcitonin, interleukin 6 (IL-6), and lactate dehydrogenase, were reported to reach $P < 0.05$. Cancer patients are also more likely to have comorbidities, which, as it becomes clear in this study, contributes seriously to the overall higher CFR[15].

A meta-analysis of 122 papers and 9 studies, including a total of 805 patients by Afshar *et al*[16], demonstrated how heterogeneous the data on mortality in cancer patients are. They showed that cancer patients are more likely to be admitted to intensive care units, need invasive ventilation, and are more likely to die. The published CFR in the analyzed studies ranges from 5.5% to 60.0%, with a pooled CFR of 21%. However, the authors warn that these data should be interpreted cautiously due to the high heterogeneity and the small number of patients in most studies[16].

Large-scale survival analysis by Li *et al*[9] based on data from United Kingdom Biobank followed 4606 cancer patients (288 positives) and 4606 noncancer patients (275 positives) for 21 mo after the SARS-CoV2 test. The cumulative CFR of the positive cancer patients was six times higher than the negative ones. The hazard ratio was assessed for each specific malignancy in the study, and the results showed that hematological malignancies, melanoma, kidney, and uterine cancer had particularly high CFRs (up to 10 times higher than the noncancer controls). The authors emphasize the importance of timely vaccination in these groups of patients[9].

In contrast to the data above, a study by Brar *et al*[17] included 585 patients, 117 with active malignancies. It showed no statistically significant difference in morbidity or mortality in cancer patients *vs* the general population. Furthermore, the authors argued that the studies claiming the opposite did not consider confounding factors such as age, sex, and comorbidities. According to this study, cytotoxic treatment within 90 d of admission is not associated with worse outcomes[17].

A team from London published a study in onco-hematology patients, where 40% (14 of 35) of patients hospitalized with COVID-19 had succumbed to the infection[18]. In general, COVID-19 appears to have an increased risk of complications and mortality in a large proportion of cancer patients. In addition, besides the virus itself, the pandemic and the restrictive measures were associated with disrupted access to medical care, hindered timely diagnosis and treatment, the lack of follow-up of many patients and lower quality of life[19]. Studies have shown that since the beginning of the pandemic, the total number of newly diagnosed cancers has dropped substantially[20]. As many authors warned, this inevitably led to an increased frequency of advanced cancers at diagnosis. Delaying diagnosis and treatment resulted in lower chances of survival[21]. Yong *et al*[22] conducted a study in Canada using microsimulation models, which estimated that for colorectal cancers, suspending primary screening for only 6 mo will increase cancer incidence by about 2200 cases, of which about 960 will be lethal over time. Consequences that otherwise would be prevented by the screening program and early detection.

Furthermore, there are many other indirect ways the COVID-19 pandemic affects cancer patients' quality of life and mortality[13]. At the same time, the standard of living, the structure and stability of the health care system, and even political factors in connection with dealing with the pandemic play roles that should not be underestimated[23]. Knowing risk factors for the severity and mortality of COVID-19, cancer patients have their unique risk factors. They may include active and progressing cancer, type of cancer, administration of cytotoxic chemotherapy, radiation therapy, impaired immune system due to leukocytopenia, low immunoglobulin levels, long-lasting immunosuppression, comorbidities, and others.

Malignancies reported as comorbidities in patients hospitalized with confirmed COVID-19 in different countries are: (1) Malignancies in 7.2% in a cohort study with 138 adults with confirmed COVID-19 pneumonia in Wuhan, China, in January 2020[24]; (2) malignancies in 8% at admission in a cohort study with 1591 patients with laboratory-confirmed COVID-19 in Lombardy, Italy between February 20 and March 18, 2020[25]; and (3) Malignancies reported in 5.6% at

admission in a cohort study with 5700 patients with confirmed COVID-19 infection hospitalized in 12 New York City hospitals between March 1 and April 4, 2020[26].

In a cohort study of 928 adults with COVID-19 and current or past cancer diagnosis, solid tumors were found in 82%, including breast (21%), hematologic (22%), prostate (16%), gastrointestinal (12%), thoracic (10%), gynecologic (5%), and renal cell carcinoma (5%)[27,28]. The estimated overall mortality in the research was 13%: 20% for patients with multiple cancers, 18% for patients with hematological malignancies, and 12% for patients with solid tumors[27].

Zhang *et al*[11] showed the COVID-19 fatality rates in subgroup analysis: (1) By cancer type: 32.9% in patients with lung cancer; 34.2% in patients with hematologic cancer; 17.2% in patients with solid cancer; and (2) By cancer treatment: 25.6% in patients with chemotherapy, 27.6% in patients with surgery, 24.3% in patients with immunotherapy, 21.3% in patients with targeted therapy, and 20.5% in patients with radiation therapy[11].

Children with cancer and positive for COVID-19 are at higher risk of severe illness than children without cancer. The cohort study found that about 20% of pediatric cancer patients with COVID-19 experienced a severe infection, compared to 1%-6% of children in the general population[29]. Among patients with hematologic malignancy and laboratory-confirmed COVID-19, mortality was reported in 34% of adults and 4% of children[4].

We can summarize that the main challenges in cancer patients regarding COVID-19 are the often immunocompromised state (*e.g.,* due to leukocytopenia, low immunoglobulin levels, long-lasting immunosuppression), the treatment (*e.g.,* severe chemotherapy, radiation therapy), progression of cancer, comorbidities, and others.

IMMUNE RESPONSE IN CANCER PATIENTS

Cancer cells induce an immune suppressive microenvironment and use various mechanisms to "escape" the body's immune response. As a systemic disease, cancer causes a wide range of functional and compositional changes in the immune system and can affect the body's defenses against various pathogens[30,31].

Dendritic cells (DCs) are antigen-presenting cells with an essential role in originating and directing cellular and humoral immune responses, converging innate and adaptive immunity. DCs have been recognized as the most potent professional antigen-presenting cells[32].

Tumors use different strategies to alter DC maturation and function, such as: (1) The ability to influence the capacity of hematopoietic progenitor cells to differentiate into functional DCs[33,34]; (2) production of various immunosuppressive factors that block the maturation of CD34+ stem cells into DCs[35]; and (3) spontaneous apoptosis of DCs in peripheral blood of patients with breast cancer[36]. Quantitative and functional DC deficiencies have been widely observed in patients with several types of cancer including breast cancer[37,38], prostate cancer[38], non-small cell lung cancer[39,40], colon cancer[41], and melanoma[42].

Data have revealed that tumors disrupt normal hematopoiesis, leading to extramedullary hematopoiesis and myeloid skewing. The three branches of terminally differentiated myeloid cells (macrophages, DCs, and granulocytes) are essential for normal innate and adaptive immune response functioning. The tumor microenvironment alters myeloid cells and can convert them into potent immunosuppressive cells[43,44]. Lymphopenia caused by disease or treatment is frequent in oncology patients and affects their prognosis[45,46].

T cells, one of the primary arms of the adaptive immune response, are also affected in oncology patients. Cancer cells express various membrane and soluble T-cell inhibitory signals. For example, programmed cell death protein-ligand 1 linking to programmed cell death protein 1 on T cells results in decreased activation, proliferation, survival, and cytotoxicity[47]. The last discovery led to the development of checkpoint inhibitors, a breakthrough in immuno-oncology, which led to the 2018 Nobel Prize for Physiology or Medicine. Indoleamine 2,3-dioxygenase, a soluble enzyme physiologically expressed in many tissues, is overproduced in some cancers leading to tryptophan depletion in the tumor microenvironment. T cells, being highly sensitive to tryptophan deprivation, suffer significant functional impairment, promoting tumor growth[48]. An increased rate of CD4+CD25+ regulatory T cells with potent immunosuppressive properties in the peripheral blood of individuals with cancer diseases has been reported[49,50].

Additionally, regulatory B cells (Bregs) are a newly designated subset of B cells that play a central role in regulating immune responses associated with inflammation, autoimmunity, and cancer. Increased Bregs express immunosuppressive properties in gastric cancer through the secretion of anti-inflammatory molecules, such as IL-10, and facilitating the conversion of T cells to regulatory T cells[44,51,52]. Additionally, tumor progression is associated with the dysfunction of natural killer cells due to the combined action of tissue-specific and systemic factors[53]. All of these immune alterations in cancer patients contribute to the differences in immune response after vaccination, including after COVID-19 vaccine administration. Before the COVID-19 pandemic, we had an experience with influenza vaccine administration in patients with oncological diseases. Infectious complications resulting from bacterial, fungal, and viral (often due to reactivation of latent disease, primarily in patients with hematological malignancies) diseases are a severe cause of morbidity and mortality in cancer patients[54]. Oncology patients receiving chemotherapy are at increased risk of influenza virus infection and serious post-influenza complications. Cancer patients are eligible for influenza vaccination, although their response may be suboptimal due to immunosuppression associated with cancer itself and/or its treatment [55,56]. Data have shown that cancer patients receiving chemotherapy can respond to influenza vaccination[57].

Breast cancer patients receiving influenza vaccination during FEC (5-fluorouracil, epirubicin, and cyclophosphamide)-containing treatment regimens have exhibited significantly lower responses to influenza virus vaccination than healthy controls. Vaccination early during the chemotherapy cycle (day 4) induces better responses than vaccination on day 16 [58]. The summary of the available evidence reveals that immunization of individuals with malignancies is critical to their care and may protect them from significant morbidity and mortality associated with vaccine-preventable diseases[59].

COVID-19 VACCINES FOR PATIENTS WITH ONCOLOGICAL DISEASES-DATA ON OUTCOMES AND EFFECTIVENESS

Several available COVID-19 vaccines are now in use all over the world. Moderate or severely immunocompromised people should receive a vaccination to protect them from severe COVID-19 disease[60,61].

The efficacy of COVID-19 vaccines in cancer patients is a question of continuous research, with most studies using immunological parameters as surrogate endpoints for clinical outcomes. Clinical trials investigating immune response after COVID-19 vaccination often use seroconversion to SARS-CoV-2 spike (S) protein as an endpoint for vaccine efficacy. Other parameters such as anti-spike antibody titers, detection of neutralizing antibodies, and cellular immune response are usually explored as secondary endpoints[62]. Some authors, however, underscore the role of neutralizing antibodies as the immunological parameter, which probably best correlates with the level of protection after COVID-19 vaccination [63-65].

Both humoral and cellular immune responses to COVID-19 vaccines differ in patients with malignancies compared to noncancer patients; this is not only attributed to the immunosuppressive nature of the oncologic disease but also to the antitumor therapy itself and its direct impact on immune cells. While patients with solid tumors have seroconversion rates similar to the general population, the most significant concern regarding post-vaccination and post-infectious COVID-19 immunity lies with hematological malignancies, especially those where lymphocyte-depletion therapy is used. In support of this is the research of Monin *et al*[66], who presented interim results of a prospective observational study that explores the immunogenicity of one compared to receiving two doses of the COVID-19 vaccine in patients with cancer by assessing the humoral immune response between 151 patients (95 with solid tumors and 56 with hematological malignancies) and 54 healthy controls. Authors reported efficacy after the first dose in 94%, 38%, and 18% of control subjects, patients with solid tumors and hematological cancer, respectively. After the second dose, the response increased to 100% in controls, 95% in patients with solid cancers, and only 60% in the group with hematological malignancies[66].

When considering post-vaccination immunity in patients with cancer, we should consider that those with hematological malignancies are expected to show different levels of antibody response to COVID-19 vaccines compared to patients with solid tumors. One of the most substantial pieces of evidence in corroboration came from the CAPTURE trial [67]. This prospective clinical study assessed the humoral response after COVID-19 vaccination in more than 700 subjects with solid tumors or hematologic neoplasms, 585 of whom did not have previous SARS-CoV-2 infection. The trial demonstrated 85% and 54% seroconversion rates for anti-spike antibodies after the second dose in patients with solid tumors and hematological malignancies, respectively. However, the response observed among participants was not the same for all SARS-CoV-2 variants[68].

The authors announced substantial differences in neutralizing antibodies concerning viral genotypes from the CAPTURE trial: 83% of patients developed detectable levels of the original SARS-CoV-2 and only 54% of the delta variant. And while nearly two-thirds (62%) of patients with solid tumors elicit humoral response against delta variant, only 31% of those with hematologic malignancies did so[67]. The prospective cohort study of immune response to COVID-19 vaccination in cancer patients CAPTURE (NCT03226886) also showed that among 585 patients, the antibody rates after two doses of BNT162b2 or AZD1222 vaccines given over 12 wk were assessed. The results showed that seroconversion was 85% and 59% after two doses in patients with solid and hematological malignancies, respectively. Neutralizing antibodies against SARS-CoV-2 VOCs were detected in a small proportion of patients, mainly with solid cancers. Vaccine-induced T-cell responses were found in 80% of patients regardless of the vaccine or type of cancer[67].

In an attempt to overcome this relatively low rate of seroconversion in patients with blood cancers, Greenberger *et al* [69] conducted a large prospective cohort trial on nearly 700 patients vaccinated with three doses of the COVID-19 vaccine. It was estimated that antibody response indeed increased with the 3rd (booster) dose, so 43% of those without detectable antibodies after the 2nd dose demonstrated humoral response after the booster. However, about 20% of all hematological patients still failed to achieve a response even with 3 doses of vaccine[69]. In contrast to the plethora of research on humoral immunity after COVID-19 vaccination in cancer patients, the cellular immune response in this setting is considerably less studied. In a review article by Rüthrich *et al*[70], the authors tried to summarize what is currently known about the issue in patients with solid tumors and hematological malignancies, comparing data from COVID-19 vaccines and other "classical" vaccines. Although the assessment of T-cell immune response in the reviewed studies varied, most research used methods based on quantifying and characterizing pathogen-specific T cells and/or estimating T-cell function by cytokine measurement[70].

Observations on immune responses in patients with hematological malignancies revealed that although this population may lack adequate levels of neutralizing viral antibodies, especially after treatment with B cell-depleting agents such as anti-cluster of differentiation 20 monoclonal antibodies, COVID-19 vaccines are still able to produce protective cellular immunity. Solid evidence for the sufficient efficacy of T-cell response comes from a trial in patients with agammaglobulinemia who demonstrated improved COVID-19 infection outcomes after vaccination. However, cellular immunity could also be impaired in this specific patient population, and some of the significant factors for this are age, disease activity, immunosuppressive treatment, and low lymphocyte counts in circulation[70].

This discordance between humoral and cellular immune response could also be seen In patients with solid tumors. In this population, T-cell responses vary among different cancer subtypes and are determined mainly by the type of systemic antitumor treatment. Various studies have demonstrated wide ranges in terms of cellular immunity achieved after COVID-19 vaccination ranging from about 50% to nearly 90% of the vaccinated cases[71,72].

However, despite being generally higher than those observed in blood cancer patients, T-cell response in those with solid tumors remains significantly lower than in healthy controls. One of the most extensive trials reporting data on immune response in patients with solid tumors receiving systemic anticancer treatment is the VOICE study[73]. After recruiting nearly 800 subjects (240 without cancer), the authors assessed cellular immunity by measuring the SARS-CoV-2

spike-specific interferon gamma T-cell response after two vaccine doses. They reported cellular responses in 67%, 66%, and 53% of patients treated with chemotherapy, immunotherapy, or chemoimmunotherapy, respectively. Another interesting trial finding was that more than 40% of patients who did not elicit a humoral immune response could develop a T-cell response, highlighting the vaccine's 'double-edge sword' efficacy in this specific population. Similar to the model observed with the humoral response, whether the cellular response is affected by a booster dose is still an open question since there are conflicting data. Some studies have reported significant enhancement of the T-cell response after the 3rd dose, whereas others refute such assertions[73].

To date, most trials reporting COVID-19 vaccine efficacy in cancer rely on immunological endpoints and not so much on clinical outcomes. However, a recent study on infection rate and outcomes in vaccinated patients with solid tumors and hematologic malignancies has raised concern that despite vaccination, these patients remain at risk of worse outcomes compared to the general population[74]. Among fully vaccinated cancer patients, who experienced breakthrough SARS-CoV-2 infection, the hospitalization rate, intensive care unit admission (or required mechanical vaccination), and death rate are 65%, 19%, and 13%, respectively. This is mainly attributed to patients' comorbidities and the much worse COVID-19 prognosis in those with hematological malignancies.

In a prospective study conducted by Goshen-Lago *et al*[75], it was shown that patients with solid tumors demonstrated short-term efficacy and safety of the BNT162b2 vaccine. A follow-up study evaluated these outcomes at 6 mo after vaccination[76]. Participants were 154 patients with solid tumors and 135 controls (health workers). At 6 mo after vaccination, 122 patients were seropositive compared with 114 controls, and the serologic titers dramatically decreased almost equally in both cohorts. Efficacy and safety evidence of BNT162b2 vaccines shows that the serological profile in cancer patients after 6 mo resembles that of the general population[76].

A similar study was conducted by Barrière *et al*[77], who evaluated the immunogenicity of the BNT162b2 vaccine in patients with solid tumors. Serological analyses were performed during the first vaccination, during the booster dose (w3-w4), and 3-4 wk after the booster dose (w6-w8). The study reported the results for 122 of 194 evaluable patients with solid tumors who had at least two doses from January 2021 to March 2021. In the first analysis (w3-w4), 58 patients had neutralizing antibodies, although the median levels were significantly lower than in the control group. In the following analysis (w6-w8), the data showed the same anti-S seroconversion rate, demonstrating impaired immunogenicity of the BNT162b2 vaccine in cancer patients[77].

Shroff *et al*[78] also compared anti-S seroconversion to the BNT162b2 mRNA vaccine in patients with solid tumors on active cytotoxic anticancer therapy with healthy control participants. Neutralizing antibodies were found in 67% of cancer patients after the first immunization, and a follow-up analysis found a threefold increase in titers after the second or third doses. European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number 2021-000291-11 was conducted in patients with solid cancers, multiple myeloma, and inflammatory bowel disease[79]. The study is a prospective, open-label, phase four trial to monitor vaccine-specific antibody and cellular responses after booster vaccination with mRNA-1273 or BNT162b2. The data show that booster vaccination against SARS-CoV-2 reverses the lack of response and early antibody weakening in immunocompromised patients.

Another study on the efficacy and safety of heterologous booster vaccination with Ad26.COV2.S after BNT162b2 mRNA vaccine in cancer patients without antibody response was conducted in 2022[80]. The assessment was done directly before vaccination and 4 wk after. Ad26.COV2.S booster vaccination resulted in a serological response in 31% of nonresponders after a double dose of BNT162b2. Clinical trials with the number NCT04368728 reported results from individuals with a history of past or active neoplasms and up to 6 mo of follow-up after dose 2 of a placebo-controlled, observer-blinded trial of the BNT162b2 vaccine[81]. In participants with past or active neoplasms, two doses of the BNT162b2 vaccine improved efficacy and safety profile as in the overall trial population. No vaccine-related deaths were reported.

One of the first evaluations of the effectiveness of vaccination against breakthrough SARS-CoV-2 infections in cancer patients at a population level was done by Lee *et al*[82]. Analysis was performed in the cancer cohort by vaccine type (BNT162b2, ChAdOx1 nCov-19, or mixed, and other), cancer type and subtype, stage, date of cancer diagnosis, and anticancer treatment or radiotherapy. Data show that vaccination with different COVID-19 vaccines is effective in people with cancer, providing varying levels of protection against SARS-CoV-2 infection. However, it is lower in cancer patients than in the general population[82].

A single-arm prospective clinical trial was conducted with 106 cancer patients by Thakkar *et al*[83]. They received two doses of mRNA followed by one dose of AD26.CoV2.S vaccine or a third dose of mRNA vaccine. The results showed that a third dose induced immunity in cancer patients. Seroconversion was also assessed in 57% of patients who did not respond to primary vaccination. A fourth dose boosted the immune response by two-thirds. Some patients have neutralizing activity against the omicron variant[83].

In conclusion, all of these studies confirm that people with cancer are at increased risk of severe COVID-19 disease, hospitalization, and death after SARS-CoV-2 infection compared to the general population. The above data show that cancer patients have impaired overall vaccine effectiveness to the approved COVID-19 vaccines. Seroconversion in them decreases faster than in the control population. Although vaccination provides different levels of protection, there should be a global prioritization of the programs to boost vaccination for cancer people, considering the impact of other treatments.

There are still a lack of data on vaccine efficacy in cancer patients concerning novel virus variants like omicron[68]. Table 1 presents the studies on the effectiveness and safety of COVID-19 vaccination with different approved COVID-19 vaccines in oncological patients with solid tumors[5,63,66,67,69-71,73,75-84,84].

Table 1 Some of the more significant studies conducted on the efficacy and safety of COVID-19 vaccination with different approved COVID-19 vaccines in oncological patients with solid tumors

Ref.	Type of vaccine	Type of study	Subjects (diagnosis, other specific characteristics)	Data on efficacy	Data on safety (main side effects)
OnCovid study group [5]	NA	A multicenter observational registry-based study	All PTs included n = 2634 (100%); PTs with advanced tumor stage n = 1244 (46%); PTs with receipt of anti-cancer therapy within 4 wk of COVID-19 diagnosis n = 1305 (51.8%); malignancy type: Breast n = 493 (18.9%); gastrointestinal n = 476 (18.2%); gynecologic/genitourinary n = 530 (20.3%); hematologic n = 357 (13.7%)	The difference in the necessity of hospitalization due to COVID-19, oxygen therapy requirement, mechanical ventilation requirement, and 14-d CFR between PTs stratified across time five phases and two major outbreaks of the pandemic; hospitalization requirement: 1st phase-64.7% to 5th phase-42.7% (P < 0.01); proportion to PTs requiring oxygen: therapy, phase 1-62.6%, to phase 5-46.0% (P < 0.001); mechanical ventilation: Phase 1-12.1% to phase 5-11.8% (P = 0.01); CFR: 1st outbreak-25.6% to-2nd outbreak 16.2% (P < 0.001)	N/A
Khoury *et al* [63], 2021	mRNA and adenoviral vector vaccines			20.2% of subjects had (95%CI) 50% protective neutralization level	N/A
Monin *et al* [66], 2021	mRNA	Prospective observational study	PTs with oncologic disease n = 151: With solid cancer n = 95; with hematological malignancy n = 56; and HCs n = 54	Surrogate marker of efficiency: Seroconversion after 1st dose: 32 of 34 (94%) HCs, 21 of 56 (38%), PTs with solid cancer, 8 of 44 (18%) PTs with hematologic malignancies; after 2nd dose: 12 of 12 (100%) HCs; 18 of 19 (95%) PTs with solid, 3 of 5 (60%) PTs with hematologic malignancies	AE: Injection site pain within 7 d following the first dose in: 23 of 65 (35%) PTs with cancer; 12 of 25 (48%) HCs; no vaccine-related deaths were reported
Greenberger *et al*[69], 2021	mRNA and adenoviral vector vaccines	Retrospective cohort study	PTs with hematologic malignancies, n = 3300		
Ehmsen *et al* [71], 2021	mRNA	Prospective cohort study (comparison between groups with different malignancies; no HCs)	PTs with cancer, n = 524, of whom: 201 (38%) with solid cancer; 323 (62%) with hematologic cancer; 524 (100%) had a blood sample drawn at a median of 36 d after the second dose of vaccine; and 247 (47%) had a second blood sample drawn 3 mo after the second dose of the vaccine	Seropositivity rate for anti-S IgG 36 d after vaccination: PTs with solid cancer 187 of 201 (93%); PTs with hematologic cancer 215 of 323 (66%); seropositivity rate for anti-S IgG 3 mo after vaccination: PTs with solid cancer-86%, PTs with hematologic cancer-53%; anti-S IgG titers; between 36-d and 3-mo samples declined from a median of 429 BAU/mL to a median of 139 BAU/mL (P = 0.03, Student's t-test); T-cell reactivity: PTs with solid cancer-92 (46%), 70 (76%) mounted both CD4+ and CD8+ T-cell responses, 21 (23%) elicited only a CD8+ T-cell response, PTs with hematologic cancer-144 (45%), 81% were positive for both CD4+ and CD8+ T cells, 26 (18%) only elicited a CD8+ T cell response, 76% of the seronegative PTs did not elicit a T-cell response; PTs with solid cancer: only 1 of the 14 (7%) seronegative PTs elicited a T-cell response; PTs with hematologic cancer: 28 of 108 (26%) PTs elicited a T-cell response	N/A
Oosting *et al* [73], 2021	mRNA	Prospective, multicenter, non-inferiority trial	Cohort A: Individuals without cancer (control cohort); cohort B: PTs with SOTs, regardless of stage and histology, treated with immunotherapy; cohort C: PTs treated with chemotherapy; and cohort D: PTs treated with chemoimmunotherapy	Presence of SARS-CoV-2-binding antibodies after the second vaccination; at 28th d, 6 mo after 12 mo after a spike-specific T-cell response was defined as a two times or more significant increase in the number of spot-forming cells	N/A
Polack *et al* [84], 2020	mRNA vaccines	Placebo-controlled, observer-blinded, pivotal efficacy trial (randomized 1:1 vaccine *vs*	All PTs included n = 43548; PTs with liver disease n = 217 (0.6%)	95% efficacy (9 vaccinated *vs* 169 controls with COVID-19); 10 cases of severe COVID-19 infection *vs* 9 in the placebo group; flares: NR	Systemic AEs: (1) Fatigue (34%-51%); (2) headache (25%-39%); (3) fever (11%), injection site reactions; (4)

		placebo)			pain (71%-83%); (5) redness and swelling (< 7%); and (6) serious AE < 4%
Fendler *et al* [67], 2021	BNT162b2 or AZD1222 vaccines (CAPTURE, NCT03226886)	Prospective cohort study	585 PTs, the seroconversion rates after two doses of BNT162b2 or AZD1222 vaccines given over 12 wk were assessed	After two doses of BNT162b2 or AZD1222 vaccines given over 12 wk, seroconversion was 85% and 59% in PTs with solid and hematological malignancies, respectively; vaccine-induced T-cell responses were found in 80% of PTs regardless of the vaccine or type of cancer	N/A
Goshen-Lago *et al* [75], 2021	BNT162b2 vaccine	Prospective study	154 PTs with SOTs and 135 HCs (health workers)	In PTs with cancer with active intravenous treatment, 79% (n = 122) of the PTs had positive serologic test results, compared with 84% (n = 114) in the control group; analysis by age, sex, or disease stage has no significant differences within the PT cohort; 15% of the seropositive PTs became seronegative after 6 mo, comparable to the control group	N/A
Waldhorn *et al*[76], 2021	BNT162b2 vaccine	Prospective study	154 PTs with SOTs and 135 controls	6 mo postvaccination, 79% of PTs and 84% of HCs were seropositive (P = 0.32); dramatically decreased serology titer	
Shroff *et al* [78], 2021	BNT162b2	Phase 1 cohort trial	53 PTs with SOTs on active cytotoxic anticancer therapy and 50 healthy cohort	Neutralizing antibodies were detected in 67% of PTs with cancer after the first immunization, followed by a threefold increase in median titers after the second dose	AEs were mild: temperature, fever, headache, redness, and swelling on the injection site
Barrière *et al* [77], 2021	BNT162b2	VMO for vaccinated PTs under active treatment in the Department of Oncology of the Saint Jean Polyclinic, Nice, France	194 evaluable PTs with SOTs and 31 HCs	58 PTs had neutralizing antibodies, although the median levels were significantly lower than those in the control group; the data demonstrating impaired immunogenicity of the BNT162b2 vaccine in immunocompromised PTs; % of efficacy was not reported	N/A
Thomas *et al* [81], 2022	BNT162b2 mRNA	Phase 3 randomized clinical trial	3813 participants had a history of neoplasm: Most common malignancies were breast (n = 460), prostate (n = 362), and melanoma (n = 223)	Vaccine efficacy was 94.4% (95%CI) after up to 6 mo of follow-up post-dose 2	N/A
Wagner *et al* [79], 2022	mRNA-1273 or BNT162b2	Prospective, open-label, phase four trail	263 PTs with SOT, n = 63), MM, n = 70, IBD, n = 130 and 66 controls	1 mo after the two-dose primary vaccination, the highest nonresponder rate was found in MM PTs (17%); 6 mo after the second dose, 18% of PTs with MM, 10% with SOT, and 4% with IBD became seronegative compared to the control group; the vaccination with mRNA-1273 led to higher antibody levels than with BNT162b2; booster vaccination increased antibody levels 8-fold in seropositive individuals and induced responses in those with undetectable pre-booster antibody levels	N/A
Lee *et al*[82], 2022	BNT162b2, ChAdOx1 nCov-19, or mixed and other	Population-based test-negative case-control study	Cancer cohort comprised 377194 individuals, of whom 42882 had breakthrough SARS-CoV-2 infections; the control population consisted of 28010955 individuals, of whom 5748708 had SARS-CoV-2 breakthrough infections	Overall vaccine effectiveness was 69.8% in the control population and 65.5% in the cancer cohort; vaccine effectiveness at 3-6 mo was lower in the cancer cohort (47.0%) than in the control population (61.4%)	N/A
Reimann *et al*[80], 2022	Ad26.COV2.S after BNT162b2 mRNA		32 oncological nonresponders to double-dose BNT162b2	The overall response rate was 31%	Mainly mild local and systemic reactions
Thakkar *et al* [83], 2023	Two doses of mRNA or one dose of AD26.CoV2.S vaccine and administered a	Single-arm prospective clinical trial	cancer PTs	A third dose of the COVID-19 vaccine induces durable immunity in cancer PTs, leading to seroconversion in 57% of PTs who did not respond to primary vaccination; 18 PTs with blood cancer and severe immune suppression had no response after three	N/A

	third dose of mRNA vaccine	doses; and the fourth dose boosted the immune response by 2/3 of PTs, with neutralizing activity against the omicron variant

AE: Adverse event; CI: Confidence interval; CFR: Circulating free RNA; COVID 19: Coronavirus disease 2019; HCs; Healthy controls; IBD: Inflammatory bowel disease; MM: Multiple myeloma; NA: Not available; N/A: Not applicable; PT: Patient; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SOT: Solid tumor; VMO: Vaccine monitoring observatory.

COVID-19 VACCINES AND CHEMOTHERAPY INTERACTIONS

People with cancer often have an increased susceptibility to infections due to various factors, including cancer itself and/or, in some cases, the applied therapy, poor nutrition, and damaged physiological barriers. In addition, the incidence of neoplasia is highest in individuals aged 65 and over. When the immune system's effectiveness is weakened, the elderly often have concomitant diseases for which they can also take medications[54,85].

Regarding cancer chemotherapy, conventional antitumor chemotherapeutic agents kill actively proliferating cells, including bone marrow cells, and myelosuppression is one of clinical oncology's most common side effects[86]. Chemotherapy-induced neutropenia is a significant cause of hematological and dose-limiting toxicities of chemotherapy [87]. Some currently available anticancer drugs, such as methotrexate and cyclophosphamide, express immunosuppressive effects and impair peripheral T cells' proliferative and/or effector functions. Methotrexate is an antimetabolite of the antifolate type developed in 1947 and is included in the World Health Organization's List of Essential Medicines. Currently, it is widely used not only in clinical oncology (in the treatment of acute lymphoblastic leukemia, acute myeloid leukemia, meningeal leukemia and lymphoma, osteosarcomas, non-Hodgkin's lymphoma, breast and bladder cancers, *etc*.) but also as a first-line treatment in autoimmune, inflammatory diseases such as rheumatoid arthritis, psoriasis and Crone's disease[88-90]. Methotrexate has been found to disturb antibody response after pneumococcal vaccination[91,92]; the drug reduces circulating T helper 17 (Th17) cells and impairs plasmablast and memory B-cell expansion following pneumococcal conjugate immunization in patients with rheumatoid arthritis[93].

Cyclophosphamide is an alkylating agent synthesized in 1958 and used for decades in clinical practice in the therapy regimens of neoplasms (malignant lymphomas, multiple myeloma, sarcoma, breast cancer, disseminated neuroblastomas, retinoblastoma, ovarian adenocarcinoma, *etc*) and as an immunosuppressive agent for the treatment of autoimmune and immune-mediated diseases such as multiple sclerosis. Cyclophosphamide shows selectivity for T cells and is an immunosuppressant to prevent transplant rejection and graft-*vs*-host complications[94]. Cyclophosphamide has been associated with suppressing helper Th1 activity and enhancing Th2 responses[95]. This drug inhibits Th1/Th17 responses and increases the cells secreting anti-inflammatory cytokines such as IL-4, IL-10, and transforming growth factor beta [96]. A single administration of low-dose cyclophosphamide selectively suppresses regulatory T cells. The low-dose cyclophosphamide promotes antitumor immunity by selectively depleting regulatory T cells and enhancing effector T cell function. However, cyclophosphamide can also increase the number of myeloid-derived suppressor cells[97,98].

Treatment with tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib applied in the treatment of chronic myeloid leukemia is associated with loss of memory B-cell subsets and impaired humoral immune responses to 23-valent polysaccharide pneumococcal vaccine, likely due to the off-target kinase inhibitory activity of these drugs[99].

CONCLUSION

Data so far show that patients with cancer are at increased risk of severe COVID-19 and developing various complications mainly due to their immunocompromised state, type of treatment and comorbidities. Although cancer patients were excluded from vaccine trials, the investigations of post-vaccinal immune responses and the effectiveness of the vaccines showed that both humoral and cellular immune responses to COVID-19 vaccines differ in patients with malignancies compared to noncancer patients, and this is being attributed not only to the immunosuppressive nature of the oncologic disease but to the antitumor therapy itself and its direct impact on immune cells.

The evidence indicates that the efficacy of vaccinations could be impaired in cancer patients in line with a reduced rate of seroconversion and shorter duration compared to healthy controls. Despite these data, when focusing on the clinical outcomes instead of immunological endpoints regarding vaccine efficacy, COVID-19 vaccines demonstrated high effectiveness in preventing severe COVID-19 and infection-related death, and safety profile with comparable to healthy controls adverse effects in patients with solid tumors and hematological malignancies.

Despite the considerably higher mortality in the cancer patients group from COVID-19 than the general population, countries with high vaccination rates have demonstrated trends toward improved survival of cancer patients early and late in the pandemic. Nevertheless, vaccination of these patients and overall vaccination of the population has proven to significantly reduce the risk of complications and mortality of COVID-19 and should be promoted worldwide.

FOOTNOTES

Author contributions: Ivanov N and Velikova T contributed to conceptualization; Krastev B contributed to methodology; Miteva DG contributed to software; Ivanov N, Alexandrova R, and Velikova T contributed to validation; Ivanov N contributed to formal analysis; Krastev B contributed to investigation; Batselova H contributed to resources; Miteva DG contributed to data curation; Ivanov N, Krastev B, and Miteva DG contributed to writing-original draft preparation; Batselova H contributed to writing-review & editing; Miteva DG contributed to visualization; Velikova T contributed to supervision, project administration, and funding acquisition; and all authors revised and approved the final version of the manuscript.

Supported by the European Union-Next Generation EU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, No. BG-RRP-2.004-0008.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Bulgaria

ORCID number: Nedelcho Ivanov 0000-0002-7471-7368; Boris Krastev 0000-0003-4196-0828; Dimitrina Georgieva Miteva 0000-0002-5931-2426; Hristiana Batselova 0000-0002-6201-848X; Radostina Alexandrova 0000-0002-7699-6479; Tsvetelina Velikova 0000-0002-0593-1272.

S-Editor: Chen YL
L-Editor: Filipodia
P-Editor: Chen YL

REFERENCES

1 **Yu J**, Ouyang W, Chua MLK, Xie C. SARS-CoV-2 Transmission in Patients With Cancer at a Tertiary Care Hospital in Wuhan, China. *JAMA Oncol* 2020; **6**: 1108-1110 [PMID: 32211820 DOI: 10.1001/jamaoncol.2020.0980]

2 **Lewis MA**. Between Scylla and Charybdis-Oncologic Decision Making in the Time of Covid-19. *N Engl J Med* 2020; **382**: 2285-2287 [PMID: 32267650 DOI: 10.1056/NEJMp2006588]

3 **ElGohary GM**, Hashmi S, Styczynski J, Kharfan-Dabaja MA, Alblooshi RM, de la Cámara R, Mohmed S, Alshaibani A, Cesaro S, Abd El-Aziz N, Almaghrabi R, Gergis U, Majhail NS, El-Gohary Y, Chemaly RF, Aljurf M, El Fakih R. The Risk and Prognosis of COVID-19 Infection in Cancer Patients: A Systematic Review and Meta-Analysis. *Hematol Oncol Stem Cell Ther* 2022; **15**: 45-53 [PMID: 32745466 DOI: 10.1016/j.hemonc.2020.07.005]

4 **Vijenthira A**, Gong IY, Fox TA, Booth S, Cook G, Fattizzo B, Martín-Moro F, Razanamahery J, Riches JC, Zwicker J, Patell R, Vekemans MC, Scarfò L, Chatzikonstantinou T, Yildiz H, Lattenist R, Mantzaris I, Wood WA, Hicks LK. Outcomes of patients with hematologic malignancies and COVID-19: a systematic review and meta-analysis of 3377 patients. *Blood* 2020; **136**: 2881-2892 [PMID: 33113551 DOI: 10.1182/blood.2020008824]

5 **OnCovid Study Group**, Pinato DJ, Patel M, Scotti L, Colomba E, Dolly S, Loizidou A, Chester J, Mukherjee U, Zambelli A, Dalla Pria A, Aguilar-Company J, Bower M, Salazar R, Bertuzzi A, Brunet J, Lambertini M, Tagliamento M, Pous A, Sita-Lumsden A, Srikandarajah K, Colomba J, Pommeret F, Seguí E, Generali D, Grisanti S, Pedrazzoli P, Rizzo G, Libertini M, Moss C, Evans JS, Russell B, Harbeck N, Vincenzi B, Biello F, Bertulli R, Ottaviani D, Liñan R, Rossi S, Carmona-García MC, Tondini C, Fox L, Baggi A, Fotia V, Parisi A, Porzio G, Queirolo P, Cruz CA, Saoudi-Gonzalez N, Felip E, Roqué Lloveras A, Newsom-Davis T, Sharkey R, Roldán E, Reyes R, Zoratto F, Earnshaw I, Ferrante D, Marco-Hernández J, Ruiz-Camps I, Gaidano G, Patriarca A, Bruna R, Sureda A, Martinez-Vila C, Sanchez de Torre A, Berardi R, Giusti R, Mazzoni F, Guida A, Rimassa L, Chiudinelli L, Franchi M, Krengli M, Santoro A, Prat A, Tabernero J, Van Hemelrijck M, Diamantis N, Gennari A, Cortellini A. Time-Dependent COVID-19 Mortality in Patients With Cancer: An Updated Analysis of the OnCovid Registry. *JAMA Oncol* 2022; **8**: 114-122 [PMID: 34817562 DOI: 10.1001/jamaoncol.2021.6199]

6 **Chen L**, Qu J, Kalyani FS, Zhang Q, Fan L, Fang Y, Li Y, Xiang C. Mesenchymal stem cell-based treatments for COVID-19: status and future perspectives for clinical applications. *Cell Mol Life Sci* 2022; **79**: 142 [PMID: 35187617 DOI: 10.1007/s00018-021-04096-y]

7 **Yuan Y**, Jiao B, Qu L, Yang D, Liu R. The development of COVID-19 treatment. *Front Immunol* 2023; **14**: 1125246 [PMID: 36776881 DOI: 10.3389/fimmu.2023.1125246]

8 **Gasparyan AY**, Ayvazyan L, Blackmore H, Kitas GD. Writing a narrative biomedical review: considerations for authors, peer reviewers, and editors. *Rheumatol Int* 2011; **31**: 1409-1417 [PMID: 21800117 DOI: 10.1007/s00296-011-1999-3]

9 **Li H**, Baldwin E, Zhang X, Kenost C, Luo W, Calhoun EA, An L, Bennett CL, Lussier YA. Comparison and impact of COVID-19 for patients with cancer: a survival analysis of fatality rate controlling for age, sex and cancer type. *BMJ Health Care Inform* 2021; **28** [PMID: 33980502 DOI: 10.1136/bmjhci-2021-100341]

10 **He W**, Chen L, Yuan G, Fang Y, Chen W, Wu D, Liang B, Lu X, Ma Y, Li L, Wang H, Chen Z, Li Q, Gale RP. COVID-19 in persons with haematological cancers. *Leukemia* 2020; **34**: 1637-1645 [PMID: 32332856 DOI: 10.1038/s41375-020-0836-7]

11 **Zhang H**, Han H, He T, Labbe KE, Hernandez AV, Chen H, Velcheti V, Stebbing J, Wong KK. Clinical Characteristics and Outcomes of COVID-19-Infected Cancer Patients: A Systematic Review and Meta-Analysis. *J Natl Cancer Inst* 2021; **113**: 371-380 [PMID: 33136163 DOI: 10.1093/jnci/djaa168]

12 **Aries JA**, Davies JK, Auer RL, Hallam SL, Montoto S, Smith M, Sevillano B, Foggo V, Wrench B, Zegocki K, Agrawal S, Le Dieu R, Truelove E, Erblich T, Araf S, Okosun J, Oakervee H, Cavenagh JD, Gribben JG, Riches JC. Clinical outcome of coronavirus disease 2019 in

haemato-oncology patients. *Br J Haematol* 2020; **190**: e64-e67 [PMID: 32420609 DOI: 10.1111/bjh.16852]

13 **Tsamakis K**, Gavriatopoulou M, Schizas D, Stravodimou A, Mougkou A, Tsiptsios D, Sioulas V, Spartalis E, Sioulas AD, Tsamakis C, Charalampakis N, Mueller C, Arya D, Zarogoulidis P, Spandidos DA, Dimopoulos MA, Papageorgiou C, Rizos E. Oncology during the COVID-19 pandemic: challenges, dilemmas and the psychosocial impact on cancer patients. *Oncol Lett* 2020; **20**: 441-447 [PMID: 32565968 DOI: 10.3892/ol.2020.11599]

14 **Iloanusi S**, Mgbere O, Essien EJ. Polypharmacy among COVID-19 patients: A systematic review. *J Am Pharm Assoc (2003)* 2021; **61**: e14-e25 [PMID: 34120855 DOI: 10.1016/j.japh.2021.05.006]

15 **Yang F**, Shi S, Zhu J, Shi J, Dai K, Chen X. Clinical characteristics and outcomes of cancer patients with COVID-19. *J Med Virol* 2020; **92**: 2067-2073 [PMID: 32369209 DOI: 10.1002/jmv.25972]

16 **Afshar ZM**, Dayani M, Naderi M, Ghanbarveisi F, Shiri S, Rajati F. Fatality rate of COVID-19 in patients with malignancies: a sytematic review and meta-analysis. *J Infect* 2020; **81**: e114-e116 [PMID: 32474042 DOI: 10.1016/j.jinf.2020.05.062]

17 **Brar G**, Pinheiro LC, Shusterman M, Swed B, Reshetnyak E, Soroka O, Chen F, Yamshon S, Vaughn J, Martin P, Paul D, Hidalgo M, Shah MA. COVID-19 Severity and Outcomes in Patients With Cancer: A Matched Cohort Study. *J Clin Oncol* 2020; **38**: 3914-3924 [PMID: 32986528 DOI: 10.1200/JCO.20.01580]

18 **Onder G**, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. *JAMA* 2020; **323**: 1775-1776 [PMID: 32203977 DOI: 10.1001/jama.2020.4683]

19 **Guven DC**, Aktas BY, Aksun MS, Ucgul E, Sahin TK, Yildirim HC, Guner G, Kertmen N, Dizdar O, Kilickap S, Aksoy S, Yalcin S, Turker A, Uckun FM, Arik Z. COVID-19 pandemic: changes in cancer admissions. *BMJ Support Palliat Care* 2020 [PMID: 32665259 DOI: 10.1136/bmjspcare-2020-002468]

20 **Skovlund CW**, Friis S, Christensen J, Nilbert MC, Mørch LS. Drop in cancer diagnosis during the COVID-19 pandemic in Denmark: assessment of impact during 2020. *Acta Oncol* 2022; **61**: 658-661 [PMID: 35020549 DOI: 10.1080/0284186X.2021.2024879]

21 **Vázquez Rosas T**, Cazap E, Delgado L, Ismael J, Bejarano S, Castro C, Castro H, Müller B, Gutiérrez-Delgado F, Santini LA, Vallejos Sologuren C. Social Distancing and Economic Crisis During COVID-19 Pandemic Reduced Cancer Control in Latin America and Will Result in Increased Late-Stage Diagnoses and Expense. *JCO Glob Oncol* 2021; **7**: 694-703 [PMID: 33999696 DOI: 10.1200/GO.21.00016]

22 **Yong JH**, Mainprize JG, Yaffe MJ, Ruan Y, Poirier AE, Coldman A, Nadeau C, Iragorri N, Hilsden RJ, Brenner DR. The impact of episodic screening interruption: COVID-19 and population-based cancer screening in Canada. *J Med Screen* 2021; **28**: 100-107 [PMID: 33241760 DOI: 10.1177/0969141320974711]

23 **Salunke AA**, Nandy K, Pathak SK, Shah J, Kamani M, Kottakota V, Thivari P, Pandey A, Patel K, Rathod P, Bhatt S, Dave P, Pandya S. Impact of COVID -19 in cancer patients on severity of disease and fatal outcomes: A systematic review and meta-analysis. *Diabetes Metab Syndr* 2020; **14**: 1431-1437 [PMID: 32755847 DOI: 10.1016/j.dsx.2020.07.037]

24 **Wang D**, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020; **323**: 1061-1069 [PMID: 32031570 DOI: 10.1001/jama.2020.1585]

25 **Grasselli G**, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, Cereda D, Coluccello A, Foti G, Fumagalli R, Iotti G, Latronico N, Lorini L, Merler S, Natalini G, Piatti A, Ranieri MV, Scandroglio AM, Storti E, Cecconi M, Pesenti A; COVID-19 Lombardy ICU Network. Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. *JAMA* 2020; **323**: 1574-1581 [PMID: 32250385 DOI: 10.1001/jama.2020.5394]

26 **Richardson S**, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW; the Northwell COVID-19 Research Consortium, Barnaby DP, Becker LB, Chelico JD, Cohen SL, Cookingham J, Coppa K, Diefenbach MA, Dominello AJ, Duer-Hefele J, Falzon L, Gitlin J, Hajizadeh N, Harvin TG, Hirschwerk DA, Kim EJ, Kozel ZM, Marrast LM, Mogavero JN, Osorio GA, Qiu M, Zanos TP. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* 2020; **323**: 2052-2059 [PMID: 32320003 DOI: 10.1001/jama.2020.6775]

27 **Kuderer NM**, Choueiri TK, Shah DP, Shyr Y, Rubinstein SM, Rivera DR, Shete S, Hsu CY, Desai A, de Lima Lopes G Jr, Grivas P, Painter CA, Peters S, Thompson MA, Bakouny Z, Batist G, Bekaii-Saab T, Bilen MA, Bouganim N, Larroya MB, Castellano D, Del Prete SA, Doroshow DB, Egan PC, Elkrief A, Farmakiotis D, Flora D, Galsky MD, Glover MJ, Griffiths EA, Gulati AP, Gupta S, Hafez N, Halfdanarson TR, Hawley JE, Hsu E, Kasi A, Khaki AR, Lemmon CA, Lewis C, Logan B, Masters T, McKay RR, Mesa RA, Morgans AK, Mulcahy MF, Panagiotou OA, Peddi P, Pennell NA, Reynolds K, Rosen LR, Rosovsky R, Salazar M, Schmidt A, Shah SA, Shaya JA, Steinharter J, Stockerl-Goldstein KE, Subbiah S, Vinh DC, Wehbe FH, Weissmann LB, Wu JT, Wulff-Burchfield E, Xie Z, Yeh A, Yu PP, Zhou AY, Zubiri L, Mishra S, Lyman GH, Rini BI, Warner JL; COVID-19 and Cancer Consortium. Clinical impact of COVID-19 on patients with cancer (CCC19): a cohort study. *Lancet* 2020; **395**: 1907-1918 [PMID: 32473681 DOI: 10.1016/S0140-6736(20)31187-9]

28 **Jee J**, Foote MB, Lumish M, Stonestrom AJ, Wills B, Narendra V, Avutu V, Murciano-Goroff YR, Chan JE, Derkach A, Philip J, Belenkaya R, Kerpelev M, Maloy M, Watson A, Fong C, Janjigian Y, Diaz LA Jr, Bolton KL, Pessin MS. Chemotherapy and COVID-19 Outcomes in Patients With Cancer. *J Clin Oncol* 2020; **38**: 3538-3546 [PMID: 32795225 DOI: 10.1200/JCO.20.01307]

29 **Mukkada S**, Bhakta N, Chantada GL, Chen Y, Vedaraju Y, Faughnan L, Homsi MR, Muniz-Talavera H, Ranadive R, Metzger M, Friedrich P, Agulnik A, Jeha S, Lam C, Dalvi R, Hessissen L, Moreira DC, Santana VM, Sullivan M, Bouffet E, Caniza MA, Devidas M, Pritchard-Jones K, Rodriguez-Galindo C; Global Registry of COVID-19 in Childhood Cancer. Global characteristics and outcomes of SARS-CoV-2 infection in children and adolescents with cancer (GRCCC): a cohort study. *Lancet Oncol* 2021; **22**: 1416-1426 [PMID: 34454651 DOI: 10.1016/S1470-2045(21)00454-X]

30 **Vinay DS**, Ryan EP, Pawelec G, Talib WH, Stagg J, Elkord E, Lichtor T, Decker WK, Whelan RL, Kumara HMCS, Signori E, Honoki K, Georgakilas AG, Amin A, Helferich WG, Boosani CS, Guha G, Ciriolo MR, Chen S, Mohammed SI, Azmi AS, Keith WN, Bilsland A, Bhakta D, Halicka D, Fujii H, Aquilano K, Ashraf SS, Nowsheen S, Yang X, Choi BK, Kwon BS. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Semin Cancer Biol* 2015; **35** Suppl: S185-S198 [PMID: 25818339 DOI: 10.1016/j.semcancer.2015.03.004]

31 **Hiam-Galvez KJ**, Allen BM, Spitzer MH. Systemic immunity in cancer. *Nat Rev Cancer* 2021; **21**: 345-359 [PMID: 33837297 DOI: 10.1038/s41568-021-00347-z]

32 **Nesmiyanov PP**. Dendritic Cells. *Encyclop of Infect and Immun* 2022; **1**: 110-117 [DOI: 10.1016/B978-0-12-818731-9.00039-2]

33 **Gabrilovich DI**, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, Kavanaugh D, Carbone DP. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat Med* 1996; **2**: 1096-1103 [PMID: 8837607 DOI: 10.1038/nm1096-1096]

34 **Menetrier-Caux C**, Montmain G, Dieu MC, Bain C, Favrot MC, Caux C, Blay JY. Inhibition of the differentiation of dendritic cells from

CD34(+) progenitors by tumor cells: role of interleukin-6 and macrophage colony-stimulating factor. *Blood* 1998; **92**: 4778-4791 [PMID: 9845545 DOI: 10.1182/blood.V92.12.4778]

35 **Kiertscher SM**, Luo J, Dubinett SM, Roth MD. Tumors promote altered maturation and early apoptosis of monocyte-derived dendritic cells. *J Immunol* 2000; **164**: 1269-1276 [PMID: 10640740 DOI: 10.4049/jimmunol.164.3.1269]

36 **Pinzon-Charry A**, Maxwell T, McGuckin MA, Schmidt C, Furnival C, López JA. Spontaneous apoptosis of blood dendritic cells in patients with breast cancer. *Breast Cancer Res* 2006; **8**: R5 [PMID: 16417648 DOI: 10.1186/bcr1361]

37 **Della Bella S**, Gennaro M, Vaccari M, Ferraris C, Nicola S, Riva A, Clerici M, Greco M, Villa ML. Altered maturation of peripheral blood dendritic cells in patients with breast cancer. *Br J Cancer* 2003; **89**: 1463-1472 [PMID: 14562018 DOI: 10.1038/sj.bjc.6601243]

38 **Mastelic-Gavillet B**, Sarivalasis A, Lozano LE, Wyss T, Inoges S, de Vries IJM, Dartiguenave F, Jichlinski P, Derrè L, Coukos G, Melero I, Harari A, Romero P, Viganó S, Kandalaft LE. Quantitative and qualitative impairments in dendritic cell subsets of patients with ovarian or prostate cancer. *Eur J Cancer* 2020; **135**: 173-182 [PMID: 32590296 DOI: 10.1016/j.ejca.2020.04.036]

39 **Tabarkiewicz J**, Rybojad P, Jablonka A, Rolinski J. CD1c+ and CD303+ dendritic cells in peripheral blood, lymph nodes and tumor tissue of patients with non-small cell lung cancer. *Oncol Rep* 2008; **19**: 237-243 [PMID: 18097601 DOI: 10.3892/or.19.1.237]

40 **Lu Y**, Xu W, Gu Y, Chang X, Wei G, Rong Z, Qin L, Chen X, Zhou F. Non-small Cell Lung Cancer Cells Modulate the Development of Human CD1c(+) Conventional Dendritic Cell Subsets Mediated by CD103 and CD205. *Front Immunol* 2019; **10**: 2829 [PMID: 31921114 DOI: 10.3389/fimmu.2019.02829]

41 **Legitimo A**, Consolini R, Failli A, Orsini G, Spisni R. Dendritic cell defects in the colorectal cancer. *Hum Vaccin Immunother* 2014; **10**: 3224-3235 [PMID: 25483675 DOI: 10.4161/hv.29857]

42 **Failli A**, Legitimo A, Orsini G, Romanini A, Consolini R. Numerical defect of circulating dendritic cell subsets and defective dendritic cell generation from monocytes of patients with advanced melanoma. *Cancer Lett* 2013; **337**: 184-192 [PMID: 23684927 DOI: 10.1016/j.canlet.2013.05.013]

43 **Gabrilovich DI**, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012; **12**: 253-268 [PMID: 22437938 DOI: 10.1038/nri3175]

44 **Wang WW**, Yuan XL, Chen H, Xie GH, Ma YH, Zheng YX, Zhou YL, Shen LS. CD19+CD24hiCD38hiBregs involved in downregulate helper T cells and upregulate regulatory T cells in gastric cancer. *Oncotarget* 2015; **6**: 33486-33499 [PMID: 26378021 DOI: 10.18632/oncotarget.5588]

45 **Ray-Coquard I**, Cropet C, Van Glabbeke M, Sebban C, Le Cesne A, Judson I, Tredan O, Verweij J, Biron P, Labidi I, Guastalla JP, Bachelot T, Perol D, Chabaud S, Hogendoorn PC, Cassier P, Dufresne A, Blay JY; European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. Lymphopenia as a prognostic factor for overall survival in advanced carcinomas, sarcomas, and lymphomas. *Cancer Res* 2009; **69**: 5383-5391 [PMID: 19549917 DOI: 10.1158/0008-5472.CAN-08-3845]

46 **Wang JL**, Ma R, Kong W, Zhao R, Wang YY. Lymphopenia in Esophageal Cancer: What Have We Learned? *Front Oncol* 2021; **11**: 625963 [PMID: 33791213 DOI: 10.3389/fonc.2021.625963]

47 **Han Y**, Liu D, Li L. PD-1/PD-L1 pathway: current researches in cancer. *Am J Cancer Res* 2020; **10**: 727-742 [PMID: 32266087]

48 **Ye Q**, Wang C, Xian J, Zhang M, Cao Y. Expression of programmed cell death protein 1 (PD-1) and indoleamine 2,3-dioxygenase (IDO) in the tumor microenvironment and in tumor-draining lymph nodes of breast cancer. *Hum Pathol* 2018; **75**: 81-90 [PMID: 29447919 DOI: 10.1016/j.humpath.2018.02.004]

49 **Wolf AM**, Wolf D, Steurer M, Gastl G, Gunsilius E, Grubeck-Loebenstein B. Increase of regulatory T cells in the peripheral blood of cancer patients. *Clin Cancer Res* 2003; **9**: 606-612 [PMID: 12576425]

50 **Liu L**, Wu G, Yao JX, Ding Q, Huang SA. CD4+CD25high regulatory cells in peripheral blood of cancer patients. *Neuro Endocrinol Lett* 2008; **29**: 240-245 [PMID: 18404145]

51 **Sarvaria A**, Madrigal JA, Saudemont A. B cell regulation in cancer and anti-tumor immunity. *Cell Mol Immunol* 2017; **14**: 662-674 [PMID: 28626234 DOI: 10.1038/cmi.2017.35]

52 **Murakami Y**, Saito H, Shimizu S, Kono Y, Shishido Y, Miyatani K, Matsunaga T, Fukumoto Y, Ashida K, Sakabe T, Nakayama Y, Fujiwara Y. Increased regulatory B cells are involved in immune evasion in patients with gastric cancer. *Sci Rep* 2019; **9**: 13083 [PMID: 31511630 DOI: 10.1038/s41598-019-49581-4]

53 **Li JH**, O'Sullivan TE. Back to the Future: Spatiotemporal Determinants of NK Cell Antitumor Function. *Front Immunol* 2021; **12**: 816658 [PMID: 35082797 DOI: 10.3389/fimmu.2021.816658]

54 **Zembower TR**. Epidemiology of infections in cancer patients. *Cancer Treat Res* 2014; **161**: 43-89 [PMID: 24706221 DOI: 10.1007/978-3-319-04220-6_2]

55 **Bitterman R**, Eliakim-Raz N, Vinograd I, Zalmanovici Trestioreanu A, Leibovici L, Paul M. Influenza vaccines in immunosuppressed adults with cancer. *Cochrane Database Syst Rev* 2018; **2**: CD008983 [PMID: 29388675 DOI: 10.1002/14651858.CD008983.pub3]

56 **Brydak LB**, Guzy J, Starzyk J, Machała M, Góźdź SS. Humoral immune response after vaccination against influenza in patients with breast cancer. *Support Care Cancer* 2001; **9**: 65-68 [PMID: 11147146 DOI: 10.1007/s005200000186]

57 **Pollyea DA**, Brown JM, Horning SJ. Utility of influenza vaccination for oncology patients. *J Clin Oncol* 2010; **28**: 2481-2490 [PMID: 20385981 DOI: 10.1200/JCO.2009.26.6908]

58 **Oates HF**, Stoker LM, Monaghan JC, Stokes GS. The beta-adrenoceptor controlling renin release. *Arch Int Pharmacodyn Ther* 1978; **234**: 205-213 [PMID: 213037 DOI: 10.1093/annonc/mdq728]

59 **Arrowood JR**, Hayney MS. Immunization recommendations for adults with cancer. *Ann Pharmacother* 2002; **36**: 1219-1229 [PMID: 12086557 DOI: 10.1345/aph.1A277]

60 **Velikova T**, Georgiev T. SARS-CoV-2 vaccines and autoimmune diseases amidst the COVID-19 crisis. *Rheumatol Int* 2021; **41**: 509-518 [PMID: 33515320 DOI: 10.1007/s00296-021-04792-9]

61 **Sen P**, Ravichandran N, Nune A, Lilleker JB, Agarwal V, Kardes S, Kim M, Day J, Milchert M, Gheita T, Salim B, Velikova T, Gracia-Ramos AE, Parodis I, Selva O'Callaghan A, Nikiphorou E, Chatterjee T, Tan AL, Cavagna L, Saavedra MA, Shinjo SK, Ziade N, Knitza J, Kuwana M, Distler O, Chinoy H, Aggarwal R, Gupta L; COVAD Study Group. COVID-19 vaccination-related adverse events among autoimmune disease patients: results from the COVAD study. *Rheumatology (Oxford)* 2022; **62**: 65-76 [PMID: 35713499 DOI: 10.1093/rheumatology/keac305]

62 **Corti C**, Antonarelli G, Scotté F, Spano JP, Barrière J, Michot JM, André F, Curigliano G. Seroconversion rate after vaccination against COVID-19 in patients with cancer-a systematic review. *Ann Oncol* 2022; **33**: 158-168 [PMID: 34718117 DOI: 10.1016/j.annonc.2021.10.014]

63 **Khoury DS**, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, Subbarao K, Kent SJ, Triccas JA, Davenport MP. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; **27**: 1205-1211 [PMID: 34002089 DOI: 10.1038/s41591-021-01377-8]

64 **Earle KA**, Ambrosino DM, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, Dull P, Plotkin SA. Evidence for antibody as a protective correlate for COVID-19 vaccines. *Vaccine* 2021; **39**: 4423-4428 [PMID: 34210573 DOI: 10.1016/j.vaccine.2021.05.063]

65 **Slabakova Y**, Gerenska D, Ivanov N, Velikova T. Immune titers of protection against severe acute respiratory syndrome coronavirus 2: are we there yet? *Explor Immunol* 2022; **2**: 9-24 [DOI: 10.37349/ei.2022.00033]

66 **Monin L**, Laing AG, Muñoz-Ruiz M, McKenzie DR, Del Molino Del Barrio I, Alaguthurai T, Domingo-Vila C, Hayday TS, Graham C, Seow J, Abdul-Jawad S, Kamdar S, Harvey-Jones E, Graham R, Cooper J, Khan M, Vidler J, Kakkassery H, Sinha S, Davis R, Dupont L, Francos Quijorna I, O'Brien-Gore C, Lee PL, Eum J, Conde Poole M, Joseph M, Davies D, Wu Y, Swampillai A, North BV, Montes A, Harries M, Rigg A, Spicer J, Malim MH, Fields P, Patten P, Di Rosa F, Papa S, Tree T, Doores KJ, Hayday AC, Irshad S. Safety and immunogenicity of one versus two doses of the COVID-19 vaccine BNT162b2 for patients with cancer: interim analysis of a prospective observational study. *Lancet Oncol* 2021; **22**: 765-778 [PMID: 33930323 DOI: 10.1016/S1470-2045(21)00213-8]

67 **Fendler A**, Shepherd STC, Au L, Wilkinson KA, Wu M, Byrne F, Cerrone M, Schmitt AM, Joharatnam-Hogan N, Shum B, Tippu Z, Rzeniewicz K, Boos LA, Harvey R, Carlyle E, Edmonds K, Del Rosario L, Sarker S, Lingard K, Mangwende M, Holt L, Ahmod H, Korteweg J, Foley T, Bazin J, Gordon W, Barber T, Emslie-Henry A, Xie W, Gerard CL, Deng D, Wall EC, Agua-Doce A, Namjou S, Caidan S, Gavrielides M, MacRae JI, Kelly G, Peat K, Kelly D, Murra A, Kelly K, O'Flaherty M, Dowdie L, Ash N, Gronthoud F, Shea RL, Gardner G, Murray D, Kinnaird F, Cui W, Pascual J, Rodney S, Mencel J, Curtis O, Stephenson C, Robinson A, Oza B, Farag S, Leslie I, Rogiers A, Iyengar S, Ethell M, Messiou C, Cunningham D, Chau I, Starling N, Turner N, Welsh L, van As N, Jones RL, Droney J, Banerjee S, Tatham KC, O'Brien M, Harrington K, Bhide S, Okines A, Reid A, Young K, Furness AJS, Pickering L, Swanton C; Crick COVID19 consortium, Gandhi S, Gamblin S, Bauer DL, Kassiotis G, Kumar S, Yousaf N, Jhanji S, Nicholson E, Howell M, Walker S, Wilkinson RJ, Larkin J, Turajlic S. Adaptive immunity and neutralizing antibodies against SARS-CoV-2 variants of concern following vaccination in patients with cancer: The CAPTURE study. *Nat Cancer* 2021; **2**: 1321-1337 [PMID: 34950880 DOI: 10.1038/s43018-021-00274-w]

68 **Miteva D**, Kitanova M, Batselova H, Lazova S, Chervenkov L, Peshevska-Sekulovska M, Sekulovski M, Gulinac M, Vasilev GV, Tomov L, Velikova T. The End or a New Era of Development of SARS-CoV-2 Virus: Genetic Variants Responsible for Severe COVID-19 and Clinical Efficacy of the Most Commonly Used Vaccines in Clinical Practice. *Vaccines (Basel)* 2023; **11** [PMID: 37514997 DOI: 10.3390/vaccines11071181]

69 **Greenberger LM**, Saltzman LA, Senefeld JW, Johnson PW, DeGennaro LJ, Nichols G. Sars-Cov-2 Antibody Levels in Blood Cancer Patients after a Third Sars-Cov-2 "Booster" Vaccination - Observational Data from the LLS National Registry. *Blood* 2021; **138** Suppl 1: 185 [DOI: 10.1182/blood-2021-151419]

70 **Rüthrich MM**, Giesen N, Mellinghoff SC, Rieger CT, von Lilienfeld-Toal M. Cellular Immune Response after Vaccination in Patients with Cancer-Review on Past and Present Experiences. *Vaccines (Basel)* 2022; **10** [PMID: 35214642 DOI: 10.3390/vaccines10020182]

71 **Ehmsen S**, Asmussen A, Jeppesen SS, Nilsson AC, Østerlev S, Vestergaard H, Justesen US, Johansen IS, Frederiksen H, Ditzel HJ. Antibody and T cell immune responses following mRNA COVID-19 vaccination in patients with cancer. *Cancer Cell* 2021; **39**: 1034-1036 [PMID: 34348121 DOI: 10.1016/j.ccell.2021.07.016]

72 **Mairhofer M**, Kausche L, Kaltenbrunner S, Ghanem R, Stegemann M, Klein K, Pammer M, Rauscher I, Salzer HJF, Doppler S, Habringer A, Paar C, Kimeswenger S, Hoetzenecker W, Lamprecht B, Lee S, Schmitt CA. Humoral and cellular immune responses in SARS-CoV-2 mRNA-vaccinated patients with cancer. *Cancer Cell* 2021; **39**: 1171-1172 [PMID: 34450047 DOI: 10.1016/j.ccell.2021.08.001]

73 **Oosting SF**, van der Veldt AAM, GeurtsvanKessel CH, Fehrmann RSN, van Binnendijk RS, Dingemans AC, Smit EF, Hiltermann TJN, den Hartog G, Jalving M, Westphal TT, Bhattacharya A, van der Heiden M, Rimmelzwaan GF, Kvistborg P, Blank CU, Koopmans MPG, Huckriede ALW, van Els CACM, Rots NY, van Baarle D, Haanen JBAG, de Vries EGE. mRNA-1273 COVID-19 vaccination in patients receiving chemotherapy, immunotherapy, or chemoimmunotherapy for solid tumours: a prospective, multicentre, non-inferiority trial. *Lancet Oncol* 2021; **22**: 1681-1691 [PMID: 34767759 DOI: 10.1016/S1470-2045(21)00574-X]

74 **Schmidt AL**, Labaki C, Hsu CY, Bakouny Z, Balanchivadze N, Berg SA, Blau S, Daher A, El Zarif T, Friese CR, Griffiths EA, Hawley JE, Hayes-Lattin B, Karivedu V, Latif T, Mavromatis BH, McKay RR, Nagaraj G, Nguyen RH, Panagiotou OA, Portuguese AJ, Puc M, Santos Dutra M, Schroeder BA, Thakkar A, Wulff-Burchfield EM, Mishra S, Farmakiotis D, Shyr Y, Warner JL, Choueiri TK; COVID-19 and Cancer Consortium. COVID-19 vaccination and breakthrough infections in patients with cancer. *Ann Oncol* 2022; **33**: 340-346 [PMID: 34958894 DOI: 10.1016/j.annonc.2021.12.006]

75 **Goshen-Lago T**, Waldhorn I, Holland R, Szwarcwort-Cohen M, Reiner-Benaim A, Shachor-Meyouhas Y, Hussein K, Fahoum L, Baruch M, Peer A, Reiter Y, Almog R, Halberthal M, Ben-Aharon I. Serologic Status and Toxic Effects of the SARS-CoV-2 BNT162b2 Vaccine in Patients Undergoing Treatment for Cancer. *JAMA Oncol* 2021; **7**: 1507-1513 [PMID: 34236381 DOI: 10.1001/jamaoncol.2021.2675]

76 **Waldhorn I**, Holland R, Goshen-Lago T, Shirman Y, Szwarcwort-Cohen M, Reiner-Benaim A, Shachor-Meyouhas Y, Hussein K, Fahoum L, Peer A, Almog R, Shaked Y, Halberthal M, Ben-Aharon I. Six-Month Efficacy and Toxicity Profile of BNT162b2 Vaccine in Cancer Patients with Solid Tumors. *Cancer Discov* 2021; **11**: 2430-2435 [PMID: 34475136 DOI: 10.1158/2159-8290.CD-21-1072]

77 **Barrière J**, Chamorey E, Adjtoutah Z, Castelnau O, Mahamat A, Marco S, Petit E, Leysalle A, Raimondi V, Carles M. Impaired immunogenicity of BNT162b2 anti-SARS-CoV-2 vaccine in patients treated for solid tumors. *Ann Oncol* 2021; **32**: 1053-1055 [PMID: 33932508 DOI: 10.1016/j.annonc.2021.04.019]

78 **Shroff RT**, Chalasani P, Wei R, Pennington D, Quirk G, Schoenle MV, Peyton KL, Uhrlaub JL, Ripperger TJ, Jergović M, Dalgai S, Wolf A, Whitmer R, Hammad H, Carrier A, Scott AJ, Nikolich-Žugich J, Worobey M, Sprissler R, Dake M, LaFleur BJ, Bhattacharya D. Immune responses to two and three doses of the BNT162b2 mRNA vaccine in adults with solid tumors. *Nat Med* 2021; **27**: 2002-2011 [PMID: 34594036 DOI: 10.1038/s41591-021-01542-z]

79 **Wagner A**, Garner-Spitzer E, Schötta AM, Orola M, Wessely A, Zwazl I, Ohradanova-Repic A, Weseslindtner L, Tajti G, Gebetsberger L, Kratzer B, Tomosel E, Kutschera M, Tobudic S, Pickl WF, Kundi M, Stockinger H, Novacek G, Reinisch W, Zielinski C, Wiedermann U. SARS-CoV-2-mRNA Booster Vaccination Reverses Non-Responsiveness and Early Antibody Waning in Immunocompromised Patients-A Phase Four Study Comparing Immune Responses in Patients With Solid Cancers, Multiple Myeloma and Inflammatory Bowel Disease. *Front Immunol* 2022; **13**: 889138 [PMID: 35634285 DOI: 10.3389/fimmu.2022.889138]

80 **Reimann P**, Ulmer H, Mutschlechner B, Benda M, Severgnini L, Volgger A, Lang T, Atzl M, Huynh M, Gasser K, Grabher C, Mink S, Fraunberger P, Petrausch U, Hartmann B, Winder T. Efficacy and safety of heterologous booster vaccination with Ad26.COV2.S after BNT162b2 mRNA COVID-19 vaccine in haemato-oncological patients with no antibody response. *Br J Haematol* 2022; **196**: 577-584 [PMID:

34872162 DOI: 10.1111/bjh.17982]

81 **Thomas SJ**, Perez JL, Lockhart SP, Hariharan S, Kitchin N, Bailey R, Liau K, Lagkadinou E, Türeci Ö, Şahin U, Xu X, Koury K, Dychter SS, Lu C, Gentile TC, Gruber WC. Efficacy and safety of the BNT162b2 mRNA COVID-19 vaccine in participants with a history of cancer: subgroup analysis of a global phase 3 randomized clinical trial. *Vaccine* 2022; **40**: 1483-1492 [PMID: 35131133 DOI: 10.1016/j.vaccine.2021.12.046]

82 **Lee LYW**, Starkey T, Ionescu MC, Little M, Tilby M, Tripathy AR, Mckenzie HS, Al-Hajji Y, Barnard M, Benny L, Burnett A, Cattell EL, Charman J, Clark JJ, Khan S, Ghafoor Q, Illsley G, Harper-Wynne C, Hattersley RJ, Lee AJX, Leonard PC, Liu JKH; NCRI Consumer Forum, Pang M, Pascoe JS, Platt JR, Potter VA, Randle A, Rigg AS, Robinson TM, Roques TW, Roux RL, Rozmanowski S, Tuthill MH, Watts I, Williams S, Iveson T, Lee SM, Middleton G, Middleton M, Protheroe A, Fittall MW, Fowler T, Johnson P. Vaccine effectiveness against COVID-19 breakthrough infections in patients with cancer (UKCCEP): a population-based test-negative case-control study. *Lancet Oncol* 2022; **23**: 748-757 [PMID: 35617989 DOI: 10.1016/S1470-2045(22)00202-9]

83 **Thakkar A**, Pradhan K, Duva B, Carreno JM, Sahu S, Thiruthuvanathan V, Campbell S, Gallego S, Bhagat TD, Rivera J, Choudhary G, Olea R, Sabalza M, Shapiro LC, Lee M, Quinn R, Mantzaris I, Chu E, Will B, Pirofski LA, Krammer F, Verma A, Halmos B. Study of efficacy and longevity of immune response to third and fourth doses of COVID-19 vaccines in patients with cancer: A single arm clinical trial. *Elife* 2023; **12** [PMID: 36975207 DOI: 10.7554/eLife.83694]

84 **Polack FP**, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Pérez Marc G, Moreira ED, Zerbini C, Bailey R, Swanson KA, Roychoudhury S, Koury K, Li P, Kalina WV, Cooper D, Frenck RW Jr, Hammitt LL, Türeci Ö, Nell H, Schaefer A, Ünal S, Tresnan DB, Mather S, Dormitzer PR, Şahin U, Jansen KU, Gruber WC; C4591001 Clinical Trial Group. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* 2020; **383**: 2603-2615 [PMID: 33301246 DOI: 10.1056/NEJMoa2034577]

85 **Haynes L**. Aging of the Immune System: Research Challenges to Enhance the Health Span of Older Adults. *Front Aging* 2020; **1**: 602108 [PMID: 35822168 DOI: 10.3389/fragi.2020.602108]

86 **Zangemeister-Wittke U**, Simon HU. Myelosuppression. In: Schwab M. Encyclopedia of Cancer. Berlin, Heidelberg: Springer, 2011: 2437-2440 [DOI: 10.1007/978-3-642-16483-5_3940]

87 **Ba Y**, Shi Y, Jiang W, Feng J, Cheng Y, Xiao L, Zhang Q, Qiu W, Xu B, Xu R, Shen B, Luo Z, Xie X, Chang J, Wang M, Li Y, Shuang Y, Niu Z, Liu B, Zhang J, Zhang L, Yao H, Xie C, Huang H, Liao W, Chen G, Zhang X, An H, Deng Y, Gong P, Xiong J, Yao Q, An X, Chen C, Wang J, Wang X, Wang Z, Xing P, Yang S, Zhou C. Current management of chemotherapy-induced neutropenia in adults: key points and new challenges: Committee of Neoplastic Supportive-Care (CONS), China Anti-Cancer Association Committee of Clinical Chemotherapy, China Anti-Cancer Association. *Cancer Biol Med* 2020; **17**: 896-909 [PMID: 33299642 DOI: 10.20892/j.issn.2095-3941.2020.0069]

88 **Weinblatt ME**, Coblyn JS, Fox DA, Fraser PA, Holdsworth DE, Glass DN, Trentham DE. Efficacy of low-dose methotrexate in rheumatoid arthritis. *N Engl J Med* 1985; **312**: 818-822 [PMID: 3883172 DOI: 10.1056/NEJM198503283121303]

89 **Alqarni AM**, Zeidler MP. How does methotrexate work? *Biochem Soc Trans* 2020; **48**: 559-567 [PMID: 32239204 DOI: 10.1042/BST20190803]

90 **Koźmiński P**, Halik PK, Chesori R, Gniazdowska E. Overview of Dual-Acting Drug Methotrexate in Different Neurological Diseases, Autoimmune Pathologies and Cancers. *Int J Mol Sci* 2020; **21** [PMID: 32423175 DOI: 10.3390/ijms21103483]

91 **Kapetanovic MC**, Roseman C, Jönsson G, Truedsson L, Saxne T, Geborek P. Antibody response is reduced following vaccination with 7-valent conjugate pneumococcal vaccine in adult methotrexate-treated patients with established arthritis, but not those treated with tumor necrosis factor inhibitors. *Arthritis Rheum* 2011; **63**: 3723-3732 [PMID: 21834061 DOI: 10.1002/art.30580]

92 **Kapetanovic MC**, Saxne T, Sjöholm A, Truedsson L, Jönsson G, Geborek P. Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis. *Rheumatology (Oxford)* 2006; **45**: 106-111 [PMID: 16287919 DOI: 10.1093/rheumatology/kei193]

93 **Nived P**, Pettersson Å, Jönsson G, Bengtsson AA, Settergren B, Skattum L, Johansson Å, Kapetanovic MC. Methotrexate reduces circulating Th17 cells and impairs plasmablast and memory B cell expansions following pneumococcal conjugate immunization in RA patients. *Sci Rep* 2021; **11**: 9199 [PMID: 33911135 DOI: 10.1038/s41598-021-88491-2]

94 **Ogino MH**, Tadi P. Cyclophosphamide. 2023 Jul 3. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan- [PMID: 31971727]

95 **Gauthier SA**, Weiner HL. Cyclophosphamide therapy for MS. *Int MS J* 2005; **12**: 52-58 [PMID: 16417815]

96 **Elkhalifa A**, Weiner H. Cyclophosphamide Treatment of MS: Current Therapeutic Approaches and Treatment Regimens. *Int MS J* 2010; **17**: 12-18 [PMID: 20663416]

97 **Ahlmann M**, Hempel G. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. *Cancer Chemother Pharmacol* 2016; **78**: 661-671 [PMID: 27646791 DOI: 10.1007/s00280-016-3152-1]

98 **Madondo MT**, Quinn M, Plebanski M. Low dose cyclophosphamide: Mechanisms of T cell modulation. *Cancer Treat Rev* 2016; **42**: 3-9 [PMID: 26620820 DOI: 10.1016/j.ctrv.2015.11.005]

99 **de Lavallade H**, Khoder A, Hart M, Sarvaria A, Sekine T, Alsuliman A, Mielke S, Bazeos A, Stringaris K, Ali S, Milojkovic D, Foroni L, Chaidos A, Cooper N, Gabriel I, Apperley J, Belsey S, Flanagan RJ, Goldman J, Shpall EJ, Kelleher P, Marin D, Rezvani K. Tyrosine kinase inhibitors impair B-cell immune responses in CML through off-target inhibition of kinases important for cell signaling. *Blood* 2013; **122**: 227-238 [PMID: 23719297 DOI: 10.1182/blood-2012-11-465039]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 October 24; 14(10): 357-444

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 10 October 24, 2023

ORIGINAL ARTICLE

Basic Study

357 Hub genes and their key effects on prognosis of Burkitt lymphoma

Xu YF, Wang GY, Zhang MY, Yang JG

373 Comprehensive analysis of disulfidptosis related genes and prognosis of gastric cancer

Li Q, Yin LK

Retrospective Study

400 Treatment of patients with multiple brain metastases by isolated radiosurgery: Toxicity and survival

de Camargo AV, de Mattos MD, Kawasaki MK, Gomes DNS, Borges ABB, Vazquez VL, Araujo RLC

Observational Study

409 Classification of patients with metastatic colorectal cancer into consensus molecular subtypes into real-world: A pilot study

González-Montero J, Burotto M, Valenzuela G, Mateluna D, Buen-Abad F, Toro J, Barajas O, Marcelain K

SCIENTOMETRICS

420 What should be the future direction of development in the field of prostate cancer with lung metastasis?

Huang ZG, Chen Y, Wu T, Yin BT, Feng X, Li SH, Li DM, Chen G, Cheng JW, He J

CASE REPORT

440 Splenic lymphangioma masquerading as splenic abscess managed by laparoscopic splenectomy: A case report

Thorat S, Shaji FM

ABOUT COVER

Peer Reviewer of *World Journal of Clinical Oncology*, Amr Ahmed El-Arabey, PhD, Assistant Professor, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al Azhar University, Nasr City 11751, Cairo, Egypt. ph.amrcapa@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJCO* as 2.8; IF without journal self cites: 2.8; 5-year IF: 3.0; Journal Citation Indicator: 0.36.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Xu Guo*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
October 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 October 24; 14(10): 357-372

DOI: 10.5306/wjco.v14.i10.357

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

Hub genes and their key effects on prognosis of Burkitt lymphoma

Yan-Feng Xu, Guan-Yun Wang, Ming-Yu Zhang, Ji-Gang Yang

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Haddadi S, Algeria; Kołat D, Poland

Received: July 19, 2023
Peer-review started: July 19, 2023
First decision: August 24, 2023
Revised: September 6, 2023
Accepted: September 18, 2023
Article in press: September 18, 2023
Published online: October 24, 2023

Yan-Feng Xu, Guan-Yun Wang, Ming-Yu Zhang, Ji-Gang Yang, Department of Nuclear Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

Corresponding author: Ji-Gang Yang, MD, PhD, Chief Doctor, Professor, Researcher, Department of Nuclear Medicine, Beijing Friendship Hospital, Capital Medical University, No. 95 Yong An Road, Xicheng District, Beijing 100050, China. yangjigang@ccmu.edu.cn

Abstract

BACKGROUND

Burkitt lymphoma (BL) is an exceptionally aggressive malignant neoplasm that arises from either the germinal center or post-germinal center B cells. Patients with BL often present with rapid tumor growth and require high-intensity multi-drug therapy combined with adequate intrathecal chemotherapy prophylaxis, however, a standard treatment program for BL has not yet been established. It is important to identify biomarkers for predicting the prognosis of BLs and discriminating patients who might benefit from the therapy. Microarray data and sequencing information from public databases could offer opportunities for the discovery of new diagnostic or therapeutic targets.

AIM

To identify hub genes and perform gene ontology (GO) and survival analysis in BL.

METHODS

Gene expression profiles and clinical traits of BL patients were collected from the Gene Expression Omnibus database. Weighted gene co-expression network analysis (WGCNA) was applied to construct gene co-expression modules, and the cytoHubba tool was used to find the hub genes. Then, the hub genes were analyzed using GO and Kyoto Encyclopedia of Genes and Genomes analysis. Additionally, a Protein-Protein Interaction network and a Genetic Interaction network were constructed. Prognostic candidate genes were identified through overall survival analysis. Finally, a nomogram was established to assess the predictive value of hub genes, and drug-gene interactions were also constructed.

RESULTS

In this study, we obtained 8 modules through WGCNA analysis, and there was a significant correlation between the yellow module and age. Then we identified 10 hub genes (*SRC*, *TLR4*, *CD40*, *STAT3*, *SELL*, *CXCL10*, *IL2RA*, *IL10RA*, *CCR7* and *FCGR2B*) by cytoHubba tool. Within these hubs, two genes were found to be associated with OS (*CXCL10*, $P = 0.029$ and *IL2RA*, $P = 0.0066$) by survival ana-

lysis. Additionally, we combined these two hub genes and age to build a nomogram. Moreover, the drugs related to *IL2RA* and *CXCL10* might have a potential therapeutic role in relapsed and refractory BL.

CONCLUSION

From WGCNA and survival analysis, we identified *CXCL10* and *IL2RA* that might be prognostic markers for BL.

Key Words: Burkitt lymphoma; Weighted gene co-expression network analysis; Microarray data; Functional enrichment analysis; Prognosis; Therapeutic target

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: This study represents the pioneering investigation of gene expression in Burkitt lymphoma (BL) using weighted gene co-expression network analysis, coupled with functional enrichment analysis. In this study, we have successfully identified and validated 10 hub genes. Survival analysis has demonstrated that the overexpression of *CXCL10* and *IL2RA* in BL may serve as robust prognostic indicators. Furthermore, an integrated mRNA signature and age nomogram potentially provide valuable prognostic insights for patients with BLs.

Citation: Xu YF, Wang GY, Zhang MY, Yang JG. Hub genes and their key effects on prognosis of Burkitt lymphoma. *World J Clin Oncol* 2023; 14(10): 357-372
URL: https://www.wjgnet.com/2218-4333/full/v14/i10/357.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i10.357

INTRODUCTION

Burkitt lymphoma (BL) is a highly aggressive B-cell non-Hodgkin's lymphoma characterized by the *t* (8; 14) chromosomal translocation involving the *MYC* oncogene and the immunoglobulin heavy chain gene (IGH)[1]. Three distinct clinical subtypes of BL have been identified: Namely endemic (African), sporadic (non-endemic), and immunodeficiency-associated. Notably, chronic Epstein-Barr virus infection plays a pivotal role in the pathogenesis of BL, particularly in the endemic subtype[2]. Endemic BL is primarily found in countries located near the equator in Africa. The estimated annual incidence of endemic BL is 3-6 cases per 100000 children in African countries[3], which is approximately 50 times higher than that in the United States[4]. Sporadic BL predominantly occurs in the United States and Western Europe. The annual incidence of BL in the United States is approximately 3 cases per 1 million individuals, while in Europe it stands at around 2.2 cases per 1 million people[5]. Immunodeficiency-associated BL primarily affects individuals with HIV infection, typically those with relatively high CD4 counts and no opportunistic infections[6].

Patients with BL frequently exhibit rapid tumor growth, spontaneous tumor lysis, and elevated levels of serum lactate dehydrogenase. Currently, patients with BL necessitate a high-intensity multi-drug regimen in conjunction with adequate intrathecal central nervous system prophylaxis. However, the absence of an established standard treatment protocol for BL persists[7]. BL is an aggressive lymphoma, which can potentially be cured; however, patients with refractory and relapsed disease have an extremely poor prognosis[8]. Therefore, it is important to identify robust biomarkers for predicting the prognosis of BLs and discriminating patients who might benefit from therapy. The development of BL depends on the constitutive expression of the MYC gene located on chromosome 8q24, which encodes the transcription factor protein MYC[9]. MYC orchestrates the expression of target genes, regulating a variety of cellular processes, including cell growth, division, apoptosis, metabolism, adhesion, and motility[10]. MYC gene rearrangements are seen in the vast majority of BLs, and factors other than MYC translocation need to be present in the process of BL. However, it is not clear why and how B cells develop genetic alterations that result in increased MYC expression and ultimately lead to BL.

The Gene Expression Omnibus (GEO) is an international public repository constructed and maintained by the National Center for Biotechnology Information[11]. At the time of writing, the GEO database hosts more than 194000 public series. Weighted gene co-expression network analysis (WGCNA) is a widely used systematic biological method for generating gene co-expression networks[12,13]. In this study, WGCNA was first used to analyze genes of BL samples mined from the GEO database. Subsequently, we identified these hub genes and conducted a functional enrichment analysis. Additionally, a survival analysis was conducted to identify an mRNA signature that exhibits a significant association with prognosis. Finally, a prognostic nomogram was established based on the combination of gene signature and clinical characteristics.

MATERIALS AND METHODS

Data collection and preprocessing

The raw gene expressions and the corresponding clinical follow-up data of GSE4475 and GSE69051 were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/. Accessed Jan 20, 2023)[14], and the two datasets were built based on the GPL96 platform (HG-U133A) and GPL14951 platform (Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip) respectively. Analysis was performed on the raw gene expression data of the BL datasets and the corresponding clinical follow-up obtained from GSE4475, which included a total of 36 BL samples. The survival data of the hub genes was verified by downloading another dataset, GSE69051, which included 77 BL samples. The mRNA sequencing data annotation information was used to match the probe with the corresponding gene and transform the gene name into a gene symbol. Probes that corresponded to more than one gene were excluded from the dataset.

Co-expression network construction

WGCNA converts gene expression data into co-expression modules, establishing relationships between genes and focusing on gene modules rather than individual genes[15]. Besides, WGCNA can identify the gene modules related to clinical traits and has been widely used in cancer research. In this study, the top 5000 most variable genes were used to construct a co-expression network by using the package of WGCNA in R[13]. The power value was filtered out during the module construction process using the WGCNA algorithm. The mean connectivity and scale independence of network modules were analyzed using the gradient test under different power values, which ranged from 1 to 20. When the degree of independence was 0.85, the appropriate power value was determined. Then, the soft threshold test was performed. In this study, the soft threshold β was 12, and the network type was "signed". The WGCNA algorithm further identified co-expression modules under these conditions. The minimum size of the gene group was set at 100 to ensure the reliability of the results for this module. Then, the correlation between the characteristics of the module-trait association module and clinical traits was visually expressed. The relationship between the expression profile and traits was analyzed to make a scatter plot between gene significance and module membership.

Hub genes identification and functional analysis

The Protein-Protein Interaction (PPI) network of the interested module was constructed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (available from https://cn.string-db.org/. Accessed 25 Jan 2023)[16] and presented by Cytoscape software. The cytoHubba tool was used to screen the hub genes. Then, the selected hub genes were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis using Database for Annotation, Visualization, and Integrated Discovery (DAVID v.6.8: available from https://david.ncifcrf.gov/. Accessed Jan 29, 2023)[17]. The possible functions were analyzed by biological processes, cellular components, and molecular functions, while the potential signal pathways were analyzed using KEGG.

Construction of hub genes PPI and genetic interaction network

The PPI network was used to analyze the hub genes at the protein level, and the STRING database (available from https://string-db.org/. Accessed Jan 31, 2023) was used to check and predict the interaction between proteins[16]. The genetic interaction (GI) network, constructed using gene function prediction, aims to understand the complex interactions between genes. We used the Gene Multiple Association Network Integration Algorithm (GeneMANIA, available from https://genemania.org/. Accessed Jan 31, 2023) to analyze the hub genes[18]. The threshold of a collective score of 0.15 was implemented.

Statistical analysis

Based on the 50th percentile cut-off value of each hub gene mRNA, patients were divided into high-expression and low-expression groups. Log-rank test and Kaplan-Meier estimation were performed to obtain log-rank P value and evaluate hub genes in overall survival (OS). Cox regression analysis was conducted to examine the association between the risk score and clinical information, and a nomogram was generated. The survival curve and nomogram were carried out by R version 4.2.1. Additionally, $P < 0.05$ was statistically significant.

Drug-gene interaction

The DGIdb database (available from https://dgidb.org/. Accessed Feb 20, 2023) was used to investigate drug-gene interaction to identify drugs associated with hub genes[19]. The interaction network was visualized *via* Cytoscape.

RESULTS

Construction and screening of BL co-expression module

In this study, we obtained the BL dataset from GSE4475, resulting in a total of 13514 gene expression values. The clinical features of the BL samples are listed in Table 1. Then, we selected a total of 5000 genes with the highest average expression values for cluster analysis. Firstly, the clustering tree of 36 samples of BL was extracted from GSE4475 (Figure 1). Secondly, we calculated the soft threshold (power value), and when the weight was equal to 12, the independence exceeded 0.85, indicating higher average connectivity (Supplementary Figure 1). By utilizing this power value for hierarchical clustering analysis and combining similar analysis results, a total of 8 modules were identified,

Table 1 Clinical features of Burkitt lymphoma patients

Clinical features	Total (*n* = 36)
Age, mean (range)	31.0 (2-90)
Gender	
Male	24
Female	11
Unknown	1
Stage	
I	4
II	10
III	5
IV	6
Unknown	11
Survival status	
Alive	20
Dead	7
Unknown	9
Ki 67	
≤ 75%	4
75%-90%	9
> 90%	22
Unknown	1
CCS	
< 10	29
≥ 10	5
Unknown	4

CCS: Chromosomal Complexity Score.

including black (1073 genes), blue (967 genes), brown (853 genes), green yellow (140 genes), grey (1019 genes), magenta (219 genes), pink (267 genes) and yellow (462 genes) (Figure 2A). Genes in grey were not included in any module, thus we analyzed the interactive relationships underlying the 7 co-expression modules (Figure 2B). Given the well-established association between age and prognosis in BL patients, we opted to investigate the module that exhibited the strongest correlation with age for subsequent analysis[20,21]. A significant correlation between the yellow module and age was discovered (Figure 3). The correlation between modules and samples is shown in Supplementary Figure 2. Finally, we conducted a scatter diagram of the correlation between the yellow module and age (Figure 4).

Hub genes identification

All of the genes from the yellow module were uploaded to the STRING database, and a PPI network was constructed using Cytoscape software (Supplementary Figure 3). And the top 10 hub genes (*SRC, TLR4, CD40, STAT3, SELL, CXCL10, IL2RA, IL10RA, CCR7* and *FCGR2B*) were screened out by cytoHubba tool. GeneMANIA showed the GI network of hub genes interaction at the mRNA expression level (Figure 5A). The STRING database generated the PPI co-expression network by analyzing the hub genes at the protein level (Figure 5B).

Functional and pathway enrichment analysis

Enrichment analyses of GO and KEGG were conducted to explore potential pathways of the hub genes. Forty-five GO-enriched terms were shown in Supplementary Table 1. The top 10 GO terms (Figure 6A) included inflammatory response, external side of plasma membrane, plasma membrane, cellular response to lipopolysaccharide, positive regulation of interleukin-12 production, receptor binding, positive regulation of MAP kinase activity, positive regulation of JNK cascade, immune response and positive regulation of humoral immune response. In the KEGG analysis, 14 pathways enriched by genes in the yellow module were shown in Supplementary Table 2, and the top 10 KEGG terms (Figure 6B)

DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 1 Clustering tree of 36 samples of Burkitt lymphoma extracted from GSE4475. Red indicated more gene expression, white less, and grey indicated deletion. CCS: Chromosomal Complexity Score.

included viral protein interaction with cytokine and cytokine receptor, cytokine-cytokine receptor interaction, toxoplasmosis, measles, tuberculosis, chemokine signaling pathway, lipid and atherosclerosis, Toll-like receptor signaling pathway, Hepatitis B and JAK-STAT signaling pathway.

Survival analysis

Additional survival analysis was conducted on the hub genes to evaluate their impact on BL patients' survival. Due to the small sample size of GSE4475, we opted for GSE69051 for survival analysis (Figure 7). Two of the 10 hub genes were significantly associated with OS: *CXCL10* (P = 0.029, Figure 7F) and *IL2RA* (P = 0.0066, Figure 7G).

Establishment of the nomogram and assessment of predictive value

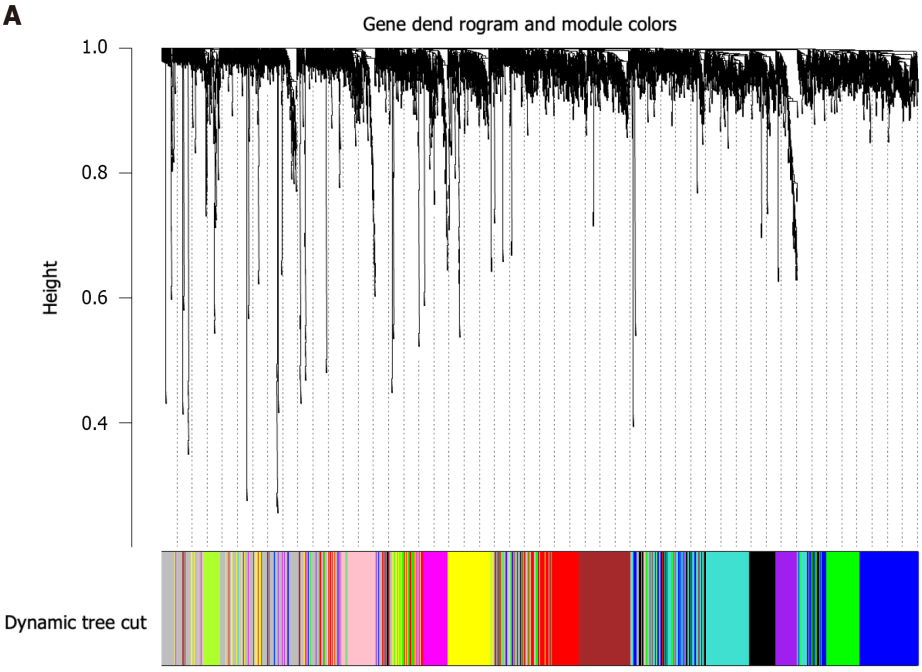
Based on the hub genes and clinical data of the patients, a nomogram was developed to predict the 1- and 3-year OS of BL patients (Figure 8A). The model had a c-index of 0.84, and the calibration curve demonstrated strong agreement between predicted and observed survival times for both 1- and 3-year OS probabilities in the BL cohort (Figure 8B).

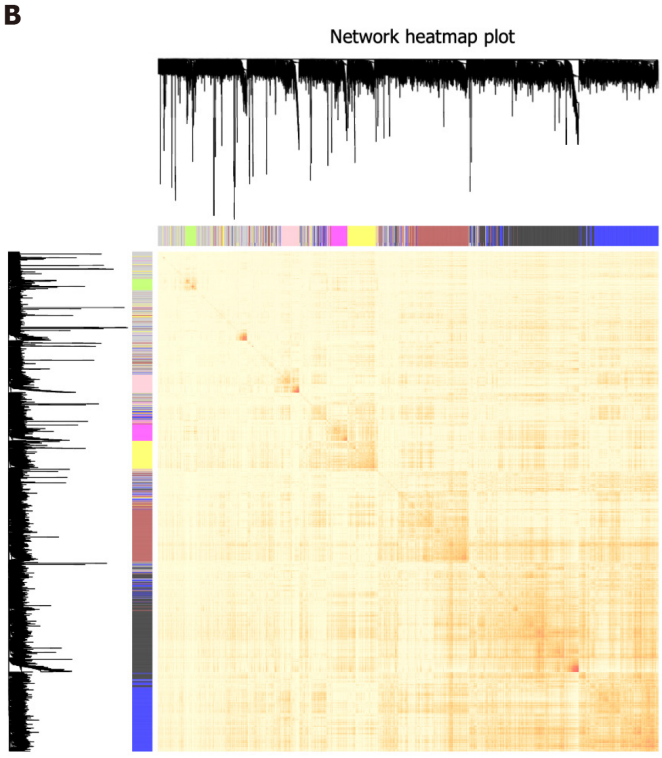
Identification of associated drugs

The drugs related to *IL2RA* and *CXCL10* were identified by the DGIdb database, as these were the only significant results from survival analysis (Figure 9). These results may provide new ideas for the treatment of BL with poor prognosis.

DISCUSSION

BL, a highly aggressive lymphoma identified and described by Denis Burkitt in the last century, continues to be the most common childhood malignancy in Africa nowadays[22]. A defining feature of BL is the translocation between the *c-MYC* gene and the *IgH* gene, which is found in 80% of cases [*t* (8; 14)], or between *c-MYC* and either the kappa or lambda light chain gene, which is found in the remaining 20% [*t* (2; 8) or *t* (8; 22)][23]. The proliferation rate and apoptosis rate of BL tumor cells are extremely high, indicating that nearly 100% of the cells are positive for Ki-67. Intensive, short-course combination chemotherapy is recommended for most BL patients. DA-EPOCH (dose-adjusted etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone) + rituximab may be an option for patients who cannot tolerate more aggressive regimens[7]. As the standard of treatment for BL has not yet been established, strictly controlled clinical trials are also recommended. The prognosis of BL patients is associated with both clinical and laboratory characteristics[8,24]. The BL international prognostic index can be used to assess the prognosis of adult patients with disseminated or immunodeficiency-related BL, but it is not currently used for stratifying BL treatment[20]. Previous studies have demonstrated associations between *MYC* rearrangements, *TCF3* mutations or *ID3* alterations (its negative regulator), *TP53* modifications, *CCND3* and *CDKN2A* changes, as well as non-antigen-dependent B cell receptor signaling (tonic B



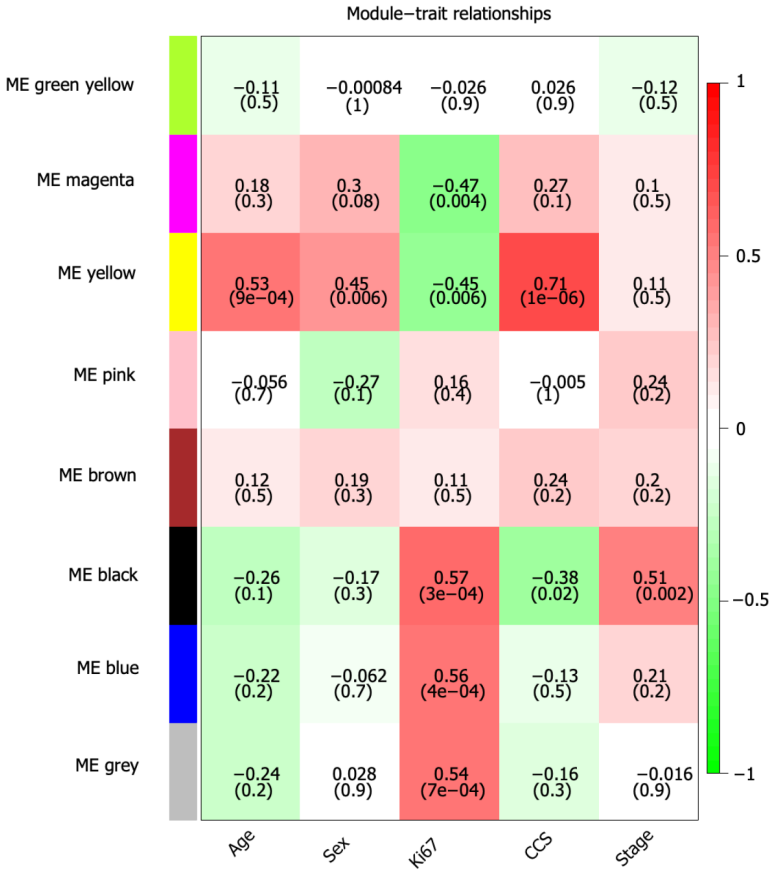


DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 2 Sample clustering to detect outliers and construction of co-expression modules. A: The constructed co-expression modules of Burkitt lymphoma genes by weighted gene co-expression network analysis; B: Interaction analysis between gene co-expression modules. The heatmap showed the Topological Overlap Matrix among genes in the analysis. Different colors on the x-axis and y-axis represented different modules. The intensity of inter-module connections was visually represented by the yellow brightness in the central region, gradually transitioning into deeper shades of orange.

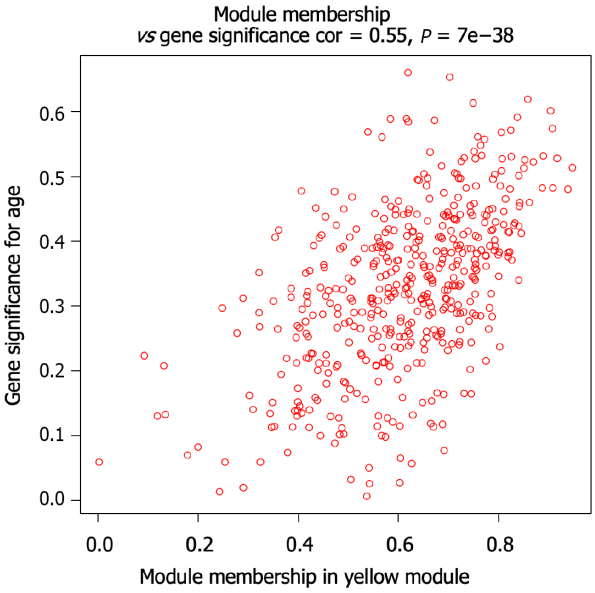
cell receptor signaling) with the development and prognosis of BL; however, a comprehensive investigation into the prognostic significance of molecular events associated with BL is lacking[25].

As a bioinformatics algorithm, WGCNA offers numerous advantages over conventional methods for differential expression analysis. It primarily focuses on elucidating co-expression patterns, facilitating the identification of biologically relevant modules comprising interconnected genes, and enabling the detection of pivotal hub genes[26-28]. So far, gene modules related to several cancers have been analyzed and verified using WGCNA[29,30].



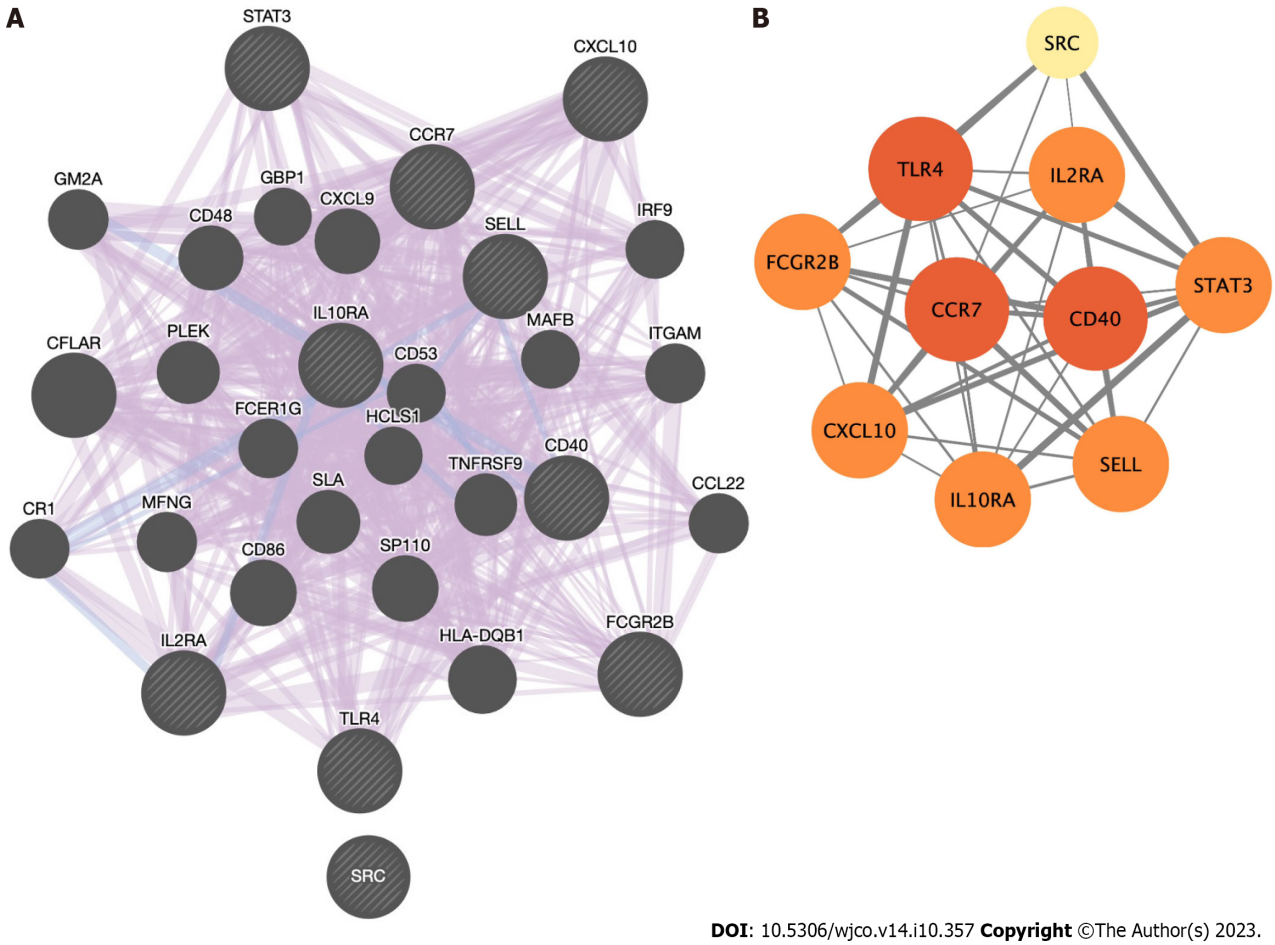
DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 3 Module-trait association. Correlation thermography between modular feature genes and clinical features of Burkitt lymphoma. Each row corresponded to a module feature, and the column corresponded to a clinical feature. Each cell contained the correlation and the corresponding *P* value. CCS: Chromosomal Complexity Score; ME: Module membership.



DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 4 The scatter plot of the correlation for an age-related gene between module membership and gene significance in the yellow module.

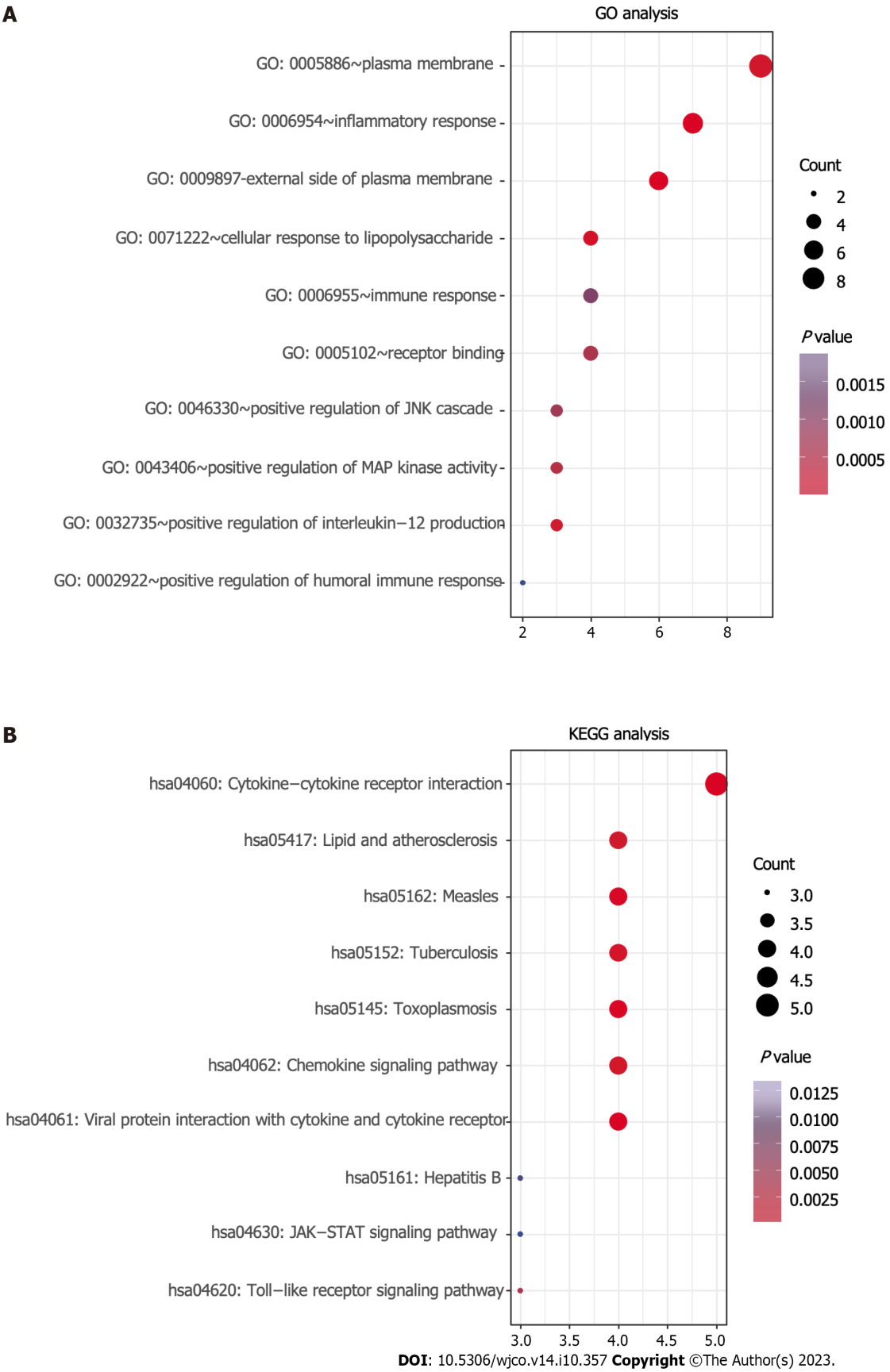


DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 5 Genetic and Protein-Protein interaction network of hub genes. A: GeneMANIA was used to construct a genetic interaction network. The black nodes with a slash represent the query gene, while the other nodes represent the predicted genes. The purple edges indicate co-expression, whereas the blue edges signify co-localization; B: A physically and functionally connected Protein-Protein Interaction network implemented common goals through Search Tool for the Retrieval of Interacting Genes/Proteins, where nodes represented proteins and edges represented pairs of interactions between proteins. Node size and color indicated richness, while edge size and color reflected combined scores.

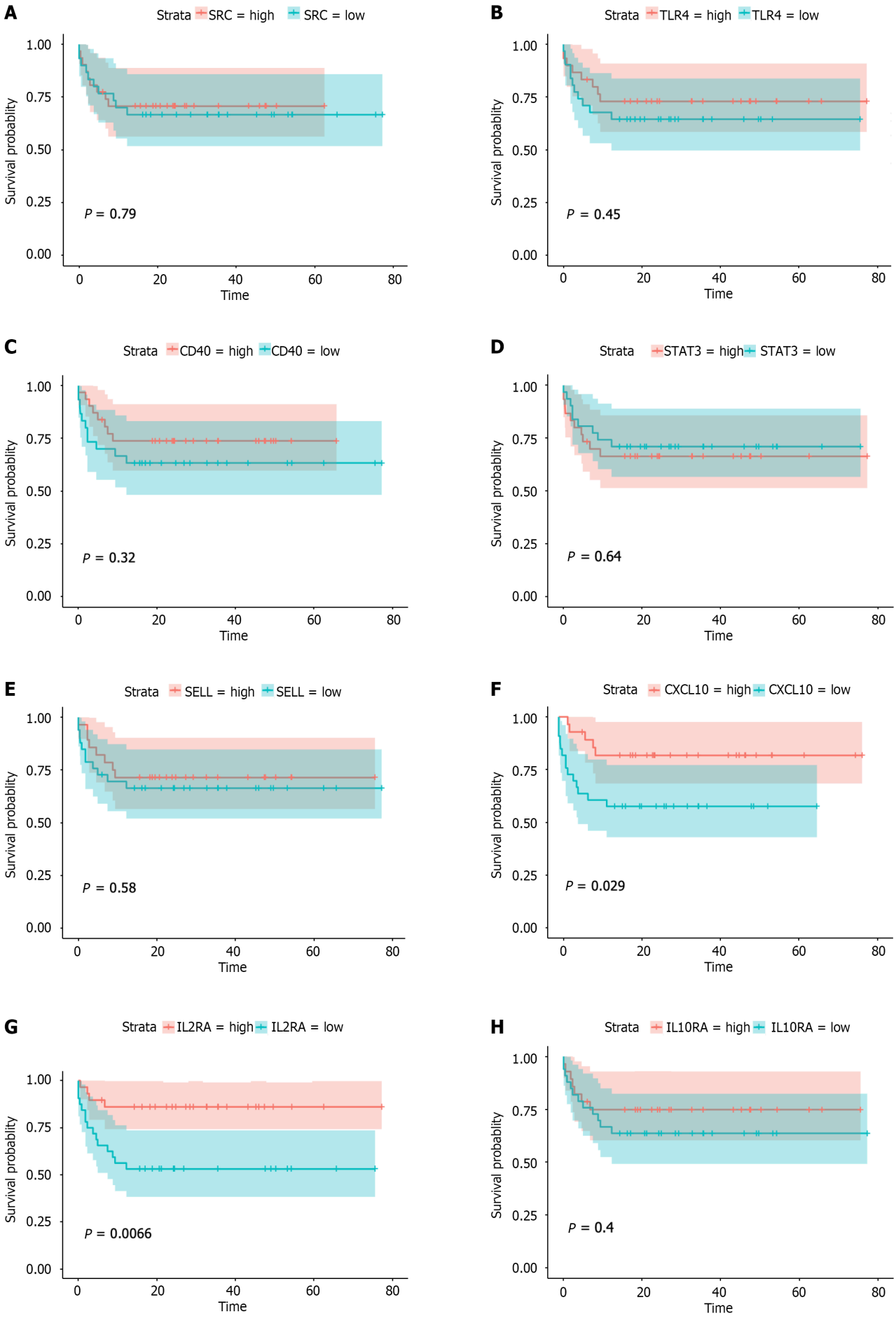
In this study, 8 modules were obtained through WGCNA. As many prior studies have shown a strong correlation between age and the prognosis of BL patients[20,21], we chose the yellow module that had the strongest correlation with age for further analysis. Ten hub genes (*SRC*, *TLR4*, *CD40*, *STAT3*, *SELL*, *CXCL10*, *IL2RA*, *IL10RA*, *CCR7* and *FCGR2B*) were identified using cytoHubba. GO and KEGG functional analyses were conducted on hub genes, and the PPI and GI analysis of these hub genes revealed their related biological functions. Based on survival analysis, CXCL10 and IL2RA have been identified as genes that affect survival. Afterward, we used a nomogram to develop a new risk assessment system for BL patients based on the aforementioned genes and age, aiming to aid in identifying high-risk groups for this disease.

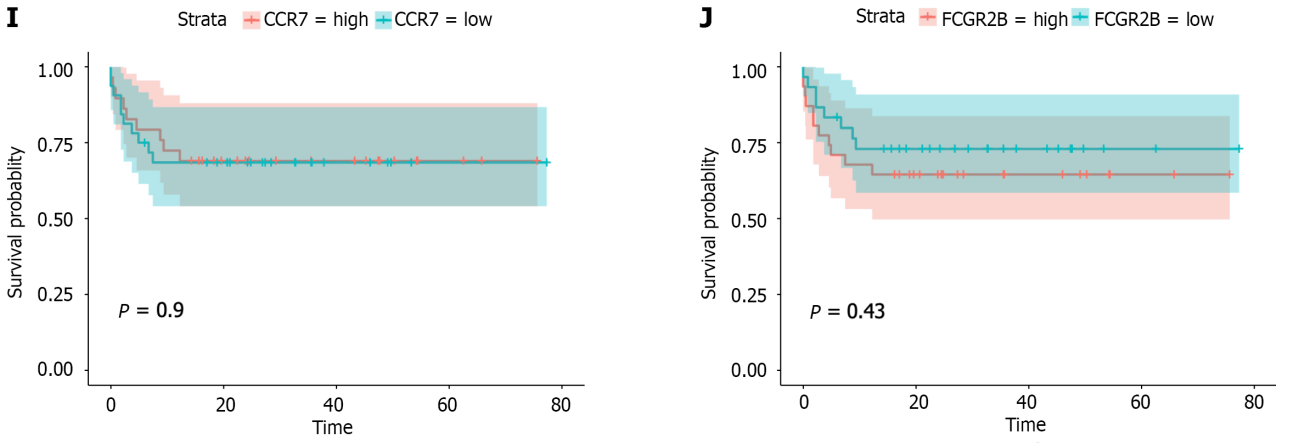
CXCL10 is one of the three ligands for *CXCR3*, which is a chemokine receptor[31]. Various studies have demonstrated that in addition to attracting CD8+ and CD4+ effector T cells to tumor sites and sites of inflammation, *CXCL10* also governs the polarization and enhances the biological functionality of these cells. This makes *CXCL10* a key chemokine driver and a valid target for the therapy of autoimmune diseases such as Inflammatory Bowel Disease, Multiple Sclerosis, Rheumatoid arthritis, and others. Previous studies have also found that chemokines and their receptors are involved in supporting tumor development and metastatic spread[32-35]. In addition to inducing effector TH1 cells, *CXCL10* has recently been proven to be associated with the recruitment of CXCR3+ CD8+ T cells to the tumor site and the induction of Granzyme B production by these cells, thereby enhancing their anti-tumor activities[36]. Barreira da Silva *et al*[37] used Dipeptidyl peptidase 4 inhibitors to increase the endogenous level of *CXCL10*, thereby suppressing experimental melanoma. It has also been demonstrated that the combination of *CXCL10* gene therapy and radiotherapy improves therapeutic efficacy in cervical cancer using a HeLa cell murine xenograft tumor model[38]. Numerous studies have demonstrated a positive correlation between increased expression of *CXCL10* at the tumor site and improved prognosis in various human cancers[39-41]. However, the biological functions of *CXCL10* in BL have not been addressed so far. Our study initially discovered that the high expression of *CXCL10* appeared to be associated with a better prognosis. In our prognostic model, *CXCL10* outperforms age, which is an accepted prognostic factor for BL. Further studies are required to investigate and validate the mechanism of *CXCL10* in BL.



DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 6 Functional enrichment analysis results of hub genes. A: The top 10 gene ontology terms of hub genes; B: The top 10 Kyoto Encyclopedia of Genes and Genomes pathways of hub genes. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.


A
Strata SRC = high SRC = low
Survival probability
1.00
0.75
0.50
0.25
0.00
P = 0.79
0 20 40 60 80
Time
B
Strata TLR4 = high TLR4 = low
Survival probability
P = 0.45
Time
C
Strata CD40 = high CD40 = low
Survival probability
P = 0.32
Time
D
Strata STAT3 = high STAT3 = low
Survival probability
P = 0.64
Time
E
Strata SELL = high SELL = low
Survival probability
P = 0.58
Time
F
Strata CXCL10 = high CXCL10 = low
Survival probability
P = 0.029
Time
G
Strata IL2RA = high IL2RA = low
Survival probability
P = 0.0066
Time
H
Strata IL10RA = high IL10RA = low
Survival probability
P = 0.4
Time



DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 7 Kaplan–Meier survival curve. A to J: Kaplan–Meier survival curve of identified hub genes in GSE69051.

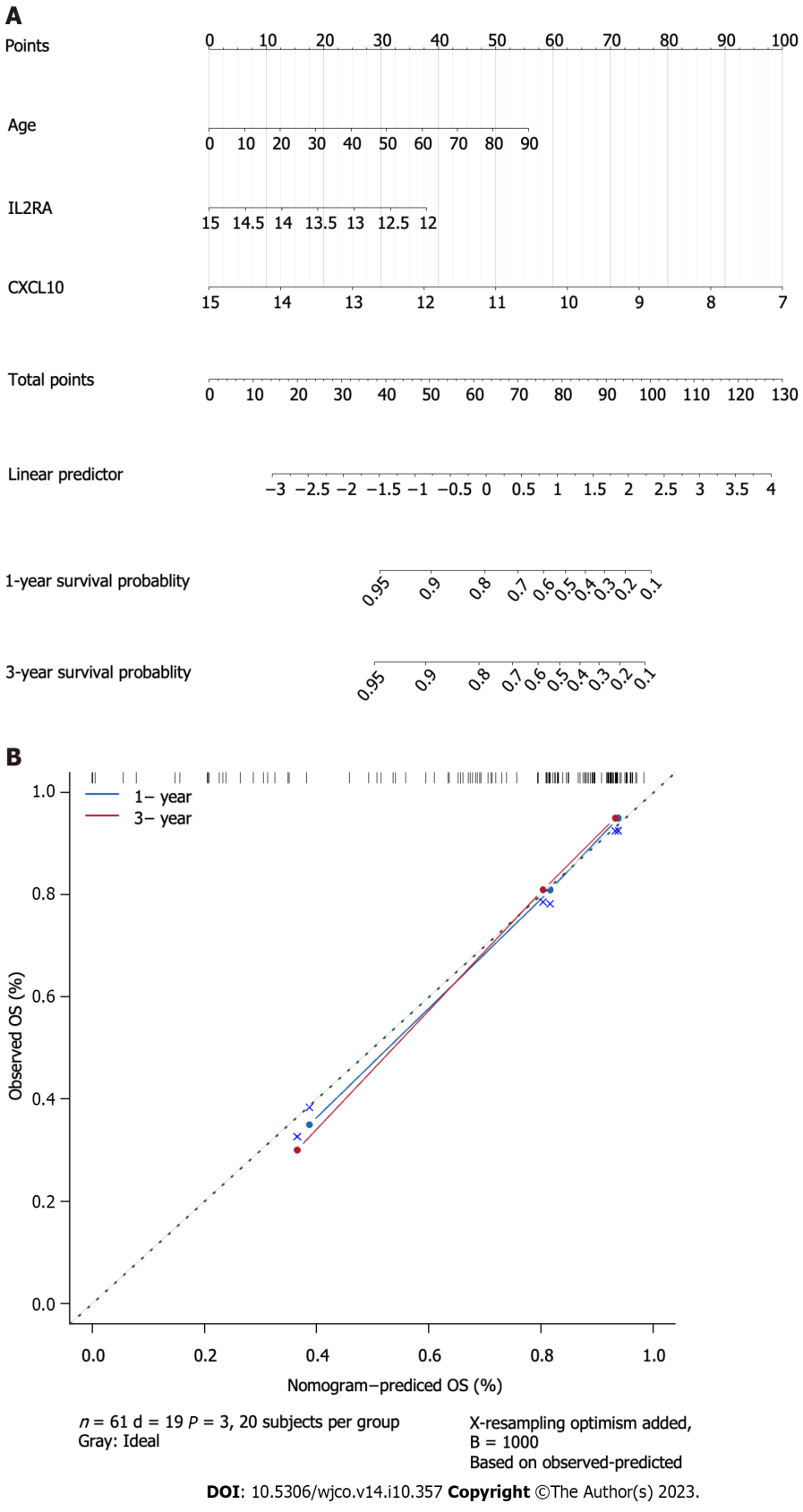
IL2RA (CD25) is a low-affinity receptor for its ligand interleukin 2 (IL2), but when combined with *IL2RB* (CD122) and *IL2RG* (CD133), it forms the high-affinity IL2 receptor[42]. The binding of IL2 to IL2 receptor activates JAK1 and JAK3, which in turn activate several pathways that regulate cell survival and proliferation, including the PI3K/AKT, RAS/RAF/MEK/ERK, and STAT5 pathways[43]. *IL2RA* expression is elevated in a variety of cancers, especially hematologic tumors[44-46]. Fell *et al*[47] studied 69 patients with leukemia, lymphoma, or multiple myeloma and found that the expression of *IL2RA* in T cells was associated with frailty independent of age. This means that patients with high *IL2RA* expression showed better tolerance to chemotherapy and thus might have a superior prognosis. However, another study demonstrated that high *IL2RA* mRNA expression was an independent and adverse prognostic factor in acute myeloid leukemia, specifically stratifying patients into a worse prognosis[48], while reports on *IL2RA* in chronic myelogenous leukemia (CML) were controversial, they described it as either a promoter or an inhibitor of CML cell proliferation and disease aggressiveness[45,46]. This study demonstrated that BL patients with high expression of *IL2RA* exhibited a better prognosis. Due to the controversial reports on the function of *IL2RA* and the lack of research on BL, further studies are required to validate the prognostic value of *IL2RA* in BL.

As a predictive statistical tool, a nomogram visually displays the significant factors that impact outcomes in multifactor regression analyses and simplifies survival probability prediction through an easy-to-understand graphical representation[49]. The construction of the nomogram model in this study is based on age, *IL2RA*, and *CXCL10*. The nomogram effectively visualizes the impact of identified hub genes and facilitates survival prediction, with the multivariate regression analysis serving as the fundamental component of this model. However, the nomogram would benefit from a validation cohort to enhance its current model. Therefore, it is recommended that additional patients with long-term follow-up be included in future studies.

Based on *CXCL10* and *IL2RA*, we have also identified some drugs that may potentially play a therapeutic role in relapsed and refractory BL, which require further research on pharmacology and treatment protocols. There are also some limitations of the present study. Firstly, the sample size may not be sufficient and could result in selection bias. Secondly, the three different clinical types of BL have the same histological features and similar clinical behavior but differ in epidemiology, clinical presentation, and genetic characteristics, which might need to be classified and analyzed separately. What's more, additional genetic and experimental studies are required to explain the mechanism and the function of these hub genes in the carcinogenesis and progression of BLs. Due to the limited experimental conditions, our study exclusively utilized data sourced from publicly available databases. However, further validation is needed in larger samples or more external datasets.

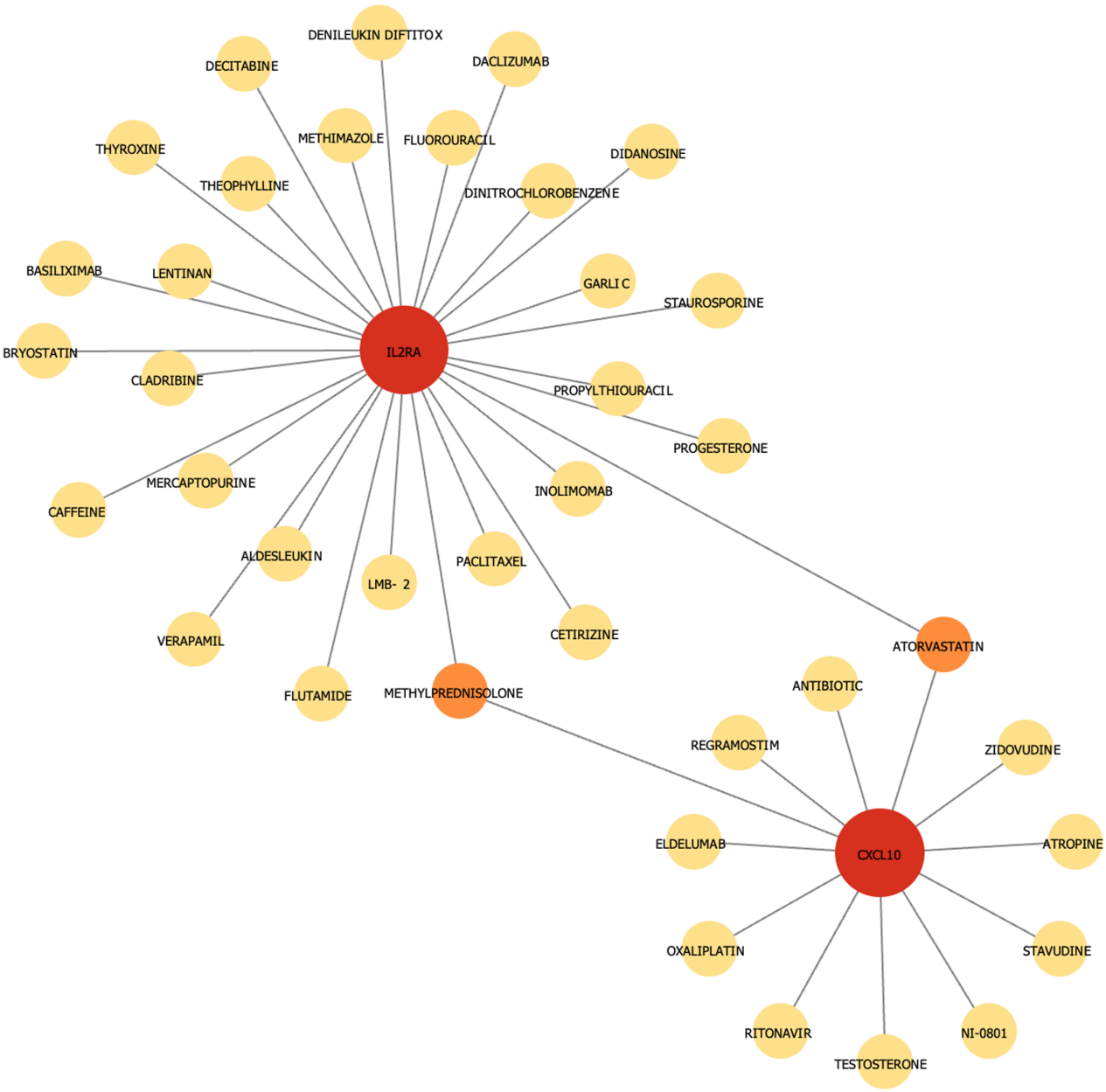
CONCLUSION

In conclusion, this study is the first to investigate gene expression in BL using WGCNA. These findings provide a framework for identifying co-expression gene modules and discriminating key pathways and hub genes in BL. In the present study, we identified and verified 10 hub genes. Survival analysis showed that overexpression of *CXCL10* and *IL2RA* in BL may serve as superior prognostic indicators. Additionally, an integrated mRNA signature and age nomogram potentially offer prognostic value for patients with BLs.



DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 8 Nomogram and calibration plot for GSE69051 cohort. A: The nomogram was constructed to predicting1, 3-year survival rate of Burkitt lymphoma patients; B: The calibration curves for predicting patient survival at 1 and 3 years in the cohort. OS: Overall survival.



DOI: 10.5306/wjco.v14.i10.357 **Copyright** ©The Author(s) 2023.

Figure 9 Drugs related to *IL2RA* and *CXCL10*.

ARTICLE HIGHLIGHTS

Research background

Burkitt lymphoma (BL) is an exceptionally aggressive malignant neoplasm originating from either the germinal center or post-germinal center B cells. However, a standardized treatment regimen for BL has yet to be established. The utilization of microarray data and sequencing information retrieved from public databases presents promising prospects for the identification of novel diagnostic or therapeutic targets.

Research motivation

It is crucial to identify biomarkers that can predict the prognosis of BLs and distinguish patients who would benefit from specific therapies.

Research objectives

The aim of our study was to identify hub genes and conduct gene ontology analysis specifically in BL, as well as perform functional enrichment analysis. Additionally, we performed survival analysis and developed a novel prognostic model incorporating candidate genes along with clinical features.

Research methods
The gene expression profiles and clinical traits of BL patients were obtained from the Gene Expression Omnibus database. Weighted gene co-expression network analysis (WGCNA) was employed to construct gene co-expression modules, while the cytoHubba tool was utilized to identify hub genes. Prognostic candidate genes were identified through overall survival (OS) analysis. A nomogram was developed to evaluate the predictive value of the hub genes.

Research results
In this study, we identified 8 modules through WGCNA analysis and found a significant correlation between the yellow module and age. By using the cytoHubba tool, we identified 10 hub genes (*SRC*, *TLR4*, *CD40*, *STAT3*, *SELL*, *CXCL10*, *IL2RA*, *IL10RA*, *CCR7*, and *FCGR2B*). Among these hubs, two genes (*CXCL10* with $P = 0.029$ and *IL2RA* with $P = 0.0066$) were associated with OS based on our survival analysis.

Research conclusions
This study is the first to investigate gene expression in BL using WGCNA. We have identified and validated 10 hub genes, demonstrating that the overexpression of *CXCL10* and *IL2RA* in BL can serve as robust prognostic indicators. Furthermore, the integration of an mRNA signature with age nomogram holds promising potential for predicting patient outcomes in BLs.

Research perspectives
Further genetic and experimental investigations are imperative to elucidate the underlying mechanism and functional significance of these hub genes in the carcinogenesis and progression of BLs.

FOOTNOTES

Author contributions: Xu YF and Yang JG designed the research; Xu YF, Wang GY and Zhang MY performed the research; Xu YF, Wang GY contributed new reagents/analytic tools; Xu YF analyzed the data; Xu YF, Zhang MY, Wang GY and Yang JG wrote the paper.

Supported by National Natural Science Foundation of China (General Program), No. 82272034.

Institutional review board statement: Institutional review board statement is not applied to our manuscript.

Institutional animal care and use committee statement: Institutional animal care and use committee statement is not applied to our manuscript.

Conflict-of-interest statement: All authors declare that they have no conflicts of interest and have never published the manuscript.

Data sharing statement: The datasets analyzed (GSE4475 and GSE69051) during this study are publicly available in the GEO database (https://www.ncbi.nlm.nih.gov/geo/), the original contributions presented in this study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author (yangjigang@ccmu.edu.cn).

ARRIVE guidelines statement: The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Guan-Yun Wang 0000-0003-1880-7163; Ji-Gang Yang 0000-0002-6489-7780.

S-Editor: Lin C
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Bishop PC**, Rao VK, Wilson WH. Burkitt's lymphoma: molecular pathogenesis and treatment. *Cancer Invest* 2000; **18**: 574-583 [PMID: 10923106 DOI: 10.3109/07357900009012197]

2 **Sall FB**, Shmakova A, Karpukhina A, Tsfasman T, Lomov N, Canoy RJ, Boutboul D, Oksenhendler E, Toure AO, Lipinski M, Wiels J, Germini D, Vassetzky Y. Epstein-Barr virus reactivation induces MYC-IGH spatial proximity and t(8;14) in B cells. *J Med Virol* 2023; **95**: e28633 [PMID: 36866703 DOI: 10.1002/jmv.28633]

3 **Magrath I**. Epidemiology: clues to the pathogenesis of Burkitt lymphoma. *Br J Haematol* 2012; **156**: 744-756 [PMID: 22260300 DOI: 10.1111/j.1365-2141.2011.09013.x]

4 **Ogwang MD**, Bhatia K, Biggar RJ, Mbulaiteye SM. Incidence and geographic distribution of endemic Burkitt lymphoma in northern Uganda revisited. *Int J Cancer* 2008; **123**: 2658-2663 [PMID: 18767045 DOI: 10.1002/ijc.23800]

5 **Sant M**, Allemani C, Tereanu C, De Angelis R, Capocaccia R, Visser O, Marcos-Gragera R, Maynadié M, Simonetti A, Lutz JM, Berrino F; HAEMACARE Working Group. Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. *Blood* 2010; **116**: 3724-3734 [PMID: 20664057 DOI: 10.1182/blood-2010-05-282632]

6 **Guech-Ongey M**, Simard EP, Anderson WF, Engels EA, Bhatia K, Devesa SS, Mbulaiteye SM. AIDS-related Burkitt lymphoma in the United States: what do age and CD4 Lymphocyte patterns tell us about etiology and/or biology? *Blood* 2010; **116**: 5600-5604 [PMID: 20813897 DOI: 10.1182/blood-2010-03-275917]

7 **Smeland S**, Blystad AK, Kvaløy SO, Ikonomou IM, Delabie J, Kvalheim G, Hammerstrøm J, Lauritzsen GF, Holte H. Treatment of Burkitt's/Burkitt-like lymphoma in adolescents and adults: a 20-year experience from the Norwegian Radium Hospital with the use of three successive regimens. *Ann Oncol* 2004; **15**: 1072-1078 [PMID: 15205201 DOI: 10.1093/annonc/mdh262]

8 **Castillo JJ**, Winer ES, Olszewski AJ. Population-based prognostic factors for survival in patients with Burkitt lymphoma: an analysis from the Surveillance, Epidemiology, and End Results database. *Cancer* 2013; **119**: 3672-3679 [PMID: 23913575 DOI: 10.1002/cncr.28264]

9 **Saleh K**, Michot JM, Camara-Clayette V, Vassetsky Y, Ribrag V. Burkitt and Burkitt-Like Lymphomas: a Systematic Review. *Curr Oncol Rep* 2020; **22**: 33 [PMID: 32144513 DOI: 10.1007/s11912-020-0898-8]

10 **Hecht JL**, Aster JC. Molecular biology of Burkitt's lymphoma. *J Clin Oncol* 2000; **18**: 3707-3721 [PMID: 11054444 DOI: 10.1200/JCO.2000.18.21.3707]

11 **Edgar R**, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* 2002; **30**: 207-210 [PMID: 11752295 DOI: 10.1093/nar/30.1.207]

12 **Botía JA**, Vandrovcova J, Forabosco P, Guelfi S, D'Sa K; United Kingdom Brain Expression Consortium, Hardy J, Lewis CM, Ryten M, Weale ME. An additional k-means clustering step improves the biological features of WGCNA gene co-expression networks. *BMC Syst Biol* 2017; **11**: 47 [PMID: 28403906 DOI: 10.1186/s12918-017-0420-6]

13 **Langfelder P**, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 2008; **9**: 559 [PMID: 19114008 DOI: 10.1186/1471-2105-9-559]

14 **Barrett T**, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res* 2013; **41**: D991-D995 [PMID: 23193258 DOI: 10.1093/nar/gks1193]

15 **Niemira M**, Collin F, Szalkowska A, Bielska A, Chwialkowska K, Reszec J, Niklinski J, Kwasniewski M, Kretowski A. Molecular Signature of Subtypes of Non-Small-Cell Lung Cancer by Large-Scale Transcriptional Profiling: Identification of Key Modules and Genes by Weighted Gene Co-Expression Network Analysis (WGCNA). *Cancers (Basel)* 2019; **12** [PMID: 31877723 DOI: 10.3390/cancers12010037]

16 **Szklarczyk D**, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 2021; **49**: D605-D612 [PMID: 33237311 DOI: 10.1093/nar/gkaa1074]

17 **Huang da W**, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 2009; **37**: 1-13 [PMID: 19033363 DOI: 10.1093/nar/gkn923]

18 **Warde-Farley D**, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, Maitland A, Mostafavi S, Montojo J, Shao Q, Wright G, Bader GD, Morris Q. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res* 2010; **38**: W214-W220 [PMID: 20576703 DOI: 10.1093/nar/gkq537]

19 **Wagner AH**, Coffman AC, Ainscough BJ, Spies NC, Skidmore ZL, Campbell KM, Krysiak K, Pan D, McMichael JF, Eldred JM, Walker JR, Wilson RK, Mardis ER, Griffith M, Griffith OL. DGIdb 2.0: mining clinically relevant drug-gene interactions. *Nucleic Acids Res* 2016; **44**: D1036-D1044 [PMID: 26531824 DOI: 10.1093/nar/gkv1165]

20 **Olszewski AJ**, Jakobsen LH, Collins GP, Cwynarski K, Bachanova V, Blum KA, Boughan KM, Bower M, Dalla Pria A, Danilov A, David KA, Diefenbach C, Ellin F, Epperla N, Farooq U, Feldman TA, Gerrie AS, Jagadeesh D, Kamdar M, Karmali R, Kassam S, Kenkre VP, Khan N, Kim SH, Klein AK, Lossos IS, Lunning MA, Martin P, Martinez-Calle N, Montoto S, Naik S, Palmisiano N, Peace D, Phillips EH, Phillips TJ, Portell CA, Reddy N, Santarsieri A, Sarraf Yazdy M, Smeland KB, Smith SE, Smith SD, Sundaram S, Zayac AS, Zhang XY, Zhu C, Cheah CY, El-Galaly TC, Evens AM. Burkitt Lymphoma International Prognostic Index. *J Clin Oncol* 2021; **39**: 1129-1138 [PMID: 33502927 DOI: 10.1200/JCO.20.03288]

21 **Lu J**, Tan H, Li B, Chen S, Xu L, Zou Y. Status and prognostic nomogram of patients with Burkitt lymphoma. *Oncol Lett* 2020; **19**: 972-984 [PMID: 31897210 DOI: 10.3892/ol.2019.11155]

22 **Ferry JA**. Burkitt's lymphoma: clinicopathologic features and differential diagnosis. *Oncologist* 2006; **11**: 375-383 [PMID: 16614233 DOI: 10.1634/theoncologist.11-4-375]

23 **Burmeister T**, Schwartz S, Horst HA, Rieder H, Gökbuget N, Hoelzer D, Thiel E. Molecular heterogeneity of sporadic adult Burkitt-type leukemia/Lymphoma as revealed by PCR and cytogenetics: correlation with morphology, immunology and clinical features. *Leukemia* 2005; **19**: 1391-1398 [PMID: 15973450 DOI: 10.1038/sj.leu.2403847]

24 **Wästerlid T**, Jonsson B, Hagberg H, Jerkeman M. Population based study of prognostic factors and treatment in adult Burkitt lymphoma: a Swedish Lymphoma Registry study. *Leuk Lymphoma* 2011; **52**: 2090-2096 [PMID: 21718134 DOI: 10.3109/10428194.2011.593274]

25 **Schmitz R**, Ceribelli M, Pittaluga S, Wright G, Staudt LM. Oncogenic mechanisms in Burkitt lymphoma. *Cold Spring Harb Perspect Med* 2014; **4** [PMID: 24492847 DOI: 10.1101/cshperspect.a014282]

26 **Fuller TF**, Ghazalpour A, Aten JE, Drake TA, Lusis AJ, Horvath S. Weighted gene coexpression network analysis strategies applied to mouse weight. *Mamm Genome* 2007; **18**: 463-472 [PMID: 17668265 DOI: 10.1007/s00335-007-9043-3]

27 **Mason MJ**, Fan G, Plath K, Zhou Q, Horvath S. Signed weighted gene co-expression network analysis of transcriptional regulation in murine embryonic stem cells. *BMC Genomics* 2009; **10**: 327 [PMID: 19619308 DOI: 10.1186/1471-2164-10-327]

28 **Saris CG**, Horvath S, van Vught PW, van Es MA, Blauw HM, Fuller TF, Langfelder P, DeYoung J, Wokke JH, Veldink JH, van den Berg LH, Ophoff RA. Weighted gene co-expression network analysis of the peripheral blood from Amyotrophic Lateral Sclerosis patients. *BMC Genomics* 2009; **10**: 405 [PMID: 19712483 DOI: 10.1186/1471-2164-10-405]

29 **Chou WC**, Cheng AL, Brotto M, Chuang CY. Visual gene-network analysis reveals the cancer gene co-expression in human endometrial cancer. *BMC Genomics* 2014; **15**: 300 [PMID: 24758163 DOI: 10.1186/1471-2164-15-300]

30 **Liu R**, Cheng Y, Yu J, Lv QL, Zhou HH. Identification and validation of gene module associated with lung cancer through coexpression network analysis. *Gene* 2015; **563**: 56-62 [PMID: 25752287 DOI: 10.1016/j.gene.2015.03.008]

31 **Karin N**, Razon H. Chemokines beyond chemo-attraction: CXCL10 and its significant role in cancer and autoimmunity. *Cytokine* 2018; **109**: 24-28 [PMID: 29449068 DOI: 10.1016/j.cyto.2018.02.012]

32 **Nagarsheth N**, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol* 2017; **17**: 559-572 [PMID: 28555670 DOI: 10.1038/nri.2017.49]

33 **Reymond N**, d'Água BB, Ridley AJ. Crossing the endothelial barrier during metastasis. *Nat Rev Cancer* 2013; **13**: 858-870 [PMID: 24263189 DOI: 10.1038/nrc3628]

34 **Nibbs RJ**, Graham GJ. Immune regulation by atypical chemokine receptors. *Nat Rev Immunol* 2013; **13**: 815-829 [PMID: 24319779 DOI: 10.1038/nri3544]

35 **Murdoch C**, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer* 2008; **8**: 618-631 [PMID: 18633355 DOI: 10.1038/nrc2444]

36 **Zumwalt TJ**, Arnold M, Goel A, Boland CR. Active secretion of CXCL10 and CCL5 from colorectal cancer microenvironments associates with GranzymeB+ CD8+ T-cell infiltration. *Oncotarget* 2015; **6**: 2981-2991 [PMID: 25671296 DOI: 10.18632/oncotarget.3205]

37 **Barreira da Silva R**, Laird ME, Yatim N, Fiette L, Ingersoll MA, Albert ML. Dipeptidylpeptidase 4 inhibition enhances lymphocyte trafficking, improving both naturally occurring tumor immunity and immunotherapy. *Nat Immunol* 2015; **16**: 850-858 [PMID: 26075911 DOI: 10.1038/ni.3201]

38 **Zhao M**, Ma Q, Xu J, Fu S, Chen L, Wang B, Wu J, Yang L. Combining CXCL10 gene therapy and radiotherapy improved therapeutic efficacy in cervical cancer HeLa cell xenograft tumor models. *Oncol Lett* 2015; **10**: 768-772 [PMID: 26622567 DOI: 10.3892/ol.2015.3281]

39 **Bronger H**, Singer J, Windmüller C, Reuning U, Zech D, Delbridge C, Dorn J, Kiechle M, Schmalfeldt B, Schmitt M, Avril S. CXCL9 and CXCL10 predict survival and are regulated by cyclooxygenase inhibition in advanced serous ovarian cancer. *Br J Cancer* 2016; **115**: 553-563 [PMID: 27490802 DOI: 10.1038/bjc.2016.172]

40 **Toiyama Y**, Fujikawa H, Kawamura M, Matsushita K, Saigusa S, Tanaka K, Inoue Y, Uchida K, Mohri Y, Kusunoki M. Evaluation of CXCL10 as a novel serum marker for predicting liver metastasis and prognosis in colorectal cancer. *Int J Oncol* 2012; **40**: 560-566 [PMID: 22038159 DOI: 10.3892/ijo.2011.1247]

41 **Liu M**, Guo S, Stiles JK. The emerging role of CXCL10 in cancer (Review). *Oncol Lett* 2011; **2**: 583-589 [PMID: 22848232 DOI: 10.3892/ol.2011.300]

42 **Flynn MJ**, Hartley JA. The emerging role of anti-CD25 directed therapies as both immune modulators and targeted agents in cancer. *Br J Haematol* 2017; **179**: 20-35 [PMID: 28556984 DOI: 10.1111/bjh.14770]

43 **Olejniczak K**, Kasprzak A. Biological properties of interleukin 2 and its role in pathogenesis of selected diseases--a review. *Med Sci Monit* 2008; **14**: RA179-RA189 [PMID: 18830208]

44 **Saito Y**, Kitamura H, Hijikata A, Tomizawa-Murasawa M, Tanaka S, Takagi S, Uchida N, Suzuki N, Sone A, Najima Y, Ozawa H, Wake A, Taniguchi S, Shultz LD, Ohara O, Ishikawa F. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. *Sci Transl Med* 2010; **2**: 17ra9 [PMID: 20371479 DOI: 10.1126/scitranslmed.3000349]

45 **Sadovnik I**, Hoelbl-Kovacic A, Herrmann H, Eisenwort G, Cerny-Reiterer S, Warsch W, Hoermann G, Greiner G, Blatt K, Peter B, Stefanzl G, Berger D, Bilban M, Herndlhofer S, Sill H, Sperr WR, Streubel B, Mannhalter C, Holyoake TL, Sexl V, Valent P. Identification of CD25 as STAT5-Dependent Growth Regulator of Leukemic Stem Cells in Ph+ CML. *Clin Cancer Res* 2016; **22**: 2051-2061 [PMID: 26607600 DOI: 10.1158/1078-0432.CCR-15-0767]

46 **Kobayashi CI**, Takubo K, Kobayashi H, Nakamura-Ishizu A, Honda H, Kataoka K, Kumano K, Akiyama H, Sudo T, Kurokawa M, Suda T. The IL-2/CD25 axis maintains distinct subsets of chronic myeloid leukemia-initiating cells. *Blood* 2014; **123**: 2540-2549 [PMID: 24574458 DOI: 10.1182/blood-2013-07-517847]

47 **Fell G**, Rosko AE, Abel GA, Dumontier C, Higby KJ, Murillo A, Neuberg DS, Burd CE, Lane AA. Peripheral blood CD3(+) T-cell gene expression biomarkers correlate with clinical frailty in patients with haematological malignancies. *Br J Haematol* 2022; **199**: 100-105 [PMID: 35766906 DOI: 10.1111/bjh.18336]

48 **Du W**, He J, Zhou W, Shu S, Li J, Liu W, Deng Y, Lu C, Lin S, Ma Y, He Y, Zheng J, Zhu J, Bai L, Li X, Yao J, Hu D, Gu S, Li H, Guo A, Huang S, Feng X. High IL2RA mRNA expression is an independent adverse prognostic biomarker in core binding factor and intermediate-risk acute myeloid leukemia. *J Transl Med* 2019; **17**: 191 [PMID: 31171000 DOI: 10.1186/s12967-019-1926-z]

49 **Raghav K**, Hwang H, Jácome AA, Bhang E, Willett A, Huey RW, Dhillon NP, Modha J, Smaglo B, Matamoros A Jr, Estrella JS, Jao J, Overman MJ, Wang X, Greco FA, Loree JM, Varadhachary GR. Development and Validation of a Novel Nomogram for Individualized Prediction of Survival in Cancer of Unknown Primary. *Clin Cancer Res* 2021; **27**: 3414-3421 [PMID: 33858857 DOI: 10.1158/1078-0432.CCR-20-4117]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 October 24; 14(10): 373-399

DOI: 10.5306/wjco.v14.i10.373

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

Comprehensive analysis of disulfidptosis related genes and prognosis of gastric cancer

Qian Li, Long-Kuan Yin

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ozturk E, Turkey; Rhoden CRB, Brazil

Received: July 30, 2023
Peer-review started: July 30, 2023
First decision: August 24, 2023
Revised: September 7, 2023
Accepted: September 18, 2023
Article in press: September 18, 2023
Published online: October 24, 2023

Qian Li, Department of Oncology, Fushun Hospital of Traditional Chinese Medicine, Zigong 643200, Sichuan Province, China

Long-Kuan Yin, Department of Gastrointestinal Surgery, Fushun People's Hospital, Zigong 643200, Sichuan Province, China

Corresponding author: Long-Kuan Yin, Doctor, Researcher, Department of Gastrointestinal Surgery, Fushun People's Hospital, No. 490 Jixiang Road, Fushi Town, Zigong 643200, Sichuan Province, China. 782286066@qq.com

Abstract

BACKGROUND

Gastric cancer (GC) is a common malignant tumor of the digestive system. Disulfidptosis is a new programmed cell death mechanism, although its specific mechanism in GC is incompletely understood.

AIM

In this study, we used bioinformatics analysis to explore a disulfidptosis-based predictive model related to GC prognosis and to identify potential therapeutic targets and sensitive drugs for GC.

METHODS

We extracted GC-related data from The Cancer Genome Atlas and Gene Expression Omnibus databases. R software (version 4.2.1) was used for correlation analysis.

RESULTS

Through the above analysis, we found that the disulfidptosis related gene may be related to the prognosis of GC. Six genes, namely, *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, and *VAMP7*, were found to constitute a predictive model for GC prognosis. *APOD* is a potential therapeutic target for treating GC. Bosutinib and other drugs are sensitive for the treatment of GC.

CONCLUSION

The results of this study indicate that disulfidptosis is related to the prognosis and treatment of GC, while *APOD* represents a potential therapeutic target for GC.

Key Words: Gastric cancer; Disulfidptosis; Drugs; Prognosis; Targets

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Gastric cancer (GC) is a common malignant tumor of the digestive system. Disulfidptosis is a new programmed cell death mechanism. The specific mechanism of disulfidptosis in GC is not fully understood. This study found that the disulfidptosis related gene may be related to the prognosis of gastric cancer. *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, *VAMP7*, these six genes constitute a predictive model for gastric cancer prognosis. *APOD* is a potential therapeutic target. Bosutinib and other drugs are sensitive for the treatment of gastric cancer.

Citation: Li Q, Yin LK. Comprehensive analysis of disulfidptosis related genes and prognosis of gastric cancer. *World J Clin Oncol* 2023; 14(10): 373-399
URL: https://www.wjgnet.com/2218-4333/full/v14/i10/373.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i10.373

INTRODUCTION

Gastric cancer (GC) is a common cause of cancer-related death worldwide, with a particularly high incidence in East Asia, such as South Korea, China, and Japan[1-9]. The early clinical symptoms of GC are not obvious and lack specificity[10-14], which leads to a low rate of early diagnosis[15-26]. Most patients with GC are diagnosed late and have a poor prognosis[27-40]. Although the diagnosis and treatment strategies for GC have gradually increased in recent decades, the prognosis of advanced GC remains poor[41-47]. Therefore, there is an urgent need to find more biomarkers as novel therapeutic targets and to develop new drugs to improve diagnosis and treatment measures and, consequently, patient survival and prognosis.

GC is a heterogeneous disease[48], with previous studies suggesting that various cell programmed death mechanisms, including ferroptosis[49-54] and cuproptosis[55-58], represent novel research directions for GC. In recent years, it has been found that disulfidptosis[59], a novel and poorly studied mechanism of programmed cell death, represents a previously uncharacterized form of cell death induced by abnormal accumulation of disulfide in cells under glucose starvation, which is different from copper death and iron death. However, its role in GC and its related mechanisms are still unclear and need to be further explored.

In this study, we analyzed the sequencing data of tumor tissues from databases such as The Cancer Genome Atlas (TCGA)[60] and Gene Expression Omnibus (GEO) (Supplementary material)[61] and 14 disulfidptosis-related gene (DRGs)[59] (*ACTN4, ACTB, CD2AP, CAPZB, DSTN, FLNA, FLNB, INF2, IQGAP1, MYH10, MYL6, MYH9, PDLIM1,* and *TLN1*). We conducted differential analysis of DRGs, as well as analyses of the tumor mutation burden (TMB)[62,63], copy variations, gene ontology (GO)[64], and the kyoto encyclopedia of genes and genomes (KEGG)[65], among others. In this paper, the mechanism of DRGs involved in the occurrence and development of GC is discussed, and new therapeutic targets and drugs that may be related to the prognosis of GC are preliminarily analyzed and screened from a new perspective.

MATERIALS AND METHODS

Data downloading and processing

Expression data, clinical data, mutation data, and copy data related to GC were downloaded and organized from TCGA database. The GSE84433 and GSE26253 datasets and their platform annotation files were downloaded from the GEO database. Data were analyzed and processed using R software (version 4.2.1) and Perl software (version 5.30.0).

Differential and prognostic analyses

GC-related data were extracted from TCGA database and analyzed in combination with the disulfidptosis-related gene. Differential analysis, mutation load analysis, copy number variation frequency analysis, and survival analysis were performed using R software.

Disulfidptosis subtype analysis

R software was used to classify all samples related to the disulfidptosis-related gene in TCGA and GEO databases for survival analysis, heatmap clustering, gene set variation analysis (GSVA), immune cell differential analysis, subtype differential analysis, and GO and KEGG enrichment analyses.

Significant differential gene subtyping, prediction model construction, and analysis

We continued to perform survival analysis, heatmap clustering, and differential analysis of the DRGs on the samples classified by differential gene subtyping. Then, we randomly divided the significant differential samples into groups and performed least absolute shrinkage and selection operator (LASSO) regression analysis and univariate and multivariate

Cox regression analyses and constructed a prognostic model. Using the prognostic model, we calculated the risk score for each patient sample using the following formula:.where $Coef_i$ is the coefficient, and X_i is the expression level of the gene. We constructed a prognostic evaluation model for overall survival based on the risk score. We then constructed a Sankey diagram and analyzed the differences in risk scores between subtypes and the differential risk of the DRGs.

Prognostic model validation

The reliability of the prognostic model was verified by survival analysis, receiver operating characteristic (ROC) curve mapping, risk curve mapping, survival state map, and clustering heatmap of model genes in each subgroup.

Nomogram construction and analysis of the correlation between risk score and immunity, as well as drug susceptibility

Next, the independent prognostic factors of GC and potential therapeutic targets were sought by constructing the column diagram, and survival analysis of potential prognostic genes was performed by Gene Expression Profiling Interactive Analysis (GEPIA). Subsequently, immune cell correlation analysis, tumor microenvironment (TME) difference analysis, waterfall map construction, tumor mutation load analysis, microsatellite instability (MSI), stem cell correlation analysis, and drug sensitivity analysis were performed for the risk score.

Immunohistochemical analysis

We conducted immunohistochemical analysis of *APOD* using the human protein atlas (HPA) network database, comparing the differences in protein expression between GC tissues and adjacent normal tissues.

Statistical analysis

All statistical analyses were performed using R software (version 4.2.1). A P-value < 0.05 was considered statistically significant.

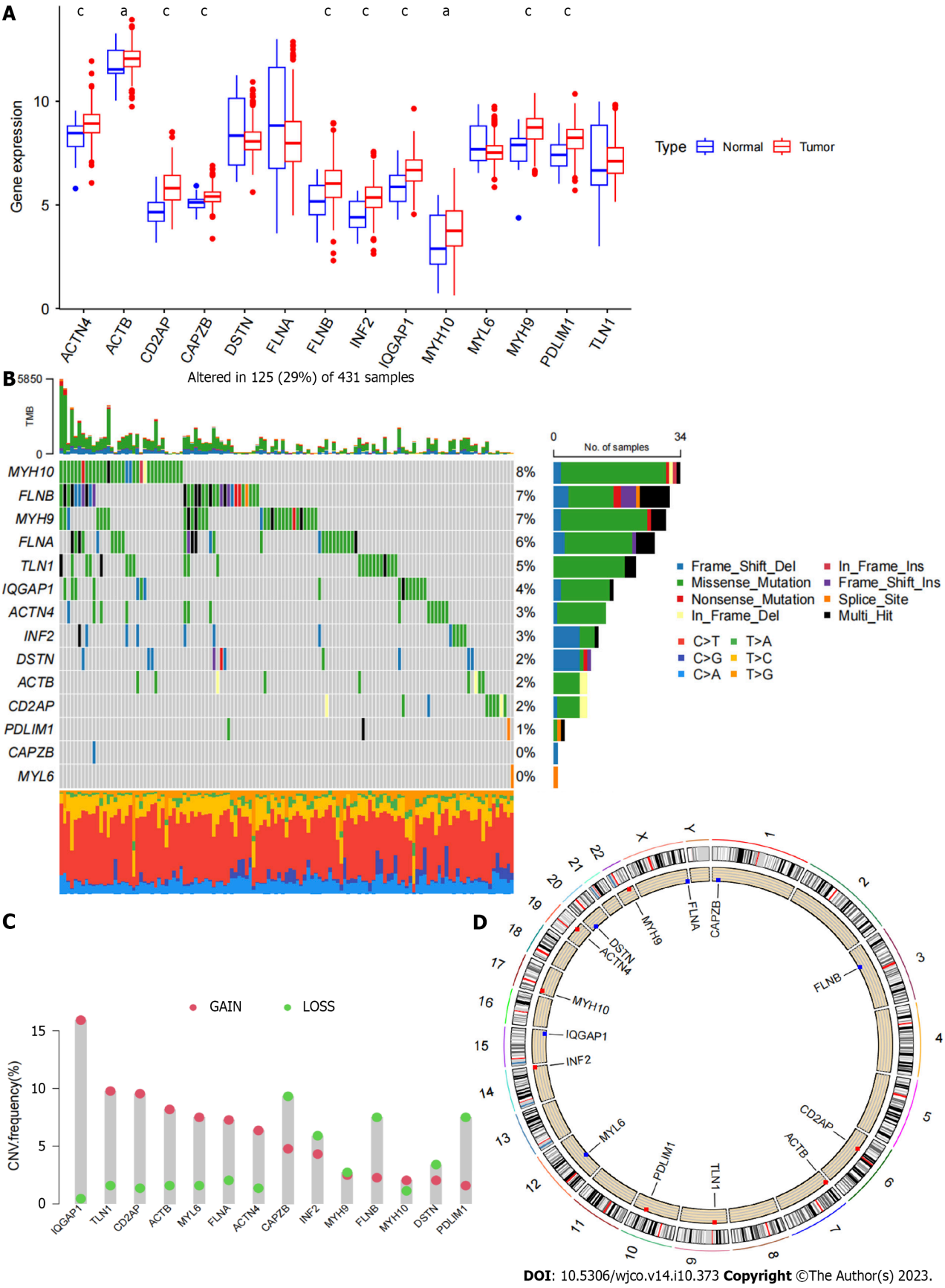
RESULTS

Difference analysis and prognosis analysis of DRGs

Difference analysis revealed that 10 DRGs, namely, *ACTN4*, *ACTB*, *CD2AP*, *CAPZB*, *FLNB*, *INF2*, *IQGAP1*, *MYH10*, *MYH9* and *PDLIM1*, were significantly different in GC samples and adjacent normal tissue samples (Figure 1A). Through mutation load analysis, copy number variation frequency analysis, and a genosphere map, we found that *CAPZB* and *MYL6* were not mutated, while *MYH10* had the most mutations. It was also found that *CAPZB* had the most deletion mutations, while *IQGAP1* had the most insertion mutations. Cyclic analysis led to the identification of disulfidptosis mutations in 14 chromosomes (Figure 1B-D). Moreover, survival analysis showed that patients with high expression of *TLN1*, *MYL6*, *MYH10*, *MYH9*, *IQGAP1*, *INF2*, *FLNA*, *DSTN*, and *ACTB* had a reduced survival time, while those with high expression of *PDLIM1* had an increased survival time (Figure 2A–J). Prognostic network diagram analysis showed that disulfidptosis-related genes, including *PDLIM1*, *FLNA*, *MYH10*, *MYL6*, and *DSTN*, were significantly correlated with the prognosis of GC (P < 0.05), and *DSTN*, *FLNA*, *MYH10*, and *MYL6* were risk factors for the prognosis of GC, while *PDLIM1* was a favorable factor for the prognosis of GC (Figure 2K).

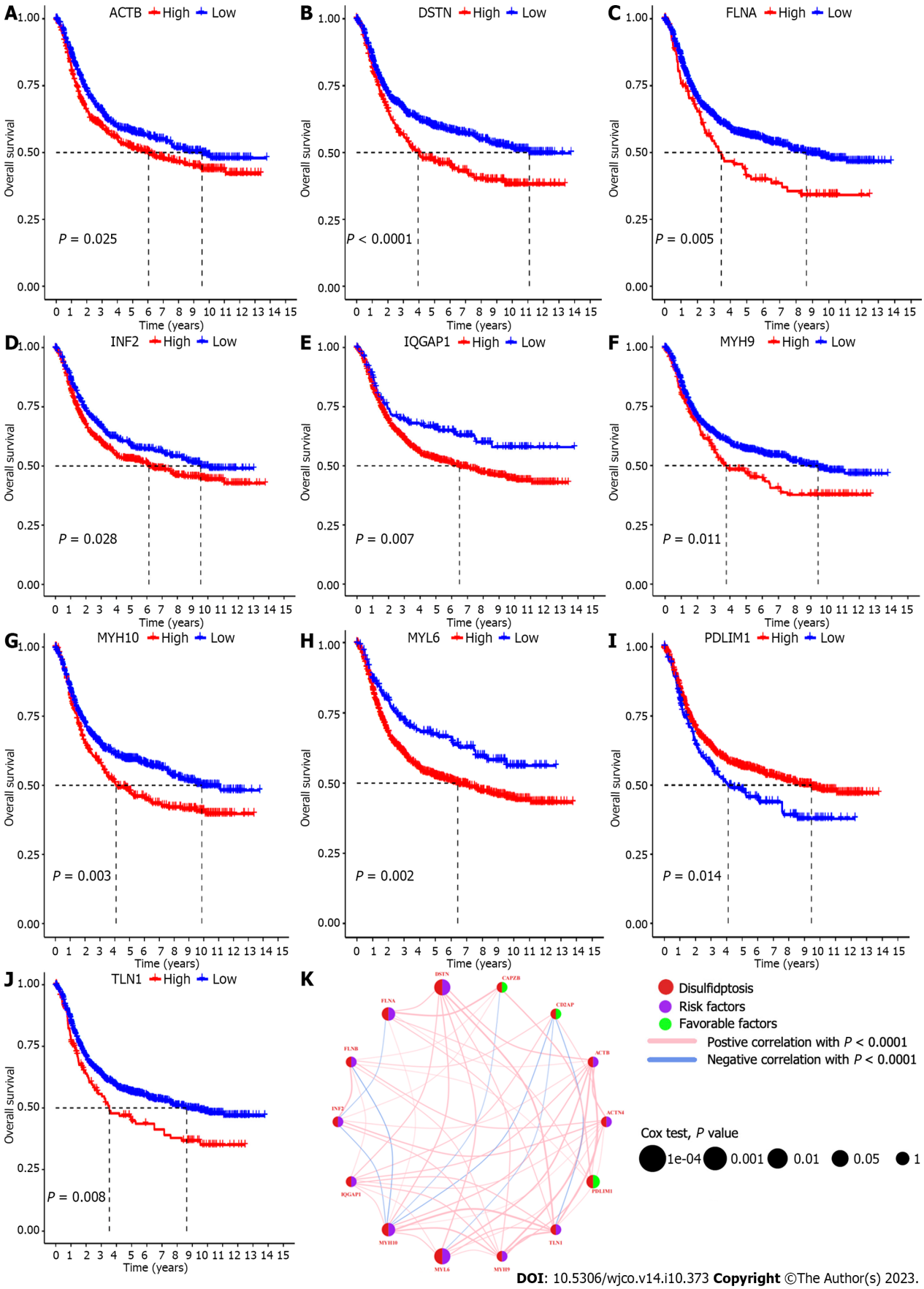
Subtyping of the DRGs and analysis through GSVA, single-sample gene set enrichment analysis, GO, and KEGG analyses

Through clustering analysis of the DRG samples, we found that the best way to divide the samples was into two subtypes, A and B (Figure 3A-D). Through survival analysis of the two subtypes, we found significant differences between the groups, P < 0.05 (Figure 3E), and through clustering heatmap analysis, we found that most DRGs were upregulated in cluster A and downregulated in cluster B (Figure 3F). Using the GSVA package in R software, we performed KEGG pathway enrichment analysis on the DRG subtyping samples and found that the significantly different pathways enriched in the two subtypes included glutamate and glutamine metabolism, extracellular matrix receptor interaction, the transforming growth factor-beta (TGF-β) signaling pathway, and the pentose phosphate pathway (Figure 4A). Through GO functional enrichment analysis of the DRG subtyping samples with the GSVA package in R, we found that the main enrichment was in the positive regulation of the transforming growth factor receptor and Wnt signaling pathways (Figure 4B). We also found significant differences in immune cells, such as activated CD4 T cells, and activated CD8 T cells, between subtypes A and B, according to the analysis of the differences in immune cells between the subtypes (Figure 5A). Subtype differential analysis led to the identification of 282 significantly different co-expressed genes between subtypes A and B (Figure 5B and C). Moreover, GO analysis of these differentially expressed genes revealed that the enriched functions of these differentially expressed genes were mainly in the extracellular matrix tissue and negative regulation of the typical Wnt signaling pathway (Figure 5D). KEGG analysis of these differentially expressed genes revealed that these genes were enriched in the TGF-β, Wnt, and MAPK signaling pathways, as well as in other pathways (Figure 5E).



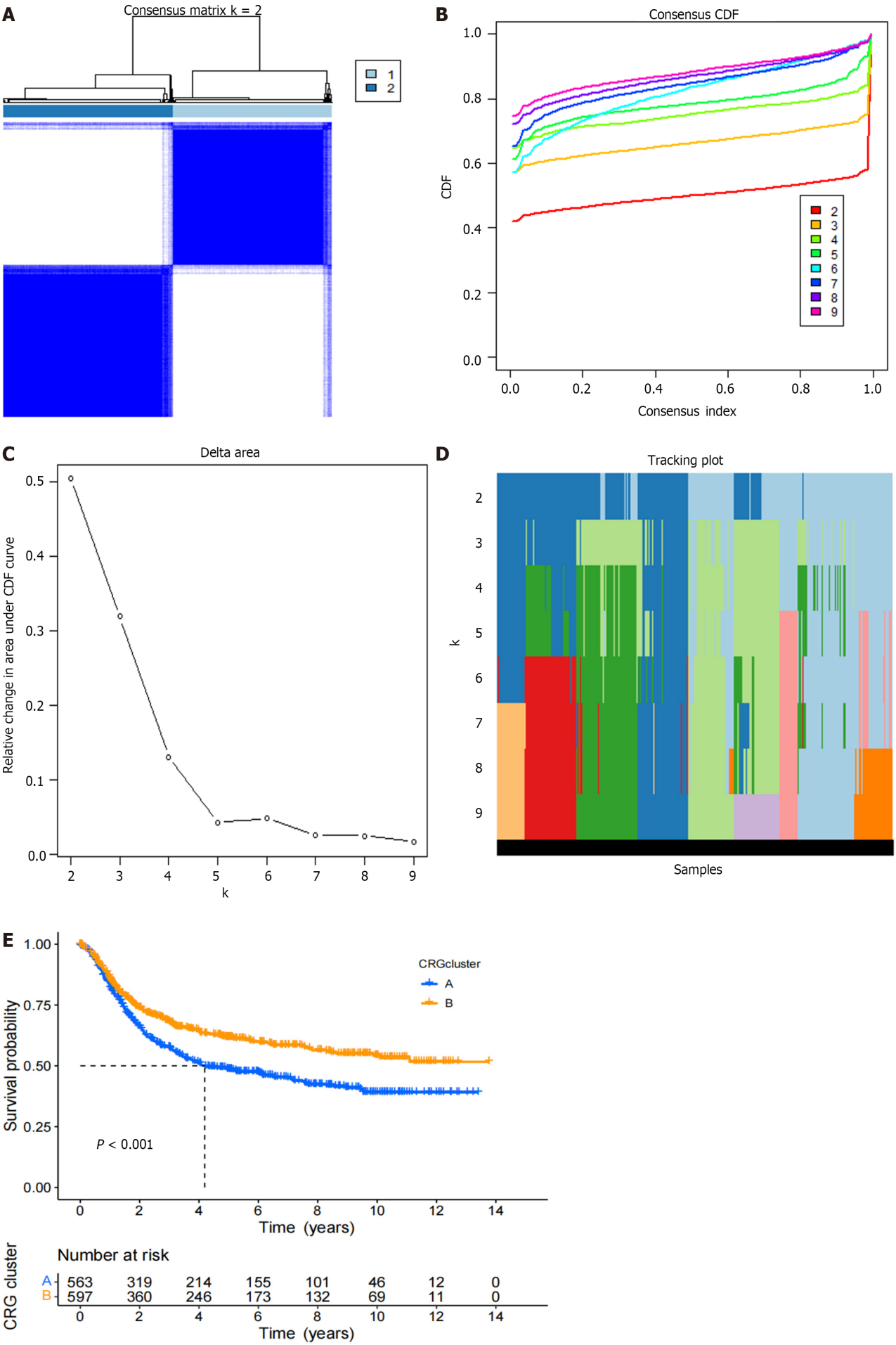
DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

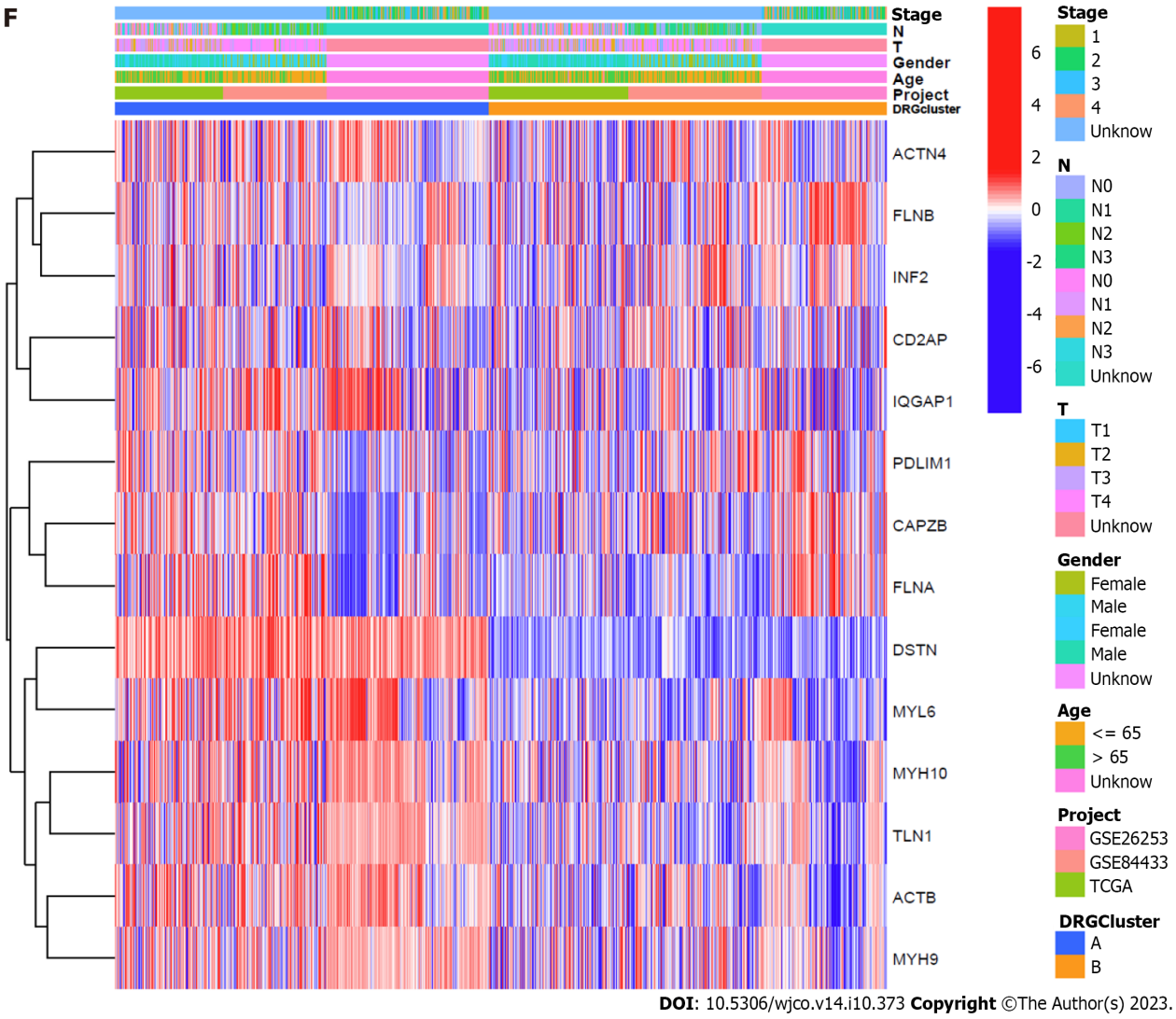
Figure 1 The results of the differential expression analysis of disulfidptosis related genes in gastric cancer and adjacent normal tissues are presented. A: Shows the difference analysis of disulfidptosis related genes in gastric cancer tissue samples and adjacent normal tissue samples; B: Shows the waterfall plot of disulfidptosis related genes mutations; C: Presents the mutation frequency of disulfidptosis related genes; D: Shows the mutation sites of disulfidptosis related genes. CNV: Copy number variation.



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

Figure 2 Screening disulfidptosis related genes related to the prognosis of gastric cancer. A-J: Show the Kaplan-Meier analysis of the survival curves of disulfidptosis related genes between high and low expression groups, and 10 disulfidptosis related genes related to gastric cancer prognosis were identified; K: Shows the COX analysis of the disulfidptosis related genes circle plot related to gastric cancer prognosis, and five significantly prognostic disulfidptosis related genes were identified.


A
Consensus matrix k = 2
1
2
B
Consensus CDF
CDF
Consensus index
2
3
4
5
6
7
8
9
C
Delta area
Relative change in area under CDF curve
k
D
Tracking plot
k
Samples
E
CRGcluster
A
B
Survival probability
P < 0.001
Time (years)
Number at risk
CRG cluster
A 563 319 214 155 101 46 12 0
B 597 360 246 173 132 69 11 0
Time (years)



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

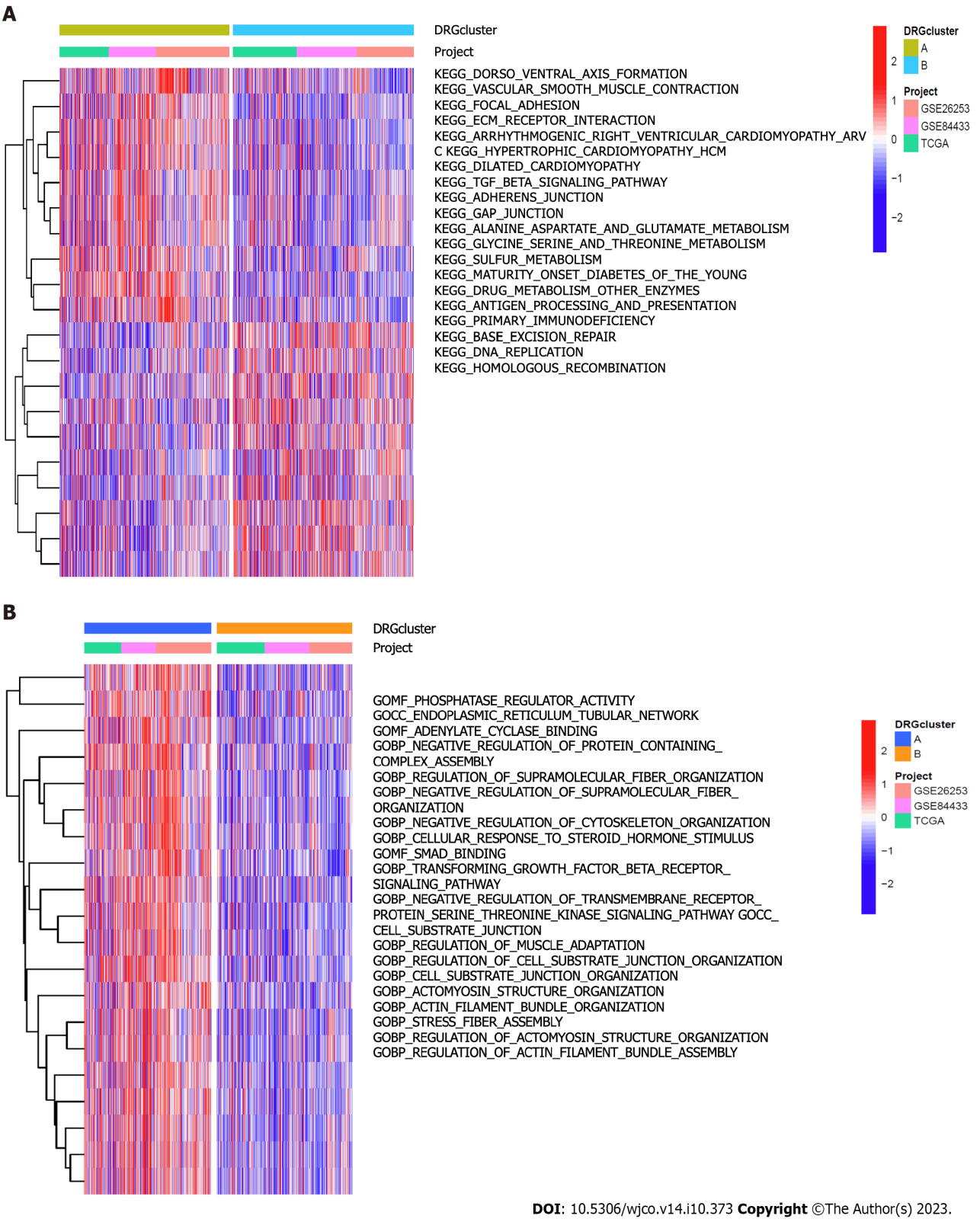
Figure 3 The sample classification, subgroup survival analysis, and differential gene heatmap related to disulfidptosis related genes are presented. A: Shows the clustering matrix plot of disulfidptosis related genes-related samples; B: Shows the clustering index plot of disulfidptosis related genes-related samples; C: Presents the relative change area under the cumulative distribution function curve; D: Shows the tracking plot of disulfidptosis related genes subgroup samples; E: Presents the survival analysis curves of disulfidptosis related genes subgroups; F: Shows the differential gene clustering heatmap between disulfidptosis related genes subgroups.

Classification and correlation analysis of significant differentially expressed genes obtained from the disulfidptosis subtype samples

The related samples of differentially expressed genes were clustered into three subtypes (Figure 6A–D). Survival analysis showed that the prognosis of subtype C was different from that of subtypes A and B, with better prognosis for subtypes B and C (Figure 6E). Heatmap analysis showed that most samples in subtype C were upregulated, while most samples in subtype B were downregulated (Figure 6F). Differential expression analysis of the DRGs in the different gene subtypes showed that the expression of the DRGs was different in subtypes A, B, and C, with $P < 0.05$ (Figure 6G). We used the create data partition package to randomly divide the samples into two groups of equal size, the training and testing groups. Then, using LASSO regression and Cox regression, we analyzed the training group samples and constructed a six-gene risk model based on the DRG subtype: Risk score = (0.164102181511909* *PLS3* expression) + (0.079055019007862* *GRP* expression) + (0.0649967121599996* *APOD* expression) + (0.0920219139298833* *SGCE* expression) + (0.107438278125729* *COL8A1* expression) + (–0.0723643090076661* *VAMP7* expression) (Figure 6H and I). The results of the risk model are shown in Supplementary Table 1. The Sankey diagram shows the distribution of samples among the different groups (Figure 7A). By evaluating the risk score for each group, we found significant differences in the risk between the groups (Figure 7B and C). By evaluating the risk score for the DRGs, we found that the expression levels of 13 DRGs differed significantly between the high and low risk groups, with nine genes showing higher expression in the high-risk group and four genes showing higher expression in the low-risk group (Figure 7D).

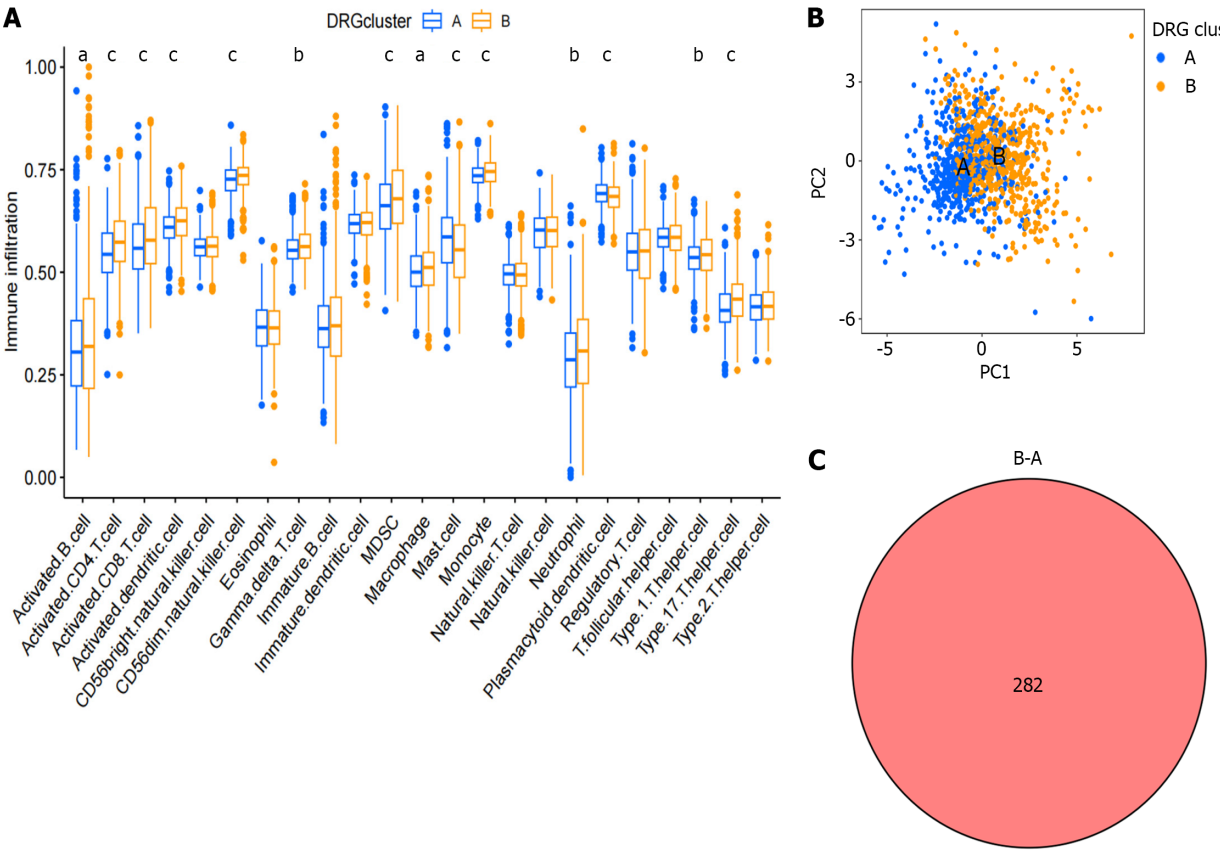
Validation results of the risk model

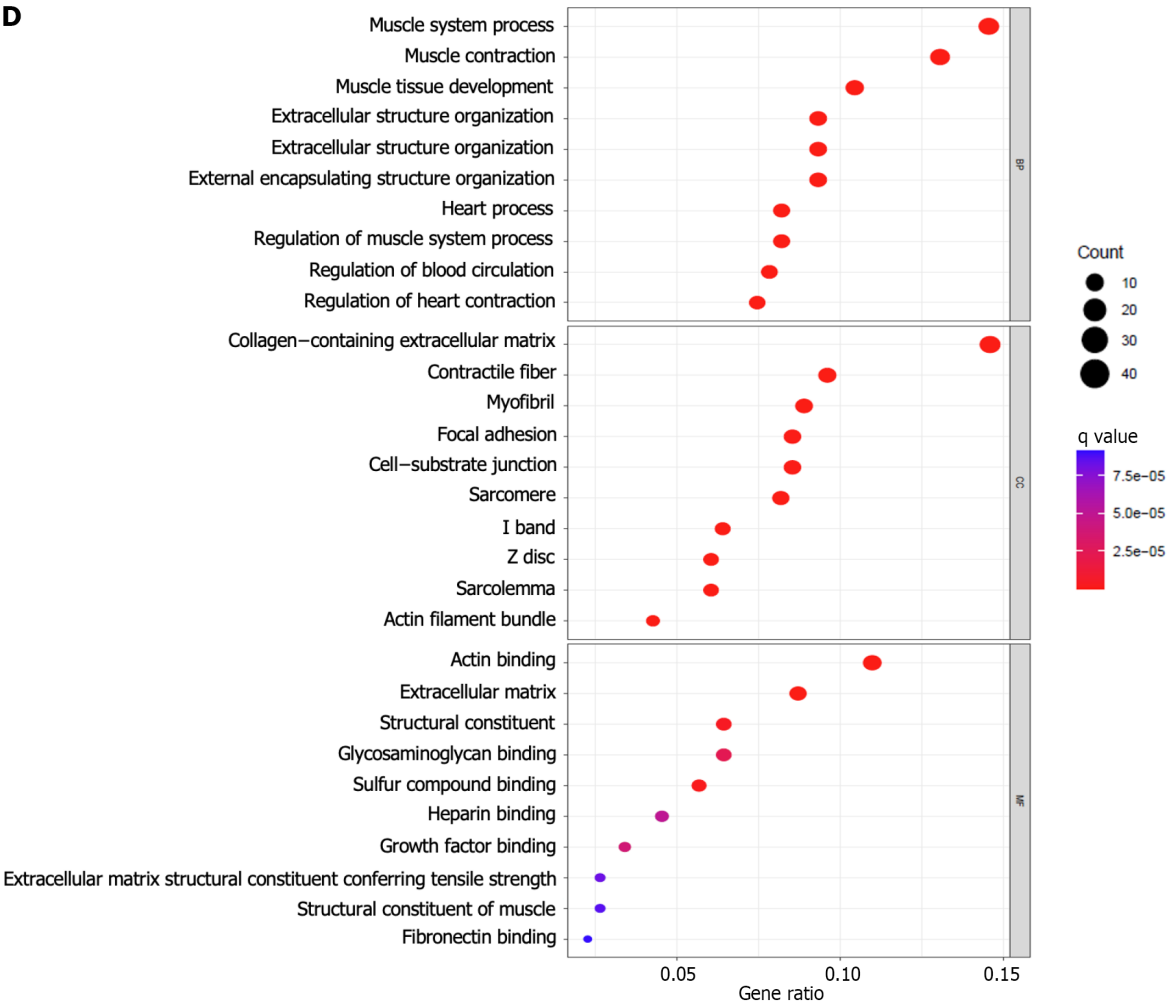
We next used the risk model to score the differential gene-related samples mentioned above and then divided them into

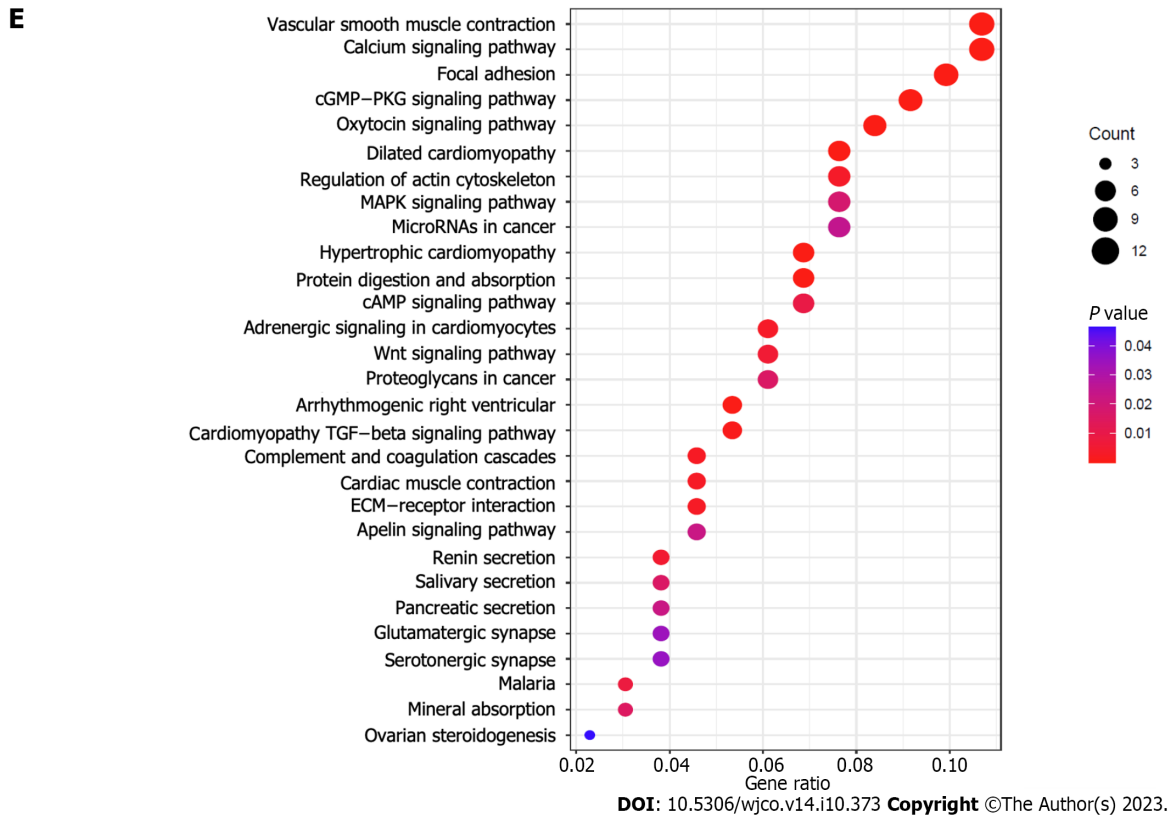


DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

Figure 4 The significantly different kyoto encyclopedia of genes and genomes pathways and gene ontology functional analysis between disulfidptosis related genes subgroups are presented. A: Shows the significantly different kyoto encyclopedia of genes and genomes pathway enrichment analysis between disulfidptosis related genes subgroups; B: Shows the significantly different gene ontology pathway enrichment analysis between disulfidptosis related genes subgroups.


A
DRGcluster A B
Immune infiltration
1.00
0.75
0.50
0.25
0.00
a c c c c b c a c c b c b c
Activated.B.cell
Activated.CD4.T.cell
Activated.CD8.T.cell
Activated.dendritic.cell
CD56bright.natural.killer.cell
CD56dim.natural.killer.cell
Eosinophil
Gamma.delta.T.cell
Immature.B.cell
Immature.dendritic.cell
MDSC
Macrophage
Mast.cell
Monocyte
Natural.killer.T.cell
Natural.killer.cell
Neutrophil
Plasmacytoid.dendritic.cell
Regulatory.T.cell
T.follicular.helper.cell
Type.1.T.helper.cell
Type.17.T.helper.cell
Type.2.T.helper.cell
B
DRG cluster
A
B
PC2
PC1
3
0
-3
-6
-5
0
5
C
B-A
282


D
Muscle system process
Muscle contraction
Muscle tissue development
Extracellular structure organization
Extracellular structure organization
External encapsulating structure organization
Heart process
Regulation of muscle system process
Regulation of blood circulation
Regulation of heart contraction
Collagen−containing extracellular matrix
Contractile fiber
Myofibril
Focal adhesion
Cell−substrate junction
Sarcomere
I band
Z disc
Sarcolemma
Actin filament bundle
Actin binding
Extracellular matrix
Structural constituent
Glycosaminoglycan binding
Sulfur compound binding
Heparin binding
Growth factor binding
Extracellular matrix structural constituent conferring tensile strength
Structural constituent of muscle
Fibronectin binding
BP
CC
MF
0.05
0.10
0.15
Gene ratio
Count
10
20
30
40
q value
7.5e−05
5.0e−05
2.5e−05



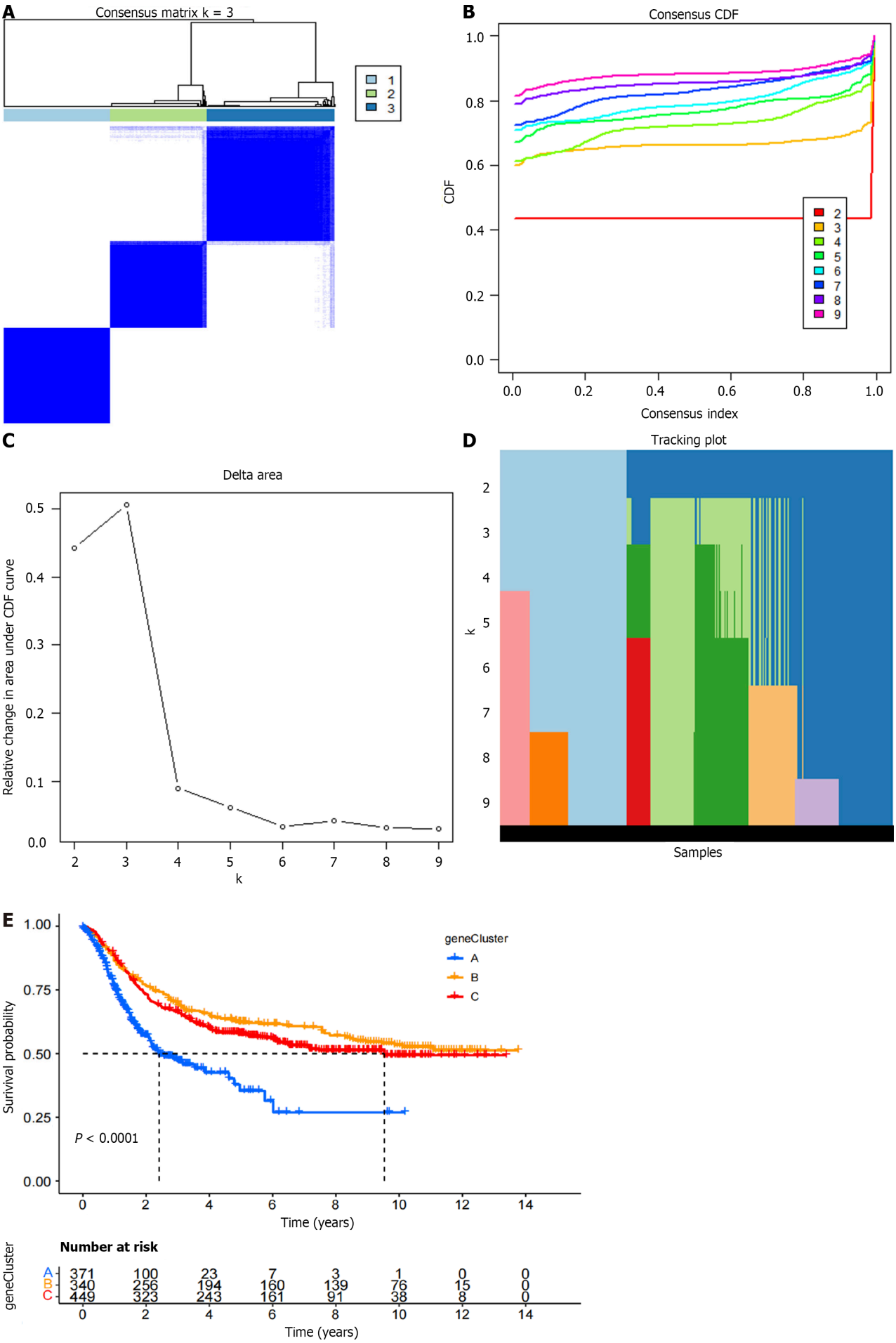
DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

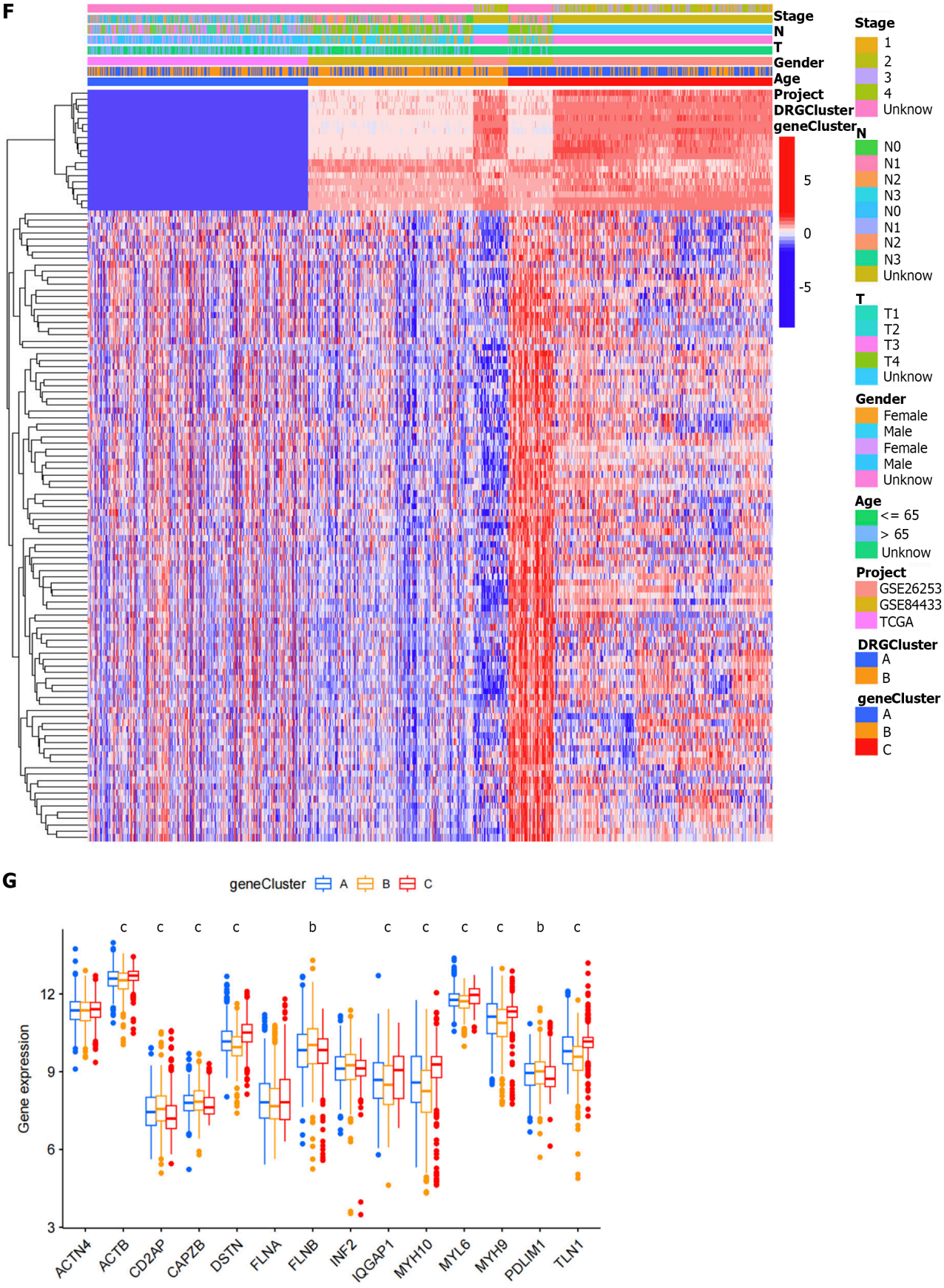
Figure 5 The immune cell differential analysis, principal component analysis analysis, significantly different genes, and gene ontology/kyoto encyclopedia of genes and genomes analysis between disulfidptosis related genes subgroups are presented. A: Shows the immune cell differential analysis between disulfidptosis related genes subgroups; B: Presents the principal component analysis analysis of disulfidptosis related genes subgroups; C: Shows the significantly different genes between disulfidptosis related genes subgroups; D: Presents the gene ontology analysis of significantly different genes between disulfidptosis related genes subgroups; E: Presents the kyoto encyclopedia of genes and genomes analysis of significantly different genes between disulfidptosis related genes subgroups.

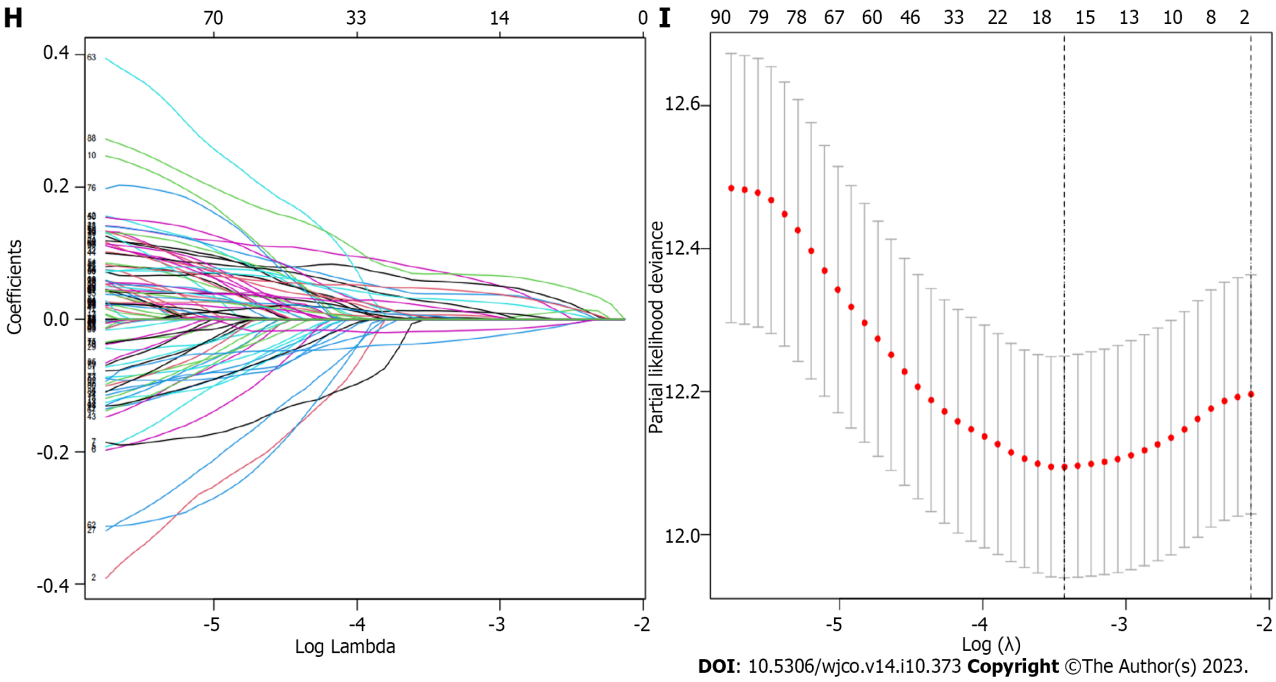
high- and low-risk groups for the overall, test set, and training set samples. We then performed survival, ROC, and risk analyses on each group. Survival analysis of each group revealed that the high-risk group had poorer prognosis than the low-risk group (Figure 8A-C). Through ROC curve analysis of each group, we found that the area under the curve values of the overall, training set, and test set samples for 1, 3, and 5 years were all > 0.05, indicating the accuracy of the model in predicting survival prognosis (Figure 8D-F). Risk curve analysis of the total, training set, and test set samples showed an increase in the number of deaths with an increase in the risk score. We also found that *VAMP7* was a low-risk gene, while *PLS3*, *GRP*, *APOD*, *SGCE*, and *COL8A1* were high-risk genes through heatmap analysis (Figure 8G-O). Comparison of the results of survival, ROC, and risk analyses among various groups showed that the results were consistent, indicating the accuracy of this risk model in predicting the prognosis of patients with GC.

Identification of potential therapeutic targets by constructing column line graphs and immune and drug sensitivity analyses

We found that *APOD*, *PLS3*, age, sex, and N staging are independent factors that impact patient prognosis, all of which are risk factors for the prognosis of patients with GC. The odds of patients surviving for 1, 3, and 5 years are 0.806, 0.527, and 0.39, respectively (Figure 9A). The correction curve shows that the predicted value of the model is close to the actual value (Figure 9B). Through immune cell analysis, we found that resting mast cells and *APOD* were significantly positively correlated. Moreover, *PLS3* was significantly positively correlated with resting mast cells (Figure 9C). We also conducted survival analysis on *APOD* and *PLS3* by GEPIA, which were found to have independent effects on the prognosis of GC through column line graph analysis, and found that the survival analysis of *APOD* showed significant differences ($P < 0.05$) (Figure 9D), while the survival analysis of *PLS3* did not indicate the presence of significant differences ($P > 0.05$) (Figure 9E). In the relationship analysis between immune cells and risk scores, we found that 13 types of immune cells were significantly correlated with risk scores (Figure 10). Through TME scoring, we found differences between high and low risk groups in terms of the Stromal Score and ESTIMATE Score, both of which were upregulated in the high-risk group (Figure 11A). The waterfall chart shows that the genes that undergo mutations in the high- and low-risk groups were consistent, while the probability of mutations occurring in the low-risk group was higher than that in the high-risk group (Figure 11B and C). Through TMB analysis, we found significant differences between the high- and low-risk groups, as well as a negative correlation between TMB and risk scores (Figure 11D and E). Through microsatellite instability analysis, we found significant differences between the microsatellite stability and MSI-high (MSI-


A
Consensus matrix k = 3
1
2
3
B
Consensus CDF
CDF
Consensus index
2
3
4
5
6
7
8
9
C
Delta area
Relative change in area under CDF curve
k
D
Tracking plot
k
Samples
E
geneCluster
A
B
C
Surivival probability
P < 0.0001
Time (years)
Number at risk
geneCluster
A 371 100 23 7 3 1 0 0
B 340 256 194 160 139 76 15 0
C 449 323 243 161 91 38 8 0
Time (years)


F
Stage
N
T
Gender
Age
Project
DRGCluster
geneCluster
5
0
-5
Stage
1
2
3
4
Unknow
N
N0
N1
N2
N3
N0
N1
N2
N3
Unknow
T
T1
T2
T3
T4
Unknow
Gender
Female
Male
Female
Male
Unknow
Age
<= 65
> 65
Unknow
Project
GSE26253
GSE84433
TCGA
DRGCluster
A
B
geneCluster
A
B
C
G
geneCluster A B C
c c c c b c c c c b c
Gene expression
12
9
6
3
ACTN4
ACTB
CD2AP
CAPZB
DSTN
FLNA
FLNB
INF2
IQGAP1
MYH10
MYL6
MYH9
PDLIM1
TLN1



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

Figure 6 The differential gene-related sample clustering matrix, clustering index, cumulative distribution function curve, tracking plot, survival curve, heat map, differential analysis of disulfidptosis related genes, lasso regression plot, and cvfit plot are presented. A: Shows the clustering matrix of differential gene-related samples; B: Presents the clustering index of differential gene-related samples; C: Shows the relative change area of the cumulative distribution function curve of differential gene-related samples; D: Presents the tracking plot of differential gene subgroups; E: Shows the survival curve of differential gene-related samples; F: Presents the heat map of differential gene-related samples; G: Presents the differential analysis of disulfidptosis related genes between differential gene-related sample groups; H: Shows the lasso regression plot; I: Presents the cvfit plot of the lasso regression.

H) groups, as well as between the MSI-H and MSI-low groups. The risk value of the MSI-H group is the lowest, and the proportion of stable samples in the high-risk group is as high as 71% (Figure 11F and G). Stem cell correlation analysis shows that RNA stemness scores (RNAss) is negatively correlated with risk scores (Figure 11H). Finally, drug analysis showed significant differences in the sensitivity of 89 drugs, including Bosutinib and Bryostatin (Figure 11I and J), between high- and low-risk groups.

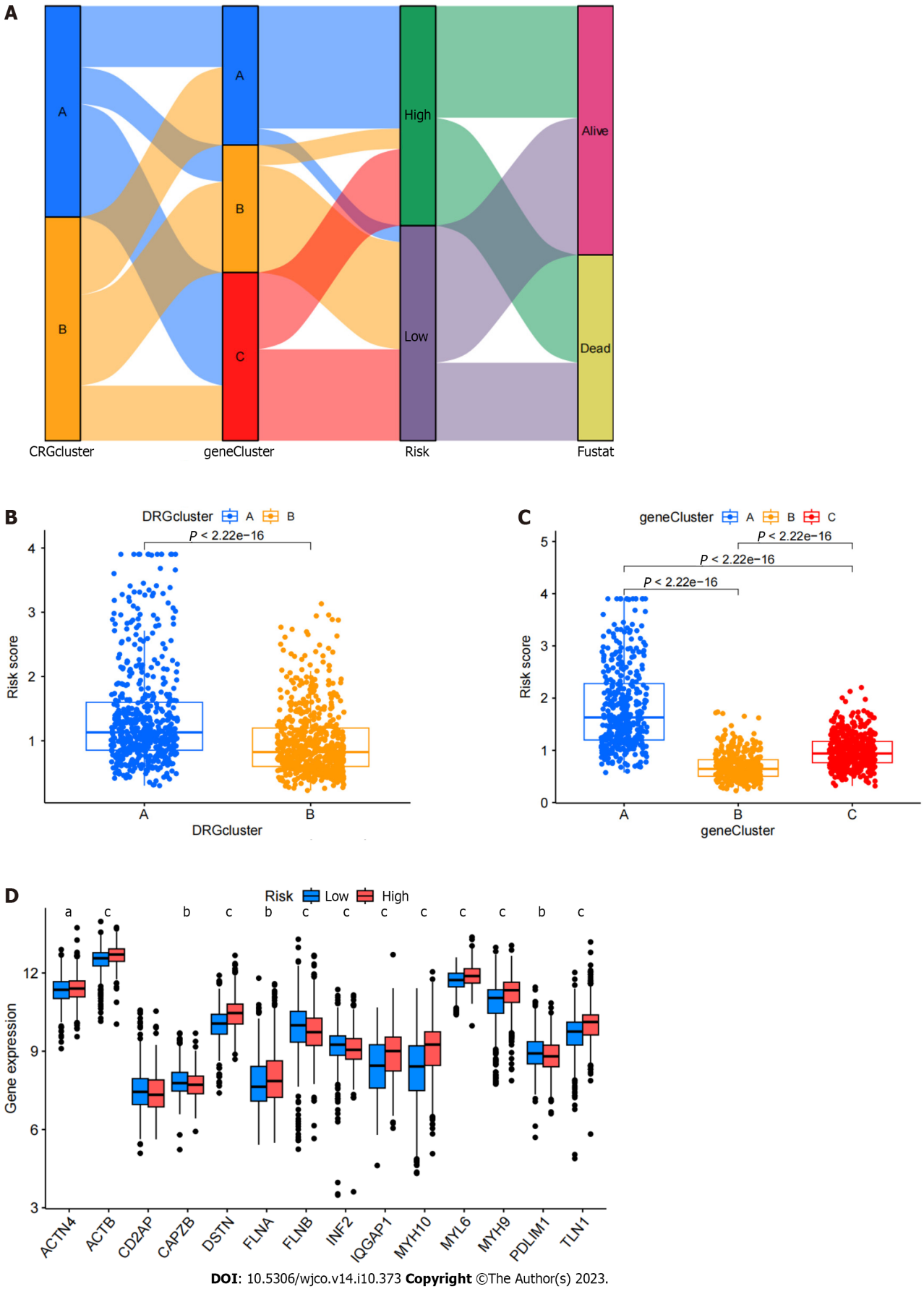
Immunohistochemical analysis

Through the HPA network database, immunohistochemical analysis of *APOD* revealed that the protein expression level of *APOD* in GC tissues was significantly higher than that in normal tissues adjacent to GC (Figure 12).

DISCUSSION

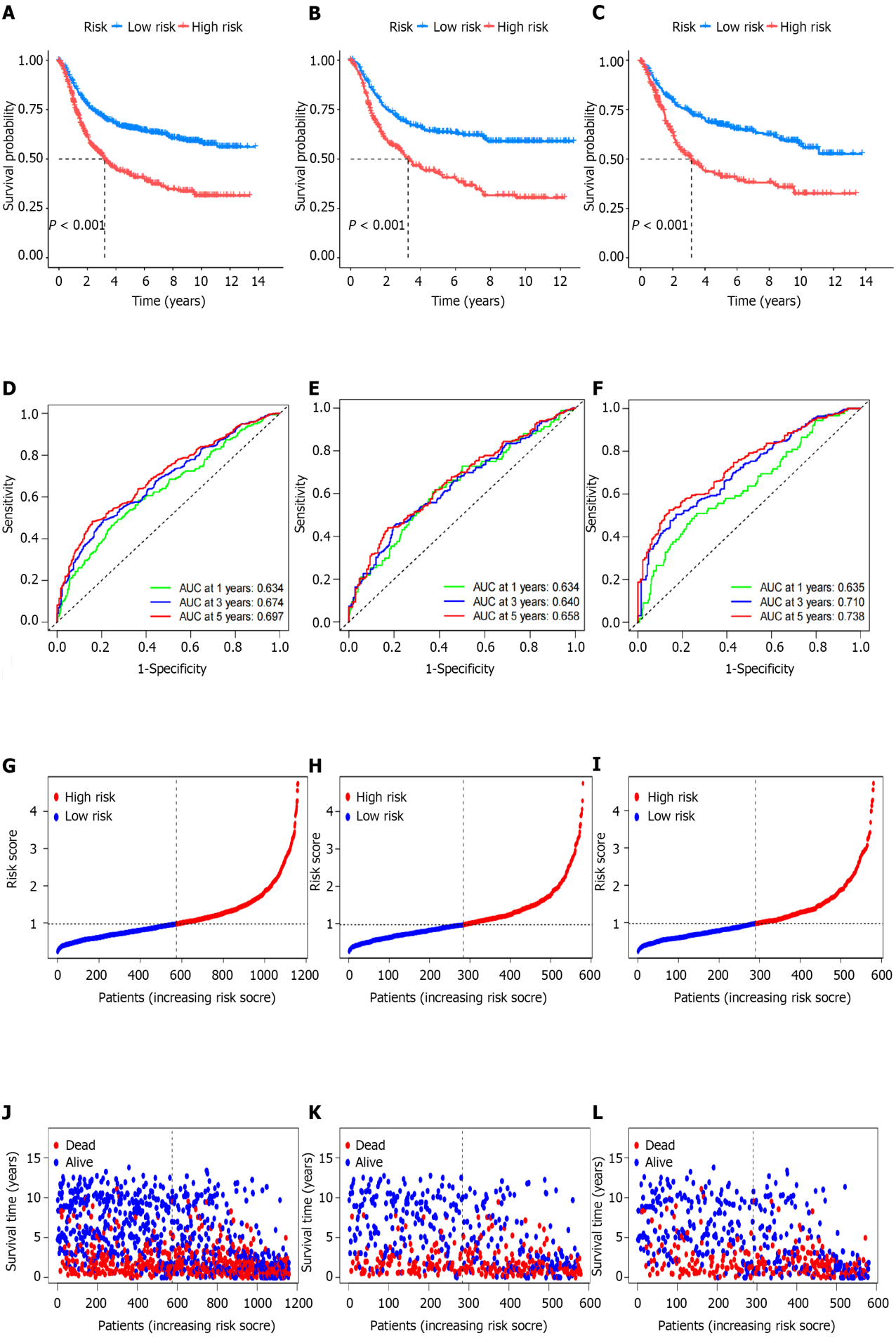
The occurrence and development of GC are complex pathological processes involving the activation and alteration of multiple genes and signaling pathways[66]. Previous studies have shown that the expression of certain genes in GC tissue and normal gastric tissue can vary[67]. In this study, we analyzed the differential expression of 10 DRGs between GC tissue and adjacent normal tissue and found significant differences between the two. By analyzing the mutation waterfall plot and mutation frequency plot of DRGs, we observed that most DRGs were mutated in GC tissue, further indicating that DRGs are differentially expressed in cancer tissue. Previous studies have found that high expression of the disulfidptosis-related gene *PDLIM1* may inhibit the proliferation, invasion, and migration of GC cells, promote apoptosis, and enhance their sensitivity to cisplatin[68]. It has also been found that the high expression of *FLNA* can lead to low survival rate and migration and invasion energy of GC cells[69]. Additionally, the disulfidptosis-related gene *MYH10* may be related to the occurrence, development, and drug resistance of ovarian cancer[70], but its role in GC requires further exploration. Moreover, previous studies have revealed that *DSTN* increases the colony formation and migration ability of tumor cells when highly expressed[71], although its relationship with GC prognosis requires further study. Study on *MYL6* revealed possible impacts on the migration of melanoma cells[72], but its relationship with GC needs further study. In this study, we found that *PDLIM1*, *FLNA*, *MYH10*, *MYL6*, and *DSTN* are significantly associated with GC prognosis ($P < 0.05$), among which, *DSTN*, *FLNA*, *MYH10*, and *MYL6* are risk factors for GC prognosis, while *PDLIM1* is a protective factor for GC prognosis. Our study further demonstrates the impact of DRGs on GC prognosis.

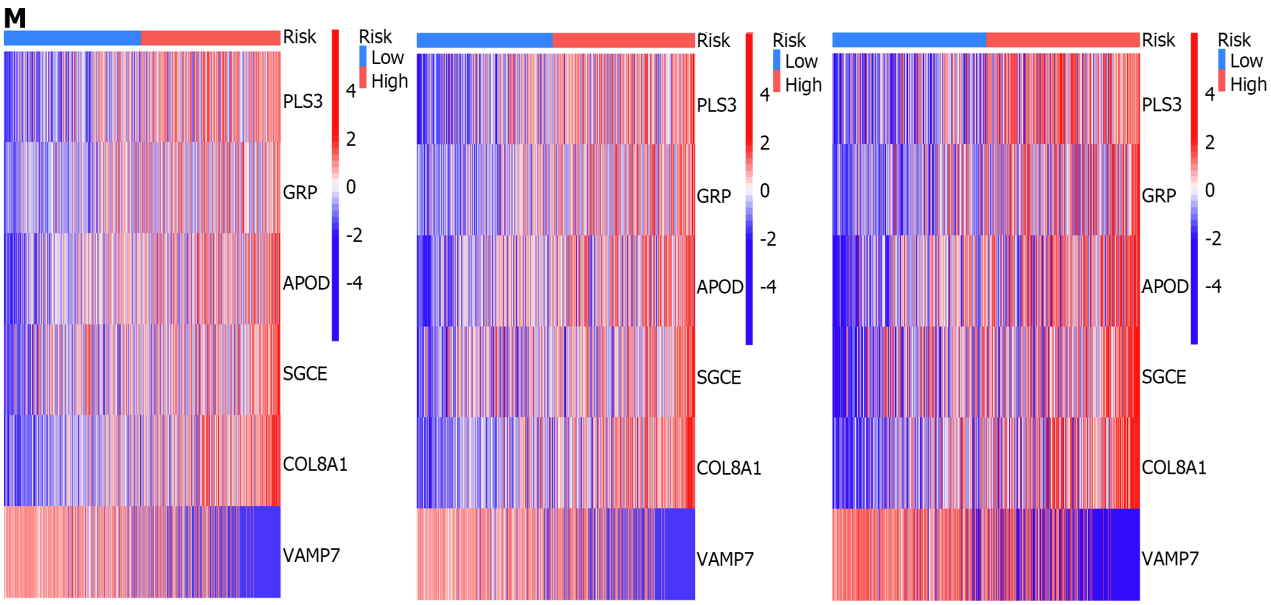
Our results revealed significant pathway and functional differences, as well as significantly different KEGG and GO pathways and functions between the two subtypes of disulfidptosis, mainly enriched in amino acid metabolism, TGF-β signaling pathway, pentose phosphate pathway, Wnt signaling pathway, and MAPK signaling pathway, among others. These functions and pathways may be related to the presence of GC. Indeed, previous studies have found that the TGF-β



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

Figure 7 Testing the reliability of prognostic models. A: A Sankey diagram of the relationships between various data is presented; B: Shows a box plot of the disulfidptosis subtype; C: Presents a box plot of gene subtypes; D: Shows the differential analysis of disulfidptosis related genes between high and low-risk groups.


A
Risk Low risk High risk
Survival probability
P < 0.001
Time (years)
B
Risk Low risk High risk
Survival probability
P < 0.001
Time (years)
C
Risk Low risk High risk
Survival probability
P < 0.001
Time (years)
D
Sensitivity
AUC at 1 years: 0.634
AUC at 3 years: 0.674
AUC at 5 years: 0.697
1-Specificity
E
Sensitivity
AUC at 1 years: 0.634
AUC at 3 years: 0.640
AUC at 5 years: 0.658
1-Specificity
F
Sensitivity
AUC at 1 years: 0.635
AUC at 3 years: 0.710
AUC at 5 years: 0.738
1-Specificity
G
High risk
Low risk
Risk score
Patients (increasing risk socre)
H
High risk
Low risk
Risk score
Patients (increasing risk socre)
I
High risk
Low risk
Risk score
Patients (increasing risk socre)
J
Dead
Alive
Survival time (years)
Patients (increasing risk socre)
K
Dead
Alive
Survival time (years)
Patients (increasing risk socre)
L
Dead
Alive
Survival time (years)
Patients (increasing risk socre)



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

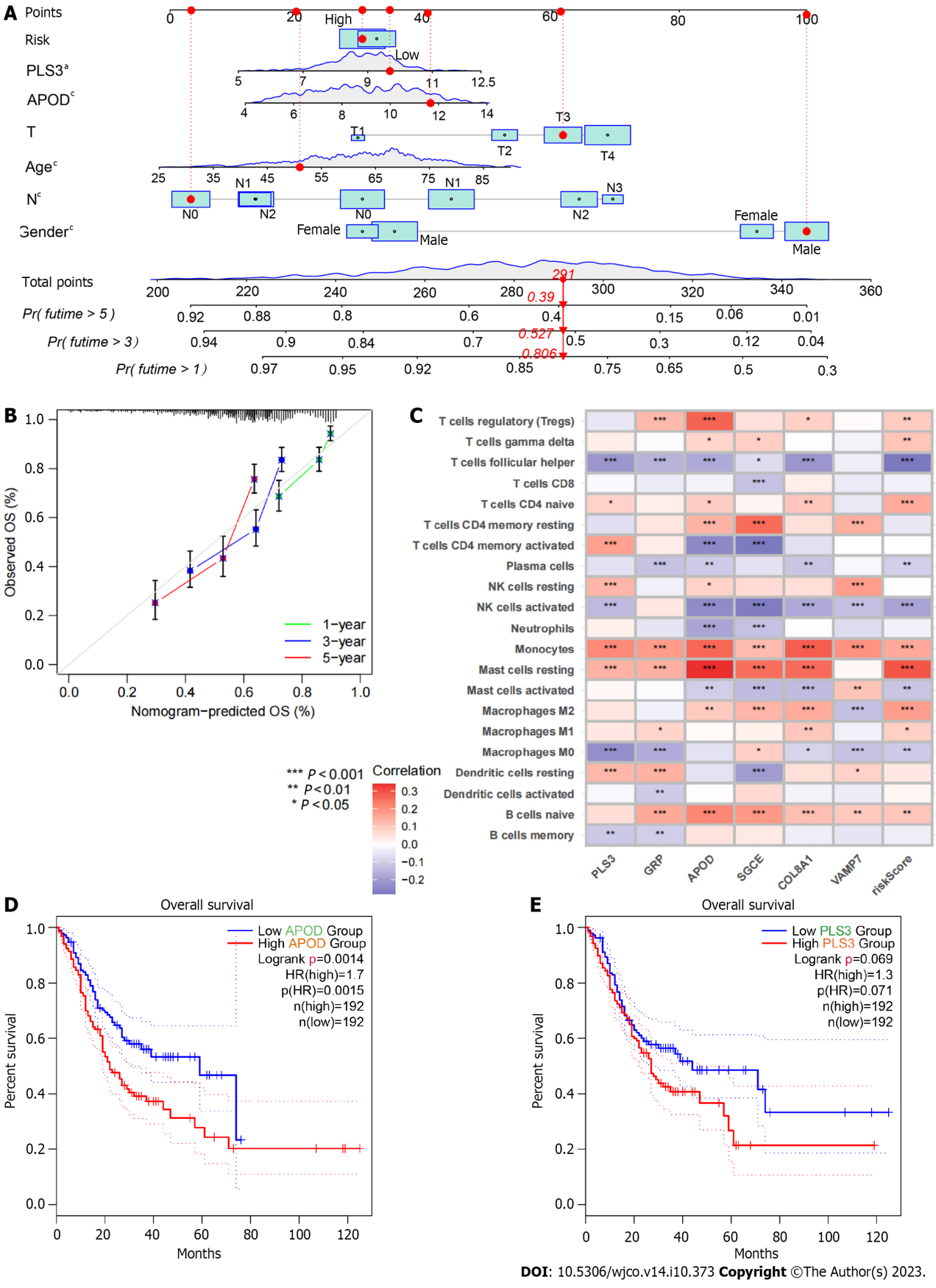
Figure 8 The accuracy of the prognostic model was verified by subgroup analysis. A-C: Survival curves between different groups are presented in panels; D-F: Show receiver operating characteristic curves between different groups; G-I: Risk curves for each group are presented; J-L: Show survival status plots for each group; M-O: Risk heat maps for each group are presented.

signaling pathway may be involved in the occurrence, invasion, proliferation, and metastasis of GC, affecting the prognosis of patients with GC[73-77]. Furthermore, some studies have suggested that the pentose phosphate pathway may be related to the proliferation of GC cells[78]. Previous studies have also found that the MAPK signaling pathway may also be involved in the occurrence, invasion, proliferation, and metastasis of GC, affecting the prognosis of GC[79-87]. Additionally, some studies have found that the Wnt signaling pathway may be involved in the metastasis, migration, invasion, and progression of GC, affecting the prognosis of GC[88-94]. In the current study, we also found differences in immune cell infiltration between the subgroups of disulfidptosis gene typing. Taken together, these findings and research suggest that DRGs affect various aspects of patients with GC, including amino acid metabolism, various signaling pathways, and immune cell infiltration, all of which may affect the survival or prognosis of patients with GC; however, the specific mechanisms and functions need to be further explored.

In this study, we used a risk model to score differentially expressed genes in overall, training set, and testing set samples, dividing them into high- and low-risk groups. Survival, ROC, and risk analyses were conducted for each group. The results showed that the high-risk group had a poorer prognosis than the low-risk group in all groups, and the result trend was consistent, further demonstrating the reliability of the model.

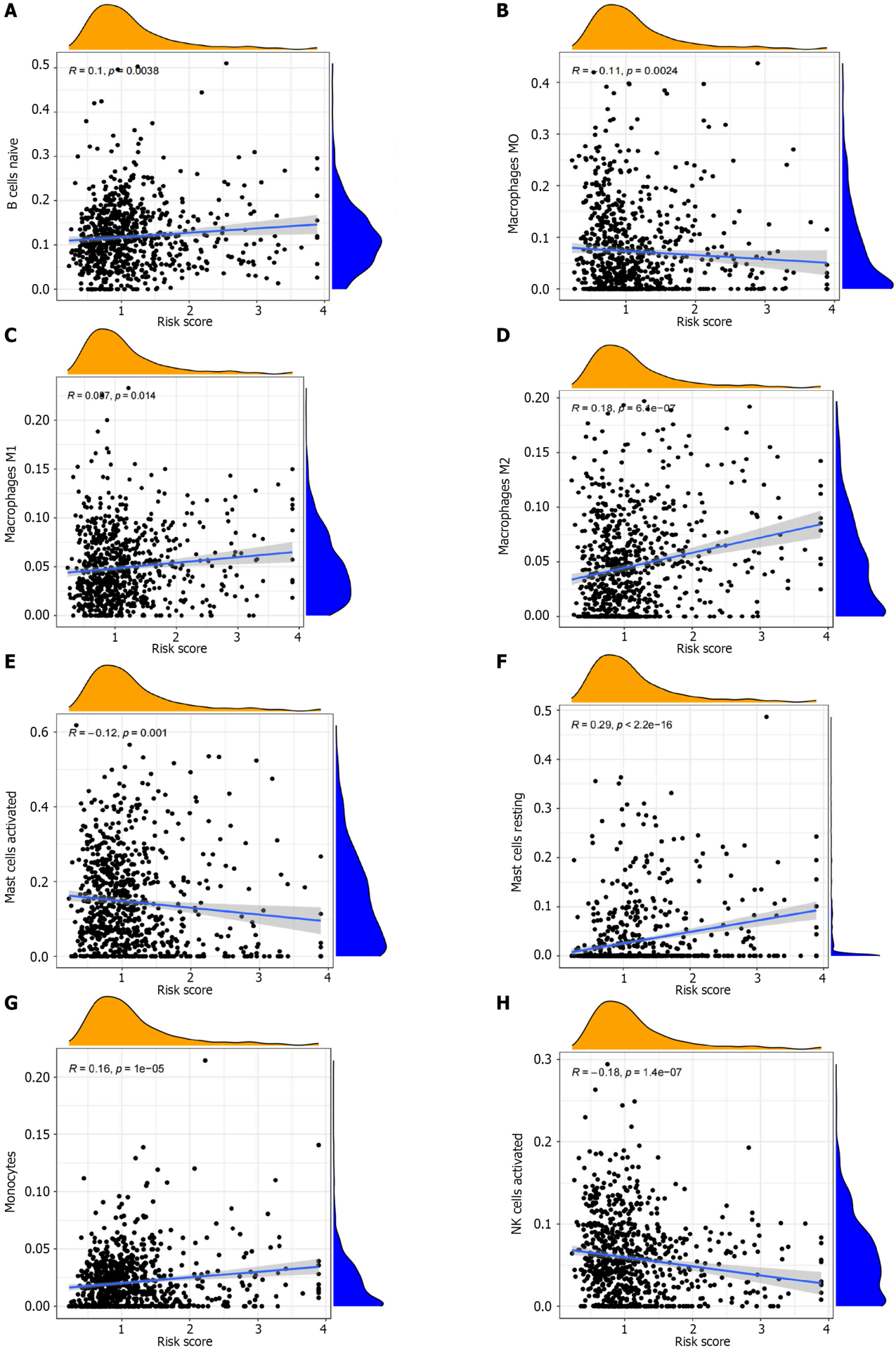
Through column line chart analysis, we revealed that *APOD*, *PLS3*, age, sex, and N stage represent independent risk factors affecting patient prognosis, all of which are risk factors for GC prognosis. Through column line chart analysis, we observed that the survival rates of patients at 1, 3, and 5 years gradually decreased, with rates of 0.806, 0.527, and 0.39, respectively; this is consistent with the trend of 1-, 3-, and 5-year survival rates in previous studies on GC, further confirming the reliability of the prognostic model[95]. Additionally, we found that *APOD* represents an independent prognostic factor for GC in this model ($P < 0.001$). Previous studies on *APOD* have found that it may be involved in the construction of multiple GC prognostic and immune prediction models[96-103], which may be related to GC prognosis. In this study, we further analyzed the protein encoded by *APOD* in the HPA network database through immunohistochemical analysis and found that its protein expression level in GC tissues was significantly higher than that in adjacent normal tissues, further indicating significant differences in *APOD* between GC tissues and adjacent normal tissues. Overall, our results suggest that *APOD* may play an important role in the occurrence and development of GC, while its expression level may be related to the prognosis of patients with GC, further suggesting that *APOD* represents a potential therapeutic target for GC.

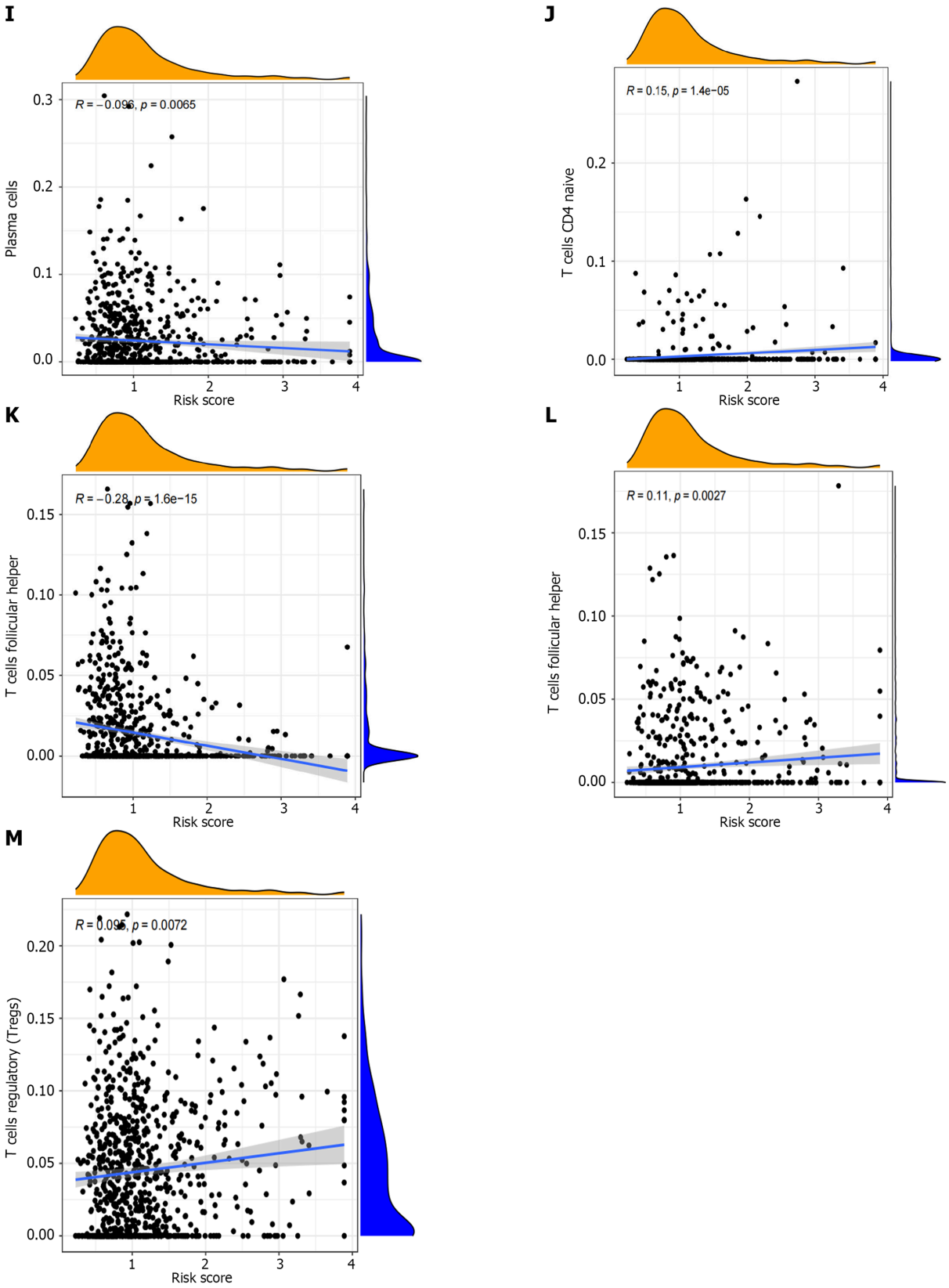
We also found that the genes constituting the GC prognosis model were related to various immune cells, indicating that the DRGs may affect the immunity of patients with GC. The heatmap of the correlation between the model genes and immune cells in this study showed a significant positive correlation between disulfidptosis *PLS3* and resting mast cells ($P < 0.001$). Indeed, previous studies have found a correlation between resting mast cells and GC[104,105], and it has been suggested that *PLS3*[106] may also be related to GC. Through the analysis of TME differences in the prognosis model, we found differences in the Stromal Score and ESTIMATE Score in the high- and low-risk groups, with both scores found to be upregulated in the high-risk group, indicating that the risk score of the prognosis model is related to the TME of GC. Through the analysis of the relationship between the risk score of the prognosis model and TMB, MSI, and stem cell correlation, we found that the risk score of the prognosis model was correlated with TMB, MSI, and RNAss. These results further indicate that the DRGs may be related to the immunity or immune therapy targets of TMB, MSI, and RNAss in patients with GC, which may affect the immune therapy effect and prognosis of patients with GC. Among them, MSI, an



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

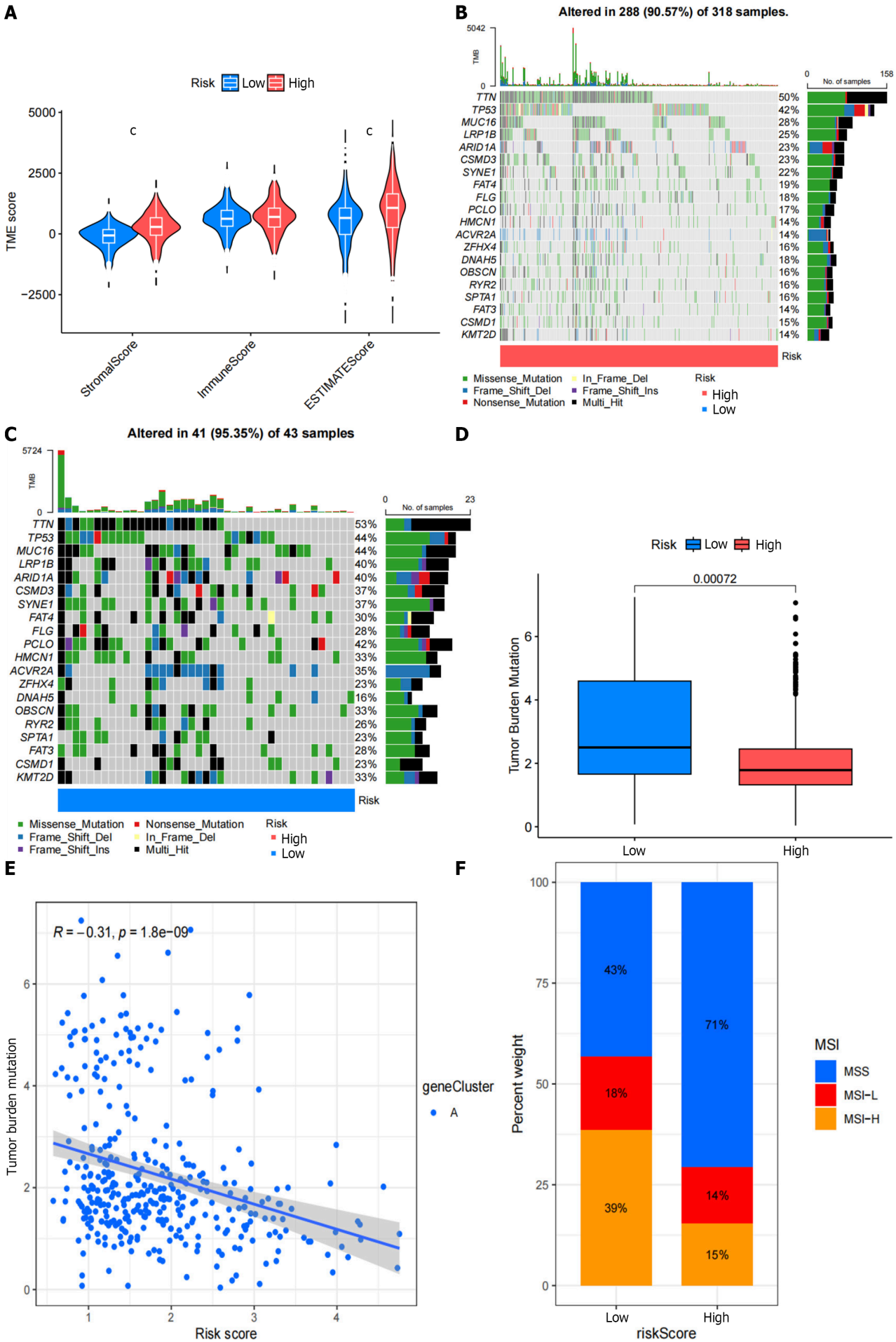
Figure 9 Further analysis of prognostic models to screen potential therapeutic targets. A: Presents a column line chart; B: Shows a calibration curve; C: Presents a heat map of the correlation between model genes and immune cells; D:The survival curve of *APOD* in gastric cancer was significantly different between high and low risk groups ($P < 0.05$); E: The survival curve of *PLS3* in gastric cancer was shown between high and low risk groups, and the results suggested that the difference was not significant ($P > 0.05$).

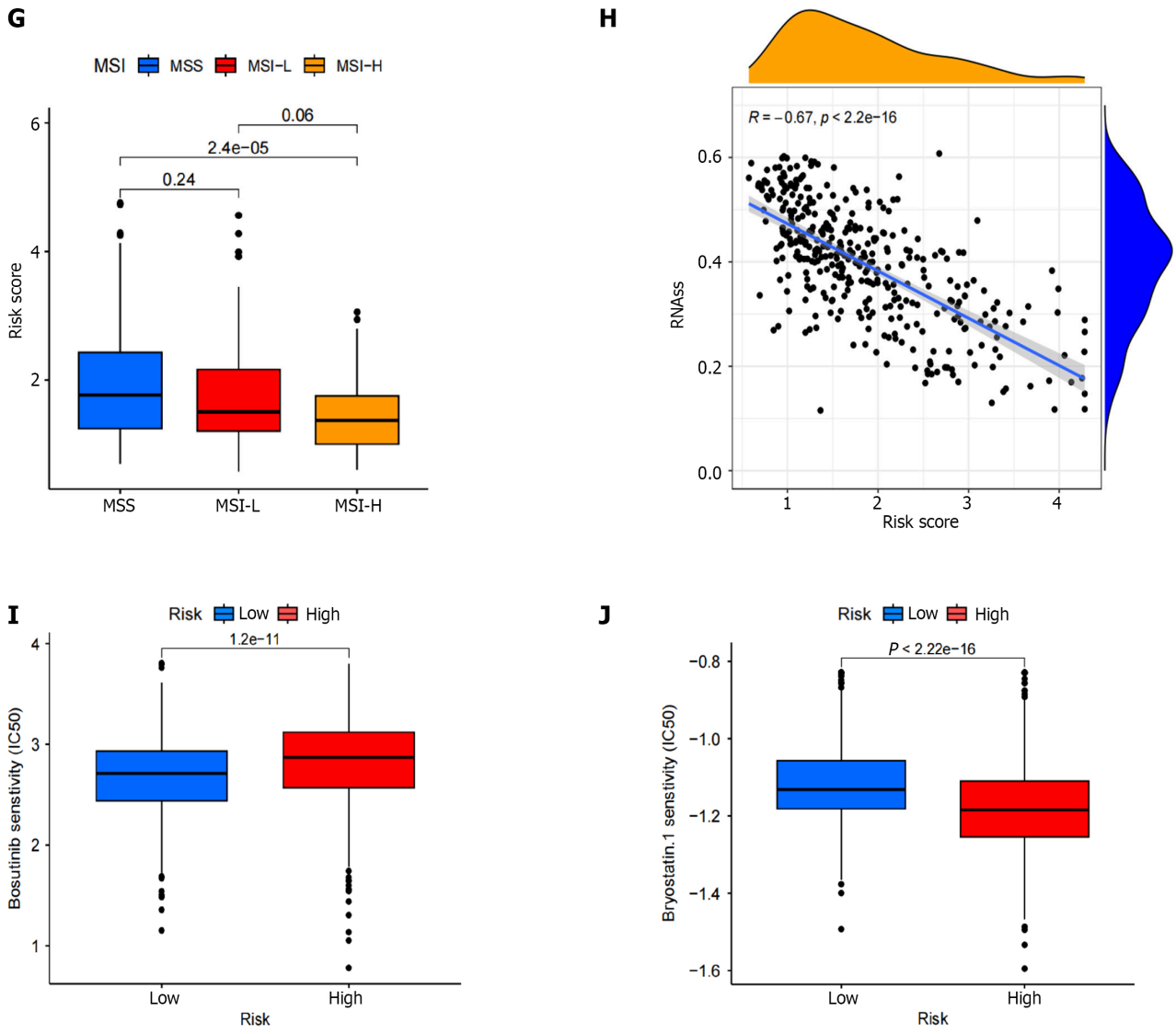

A
R = 0.1, p = 0.0038
B cells naive
Risk score
B
R = 0.11, p = 0.0024
Macrophages M0
Risk score
C
R = 0.087, p = 0.014
Macrophages M1
Risk score
D
R = 0.18, p = 6.4e−07
Macrophages M2
Risk score
E
R = −0.12, p = 0.001
Mast cells activated
Risk score
F
R = 0.29, p < 2.2e−16
Mast cells resting
Risk score
G
R = 0.16, p = 1e−05
Monocytes
Risk score
H
R = −0.18, p = 1.4e−07
NK cells activated
Risk score



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

Figure 10 The correlation between immune cells and risk scores is analyzed. A: There was a positive correlation between B cells naive and risk score; B: The result shows that macrophages M0 is negatively correlated with risk score; C: The results showed that macrophages M1 was positively correlated with risk score; D: The results showed that macrophages M2 was positively correlated with risk score; E: There was a negative correlation between mast cells activated and risk score; F: The results showed that mast cells reting was positively correlated with risk score; G: There was a positive correlation between monocytes and risk score; H: There was a negative correlation between natural killer cells activated and risk score; I: There was a negative correlation between plasma cells and risk score; J: There was a positive correlation between T cells CD4 naive and risk score; K: It showed that T cells follicular helper was negatively correlated with risk score; L: There was a positive correlation between T cells gamma delta and risk score; M: There was a positive correlation between T cells regulation and risk score.


A
Risk Low High
TME score
5000
2500
0
−2500
c
c
StromalScore
ImmuneScore
ESTIMATEScore
B
Altered in 288 (90.57%) of 318 samples.
TMB
5042
0
No. of samples
0
158
TTN 50%
TP53 42%
MUC16 28%
LRP1B 25%
ARID1A 23%
CSMD3 23%
SYNE1 22%
FAT4 19%
FLG 18%
PCLO 17%
HMCN1 14%
ACVR2A 14%
ZFHX4 16%
DNAH5 18%
OBSCN 16%
RYR2 16%
SPTA1 16%
FAT3 14%
CSMD1 15%
KMT2D 14%
Risk
Missense_Mutation
Frame_Shift_Del
Nonsense_Mutation
In_Frame_Del
Frame_Shift_Ins
Multi_Hit
Risk
High
Low
C
Altered in 41 (95.35%) of 43 samples
TMB
5724
0
No. of samples
0
23
TTN 53%
TP53 44%
MUC16 44%
LRP1B 40%
ARID1A 40%
CSMD3 37%
SYNE1 37%
FAT4 30%
FLG 28%
PCLO 42%
HMCN1 33%
ACVR2A 35%
ZFHX4 23%
DNAH5 16%
OBSCN 33%
RYR2 26%
SPTA1 23%
FAT3 28%
CSMD1 23%
KMT2D 33%
Risk
Missense_Mutation
Frame_Shift_Del
Frame_Shift_Ins
Nonsense_Mutation
In_Frame_Del
Multi_Hit
Risk
High
Low
D
Risk Low High
0.00072
Tumor Burden Mutation
0
2
4
6
Low
High
E
R = −0.31, p = 1.8e−09
Tumor burden mutation
0
2
4
6
1
2
3
4
Risk score
geneCluster
A
F
Percent weight
0
25
50
75
100
43%
18%
39%
71%
14%
15%
Low
High
riskScore
MSI
MSS
MSI-L
MSI-H



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

Figure 11 The correlation between the prognostic scoring model and tumor microenvironment, microsatellite instability, and RNAss, as well as drug sensitivity analysis, is presented. A: Shows the tumor microenvironment score for high and low-risk groups; B and C: Present waterfall plots of mutations for high and low-risk groups; D: Analyzes the differences in tumor mutation burden between high and low-risk groups; E: Shows the relationship between tumor mutation burden and risk score; F and G: Present microsatellite instability analysis for high and low-risk groups; H: Analyzes the correlation between stem cells and risk score; I and J: Present drug sensitivity analysis for drugs such as Bosutinib and Bryostatin.

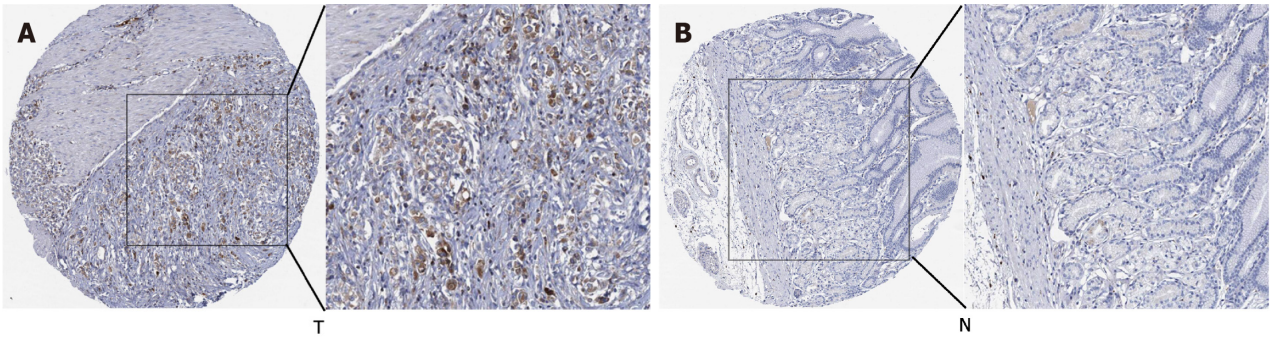
immune therapy target, has been found to affect the treatment and prognosis of patients with GC in previous studies[107-111], while the significant correlation between the GC risk prediction model established in this study and MSI indicates that MSI-targeted treatment may be meaningful for the treatment and prognosis of patients with GC. This further indicates the correlation between disulfidptosis and the immunity or immune therapy targets of patients with GC.

The results of our drug sensitivity analysis revealed that 89 drugs, including Bosutinib and Bryostatin, were significantly correlated with the sensitivity of GC treatment. Previous studies have found that Bryostatin can enhance the effect of paclitaxel in the treatment of GC[112], while others have found that Bosutinib may inhibit the migration of GC cells[113]. These results suggest that Bosutinib may have therapeutic effects on GC. The high sensitivity of Bosutinib and Bryostatin to GC found in this study suggests that they may be useful drugs for the treatment of GC. Therefore, the 89 drugs represented by Bosutinib in this study may be potential drugs for the treatment of GC.

CONCLUSION

In conclusion, our findings suggest that the DRGs and their submolecules may have an impact on immunity, immunotherapy targets, signaling pathways, and drug sensitivity in patients with GC. DRGs, including *PDLIM1*, *FLNA*, *MYH10*, *MYL6*, and *DSTN*, may be related to the prognosis of GC. Six genes, namely, *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, and *VAMP7*, constituted a prognostic model of GC associated with DRG. *APOD* may be a potential target for the treatment of

GC, while 89 drugs, including Bosutinib and Bryostatin, may be potential drugs for the treatment of GC.



DOI: 10.5306/wjco.v14.i10.373 **Copyright** ©The Author(s) 2023.

Figure 12 The immunohistochemical analysis of the *APOD* gene based on human protein atlas is presented. A: Tumor tissue; B: Normal tissue. T: Tumor tissue; B: Normal tissue.

ARTICLE HIGHLIGHTS

Research background

Gastric cancer (GC) is one of the most common malignant tumors, although its pathogenesis remains unclear.

Research motivation

For the first time, in the current study, we constructed a new GC prognostic model based on the sub-group analysis of disulfidptosis-related genes (DRGs) and explored treatment targets and sensitive drugs.

Research objectives

The aims of this study were to explore a new GC prognostic model based on the sub-group analysis of DRGs and explore treatment targets and sensitive drugs.

Research methods

In this study, a bioinformatics strategy was used to extract GC-related data from The Cancer Genome Atlas and Gene Expression Omnibus databases, while R software (version 4.2.1) was used for correlation analysis.

Research results

Through the above analysis, we found that the didisulfidptosis-related gene may be related to the prognosis of GC. Six genes, namely, *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, and *VAMP7*, constitute a predictive model for GC prognosis. *APOD* is a potential therapeutic target. Bosutinib and other drugs are suitable for the treatment of GC.

Research conclusions

The results of this study indicate that didisulfidptosis is related to the prognosis and treatment of GC. Additionally, *APOD* can be used as a potential therapeutic target for GC.

Research perspectives

Six genes, namely, *PLS3*, *GRP*, *APOD*, *SGCE*, *COL8A1*, and *VAMP7*, constitute a predictive model for GC prognosis. *APOD* is a potential therapeutic target for treating GC. Bosutinib and other drugs are suitable for the treatment of GC, although this requires further confirmation through molecular biology and clinical experiments.

FOOTNOTES

Author contributions: Li Q contributed to this work; Yin LK and Li Q prepared for the figures and tables; and all authors have approved the final manuscript.

Institutional review board statement: The data supporting the results of this study are available from Gene Expression Omnibus database (GSE84433andGSE26253) and the expression data, clinical data, mutation data, and copy data related to gastric cancer from The Cancer Genome Atlas database.

Conflict-of-interest statement: The authors deny any conflict of interest.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Qian Li 0009-0006-9250-0521; Long-Kuan Yin 0000-0001-5974-5443.

S-Editor: Qu XL
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Li Y**, Hu X, Lin R, Zhou G, Zhao L, Zhao D, Zhang Y, Li W, Ma P, Ren H, Liao X, Niu P, Wang T, Zhang X, Wang W, Gao R, Li Q, Church G, He J, Chen Y. Single-cell landscape reveals active cell subtypes and their interaction in the tumor microenvironment of gastric cancer. *Theranostics* 2022; **12**: 3818-3833 [PMID: 35664061 DOI: 10.7150/thno.71833]
2 **Chang X**, Ge X, Zhang Y, Xue X. The current management and biomarkers of immunotherapy in advanced gastric cancer. *Medicine (Baltimore)* 2022; **101**: e29304 [PMID: 35623069 DOI: 10.1097/MD.0000000000029304]
3 **Zhang Y**, Yu J. The role of MRI in the diagnosis and treatment of gastric cancer. *Diagn Interv Radiol* 2020; **26**: 176-182 [PMID: 32209504 DOI: 10.5152/dir.2019.19375]
4 **Smyth EC**, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet* 2020; **396**: 635-648 [PMID: 32861308 DOI: 10.1016/S0140-6736(20)31288-5]
5 **Machlowska J**, Baj J, Sitarz M, Maciejewski R, Sitarz R. Gastric Cancer: Epidemiology, Risk Factors, Classification, Genomic Characteristics and Treatment Strategies. *Int J Mol Sci* 2020; **21** [PMID: 32512697 DOI: 10.3390/ijms21114012]
6 **Zhao Q**, Cao L, Guan L, Bie L, Wang S, Xie B, Chen X, Shen X, Cao F. Immunotherapy for gastric cancer: dilemmas and prospect. *Brief Funct Genomics* 2019; **18**: 107-112 [PMID: 30388190 DOI: 10.1093/bfgp/ely019]
7 **Wu SL**, Zhang Y, Fu Y, Li J, Wang JS. Gastric cancer incidence, mortality and burden in adolescents and young adults: a time-trend analysis and comparison among China, South Korea, Japan and the USA. *BMJ Open* 2022; **12**: e061038 [PMID: 35863834 DOI: 10.1136/bmjopen-2022-061038]
8 **Yang X**, Zhang T, Zhang H, Sang S, Chen H, Zuo X. Temporal trend of gastric cancer burden along with its risk factors in China from 1990 to 2019, and projections until 2030: comparison with Japan, South Korea, and Mongolia. *Biomark Res* 2021; **9**: 84 [PMID: 34784961 DOI: 10.1186/s40364-021-00340-6]
9 **Gao M**, Yin H, Fei ZW. Clinical application of microRNA in gastric cancer in Eastern Asian area. *World J Gastroenterol* 2013; **19**: 2019-2027 [PMID: 23599620 DOI: 10.3748/wjg.v19.i13.2019]
10 **Tang D**, Liu S, Shen H, Deng G, Zeng S. Extracellular Vesicles Promote the Formation of Pre-Metastasis Niche in Gastric Cancer. *Front Immunol* 2022; **13**: 813015 [PMID: 35173726 DOI: 10.3389/fimmu.2022.813015]
11 **Gu X**, Zhang Y, Huang Y, Ju S. Comprehensive Evaluation of Serum tRF-17-WS7K092 as a Promising Biomarker for the Diagnosis of Gastric Cancer. *J Oncol* 2022; **2022**: 8438726 [PMID: 36245992 DOI: 10.1155/2022/8438726]
12 **Titov SE**, Anishchenko VV, Poloz TL, Veryaskina YA, Arkhipova AA, Ustinov SN. [Differential diagnostics of gastric cancer and precancerous changes of the gastric mucosa using analysis of expression of six microRNAS.]. *Klin Lab Diagn* 2020; **65**: 131-136 [PMID: 32159312 DOI: 10.18821/0869-2084-2020-65-2-131-136]
13 **Tong H**, Wang Y, Li Y, Liu S, Chi C, Liu D, Guo L, Li E, Wang C. Volatile organic metabolites identify patients with gastric carcinoma, gastric ulcer, or gastritis and control patients. *Cancer Cell Int* 2017; **17**: 108 [PMID: 29200968 DOI: 10.1186/s12935-017-0475-x]
14 **Zhao Y**, Zhou T, Li A, Yao H, He F, Wang L, Si J. A potential role of collagens expression in distinguishing between premalignant and malignant lesions in stomach. *Anat Rec (Hoboken)* 2009; **292**: 692-700 [PMID: 19306436 DOI: 10.1002/ar.20874]
15 **Qi M**, Liu D, Wang H, Bianba C, Ji W. Correlation of Single Nucleotide Gene Polymorphisms and Gastric Cancer Based on Magnetic Nanoparticles. *J Nanosci Nanotechnol* 2021; **21**: 928-934 [PMID: 33183426 DOI: 10.1166/jnn.2021.18628]
16 **Jiang L**, Gong X, Liao W, Lv N, Yan R. Molecular targeted treatment and drug delivery system for gastric cancer. *J Cancer Res Clin Oncol* 2021; **147**: 973-986 [PMID: 33550445 DOI: 10.1007/s00432-021-03520-x]
17 **Jiang H**, Hu K, Xia Y, Liang L, Zhu X. Long Noncoding RNA KLF3-AS1 Acts as an Endogenous RNA of miR-223 to Attenuate Gastric Cancer Progression and Chemoresistance. *Front Oncol* 2021; **11**: 704339 [PMID: 34745937 DOI: 10.3389/fonc.2021.704339]
18 **Guo XM**, Zhao HY, Shi ZY, Wang Y, Jin ML. [Application and Progress of Convolutional Neural Network-based Pathological Diagnosis of Gastric Cancer]. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2021; **52**: 166-169 [PMID: 33829686 DOI: 10.12182/20210360501]
19 **Chen R**, Yang M, Huang W, Wang B. Cascades between miRNAs, lncRNAs and the NF-κB signaling pathway in gastric cancer (Review). *Exp Ther Med* 2021; **22**: 769 [PMID: 34055068 DOI: 10.3892/etm.2021.10201]
20 **Chen J**, Liu Z, Gao G, Mo Y, Zhou H, Huang W, Wu L, He X, Ding J, Luo C, Long H, Feng J, Sun Y, Guan X. Efficacy of circulating microRNA-130b and blood routine parameters in the early diagnosis of gastric cancer. *Oncol Lett* 2021; **22**: 725 [PMID: 34429765 DOI: 10.3892/ol.2021.12986]
21 **Wang H**, Li J. A systematic review and meta-analysis protocol of clinical characteristics and prognostic significance of mammalian target of rapamycin for gastric cancer patients. *Medicine (Baltimore)* 2020; **99**: e21138 [PMID: 32769866 DOI: 10.1097/MD.0000000000021138]
22 **Shafabakhsh R**, Yousefi B, Asemi Z, Nikfar B, Mansournia MA, Hallajzadeh J. Chitosan: A compound for drug delivery system in gastric cancer-a review. *Carbohydr Polym* 2020; **242**: 116403 [PMID: 32564837 DOI: 10.1016/j.carbpol.2020.116403]
23 **Tan Z**. Recent Advances in the Surgical Treatment of Advanced Gastric Cancer: A Review. *Med Sci Monit* 2019; **25**: 3537-3541 [PMID:

31080234 DOI: 10.12659/MSM.916475]
24 **Zou WB**, Yang F, Li ZS. [How to improve the diagnosis rate of early gastric cancer in China]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2015; **44**: 9-14 [PMID: 25851969 DOI: 10.3785/j.issn.1008-9292.2015.01.002]
25 **Mu YP**, Sun WJ, Lu CW, Su XL. MicroRNAs May Serve as Emerging Molecular Biomarkers for Diagnosis and Prognostic Assessment or as Targets for Therapy in Gastric Cancer. *Asian Pac J Cancer Prev* 2015; **16**: 4813-4820 [PMID: 26163596 DOI: 10.7314/apjcp.2015.16.12.4813]
26 **Zheng G**, Xiong Y, Xu W, Wang Y, Chen F, Wang Z, Yan Z. A two-microRNA signature as a potential biomarker for early gastric cancer. *Oncol Lett* 2014; **7**: 679-684 [PMID: 24527072 DOI: 10.3892/ol.2014.1797]
27 **Xiang Z**, Zhou X, Mranda GM, Xue Y, Wang Y, Wei T, Liu J, Ding Y. Identification of the ferroptosis-related ceRNA network related to prognosis and tumor immunity for gastric cancer. *Aging (Albany NY)* 2022; **14**: 5768-5782 [PMID: 35835721 DOI: 10.18632/aging.204176]
28 **White JR**, Banks M. Identifying the pre-malignant stomach: from guidelines to practice. *Transl Gastroenterol Hepatol* 2022; **7**: 8 [PMID: 35243117 DOI: 10.21037/tgh.2020.03.03]
29 **Sukri A**, Hanafiah A, Kosai NR. The Roles of Immune Cells in Gastric Cancer: Anti-Cancer or Pro-Cancer? *Cancers (Basel)* 2022; **14** [PMID: 36010915 DOI: 10.3390/cancers14163922]
30 **Niu X**, Ren L, Hu A, Zhang S, Qi H. Identification of Potential Diagnostic and Prognostic Biomarkers for Gastric Cancer Based on Bioinformatic Analysis. *Front Genet* 2022; **13**: 862105 [PMID: 35368700 DOI: 10.3389/fgene.2022.862105]
31 **Koopaie M**, Ghafourian M, Manifar S, Younespour S, Davoudi M, Kolahdooz S, Shirkhoda M. Evaluation of CSTB and DMBT1 expression in saliva of gastric cancer patients and controls. *BMC Cancer* 2022; **22**: 473 [PMID: 35488257 DOI: 10.1186/s12885-022-09570-9]
32 **Hassen G**, Kasar A, Jain N, Berry S, Dave J, Zouetr M, Priyanka Ganapathiraju VLN, Kurapati T, Oshai S, Saad M, Pathan J, Kamat S, Tirupathi R, Patel UK, Rana RK. Programmed Death-Ligand 1 (PD-L1) Positivity and Factors Associated with Poor Prognosis in Patients with Gastric Cancer: An Umbrella Meta-Analysis. *Cureus* 2022; **14**: e23845 [PMID: 35530821 DOI: 10.7759/cureus.23845]
33 **Waddingham W**, Nieuwenburg SAV, Carlson S, Rodriguez-Justo M, Spaander M, Kuipers EJ, Jansen M, Graham DG, Banks M. Recent advances in the detection and management of early gastric cancer and its precursors. *Frontline Gastroenterol* 2021; **12**: 322-331 [PMID: 34249318 DOI: 10.1136/flgastro-2018-101089]
34 **Feng H**, Liu X. Interaction between ACOT7 and LncRNA NMRAL2P *via* Methylation Regulates Gastric Cancer Progression. *Yonsei Med J* 2020; **61**: 471-481 [PMID: 32469171 DOI: 10.3349/ymj.2020.61.6.471]
35 **Canale M**, Casadei-Gardini A, Ulivi P, Arechederra M, Berasain C, Lollini PL, Fernández-Barrena MG, Avila MA. Epigenetic Mechanisms in Gastric Cancer: Potential New Therapeutic Opportunities. *Int J Mol Sci* 2020; **21** [PMID: 32752096 DOI: 10.3390/ijms21155500]
36 **Negovan A**, Iancu M, Fülöp E, Bănescu C. Helicobacter pylori and cytokine gene variants as predictors of premalignant gastric lesions. *World J Gastroenterol* 2019; **25**: 4105-4124 [PMID: 31435167 DOI: 10.3748/wjg.v25.i30.4105]
37 **Gu X**, Zhang Q, Zhang W, Zhu L. Curcumin inhibits liver metastasis of gastric cancer through reducing circulating tumor cells. *Aging (Albany NY)* 2019; **11**: 1501-1509 [PMID: 30844765 DOI: 10.18632/aging.101848]
38 **Cen D**, Huang H, Yang L, Guo K, Zhang J. Long noncoding RNA STXBP5-AS1 inhibits cell proliferation, migration, and invasion through inhibiting the PI3K/AKT signaling pathway in gastric cancer cells. *Onco Targets Ther* 2019; **12**: 1929-1936 [PMID: 30881044 DOI: 10.2147/OTT.S194463]
39 **Banks M**, Graham D, Jansen M, Gotoda T, Coda S, di Pietro M, Uedo N, Bhandari P, Pritchard DM, Kuipers EJ, Rodriguez-Justo M, Novelli MR, Ragunath K, Shepherd N, Dinis-Ribeiro M. British Society of Gastroenterology guidelines on the diagnosis and management of patients at risk of gastric adenocarcinoma. *Gut* 2019; **68**: 1545-1575 [PMID: 31278206 DOI: 10.1136/gutjnl-2018-318126]
40 **Yang J**, Bo L, Han T, Ding D, Nie M, Yin K. Pathway- and clinical-factor-based risk model predicts the prognosis of patients with gastric cancer. *Mol Med Rep* 2018; **17**: 6345-6356 [PMID: 29532879 DOI: 10.3892/mmr.2018.8722]
41 **Zhang Z**, Wu H, Chong W, Shang L, Jing C, Li L. Liquid biopsy in gastric cancer: predictive and prognostic biomarkers. *Cell Death Dis* 2022; **13**: 903 [PMID: 36302755 DOI: 10.1038/s41419-022-05350-2]
42 **Jin X**, Liu Z, Yang D, Yin K, Chang X. Recent Progress and Future Perspectives of Immunotherapy in Advanced Gastric Cancer. *Front Immunol* 2022; **13**: 948647 [PMID: 35844558 DOI: 10.3389/fimmu.2022.948647]
43 **Ajani JA**, D'Amico TA, Bentrem DJ, Chao J, Cooke D, Corvera C, Das P, Enzinger PC, Enzler T, Fanta P, Farjah F, Gerdes H, Gibson MK, Hochwald S, Hofstetter WL, Ilson DH, Keswani RN, Kim S, Kleinberg LR, Klempner SJ, Lacy J, Ly QP, Matkowskyj KA, McNamara M, Mulcahy MF, Outlaw D, Park H, Perry KA, Pimiento J, Poultsides GA, Reznik S, Roses RE, Strong VE, Su S, Wang HL, Wiesner G, Willett CG, Yakoub D, Yoon H, McMillian N, Pluchino LA. Gastric Cancer, Version 2.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2022; **20**: 167-192 [PMID: 35130500 DOI: 10.6004/jnccn.2022.0008]
44 **Necula L**, Matei L, Dragu D, Neagu AI, Mambet C, Nedeianu S, Bleotu C, Diaconu CC, Chivu-Economescu M. Recent advances in gastric cancer early diagnosis. *World J Gastroenterol* 2019; **25**: 2029-2044 [PMID: 31114131 DOI: 10.3748/wjg.v25.i17.2029]
45 **Coutzac C**, Pernot S, Chaput N, Zaanan A. Immunotherapy in advanced gastric cancer, is it the future? *Crit Rev Oncol Hematol* 2019; **133**: 25-32 [PMID: 30661655 DOI: 10.1016/j.critrevonc.2018.10.007]
46 **Ratti M**, Lampis A, Hahne JC, Passalacqua R, Valeri N. Microsatellite instability in gastric cancer: molecular bases, clinical perspectives, and new treatment approaches. *Cell Mol Life Sci* 2018; **75**: 4151-4162 [PMID: 30173350 DOI: 10.1007/s00018-018-2906-9]
47 **Alessandrini L**, Manchi M, De Re V, Dolcetti R, Canzonieri V. Proposed Molecular and miRNA Classification of Gastric Cancer. *Int J Mol Sci* 2018; **19** [PMID: 29882766 DOI: 10.3390/ijms19061683]
48 **Massarrat S**, Stolte M. Development of gastric cancer and its prevention. *Arch Iran Med* 2014; **17**: 514-520 [PMID: 24979566]
49 **Song S**, Shu P. Expression of ferroptosis-related gene correlates with immune microenvironment and predicts prognosis in gastric cancer. *Sci Rep* 2022; **12**: 8785 [PMID: 35610340 DOI: 10.1038/s41598-022-12800-6]
50 **Ouyang S**, Li H, Lou L, Huang Q, Zhang Z, Mo J, Li M, Lu J, Zhu K, Chu Y, Ding W, Zhu J, Lin Z, Zhong L, Wang J, Yue P, Turkson J, Liu P, Wang Y, Zhang X. Inhibition of STAT3-ferroptosis negative regulatory axis suppresses tumor growth and alleviates chemoresistance in gastric cancer. *Redox Biol* 2022; **52**: 102317 [PMID: 35483272 DOI: 10.1016/j.redox.2022.102317]
51 **Yao F**, Zhan Y, Pu Z, Lu Y, Chen J, Deng J, Wu Z, Chen B, Tian K, Ni Y, Mou L. LncRNAs Target Ferroptosis-Related Genes and Impair Activation of CD4(+) T Cell in Gastric Cancer. *Front Cell Dev Biol* 2021; **9**: 797339 [PMID: 34966745 DOI: 10.3389/fcell.2021.797339]
52 **Xiao S**, Liu X, Yuan L, Wang F. A Ferroptosis-Related lncRNAs Signature Predicts Prognosis and Therapeutic Response of Gastric Cancer. *Front Cell Dev Biol* 2021; **9**: 736682 [PMID: 34926441 DOI: 10.3389/fcell.2021.736682]
53 **Huo J**, Wu L, Zang Y. Eight-gene prognostic signature associated with hypoxia and ferroptosis for gastric cancer with general applicability.

Epigenomics 2021; **13**: 875-890 [PMID: 33942671 DOI: 10.2217/epi-2020-0411]

54 **Chen W**, Feng Z, Huang J, Fu P, Xiong J, Cao Y, Liu Y, Tu Y, Li Z, Jie Z, Xiao T. Identification of Ferroptosis-Related Long Noncoding RNA and Construction of a Novel Prognostic Signature for Gastric Cancer. *Dis Markers* 2021; **2021**: 7724997 [PMID: 34394774 DOI: 10.1155/2021/7724997]

55 **Wang J**, Qin D, Tao Z, Wang B, Xie Y, Wang Y, Li B, Cao J, Qiao X, Zhong S, Hu X. Identification of cuproptosis-related subtypes, construction of a prognosis model, and tumor microenvironment landscape in gastric cancer. *Front Immunol* 2022; **13**: 1056932 [PMID: 36479114 DOI: 10.3389/fimmu.2022.1056932]

56 **Song X**, Hou L, Zhao Y, Guan Q, Li Z. Metal-dependent programmed cell death-related lncRNA prognostic signatures and natural drug sensitivity prediction for gastric cancer. *Front Pharmacol* 2022; **13**: 1039499 [PMID: 36339625 DOI: 10.3389/fphar.2022.1039499]

57 **Nie H**, Wang H, Zhang M, Ning Y, Chen X, Zhang Z, Hu X, Zhao Q, Chen P, Fang J, Wang F. Comprehensive analysis of cuproptosis-related genes in prognosis, tumor microenvironment infiltration, and immunotherapy response in gastric cancer. *J Cancer Res Clin Oncol* 2023; **149**: 5453-5468 [PMID: 36462036 DOI: 10.1007/s00432-022-04474-4]

58 **Huang J**, Chen M, Pei W, Xu Z, Ning J, Chen C. Distinct tumor microenvironment landscapes in gastric cancer classified by cuproptosis-related lncRNAs. *J Cancer* 2022; **13**: 3687-3700 [PMID: 36606199 DOI: 10.7150/jca.79640]

59 **Liu X**, Nie L, Zhang Y, Yan Y, Wang C, Colic M, Olszewski K, Horbath A, Chen X, Lei G, Mao C, Wu S, Zhuang L, Poyurovsky MV, James You M, Hart T, Billadeau DD, Chen J, Gan B. Actin cytoskeleton vulnerability to disulfide stress mediates disulfidptosis. *Nat Cell Biol* 2023; **25**: 404-414 [PMID: 36747082 DOI: 10.1038/s41556-023-01091-2]

60 **Tomczak K**, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemp Oncol (Pozn)* 2015; **19**: A68-A77 [PMID: 25691825 DOI: 10.5114/wo.2014.47136]

61 **Barrett T**, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Res* 2013; **41**: D991-D995 [PMID: 23193258 DOI: 10.1093/nar/gks1193]

62 **Addeo A**, Friedlaender A, Banna GL, Weiss GJ. TMB or not TMB as a biomarker: That is the question. *Crit Rev Oncol Hematol* 2021; **163**: 103374 [PMID: 34087341 DOI: 10.1016/j.critrevonc.2021.103374]

63 **Liu L**, Bai X, Wang J, Tang XR, Wu DH, Du SS, Du XJ, Zhang YW, Zhu HB, Fang Y, Guo ZQ, Zeng Q, Guo XJ, Liu Z, Dong ZY. Combination of TMB and CNA Stratifies Prognostic and Predictive Responses to Immunotherapy Across Metastatic Cancer. *Clin Cancer Res* 2019; **25**: 7413-7423 [PMID: 31515453 DOI: 10.1158/1078-0432.CCR-19-0558]

64 **Gene Ontology Consortium**. Gene Ontology Consortium: going forward. *Nucleic Acids Res* 2015; **43**: D1049-D1056 [PMID: 25428369 DOI: 10.1093/nar/gku1179]

65 **Kanehisa M**, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 2000; **28**: 27-30 [PMID: 10592173 DOI: 10.1093/nar/28.1.27]

66 **Tao W**, Li Y, Zhu M, Li C, Li P. LncRNA NORAD Promotes Proliferation And Inhibits Apoptosis Of Gastric Cancer By Regulating miR-214/Akt/mTOR Axis. *Onco Targets Ther* 2019; **12**: 8841-8851 [PMID: 31802897 DOI: 10.2147/OTT.S216862]

67 **Sung H**, Hu N, Yang HH, Giffen CA, Zhu B, Song L, Su H, Wang C, Parisi DM, Goldstein AM, Taylor PR, Hyland PL. Association of high-evidence gastric cancer susceptibility loci and somatic gene expression levels with survival. *Carcinogenesis* 2017; **38**: 1119-1128 [PMID: 29028942 DOI: 10.1093/carcin/bgx090]

68 **Tan Y**, Li Y, Zhu H, Wu X, Mei K, Li P, Yang Q. miR-187/PDLIM1 Gets Involved in Gastric Cancer Progression and Cisplatin Sensitivity of Cisplatin by Mediating the Hippo-YAP Signaling Pathway. *J Oncol* 2022; **2022**: 5456016 [PMID: 36164345 DOI: 10.1155/2022/5456016]

69 **Sun GG**, Sheng SH, Jing SW, Hu WN. An antiproliferative gene FLNA regulates migration and invasion of gastric carcinoma cell in vitro and its clinical significance. *Tumour Biol* 2014; **35**: 2641-2648 [PMID: 24241900 DOI: 10.1007/s13277-013-1347-1]

70 **Liu L**, Chen C, Liu P, Li J, Pang Z, Zhu J, Lin Z, Zhou H, Xie Y, Lan T, Chen ZS, Zeng Z, Fang W. MYH10 Combines with MYH9 to Recruit USP45 by Deubiquitinating Snail and Promotes Serous Ovarian Cancer Carcinogenesis, Progression, and Cisplatin Resistance. *Adv Sci (Weinh)* 2023; **10**: e2203423 [PMID: 36929633 DOI: 10.1002/advs.202203423]

71 **Yu J**, Liang QY, Wang J, Cheng Y, Wang S, Poon TC, Go MY, Tao Q, Chang Z, Sung JJ. Zinc-finger protein 331, a novel putative tumor suppressor, suppresses growth and invasiveness of gastric cancer. *Oncogene* 2013; **32**: 307-317 [PMID: 22370639 DOI: 10.1038/onc.2012.54]

72 **Vierthaler M**, Sun Q, Wang Y, Steinfass T, Poelchen J, Hielscher T, Novak D, Umansky V, Utikal J. ADCK2 Knockdown Affects the Migration of Melanoma Cells *via* MYL6. *Cancers (Basel)* 2022; **14** [PMID: 35205819 DOI: 10.3390/cancers14041071]

73 **Kato S**, Shiozaki A, Kudou M, Shimizu H, Kosuga T, Ohashi T, Arita T, Konishi H, Komatsu S, Kubota T, Fujiwara H, Okamoto K, Kishimoto M, Konishi E, Otsuji E. TRPV2 Promotes Cell Migration and Invasion in Gastric Cancer *via* the Transforming Growth Factor-β Signaling Pathway. *Ann Surg Oncol* 2022; **29**: 2944-2956 [PMID: 34855064 DOI: 10.1245/s10434-021-11132-5]

74 **Jiang H**, Ma P, Duan Z, Liu Y, Shen S, Mi Y, Fan D. Ginsenoside Rh4 Suppresses Metastasis of Gastric Cancer *via* SIX1-Dependent TGF-β/Smad2/3 Signaling Pathway. *Nutrients* 2022; **14** [PMID: 35458126 DOI: 10.3390/nu14081564]

75 **Zhang X**, Wu J. LINC00665 promotes cell proliferation, invasion, and metastasis by activating the TGF-β pathway in gastric cancer. *Pathol Res Pract* 2021; **224**: 153492 [PMID: 34091388 DOI: 10.1016/j.prp.2021.153492]

76 **Xiong R**, Yin T, Gao JL, Yuan YF. HOXD9 Activates the TGF-β/Smad Signaling Pathway to Promote Gastric Cancer. *Onco Targets Ther* 2020; **13**: 2163-2172 [PMID: 32210582 DOI: 10.2147/OTT.S234829]

77 **Xiong R**, Gao JL, Yin T. G3BP1 activates the TGF-β/Smad signaling pathway to promote gastric cancer. *Onco Targets Ther* 2019; **12**: 7149-7156 [PMID: 31564899 DOI: 10.2147/OTT.S213728]

78 **Tao L**, Yu H, Liang R, Jia R, Wang J, Jiang K, Wang Z. Rev-erbα inhibits proliferation by reducing glycolytic flux and pentose phosphate pathway in human gastric cancer cells. *Oncogenesis* 2019; **8**: 57 [PMID: 31591390 DOI: 10.1038/s41389-019-0168-5]

79 **Xia S**, Ji L, Tang L, Zhang L, Zhang X, Tang Q, Feng Z, Lu L. Proteasome Subunit Alpha Type 7 Promotes Proliferation and Metastasis of Gastric Cancer Through MAPK Signaling Pathway. *Dig Dis Sci* 2022; **67**: 880-891 [PMID: 33721161 DOI: 10.1007/s10620-021-06903-9]

80 **Feng X**, Song Z, Huang Q, Jia J, Zhang L, Zhu M, Qian J. AIM2 Promotes Gastric Cancer Cell Proliferation *via* the MAPK Signaling Pathway. *J Healthc Eng* 2022; **2022**: 8756844 [PMID: 35432843 DOI: 10.1155/2022/8756844]

81 **Xu W**, Zhou B, Wang J, Tang L, Hu Q, Chen H, Zheng J, Yan F. tRNA-Derived Fragment tRF-Glu-TTC-027 Regulates the Progression of Gastric Carcinoma *via* MAPK Signaling Pathway. *Front Oncol* 2021; **11**: 733763 [PMID: 34497772 DOI: 10.3389/fonc.2021.733763]

82 **Wu S**, Chen M, Huang J, Zhang F, Lv Z, Jia Y, Cui YZ, Sun LZ, Wang Y, Tang Y, Verhoeft KR, Li Y, Qin Y, Lin X, Guan XY, Lam KO. ORAI2 Promotes Gastric Cancer Tumorigenicity and Metastasis through PI3K/Akt Signaling and MAPK-Dependent Focal Adhesion

Disassembly. *Cancer Res* 2021; **81**: 986-1000 [PMID: 33310726 DOI: 10.1158/0008-5472.CAN-20-0049]
83 **Wang JF**, Zhao K, Chen YY, Qiu Y, Zhu JH, Li BP, Wang Z, Chen JQ. NKCC1 promotes proliferation, invasion and migration in human gastric cancer cells *via* activation of the MAPK-JNK/EMT signaling pathway. *J Cancer* 2021; **12**: 253-263 [PMID: 33391422 DOI: 10.7150/jca.49709]
84 **Zhang Q**, Wang X, Cao S, Sun Y, He X, Jiang B, Yu Y, Duan J, Qiu F, Kang N. Berberine represses human gastric cancer cell growth in vitro and in vivo by inducing cytostatic autophagy *via* inhibition of MAPK/mTOR/p70S6K and Akt signaling pathways. *Biomed Pharmacother* 2020; **128**: 110245 [PMID: 32454290 DOI: 10.1016/j.biopha.2020.110245]
85 **Xu B**, Li S, Fang Y, Zou Y, Song D, Zhang S, Cai Y. Proprotein Convertase Subtilisin/Kexin Type 9 Promotes Gastric Cancer Metastasis and Suppresses Apoptosis by Facilitating MAPK Signaling Pathway Through HSP70 Up-Regulation. *Front Oncol* 2020; **10**: 609663 [PMID: 33489919 DOI: 10.3389/fonc.2020.609663]
86 **Xiang Z**, Li J, Song S, Wang J, Cai W, Hu W, Ji J, Zhu Z, Zang L, Yan R, Yu Y. A positive feedback between IDO1 metabolite and COL12A1 *via* MAPK pathway to promote gastric cancer metastasis. *J Exp Clin Cancer Res* 2019; **38**: 314 [PMID: 31315643 DOI: 10.1186/s13046-019-1318-5]
87 **Du F**, Sun L, Chu Y, Li T, Lei C, Wang X, Jiang M, Min Y, Lu Y, Zhao X, Nie Y, Fan D. DDIT4 promotes gastric cancer proliferation and tumorigenesis through the p53 and MAPK pathways. *Cancer Commun (Lond)* 2018; **38**: 45 [PMID: 29976242 DOI: 10.1186/s40880-018-0315-y]
88 **Peng Y**, Xu Y, Zhang X, Deng S, Yuan Y, Luo X, Hossain MT, Zhu X, Du K, Hu F, Chen Y, Chang S, Feng X, Fan X, Ashktorab H, Smoot D, Meltzer SJ, Hou G, Wei Y, Li S, Qin Y, Jin Z. A novel protein AXIN1-295aa encoded by circAXIN1 activates the Wnt/β-catenin signaling pathway to promote gastric cancer progression. *Mol Cancer* 2021; **20**: 158 [PMID: 34863211 DOI: 10.1186/s12943-021-01457-w]
89 **Liu H**, Fredimoses M, Niu P, Liu T, Qiao Y, Tian X, Chen X, Kim DJ, Li X, Liu K, Dong Z. EPRS/GluRS promotes gastric cancer development *via* WNT/GSK-3β/β-catenin signaling pathway. *Gastric Cancer* 2021; **24**: 1021-1036 [PMID: 33740160 DOI: 10.1007/s10120-021-01180-x]
90 **Li H**, Zhao J, Sun J, Tian C, Jiang Q, Ding C, Gan Q, Shu P, Wang X, Qin J, Sun Y. Demethylation of the SFRP4 Promoter Drives Gastric Cancer Progression *via* the Wnt Pathway. *Mol Cancer Res* 2021; **19**: 1454-1464 [PMID: 34016745 DOI: 10.1158/1541-7786.MCR-20-0933]
91 **Guo Q**, Xu J, Huang Z, Yao Q, Chen F, Liu H, Zhang Z, Lin J. ADMA mediates gastric cancer cell migration and invasion *via* Wnt/β-catenin signaling pathway. *Clin Transl Oncol* 2021; **23**: 325-334 [PMID: 32607811 DOI: 10.1007/s12094-020-02422-7]
92 **Tian S**, Peng P, Li J, Deng H, Zhan N, Zeng Z, Dong W. SERPINH1 regulates EMT and gastric cancer metastasis *via* the Wnt/β-catenin signaling pathway. *Aging (Albany NY)* 2020; **12**: 3574-3593 [PMID: 32091407 DOI: 10.18632/aging.102831]
93 **Yue B**, Liu C, Sun H, Liu M, Song C, Cui R, Qiu S, Zhong M. A Positive Feed-Forward Loop between LncRNA-CYTOR and Wnt/β-Catenin Signaling Promotes Metastasis of Colon Cancer. *Mol Ther* 2018; **26**: 1287-1298 [PMID: 29606502 DOI: 10.1016/j.ymthe.2018.02.024]
94 **Yang XZ**, Cheng TT, He QJ, Lei ZY, Chi J, Tang Z, Liao QX, Zhang H, Zeng LS, Cui SZ. LINC01133 as ceRNA inhibits gastric cancer progression by sponging miR-106a-3p to regulate APC expression and the Wnt/β-catenin pathway. *Mol Cancer* 2018; **17**: 126 [PMID: 30134915 DOI: 10.1186/s12943-018-0874-1]
95 **Xiaobin C**, Zhaojun X, Tao L, Tianzeng D, Xuemei H, Fan Z, Chunyin H, Jianqiang H, Chen L. Analysis of Related Risk Factors and Prognostic Factors of Gastric Cancer with Bone Metastasis: A SEER-Based Study. *J Immunol Res* 2022; **2022**: 3251051 [PMID: 35211630 DOI: 10.1155/2022/3251051]
96 **Song L**, Wang S, Li Q, Lu Y, Yang R, Feng X. Identification and Validation of a m5C RNA Modification-Related Gene Signature for Predicting Prognosis and Immunotherapeutic Efficiency of Gastric Cancer. *J Oncol* 2023; **2023**: 9931419 [PMID: 36936373 DOI: 10.1155/2023/9931419]
97 **Khan M**, Lin J, Wang B, Chen C, Huang Z, Tian Y, Yuan Y, Bu J. A novel necroptosis-related gene index for predicting prognosis and a cold tumor immune microenvironment in stomach adenocarcinoma. *Front Immunol* 2022; **13**: 968165 [PMID: 36389725 DOI: 10.3389/fimmu.2022.968165]
98 **Huo J**, Guan G, Cai J, Wu L. Integrated analysis of 1804 samples of six centers to construct and validate a robust immune-related prognostic signature associated with stromal cell abundance in tumor microenvironment for gastric cancer. *World J Surg Oncol* 2022; **20**: 4 [PMID: 34983559 DOI: 10.1186/s12957-021-02485-y]
99 **Dong R**, Chen S, Lu F, Zheng N, Peng G, Li Y, Yang P, Wen H, Qiu Q, Wang Y, Wu H, Liu M. Models for Predicting Response to Immunotherapy and Prognosis in Patients with Gastric Cancer: DNA Damage Response Genes. *Biomed Res Int* 2022; **2022**: 4909544 [PMID: 36578802 DOI: 10.1155/2022/4909544]
100 **Sun Q**, Guo D, Li S, Xu Y, Jiang M, Li Y, Duan H, Zhuo W, Liu W, Zhu S, Wang L, Zhou T. Combining gene expression signature with clinical features for survival stratification of gastric cancer. *Genomics* 2021; **113**: 2683-2694 [PMID: 34129933 DOI: 10.1016/j.ygeno.2021.06.018]
101 **Huo J**, Wu L, Zang Y. Construction and Validation of a Universal Applicable Prognostic Signature for Gastric Cancer Based on Seven Immune-Related Gene Correlated With Tumor Associated Macrophages. *Front Oncol* 2021; **11**: 635324 [PMID: 34178625 DOI: 10.3389/fonc.2021.635324]
102 **Guo X**, Liang X, Wang Y, Cheng A, Zhang H, Qin C, Wang Z. Significance of Tumor Mutation Burden Combined With Immune Infiltrates in the Progression and Prognosis of Advanced Gastric Cancer. *Front Genet* 2021; **12**: 642608 [PMID: 34306002 DOI: 10.3389/fgene.2021.642608]
103 **Hu C**, Zhou Y, Liu C, Kang Y. A novel scoring system for gastric cancer risk assessment based on the expression of three CLIP4 DNA methylation-associated genes. *Int J Oncol* 2018; **53**: 633-643 [PMID: 29901187 DOI: 10.3892/ijo.2018.4433]
104 **Huang J**, Song J, Li X, Liu S, Huang W, Shen Z, Cheng Y, Kou S, Gao Z, Tian Y, Hu J. Analysis and prognostic significance of tumour immune infiltrates and immune microenvironment of m6A-related lncRNAs in patients with gastric cancer. *BMC Med Genomics* 2022; **15**: 164 [PMID: 35879790 DOI: 10.1186/s12920-022-01318-5]
105 **Zhao Y**, Hu S, Zhang J, Cai Z, Wang S, Liu M, Dai J, Gao Y. Glucoside xylosyltransferase 2 as a diagnostic and prognostic marker in gastric cancer *via* comprehensive analysis. *Bioengineered* 2021; **12**: 5641-5654 [PMID: 34506251 DOI: 10.1080/21655979.2021.1967067]
106 **Cao L**, Wang S, Zhang Y, Wong KC, Nakatsu G, Wang X, Wong S, Ji J, Yu J. Zinc-finger protein 471 suppresses gastric cancer through transcriptionally repressing downstream oncogenic PLS3 and TFAP2A. *Oncogene* 2018; **37**: 3601-3616 [PMID: 29610526 DOI: 10.1038/s41388-018-0220-5]
107 **Yang Y**, Shi Z, Bai R, Hu W. Heterogeneity of MSI-H gastric cancer identifies a subtype with worse survival. *J Med Genet* 2021; **58**: 12-19 [PMID: 32170001 DOI: 10.1136/jmedgenet-2019-106609]

108 **Pietrantonio F**, Randon G, Di Bartolomeo M, Luciani A, Chao J, Smyth EC, Petrelli F. Predictive role of microsatellite instability for PD-1 blockade in patients with advanced gastric cancer: a meta-analysis of randomized clinical trials. *ESMO Open* 2021; **6**: 100036 [PMID: 33460964 DOI: 10.1016/j.esmoop.2020.100036]

109 **Kwon M**, An M, Klempner SJ, Lee H, Kim KM, Sa JK, Cho HJ, Hong JY, Lee T, Min YW, Kim TJ, Min BH, Park WY, Kang WK, Kim KT, Kim ST, Lee J. Determinants of Response and Intrinsic Resistance to PD-1 Blockade in Microsatellite Instability-High Gastric Cancer. *Cancer Discov* 2021; **11**: 2168-2185 [PMID: 33846173 DOI: 10.1158/2159-8290.CD-21-0219]

110 **Chao J**, Fuchs CS, Shitara K, Tabernero J, Muro K, Van Cutsem E, Bang YJ, De Vita F, Landers G, Yen CJ, Chau I, Elme A, Lee J, Özgüroglu M, Catenacci D, Yoon HH, Chen E, Adelberg D, Shih CS, Shah S, Bhagia P, Wainberg ZA. Assessment of Pembrolizumab Therapy for the Treatment of Microsatellite Instability-High Gastric or Gastroesophageal Junction Cancer Among Patients in the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 Clinical Trials. *JAMA Oncol* 2021; **7**: 895-902 [PMID: 33792646 DOI: 10.1001/jamaoncol.2021.0275]

111 **Pietrantonio F**, Miceli R, Raimondi A, Kim YW, Kang WK, Langley RE, Choi YY, Kim KM, Nankivell MG, Morano F, Wotherspoon A, Valeri N, Kook MC, An JY, Grabsch HI, Fucà G, Noh SH, Sohn TS, Kim S, Di Bartolomeo M, Cunningham D, Lee J, Cheong JH, Smyth EC. Individual Patient Data Meta-Analysis of the Value of Microsatellite Instability As a Biomarker in Gastric Cancer. *J Clin Oncol* 2019; **37**: 3392-3400 [PMID: 31513484 DOI: 10.1200/JCO.19.01124]

112 **Ajani JA**, Jiang Y, Faust J, Chang BB, Ho L, Yao JC, Rousey S, Dakhil S, Cherny RC, Craig C, Bleyer A. A multi-center phase II study of sequential paclitaxel and bryostatin-1 (NSC 339555) in patients with untreated, advanced gastric or gastroesophageal junction adenocarcinoma. *Invest New Drugs* 2006; **24**: 353-357 [PMID: 16683077 DOI: 10.1007/s10637-006-6452-1]

113 **Tseng LL**, Cheng HH, Yeh TS, Huang SC, Syu YY, Chuu CP, Yuh CH, Kung HJ, Wang WC. Targeting the histone demethylase PHF8-mediated PKCα-Src-PTEN axis in HER2-negative gastric cancer. *Proc Natl Acad Sci U S A* 2020; **117**: 24859-24866 [PMID: 32958674 DOI: 10.1073/pnas.1919766117]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 October 24; 14(10): 400-408

DOI: 10.5306/wjco.v14.i10.400

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

Treatment of patients with multiple brain metastases by isolated radiosurgery: Toxicity and survival

André Vinícius de Camargo, Marcos Duarte de Mattos, Murilo Kenji Kawasaki, Danilo Nascimento Salviano Gomes, Allisson Bruno Barcelos Borges, Vinicius de Lima Vazquez, Raphael L C Araujo

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): E

P-Reviewer: Bergsma H, Netherlands; Kupeli S, Turkey; Tang H, China

Received: June 27, 2023
Peer-review started: June 27, 2023
First decision: August 10, 2023
Revised: September 1, 2023
Accepted: September 22, 2023
Article in press: September 22, 2023
Published online: October 24, 2023

André Vinícius de Camargo, Marcos Duarte de Mattos, Murilo Kenji Kawasaki, Danilo Nascimento Salviano Gomes, Department of Radiotherapy, Barretos Cancer Hospital, São Paulo, Barretos 14784-400, Brazil

Allisson Bruno Barcelos Borges, Department of Radiation Therapy, Hospital DF Star Rede D´Or, Brasília 70390-140, Brazil

Vinicius de Lima Vazquez, Department of Surgery, Barretos Cancer Hospital, São Paulo, Barretos 14784-400, Brazil

Raphael L C Araujo, Department of Surgery, Universidade Federal de São Paulo, São Paulo 04024-002, Brazil

Raphael L C Araujo, IEP, Barretos Cancer Hospital, São Paulo, Barretos 14784-400, Brazil

Corresponding author: Raphael L C Araujo, MD, PhD, Adjunct Professor, Surgical Oncologist, Department of Surgery, Universidade Federal de São Paulo, No. 715 Napoleão de Barros Street, São Paulo 04024-002, Brazil. raphaellcaraujo@gmail.com

Abstract

BACKGROUND

Radiosurgery for multiple brain metastases has been more reported recently without using whole-brain radiotherapy. Nevertheless, the sparsity of the data still claims more information about toxicity and survival and their association with both dosimetric and geometric aspects of this treatment.

AIM

To assess the toxicity and survival outcome of radiosurgery in patients with multiple (four or more lesions) brain metastases.

METHODS

In a single institution, data were collected retrospectively from patients who underwent radiosurgery to treat brain metastases from diverse primary sites. Patients with 4-21 brain metastases were treated with a single fraction with a dose of 18 Gy or 20 Gy. The clinical variables collected were relevant to toxicity, survival, treatment response, planning, and dosimetric variables. The Spearman's rank correlation coefficients, Mann-Whitney test, Kruskal-Wallis test, and Log-

rank test were used according to the type of variable and outcomes.

RESULTS

From August 2017 to February 2020, 55 patients were evaluated. Headache was the most common complaint (38.2%). The median overall survival (OS) for patients with karnofsky performance status (KPS) > 70 was 8.9 mo, and this was 3.6 mo for those with KPS ≤ 70 (P = 0.047). Patients with treated lesions had a median progression-free survival of 7.6 mo. There were no differences in OS (19.7 *vs* 9.5 mo) or progression-free survival (10.6 *vs* 6.3 mo) based on prior irradiation. There was no correlation found between reported toxicities and planning, dosimetric, and geometric variables, implying that no additional significant toxicity risks appear to be added to the treatment of multiple (four or more) lesions.

CONCLUSION

No associations were found between the evaluated toxicities and the planning dosimetric parameters, and no differences in survival rates were detected based on previous treatment status.

Key Words: Radiosurgery; Brain metastases; Radiotherapy; Survival; Toxicity; Cancer

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Toxicity and survival outcome of radiosurgery in patients with multiple brain metastases (≥ 4) were evaluated. A total of 55 patients were evaluated; headache was the most common complaint, but no associations were found between the evaluated toxicities and the planning and dosimetric parameters. The median overall survival found was 10 mo and the survival of the group that did not undergo irradiation before radiosurgery was 9.5 mo. The results are equivalent to those found by authors who evaluated patients with up to four lesions. Our data demonstrate the safe use of isolated stereotactic radiosurgery to treat patients with four or more brain metastases.

Citation: de Camargo AV, de Mattos MD, Kawasaki MK, Gomes DNS, Borges ABB, Vazquez VL, Araujo RLC. Treatment of patients with multiple brain metastases by isolated radiosurgery: Toxicity and survival. *World J Clin Oncol* 2023; 14(10): 400-408
URL: https://www.wjgnet.com/2218-4333/full/v14/i10/400.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i10.400

INTRODUCTION

It is estimated that 19.3 million new cancer cases and 10 million deaths occurred in 2020. Breast (11.7%) and lung (11.4%) cancers are among the most common cancer cases, causing 2.5 million deaths (24.9% of all cancer deaths)[1]. Besides being the most prevalent in the population, they are the most prevalent cancer types that evolve into brain metastasis due to their favorable microenvironment for brain metastases development[2,3].

The main radiotherapy technique used in brain metastasis is stereotactic radiosurgery (SRS) performed in a linear accelerator (LA). Thus, it is necessary to determine whether the treatment of multiple brain metastases by isolated radiotherapy is safe and non-inferior to the treatment of one or few lesions, regarding toxicity and survival[4-7] and if previous treatment, such as whole brain radiotherapy (WBRT), is beneficial before radiosurgery[8,9]. Moreover, to determine which therapy is appropriate for each patient's prognosis, it is also important to estimate the survival rate of patients with brain metastasis through prognostic factors such as Karnofsky performance status (KPS), diagnosis-specific graded prognostic assessment (DS-GPA), score index for radiosurgery (SIR), and recursive partitioning analyses (RPA), to determine which therapy is adequate for the prognosis of each patient[10-14].

This work aimed to evaluate the toxicity of isolated radiosurgery in patients with multiple brain metastases (≥ 4 lesions). In addition, overall survival and progression-free survival were evaluated, and survival was correlated with the prognostic index.

MATERIALS AND METHODS

Retrospective data were collected from 55 patients who underwent radiosurgery at Barretos Cancer Hospital from August 2017 to February 2020. Patients who presented with 4-21 brain metastases delineated with the aid of magnetic resonance (MR) were treated in a single fraction with a dose of 18 Gy or 20 Gy. Patients who met the inclusion criteria were included regardless of previous systemic and primary local treatment since all of them received radiation therapy for four or more brain lesions in palliative manners, and the main outcomes were either local or systemic toxicity. A frameless immobilization system was used for simulation and treatment. Simulation computed tomography with a slice thickness of 1.25 mm was used for all plannings.

All lesions were treated on a Varian TrueBeam® ™ STX Varian Medical Systems LA with high-definition mulitleaf (120-leaf) collimator and planned with an Eclipse® treatment planning system (Varian Medical System Inc, version 13.6). The calculation algorithm used was the anisotropic analytical algorithm with a 1.25 mm calculation grid and heterogeneity correction. VMAT (RapidArc®, Varian Medical System, Inc.) treatment technique was used for all cases with a planning target volume (PTV) margin of 1 mm from the gross target volume contour. Before treatment, a cone-beam computed tomography scan was performed. Planning was carried out by the Department of Radiation Oncology with many physicists and radiation oncologists in who followed the institutional protocol of dose constraints in the organs at risk and of coverage of targets.

The following toxicities were collected: Headache, convulsion, focal deficit, drop in the level of consciousness, fatigue, nausea or vomiting, and mental confusion. They were based on the Common Terminology Criteria for Adverse Events. The patient's first complaint after radiosurgery was selected.

Prognostic factors were also collected: Initial KPS and that at the first follow-up after radiosurgery, DS-GPA, SIR, and RPA. In addition, age, gender, and the International Classification of Disease of the primary tumor were also surveyed.

Dosimetric variables included were V5Gy, V8Gy, V10Gy, V12Gy, V14Gy, conformity index (CI), heterogeneity index (HI), dmax, and 50% isodose CI (CI_R50). The VxGy represents the volume of the "x" Gy dose that the normal brain minus PTV received. The CI was calculated by the ratio between the volume of the prescription isodose and the volume of the PTVs: $V_{presc_isodose}/V_{PTVs.}$ The HI was calculated as $(D_{2\%}-D_{98\%})/D_{50\%}$[15]. Dmax is the maximum point dose of the plan and the CI_R50 is the ratio between the volume of the 50% isodose line and the volume of the PTVs.

The geometric variables collected were the number of lesions, total target volumes, the smallest and largest target volumes, and the distance between the isocenter and the most distant lesion. The distance between the isocenter and the most distant lesion was determined using the coordinates of the lesion center and its respective isocenter. The calculation was according to equation 1. In cases where there was more than one isocenter, the distance was measured between the isocenter and the most distant lesion that its arcs was treated, as demonstrated in the Supplementary material.

Technical variables included were the total number of arcs, coplanar or non-coplanar arcs, number of non-coplanar arcs if used, and number of isocenters. The correlations between the dosimetric, geometric, and technical variables collected for this work were previously published by our group[16].

We reported the response in treated lesions as complete response, partial response, stable disease, progressive disease, or radionecrosis. Complete response indicated complete remission of all lesions; partial response indicated that some lesions entered complete remission, while others remained stable; stable disease indicated that all lesions remained the same size; progressive disease indicated that at least one of the lesions enlarged in size; and radionecrosis indicated that at least one lesion went through necrosis due to radiation. Information on the location of new lesions was also reported as either parenchymal or meningeal.

The initial date of treatment was used for estimating the overall and progression-free survival rates and for the outcomes, respectively, the date of death or the date of the last information obtained in the medical records after the treatment, and the date of the MR in which the progression of the treated lesions was detected or the date of the MR in which the appearance of new lesions was detected were used. Survival rates were calculated based on the data of 53 patients with assessable clinical records.

In this study, radiosurgery treatment of multiple brain metastasis (≥ 4) delivered in a isolated manner and at a single dose was referred to as radiosurgery. Whenever the patient underwent radiosurgery in more than one course of treatment, we would consider the first radiosurgery with four or more lesions. To evaluate if the previous treatment influenced survival rates, patients were divided into two groups: No previous irradiation (NP) and irradiation before radiosurgery (P).

Comparisons of toxicities between categories or between different groups of patients were made using chi-square tests or Fisher exact tests, and Mann-Whitney tests for continuous variables, and the relation between prognostic factors and age was evaluated using Mann-Whitney or Kruskal-Wallis tests. The results are presented as proportions or median and interquartile when appropriate. Spearman's correlation coefficient was used to determine if there was a correlation between toxicities and dosimetric and geometric variables. KPS comparison was made using a marginal homogeneity test. Survival was estimated by the Kaplan-Meier method and the curves of each category were compared using the Log-rank test. Statistical relevance was considered if $P < 0.05$. Data were collected and managed using the research electronic data capture platform[17] and analyzed using the software SSPS® (v. 20).

RESULTS

The descriptive characteristics of groups NP and P are displayed in Table 1. Briefly, the most prescribed dosage was 18 Gy (83.6%), and 67.3% of patients were female. Of the 55 patients who underwent radiosurgery, 32 (58.2%) declared feeling some toxicity, with headaches (38.2%) being the most frequent. Incidence rates for each toxicity are shown in Table 2.

The number of reported cases of toxicity as a function of time after treatment is shown in Table 3. It was observed that the highest incidence (40.6%) occurred between the first and third month after treatment. To deal with the heterogeneity of patients who had nervous system irradiation more than once, they were divided into four groups: (1) Patients with ≥ 4 lesions who underwent radiosurgery only; (2) patients with previous either WBRT or SRS with less than four lesions or fractionated stereotactic radiotherapy (SRT); (3) patients that underwent irradiation after radiosurgery and reported side effects after the second irradiation; and (4) patients that were irradiated before and after radiosurgery and reported some toxicities after the last irradiation.

Table 1 Descriptive characteristics of 55 patients treated by radiosurgery for multiple brain metastases (%)

Characteristic	NP (*n* = 35)	*P* (*n* = 20)	*P* value
Age at treatment (yr)[1]	62 (51-67)	53 (42-58)	0.032
Age (yr)			0.162
< 55	15 (42.9)	13 (65)	
≥ 55	20 (57.1)	7 (35)	
Gender			0.391
Female	22 (62.9)	15 (75)	
Male	13 (37.1)	5 (25)	
Number of lesions[1]	5 (4-7)	5 (4-7)	0.89
Target volumes[1]	5.4 (2.2-9.9)	2.9 (1.63-4.3)	0.08
Primary site			0.85
Lung	16 (45.7)	7 (35)	
Breast	8 (22.9)	7 (35)	
Melanoma	8 (22.9)	6 (30)	
Others	3 (8.6)	0	
KPS	*n* = 34	*n* = 20	0.194
≤ 70	6 (17.6)	7 (35)	
> 70	28 (82.4)	13 (65)	
DS-GPA	*n* = 31	*n* = 20	0.7
0-1	11 (35.5)	8 (40)	
1.5-2	14 (45.2)	7 (35)	
2.5-3	4 (12.9)	2 (10)	
3-4	2 (6.5)	3 (15)	
RPA	*n* = 34	*n* = 20	0.751
Class 1	2 (5.9)	1 (5)	
Class 2	28 (82.4)	15 (75)	
Class 3	4 (11.8)	4 (20)	
SIR[1]	*n* = 34	*n* = 20	0.104
	4 (4-6)	5 (4-6.5)	
Prescription dose (Gy)			0.133
20	8 (22.9)	1 (5)	
18	27 (77.1)	19 (95)	

[1]Median and interquartile range (p25-p75).

P < 0.05 was considered significant. P: Previous irradiation before radiosurgery; NP: No previous irradiation; KPS: Karnofsky performance status; DS-GPA: Diagnosis-specific graded prognostic assessment; RPA: Recursive partitioning analyses; SIR: Score index for radiosurgery.

The proportion of patients per technique that had one irradiation before SRS of multiple lesions was ten for WBRT, seven for SRS with less than four lesions, and eight for SRT. Five patients did two previous irradiations before treating multiple brain metastases (≥ 4 lesions) by SRS. The proportion of patients in each group that reported toxicity was 17 of 18 patients (94.4%) in group 1; 7 of 11 (12.7%) in group 2; 3 of 17 (17.6%) in group 3; and 5 of 9 (55.6%) in group 4.

The incidence of toxicities in each patient group is presented in Table 4. Despite the higher incidence in group 1, no statistical relevance was found between the four groups regarding the seven toxicities. There was also no difference in the toxicities among the different categories of DS-GPA, RPA, and SIR.

Regarding the response of treated lesions and the emergence of new lesions, there were no differences observed between the P and NP groups ($P = 0.643$ and $P = 0.412$, respectively). A single patient had a complete response, 17 had partial responses, seven were stable, 15 had progression, and six presented radionecrosis (half in each group).

Table 2 Incidence of toxicities

Toxicity	*n* of patients	Percentage (%)
Headache	21	38.2
Convulsion	4	7.3
Focal deficit	5	9.1
Drop in level of consciousness	3	5.5
Fatigue	8	14.5
Nausea or vomiting	6	10.9
Mental confusion	1	1.8

Table 3 Number of reported cases of toxicity per period after treatment

Period (mo)	*n* of patients	Percentage
$t < 1$	4	12.5
$1 \leq t < 3$	13	40.6
$3 \leq t < 6$	6	18.8
$6 \leq t < 9$	6	18.8
$9 \leq t < 12$	2	6.2
$t \geq 12$	1	3.1

Table 4 Incidence of toxicity in each group of patients, *n* (%)

	Group 1	Group 2	Group 3	Group 4
Headache	10 (58.8)	4 (57.1)	2 (66.7)	5 (100)
Convulsion	1 (5.9)	1 (14.3)	1 (33.3)	1 (20)
Focal deficit	3 (17.6)	1 (14.3)		1 (20)
Drop in level of consciousness	2 (11.8)		1 (33.3)	
Fatigue	6 (35.3)	1 (14.3)	1 (33.3)	
Nausea or vomiting	5 (29.4)	1 (14.3)		
Mental confusion	1 (5.9)			
Total	28	8	5	7

Despite the higher number of patients that presented new lesions in group NP (18) compared to group P (11), there were no differences between the two groups. The number of patients with new lesions was 29, and 17 patients did not develop new lesions. According to location, new parenchymal lesions (26) were more frequent than meningeal ones (3).

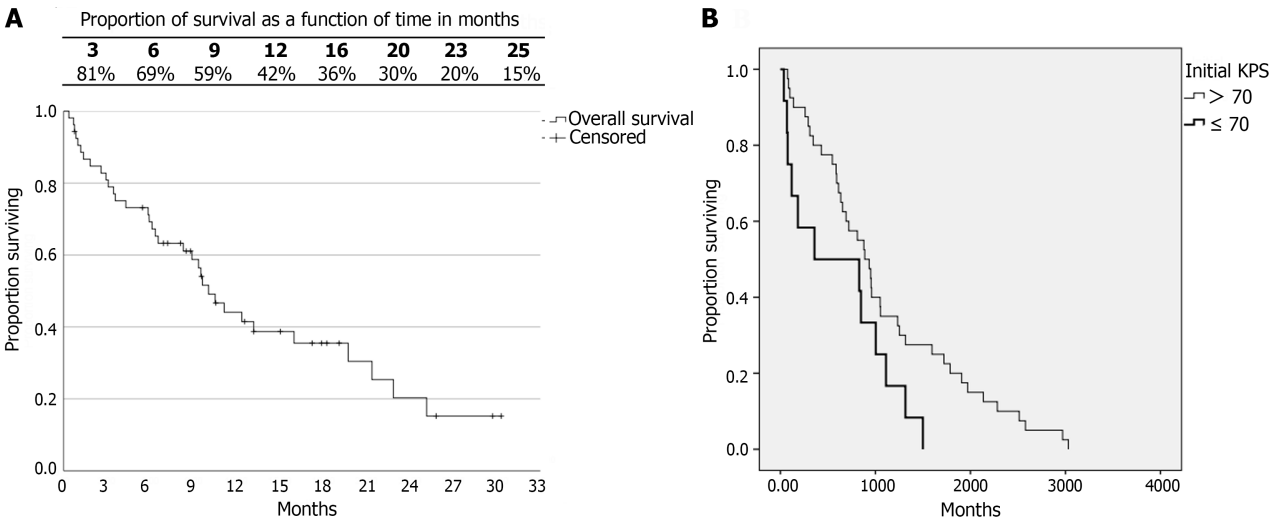
Comparing the initial KPS with that evaluated in the first consult after treatment, a relevant difference was observed between them ($P = 0.033$). The percentage of patients whose KPS decreased after treatment was 39.6%, and 60.4% of patients improved or maintained their KPS.

No statistical correlation was observed between dosimetric and geometric variables and toxicities. The descriptive statistics of dosimetric, geometric, and technical variables have previously been published by our group[16].

The average overall survival (OS) was 13.3 mo, and the median was 10 mo. The life expectancy over time can be observed in Figure 1. It is noteworthy that the survival rate at 12 mo was 42%. Of 53 patients, 78% died, and of those, only 10 patients (18.9%) were due to neurological causes.

No differences were observed in the OS rates between groups NP and P. The median survival in group NP was 9.5 mo, and in group P it was 19.7 mo ($P = 0.110$). Considering patients with KPS > 70 and KPS ≤ 70, a difference was observed in OS ($P = 0.047$) (Figure 1B). The median survival of the group with KPS > 70 was 8.9 mo, and in the group with KPS ≤ 70 it was 3.6 mo. No differences were observed in the survival of DS-GPA ($P = 0.547$), RPA ($P = 0.113$), and SIR categories 0 to 4 and 5 to 10 ($P = 0.586$).

The OS of patients was categorized into two groups for each variable for analysis. No difference between them was found: Number of lesions ($P = 0.840$), $n < 6$ (10.5 mo) and $n \geq 6$ (9.3 mo); volume of targets ($P = 0.786$), v < 5 cc (10.5 mo)



DOI: 10.5306/wjco.v14.i10.400 **Copyright** ©The Author(s) 2023.

Figure 1 Kaplan-Meyer curves for overall survival of 53 patients treated by radiosurgery for multiple brain metastases. A: Overall survival (OS); B: OS according to Karnofsky performance status (KPS) > 70 and KPS ≤ 70.

and v ≥ 5 cc (9.5 mo); V12Gy ≤ 10 cc (11.1 mo) and V12Gy > 10 cc (9.6 mo) (P = 0.693); CI_R50 ≤ 8 (13.2 mo) and CI_R50 > 8 (9.6 mo) (P = 0.655).

The median progression-free survival of patients with treated lesions (PFSL) was 7.6 mo. No differences (P = 0.293) were found between groups NP and P. The median PFSL of group NP was 6.3 mo, and in group P it was 10.6 mo. The curves for the PFSL of both groups are displayed in Figure 2A. The median survival free from the appearance of new lesions was 6 mo. No difference was observed between groups NP and P (P = 0.188). The median for group NP was 4.5 mo, and for group P it was 8.9 mo. The curves of survival free from the appearance of new lesions in both groups are displayed in Figure 2B.

DISCUSSION

It was observed that in group 1, a higher proportion (94.4%) of patients reported grievances and a higher number of different toxicities. Nevertheless, no difference was observed between groups when comparing their toxicity incidence. Besides, the toxicities reported varied regarding their start point. One of the patients reported a grievance a year after treatment, thus rendering it difficult to classify it as a side effect of radiosurgery.

Analyzing the responses of treated lesions, six patients developed radionecrosis. As discussed by Blonigen *et al*[18], V10Gy and V12Gy can be predictors of radionecrosis. The median of V10Gy and V12Gy of those six patients was 27.8 cc (9.7-45.5 cc) and 17.6 cc (6.2-27.4 cc), respectively (only a single patient had the dosage of 20Gy as prescription).

The median OS found by Chang *et al*[8] was 9.2 mo and the median survival of patients treated only by SRS was 15.2 mo. Aoyama *et al*[9] obtained a median OS of 8 mo on the arm of patients treated only by SRS. Brown *et al*[19] found a median OS of 13.5 mo and a median survival of 10.4 mo for patients treated only by SRS.

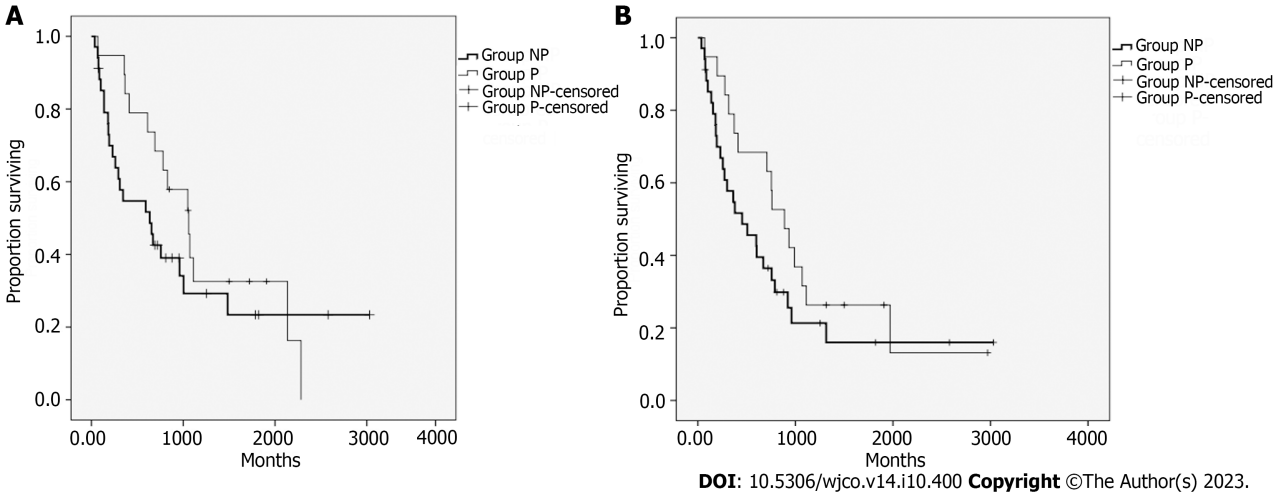
Sahgal *et al*[20] found a median OS of 10 mo for the group that only received SRS. The median time to local failure and development of new lesions was 6.6 mo and 4.7 mo, respectively. This last result matches the PFSL and the development of new lesions in this study. The four aforementioned studies compared patients who underwent SRS alone with patients treated by SRS + WBRT.

Scorsetti *et al*[21] observed a median OS of 16.2 mo and a 12-mo survival rate of 65.3% in the group of patients who underwent only SRS with an LA. They also indicated that 27 of the 130 patients (20.8%) included in that study presented symptomatic radionecrosis. The incidence obtained in that study was 10.9%.

Differently from the studies mentioned before, in which treated patients had up to four lesions, the current study evaluated patients with 4 to 21 lesions and, despite the underestimated OS (group P began treatment of metastasis before the studied SRS), it was observed that survival values are similar to studies with up to four lesions, especially when analyzing the global survival of all patients, and the survival of patients without previous irradiation, whose comparison is possible with the aforementioned studies.

Among the prognostic indexes, despite the predictive power of survival from DS-GPA, RPA, and SIR[11-14,22] being better than KPS for patients with brain metastasis, only KPS showed a difference in OS (P = 0.047) between patients with KPS > 70 and KPS ≤ 70. This likely occurred due to KPS considering only the clinical condition of patients, whereas other indexes also consider specific parameters of patients with brain metastasis that were not discretized in the analysis, such as the primary site of disease, number of lesions, and systemic diseases, among others.

Regarding the number of patients whose KPS decreased, it is important to note that metastatic patients have systemic diseases that worsen the clinical outcome. Therefore, we cannot contribute the decline of KPS to SRS, which is corrob-



DOI: 10.5306/wjco.v14.i10.400 **Copyright** ©The Author(s) 2023.

Figure 2 Kaplan-Meyer curves for progression-free survival. A: Progression-free survival (PFS) of 34 patients with treated lesions who did not undergo previous irradiation (NP group) and 19 patients who underwent prior irradiation (P group); B: PFS addressing the appearance of new lesions in both NP and P groups.

orated by the low death number due to neurological causes.

According to dosimetric, geometric, and technical variables, the lack of correlation with toxicities does not imply they do not impact each other, especially considering dosimetric variables used for planning approval. It is known that the volume of targets, number of lesions, distance between lesions, and the isocenter impact these plan evaluation indexes[16, 23-25]. What can be observed is that the indication of isolated radiosurgery for multiple brain metastases was safe, considering that the technique achieved dosimetric values good enough not to cause collateral effects.

This study has limitations inherent to the retrospective cohort model where selection and information biases cannot be discarded. There were patients subjected to multiple irradiation techniques before the SRS in this study, and many of the patients were also under systemic treatment, which may interfere with the clinical results. In addition, all of them received some or many kinds of local and systemic treatment for many types of tumors, since in our institution the radiosurgery for four or more lesions was reserved for local control in a palliative manner and usually failed for previous treatments. Regarding the toxicities, precise graduation was not possible to obtain and therefore, they were not differentiated. Although our study had some missing data for clinical variables, they represent less than 4%, which seems acceptable for a retrospective study[26]. If factors were significantly associated with outcomes in univariate analysis, and they were not as demonstrated in Table 1, they would be entered into a multivariate analysis, but it was not possible.

The planning was performed by different personnel, with distinct dose prescriptions and, in some patients with one or more lesions (more significant volumes), planned with three fractions but, even in these cases, there were also four or more lesions treated with a single dose. Considering the prescription, we tested the difference between the doses of 18 and 20 Gy regarding geometric, dosimetric, and technical variables, and no differences were observed.

CONCLUSION

Our data demonstrate the safe use of isolated SRS to treat patients with four or more brain metastases, with no significant association between dosimetric, geometric, or clinical parameters and the related toxicities.

ARTICLE HIGHLIGHTS

Research background

Radiosurgery for multiple brain metastases has been more reported recently without using whole-brain radiotherapy, but mainly for oligometastatic scenarios (up to 3-4 lesions). Nevertheless, the sparsity of the data still claims more information about toxicity and survival and their association with both dosimetric and geometric aspects of this treatment, especially for the presence of more lesions or in patients with previous irradiation.

Research motivation

To evaluate the toxicity of treatment offered for patients with four or more lesions.

Research objectives

To assess associations of toxicity and survival outcome of stereotactic radiosurgery (SRS) among patients with four or more brain lesions with or without previous brain irradiation.

Research methods
Retrospective cohort.

Research results
Neither difference in toxicity nor survival was detected when comparing patients who underwent SRS for four or more brain lesions with or without previous brain irradiation.

Research conclusions
This retrospective study did not detect differences in toxicity for this population with or without previous irradiation, suggesting that the use of SRS for four or more brain lesions with or without previous brain irradiation is safe.

Research perspectives
This study claims for more data in larger studies in a prospective manner to better address this question.

FOOTNOTES

Author contributions: de Camargo AV, Borges ABB, Vazquez VL, and Araujo RLC contributed to conceptualization; de Camargo AV, de Mattos MD, Kawasaki MK, Gomes DNS, and Borges ABB contributed to data collection; de Camargo AV and Araujo RLC contributed to data analysis; all authors have read and approved the final manuscript.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Barretos Cancer Hospital.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Brazil

ORCID number: André Vinícius de Camargo 0000-0001-7153-6724; Marcos Duarte de Mattos 0000-0002-5069-8051; Murilo Kenji Kawasaki 0000-0001-5151-5221; Danilo Nascimento Salviano Gomes 0000-0002-7909-0728; Allisson Bruno Barcelos Borges 0000-0003-3868-4420; Vinicius de Lima Vazquez 0000-0002-0325-5514; Raphael L C Araujo 0000-0002-7834-5944.

S-Editor: Qu XL
L-Editor: Wang TQ
P-Editor: Cai YX

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
2 **Huh HD**, Sub Y, Oh J, Kim YE, Lee JY, Kim HR, Lee S, Lee H, Pak S, Amos SE, Vahala D, Park JH, Shin JE, Park SY, Kim HS, Roh YH, Lee HW, Guan KL, Choi YS, Jeong J, Choi J, Roe JS, Gee HY, Park HW. Reprogramming anchorage dependency by adherent-to-suspension transition promotes metastatic dissemination. *Mol Cancer* 2023; **22**: 63 [PMID: 36991428 DOI: 10.1186/s12943-023-01753-7]
3 **Zou Y**, Ye F, Kong Y, Hu X, Deng X, Xie J, Song C, Ou X, Wu S, Wu L, Xie Y, Tian W, Tang Y, Wong CW, Chen ZS, Xie X, Tang H. The Single-Cell Landscape of Intratumoral Heterogeneity and The Immunosuppressive Microenvironment in Liver and Brain Metastases of Breast Cancer. *Adv Sci (Weinh)* 2023; **10**: e2203699 [PMID: 36529697 DOI: 10.1002/advs.202203699]
4 **Yamamoto M**, Serizawa T, Shuto T, Akabane A, Higuchi Y, Kawagishi J, Yamanaka K, Sato Y, Jokura H, Yomo S, Nagano O, Kenai H, Moriki A, Suzuki S, Kida Y, Iwai Y, Hayashi M, Onishi H, Gondo M, Sato M, Akimitsu T, Kubo K, Kikuchi Y, Shibasaki T, Goto T, Takanashi M, Mori Y, Takakura K, Saeki N, Kunieda E, Aoyama H, Momoshima S, Tsuchiya K. Stereotactic radiosurgery for patients with multiple brain metastases (JLGK0901): a multi-institutional prospective observational study. *Lancet Oncol* 2014; **15**: 387-395 [PMID: 24621620 DOI: 10.1016/S1470-2045(14)70061-0]
5 **Yamamoto M**, Kawabe T, Sato Y, Higuchi Y, Nariai T, Watanabe S, Kasuya H. Stereotactic radiosurgery for patients with multiple brain metastases: a case-matched study comparing treatment results for patients with 2-9 versus 10 or more tumors. *J Neurosurg* 2014; **121** Suppl: 16-25 [PMID: 25434933 DOI: 10.3171/2014.8.GKS141421]
6 **Minniti G**, Clarke E, Lanzetta G, Osti MF, Trasimeni G, Bozzao A, Romano A, Enrici RM. Stereotactic radiosurgery for brain metastases: analysis of outcome and risk of brain radionecrosis. *Radiat Oncol* 2011; **6**: 48 [PMID: 21575163 DOI: 10.1186/1748-717X-6-48]

7 **Fokas E**, Henzel M, Surber G, Kleinert G, Hamm K, Engenhart-Cabillic R. Stereotactic radiosurgery and fractionated stereotactic radiotherapy: comparison of efficacy and toxicity in 260 patients with brain metastases. *J Neurooncol* 2012; **109**: 91-98 [PMID: 22528795 DOI: 10.1007/s11060-012-0868-6]

8 **Chang EL**, Wefel JS, Hess KR, Allen PK, Lang FF, Kornguth DG, Arbuckle RB, Swint JM, Shiu AS, Maor MH, Meyers CA. Neurocognition in patients with brain metastases treated with radiosurgery or radiosurgery plus whole-brain irradiation: a randomised controlled trial. *Lancet Oncol* 2009; **10**: 1037-1044 [PMID: 19801201 DOI: 10.1016/S1470-2045(09)70263-3]

9 **Aoyama H**, Shirato H, Tago M, Nakagawa K, Toyoda T, Hatano K, Kenjyo M, Oya N, Hirota S, Shioura H, Kunieda E, Inomata T, Hayakawa K, Katoh N, Kobashi G. Stereotactic radiosurgery plus whole-brain radiation therapy vs stereotactic radiosurgery alone for treatment of brain metastases: a randomized controlled trial. *JAMA* 2006; **295**: 2483-2491 [PMID: 16757720 DOI: 10.1001/jama.295.21.2483]

10 **Reali A**, Allis S, Girardi A, Verna R, Bianco L, Redda MG. Is Karnofsky Performance Status Correlate with Better Overall Survival in Palliative Conformal Whole Brain Radiotherapy? Our Experience. *Indian J Palliat Care* 2015; **21**: 311-316 [PMID: 26600700 DOI: 10.4103/0973-1075.164891]

11 **Sperduto PW**, Kased N, Roberge D, Xu Z, Shanley R, Luo X, Sneed PK, Chao ST, Weil RJ, Suh J, Bhatt A, Jensen AW, Brown PD, Shih HA, Kirkpatrick J, Gaspar LE, Fiveash JB, Chiang V, Knisely JP, Sperduto CM, Lin N, Mehta M. Summary report on the graded prognostic assessment: an accurate and facile diagnosis-specific tool to estimate survival for patients with brain metastases. *J Clin Oncol* 2012; **30**: 419-425 [PMID: 22203767 DOI: 10.1200/JCO.2011.38.0527]

12 **Nagtegaal SHJ**, Claes A, Suijkerbuijk KPM, Schramel FMNH, Snijders TJ, Verhoeff JJC. Comparing survival predicted by the diagnosis-specific Graded Prognostic Assessment (DS-GPA) to actual survival in patients with 1-10 brain metastases treated with stereotactic radiosurgery. *Radiother Oncol* 2019; **138**: 173-179 [PMID: 31302392 DOI: 10.1016/j.radonc.2019.06.033]

13 **Weltman E**, Brandt RA, Hanriot RM, Luz FP, Chen MJ, Cruz JC, Wajsbrot DB, Nadalin W. Validating the SIR: a better prognostic score index for patients with brain metastases treated with stereotactic radiosurgery. *Int. J. Radiat. Oncol***5**: S268-S269 [DOI: 10.1016/j.ijrobp.2005.07.460]

14 **Gaspar L**, Scott C, Rotman M, Asbell S, Phillips T, Wasserman T, McKenna WG, Byhardt R. Recursive partitioning analysis (RPA) of prognostic factors in three Radiation Therapy Oncology Group (RTOG) brain metastases trials. *Int J Radiat Oncol Biol Phys* 1997; **37**: 745-751 [PMID: 9128946 DOI: 10.1016/S0360-3016(96)00619-0]

15 **International Commission on Radiation Units and Measurements (ICRU)**. Feb 16, 2021. [cited 16 February 2021]. Available from: https://icru.org/testing/reports/prescribing-recording-and-reporting-intensitymodulated-photon-beam-therapy-imrt-icru-report-83

16 **de Camargo AV**, Cao M, da Silva DDCSA, de Araújo RLC. Evaluation of the correlation between dosimetric, geometric, and technical parameters of radiosurgery planning for multiple brain metastases. *J Appl Clin Med Phys* 2021; **22**: 83-92 [PMID: 34212482 DOI: 10.1002/acm2.13326]

17 **Harris PA**, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; **42**: 377-381 [PMID: 18929686 DOI: 10.1016/j.jbi.2008.08.010]

18 **Blonigen BJ**, Steinmetz RD, Levin L, Lamba MA, Warnick RE, Breneman JC. Irradiated volume as a predictor of brain radionecrosis after linear accelerator stereotactic radiosurgery. *Int J Radiat Oncol Biol Phys* 2010; **77**: 996-1001 [PMID: 19783374 DOI: 10.1016/j.ijrobp.2009.06.006]

19 **Brown PD**, Jaeckle K, Ballman KV, Farace E, Cerhan JH, Anderson SK, Carrero XW, Barker FG 2nd, Deming R, Burri SH, Ménard C, Chung C, Stieber VW, Pollock BE, Galanis E, Buckner JC, Asher AL. Effect of Radiosurgery Alone vs Radiosurgery With Whole Brain Radiation Therapy on Cognitive Function in Patients With 1 to 3 Brain Metastases: A Randomized Clinical Trial. *JAMA* 2016; **316**: 401-409 [PMID: 27458945 DOI: 10.1001/jama.2016.9839]

20 **Sahgal A**, Aoyama H, Kocher M, Neupane B, Collette S, Tago M, Shaw P, Beyene J, Chang EL. Phase 3 trials of stereotactic radiosurgery with or without whole-brain radiation therapy for 1 to 4 brain metastases: individual patient data meta-analysis. *Int J Radiat Oncol Biol Phys* 2015; **91**: 710-717 [PMID: 25752382 DOI: 10.1016/j.ijrobp.2014.10.024]

21 **Scorsetti M**, Navarria P, Cozzi L, Clerici E, Bellu L, Franceschini D, Marzo AM, Franzese C, Torri V, Reggiori G, Lobefalo F, Raspagliesi L, Attuati L, Pessina F, Franzini A, Picozzi P, Tomatis S. Radiosurgery of limited brain metastases from primary solid tumor: results of the randomized phase III trial (NCT02355613) comparing treatments executed with a specialized or a C-arm linac-based platform. *Radiat Oncol* 2023; **18**: 28 [PMID: 36750848 DOI: 10.1186/s13014-023-02216-5]

22 **Nieder C**, Andratschke NH, Geinitz H, Grosu AL. Diagnosis-specific graded prognostic assessment score is valid in patients with brain metastases treated in routine clinical practice in two European countries. *Med Sci Monit* 2012; **18**: CR450-CR455 [PMID: 22739735 DOI: 10.12659/MSM.883213]

23 **Narayanasamy G**, Smith A, Van Meter E, McGarry R, Molloy JA. Total target volume is a better predictor of whole brain dose from gamma stereotactic radiosurgery than the number, shape, or location of the lesions. *Med Phys* 2013; **40**: 091714 [PMID: 24007147 DOI: 10.1118/1.4818825]

24 **Roper J**, Chanyavanich V, Betzel G, Switchenko J, Dhabaan A. Single-Isocenter Multiple-Target Stereotactic Radiosurgery: Risk of Compromised Coverage. *Int J Radiat Oncol Biol Phys* 2015; **93**: 540-546 [PMID: 26460996 DOI: 10.1016/j.ijrobp.2015.07.2262]

25 **Selvan KT**, Padma G, Revathy MK, Nambi Raj NA, Senthilnathan K, Babu PR. Dosimetric Effect of Rotational Setup Errors in Single-Isocenter Volumetric-Modulated Arc Therapy of Multiple Brain Metastases. *J Med Phys* 2019; **44**: 84-90 [PMID: 31359925 DOI: 10.4103/jmp.JMP_103_18]

26 **Slim K**, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (minors): development and validation of a new instrument. *ANZ J Surg* 2003; **73**: 712-716 [PMID: 12956787 DOI: 10.1046/j.1445-2197.2003.02748.x]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 October 24; 14(10): 409-419

DOI: 10.5306/wjco.v14.i10.409

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Observational Study

Classification of patients with metastatic colorectal cancer into consensus molecular subtypes into real-world: A pilot study

Jaime González-Montero, Mauricio Burotto, Guillermo Valenzuela, Debora Mateluna, Florencia Buen-Abad, Jessica Toro, Olga Barajas, Katherine Marcelain

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gu GL, China; Li L, China

Received: July 25, 2023
Peer-review started: July 25, 2023
First decision: September 13, 2023
Revised: September 24, 2023
Accepted: October 8, 2023
Article in press: October 8, 2023
Published online: October 24, 2023

Jaime González-Montero, Mauricio Burotto, Bradford Hill Clinical Research Center, Santiago 8420383, Chile

Jaime González-Montero, Guillermo Valenzuela, Debora Mateluna, Florencia Buen-Abad, Jessica Toro, Olga Barajas, Katherine Marcelain, Basic and Clinical Oncology Department, University of Chile, Santiago 8380453, Chile

Corresponding author: Jaime González-Montero, MD, PhD, Assistant Professor, Basic and Clinical Oncology Deparment, University of Chile. Bradford Hill Clinical Research Center. Independencia 1027, Casilla 70058, Santiago 8380453, Chile. jagonzalez@ug.uchile.cl

Abstract

BACKGROUND

Colorectal cancer is a complex disease with high mortality rates. Over time, the treatment of metastatic colorectal cancer (mCRC) has gradually improved due to the development of modern chemotherapy and targeted therapy regimens. However, due to the inherent heterogeneity of this condition, identifying reliable predictive biomarkers for targeted therapies remains challenging. A recent promising classification system—the consensus molecular subtype (CMS) system—offers the potential to categorize mCRC patients based on their unique biological and molecular characteristics. Four distinct CMS categories have been defined: immune (CMS1), canonical (CMS2), metabolic (CMS3), and mesenchymal (CMS4). Nevertheless, there is currently no standardized protocol for accurately classifying patients into CMS categories. To address this challenge, reverse transcription polymerase chain reaction (RT-qPCR) and next-generation genomic sequencing (NGS) techniques may hold promise for precisely classifying mCRC patients into their CMSs.

AIM

To investigate if mCRC patients can be classified into CMS categories using a standardized molecular biology workflow.

METHODS

This observational study was conducted at the University of Chile Clinical Hospital and included patients with unresectable mCRC who were undergoing systemic treatment with chemotherapy and/or targeted therapy. Molecular

biology techniques were employed to analyse primary tumour samples from these patients. RT-qPCR was utilized to assess the expression of genes associated with fibrosis (TGF-β and β-catenin) and cell growth pathways (c-MYC). NGS using a 25-gene panel (TumorSec) was performed to identify specific genomic mutations. The patients were then classified into one of the four CMS categories according to the clinical consensus of a Tumour Board. Informed consent was obtained from all the patients prior to their participation in this study. All techniques were conducted at University of Chile.

RESULTS

Twenty-six patients were studied with the techniques and then evaluated by the Tumour Board to determine the specific CMS. Among them, 23% (n = 6), 19% (n = 5), 31% (n = 8), and 19% (n = 5) were classified as CMS1, CMS2, CMS3, and CMS4, respectively. Additionally, 8% of patients (n = 2) could not be classified into any of the four CMS categories. The median overall survival of the total sample was 28 mo, and for CMS1, CMS2, CMS3 and CMS4 it was 11, 20, 30 and 45 mo respectively, with no statistically significant differences between groups.

CONCLUSION

A molecular biology workflow and clinical consensus analysis can be used to accurately classify mCRC patients. This classification process, which divides patients into the four CMS categories, holds significant potential for improving research strategies and targeted therapies tailored to the specific characteristics of mCRC.

Key Words: Metastatic colorectal cancer; Targeted therapy; Consensus molecular subtypes; Personalized medicine

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Colorectal cancer is molecularly heterogeneous. Consensus molecular subtype classification sheds light on its biology, potentially guiding targeted therapy selection. However, an optimal consensus molecular subtype classification mechanism remains elusive. This workflow, which combines reverse transcription polymerase chain reaction and next-generation sequencing, introduces a novel approach for molecular patient classification. We aim to use these techniques to improve the precision of tumour subtyping.

Citation: González-Montero J, Burotto M, Valenzuela G, Mateluna D, Buen-Abad F, Toro J, Barajas O, Marcelain K. Classification of patients with metastatic colorectal cancer into consensus molecular subtypes into real-world: A pilot study. *World J Clin Oncol* 2023; 14(10): 409-419

URL: https://www.wjgnet.com/2218-4333/full/v14/i10/409.htm

DOI: https://dx.doi.org/10.5306/wjco.v14.i10.409

INTRODUCTION

Colorectal cancer (CRC) exhibits high incidence and mortality rates. At the time of diagnosis, approximately 25% of patients already present with metastatic disease, while 50% of those initially diagnosed with localized stages later develop disseminated disease[1]. Recent years have seen significant advancements in systemic therapies for metastatic colorectal cancer (mCRC) patients, including diverse combination chemotherapy regimens, targeted therapy, immunotherapy, and multi-kinase inhibitors[2]. Despite these improvements, patients' responses remain variable and unpredictable due to the molecular heterogeneity of this disease. Thus, it is imperative to identify specific mutations for a personalized treatment approach[3].

Numerous efforts have attempted to identify distinct molecular mCRC phenotypes. In 2015, bioinformatic studies revealed a promising classification system with four consensus molecular subtypes (CMS)[4]. This classification system has gained widespread clinical acceptance and is currently guiding various ongoing clinical trials[5]. The four CMS are as follows: CMS1, or immune subtype, primarily affects young patients and exhibits rapid progression and resistance to conventional therapies. This subtype may benefit from aggressive chemotherapy and, potentially, immunotherapy. CMS2, or canonical subtype, is characterized by mutations in specific pathways linked to cellular metabolism. CMS3, or metabolic subtype, is characterized by mutations in pathways responsible for cellular metabolism, with a high prevalence of *KRAS* pathway mutations. Finally, CMS4, or mesenchymal subtype, is associated with mutations in fibrogenesis and epithelial-mesenchymal transition pathways, leading to a poor prognosis and a higher incidence of metastasis[5]. To date, there is no established methodology for effectively classifying patients into CMS categories. However, given that each CMS is linked to distinct patterns of mutations and gene expression, it is plausible that a molecular biology workflow designed to identify specific mutations could help accurately classify patients into different CMS groups[6]. Therefore, the objective of this study was to establish a workflow for assigning mCRC patients to CMS categories using reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and next-generation sequencing (NGS) techniques.

MATERIALS AND METHODS

Study design and participants

In this observational study conducted between 2020 and 2023, we analyzed primary tumor tissue samples from mCRC patients who were receiving systemic treatment at the University of Chile Clinical Hospital. Colon or rectal tissue samples were collected through colonoscopy or surgical procedures. The samples were processed and stored according to protocols established by the Biobank of Tissues and Fluids at the University of Chile (http://biobanco.uchile.cl/). Both formalin-fixed paraffin-embedded (FFPE) tissue biopsies and fresh neoplastic tissue (frozen without fixation) were examined.

The inclusion criteria for this study were as follows: Patients diagnosed with unresectable mCRC (colon or rectal cancer) confirmed through histological diagnosis. Undergoing treatment at the University of Chile Clinical Hospital. Receiving systemic therapy in accordance with international clinical guidelines (National Comprehensive Cancer Network[7] and European Society of Medical Oncology)[8]. Treatment regimens included chemotherapy (FOLFOX, CAPOX, or FOLFIRI) and targeted therapy (bevacizumab, aflibercept, cetuximab, panitumumab, regorafenib, and TAS102). Chemotherapy and targeted therapy regimens were selected by the physicians on a case-by-case basis.

The exclusion criteria for this study were as follows: Patients who underwent the removal of metastases (metastasectomy) before enrollment. Any comorbidity leading to a life expectancy of less than six months. Inability to maintain clinical follow-up.

RT-qPCR

The expression of TGF-β, β-catenin, and c-MYC was investigated as follows: RNA was extracted from FFPE tissue using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Invitrogen). Subsequently, the concentration of each RNA sample was determined using the Quant-iT™ RiboGreen™ RNA Reagent Kit (Invitrogen) on a Cytation 3 instrument (BioTek). RNA (1000) ng was then used to prepare cDNA with the AffinityScript qPCR cDNA Synthesis Kit (Agilent) according to the manufacturer's instructions. Amplifications by qPCR (real-time PCR) was conducted in triplicate using the Brilliant II SYBR Green qPCR Master Mix kit (Agilent) on an Eco Real-time PCR System (Illumina). The following cycling conditions were used: an initial denaturation step at 95°C for 10 min, then 40 cycles of amplification (each cycle is 10 s at 95°C, 30 s at 60°C and 15s at 72°C). A melting curve for each primer ensured amplification of a single product. Finally, six FFPE non-tumour tissue samples treated in the same manner as the FFPE tumour tissues from each patient were included as controls. The relative expression was calculated using the ΔΔCt method[9] and normalized using expression levels of reference genes: B2M, PPIA, and RPLP0. Table 1 presents a summary of the primers used to conduct the RT-qPCR experiments[10-15].

NGS

The presence of genomic mutations was assessed using a 25-gene panel (TumorSec) as described by our team[16]. The RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE was utilized to extract genomic deoxyribonucleic acid (DNA) from FFPE samples. Briefly, samples were incubated with 1 mL of Histo-Clear at 50°C for 3 min to remove paraffin. The supernatant was then removed, followed by two ethanol washes, and the residual ethanol was evaporated using a SpeedVAC (Thermo Scientific). The samples were then incubated overnight in a digestion solution containing proteases. The next day, the samples were incubated at 80°C for 15 min and an isolation additive was added and centrifuged. Subsequently, the supernatant was transferred to a filter column and centrifuged to isolate the RNA, which was then treated with DNase. The column contained the DNA, which was subsequently treated with RNase. The DNA and RNA were washed with wash buffers and eluted in elution buffer in separate tubes.

Quantification and quality analysis: The purity and quantity of DNA and RNA were determined by measuring absorbance at 260/280 nm with the PicoGreen assay (Quant-iT™ PicoGreen® dsDNA, Invitrogen) and the Quant-iT™ RiboGreen™ RNA Reagent Kit, respectively, on a Cytation 3 instrument (Biotek). Additionally, DNA quality analysis was conducted by measuring fragment size with the HS Genomic DNA Analysis Kit (DNF-488) (Agilent) on a Fragment Analyzer instrument (Agilent). As the extraction of genomic DNA from FFPE samples often results in low yields and degradation ranging from more than 1000 bp to less than 200 bp, fragments less than 200 bp were not used for library preparation due to excessive degradation. To ensure adequate DNA quantity, a minimum of four, 6-μm FFPE sections per patient were used for sequencing. Moreover, each sample needed to contain more than 20% tumour content.

Library preparation: The KAPA HyperPlus Library Preparation Kit (Kapa Biosystems) was utilized to prepare DNA libraries. Library sizes and concentrations were verified for quality control purposes. The 260/280 nm ratio was measured with Cytation equipment and quantification was carried out using the Quant-iT™ PicoGreen™ dsDNA Assay Kit. Furthermore, library sizes were visualized using the HS NGS Analysis Kit in a Fragment Analyzer instrument.

NGS: NGS was conducted following a protocol previously published by our team[9]. For sequencing, an equimolar pool of libraries (4 nM) was prepared, diluted, and denatured to achieve a final concentration of 9.4–9.5 pM according to guidelines in the "MiSeq System Denature and Dilute Libraries Guide" (Illumina). Paired-end sequencing (300 cycles) was performed using the Illumina MiSeq System (MiSeq Reagent Kits v2). Finally, bioinformatics analysis was conducted.

Classification of patients into CMS categories

Given the absence of a singular marker that differentiates each of the four CMS categories on its own, we developed a comprehensive protocol involving analysis by a Tumour Board consisting of experts in Molecular and Medical Oncology.

Table 1 Primers employed for reverse transcription-quantitative polymerase chain reaction experiments to determine the expression of β-catenin, c-Myc and TFG-β, and the genes used as reference genes

Name	Primer	Sequence	Product Lenght	Ref.
TGF-β	Forward	5′- TACCTGAACCCGTGTTGCTCTC-3′	122	[10]
	Reverse	5′- GTTGCTGAGGTATCGCCAGGA-3′		
β-catenin	Forward	5′- CACAAGCAGAGTGCTGAAGGTG-3′	146	[11]
	Reverse	5′- GATTCCTGAGAGTCCAAAGACAG-3′		
c-MYC	Forward	5′-GCCACGTCTCCACACATCAG-3′	132	[12]
	Reverse	5′-TGGTGCATTTTCGGTTGTTG-3′		
B2M	Forward	5′-GTGCTCGCGCTACTCTCTC-3′	150	[13]
	Reverse	5′-GTCAACTTCAATGTCGGAT-3′		
PPIA	Forward	5′-GCAAATGCTGGACCCAACACAAAT-3′	174	[14]
	Reverse	5′-AATGGTGATCTTCTTGCTGGTCTTG-3′		
RPLP0	Forward	5′-GCAATGTTGCCAGTGTCTG-3′	142	[15]
	Reverse	5′-GCCTTGACCTTTTCAGCAA-3′		

Each case was individually assessed and the CMS was determined based on the criteria defined by Guinney *et al*[4]. The Tumour Board relied on patients' clinical characteristics, mismatch repair (MMR) expression, and RT-qPCR and NGS results. Each patient's CMS was determined by consensus among all committee members. Patients for whom a CMS consensus could not be reached were considered unclassifiable.

The Tumour Board employed the following criteria to classify each patient into one of the four CMS categories. It is important to note that none of these elements individually serve as a specific CMS marker; instead, classifications were based on the combination of multiple elements and reached through tumour board consensus. CMS1: presence of *BRAF* mutation; MMR protein deficiency; low TGF-β, β-catenin, and c-MYC mRNA expression; and absence of *APC* or *KRAS* mutations. CMS2 and CMS3: presence of *APC* mutation; absence of *BRAF* mutation (with a predominance of *KRAS* mutations in CMS3); MMR-proficient; low TGF-β and β-catenin mRNA expression; and high c-MYC mRNA expression. CMS4: MMR-proficient; high expression of TGF-β and β-catenin mRNA; low expression of c-MYC mRNA; and presence of non-categorical mutations identified through NGS[6].

Ethics

All procedures conducted in this study were in full compliance with the ethical standards set by the Institutional and National Research Committee, as well as the principles outlined in the 1964 Declaration of Helsinki and its subsequent amendments. Ethical approval for this study was obtained from the Ethics Committee of the University of Chile Clinical Hospital and Faculty of Medicine prior to beginning the research. Informed written consent was obtained from all patients before their participation in the trial.

Statistics

Results are presented as the number and percentage of total patients included in this study. To determine the appropriate sample size, we considered the estimated prevalence of each mCRC CMS. According to previous work[4], the expected prevalence of each CMS is approximately 20%–25%. A sample size of 25 patients was deemed sufficient to analyze the prevalence and distribution of the different CMS categories. Indeed, prior research has utilized sample sizes of 20–30 patients; thus, a sample size of 25 patients is consistent with the literature. For the overall survival analysis of the studied patients, log-rank test was conducted using GraphPad Prism 10.0 software.

RESULTS

Between 2020 and 2023, a total of 26 patients with unresectable mCRC undergoing systemic treatment at the University of Chile Clinical Hospital were included in this study. Table 2 presents the demographic and clinical characteristics of the patients, including age, gender, primary tumour site, and the presence or absence of MMR proteins. Each patient is identified with a number from 1–26.

Molecular studies

Table 2 illustrates the results of an RT-qPCR-based gene expression analysis of TGF-β, β-catenin, and c-MYC in each of the patients studied. It is observed that the expression of these three genes is heterogeneous among patients. Table 3 provides a comprehensive overview of the mutations identified with the 25-gene TumorSec panel. The most frequently observed

Table 2 Clinical characteristics, overall survival, and reverse transcription-quantitative polymerase chain reaction results of the *n* = 26 patients included in the final analysis

Patient number	Age	Gender	Site of primary cancer	Overall survival (mo)	Miss-match repair proteins expression	β-catenin expression (RT-qPCR) (relative expression with respect to reference gene average)	c-MYC expression (RT-qPCR) (relative expression with respect to reference gene average)	TGF-β expression (RT-qPCR) (relative expression with respect to reference gene average)	CMS
1	69	Male	Sigmoid	5	Proficient	0.185	2.864	0.201	CMS2
2	85	Female	Right colon	31	Proficient	0.100	0.352	0.169	CMS1
3	68	Female	Rectal and sigmoid	12	Proficient	0.042	0.384	0.076	CMS3
4	57	Male	Rectal and sigmoid	34	Proficient	2.684	18.817	9.778	CMS3
5	45	Female	Transverse	40	Proficient	1.812	19.445	5.231	CMS2
6	62	Male	Rectum	28	Proficient	0.010	4.401	0.973	CMS3
7	54	Male	Rectum	20	Proficient	0.301	3.234	1.433	CMS2
8	55	Male	Sigmoid	53	Proficient	0.080	1.870	11.718	CMS4
9	73	Male	Sigmoid	62	Proficient	0.038	0.645	0.461	CMS1
10	79	Male	Rectum	40	Proficient	0.121	2.080	3.513	CMS3
11	56	Female	Right colon	29	Proficient	0.235	3.799	14.700	CMS4
12	66	Female	Right colon	10	Proficient	0.351	6.004	76.116	CMS4
13	53	Male	Sigmoid	52	Proficient	0.233	3.863	2.688	CMS4
14	75	Male	Sigmoid	35	Proficient	0.089	0.760	0.205	CMS3
15	63	Male	Right colon	32	Proficient	0.089	0.760	0.466	CMS3
16	48	Female	Sigmoid	28	Proficient	0.038	1.110	0.498	CMS3
17	53	Female	Rectum	20	Proficient	0.084	1.124	0.801	Not classifiable
18	71	Female	Right colon	12	Proficient	0.083	1.540	0.897	CMS1
19	61	Female	Sigmoid	45	Proficient	0.106	9.208	6.820	CMS4
20	71	Male	Rectum	10	Proficient	0.013	0.855	0.065	CMS3
21	49	Female	Sigmoid	6	Deficient	0.063	2.968	1.871	CMS1
22	74	Male	Right colon	11	Deficient	0.047	0.552	0.249	CMS1
23	65	Female	Rectum	39	Proficient	0.059	0.828	0.084	Not classifiable
24	59	Female	Sigmoid	8	Proficient	0.045	1.324	0.152	CMS2
25	54	Male	Sigmoid	5	Deficient	0.036	0.543	0.127	CMS1
26	69	Male	Sigmoid	22	Proficient	0.192	5.025	2.654	CMS2

Each patient is individually identified in the first column on the left with a sequential number ranging from 1 to 26. Additionally, the consensus molecular subtype assigned based on the Tumour Board analysis is provided. RT-qPCR: reverse transcription-quantitative polymerase chain reaction; TGF-β: Transforming growth factor beta; CMS: consensus molecular subtype.

mutations were in *KRAS*, *TP53* and *ARID1A*. All observed mutations were single nucleotide variants (SNVs) and two patients possessed deletions.

Classification of patients into CMS categories

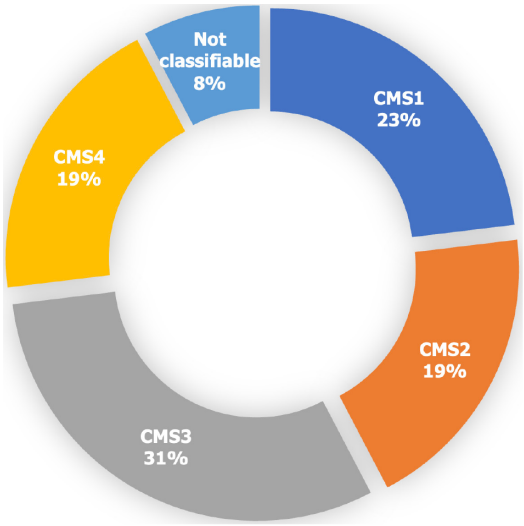
Out of the 26 patients analyzed, a specific CMS could be identified for 24 patients (92%) by clinical consensus by the Tumour Board. Two patients (8%) were found to be unclassifiable. Figure 1 illustrates the distribution of patients across

Table 3 Mutations identified in the *n* = 26 patients included in the final analysis through massive genomic sequencing using the TumorSec panel

Patient number	Mutation	Mutation variant classification	Affected protein	Variant type
1	TSC2	Missense	p.R1729C	SNV
	TP53	Missense	p.R175H	SNV
2	KRAS	Missense	p.G12C	SNV
3	KRAS	Missense	p.G12V	SNV
	TP53	Missense	p.R175H	SNV
4	KRAS	Missense	p.Q61H	SNV
	PIK3CA	Missense	p.E545G	SNV
5	TP53	Missense	p.P152L	SNV
6	KRAS	Missense	p.G12D	SNV
7	BRCA2	Missense	p.K584E	SNV
	ARID1A	Nonsense	p.Q1584	SNV
8	KRAS	Missense	p.N116H	SNV
	TP53	Missense	p.R175H	SNV
	PIK3CA	Missense	p.H1047R	SNV
	BRAF	Missense	p.N581Y	SNV
9	BRCA2	Frameshift (deletion)	p.N863Ifs11	SNV
	ARID1A	Frameshift (deletion)	p.P1326Rfs155	SNV
	PIK3CA	Missense	p.H1047R	SNV
10	PTEN	Nonsense	p.Y225	SNV
	KRAS	Missense	p.G12C	SNV
	TP53	Frameshift (insertion)	p.Q317Pfs20	SNV
11	KRAS	Missense	p.Q61H	SNV
12	KRAS	Missense	p.G12D	SNV
	TP53	Missense	p.R280K	SNV
13	TP53	Missense	p.R273H	SNV
14	KRAS	Missense	p.G12D	SNV
	TP53	Missense	p.P278L	SNV
15	KRAS	Missense	p.K117N	SNV
	TP53	Missense	p.R282W	SNV
16	KRAS	Missense	p.G12D	SNV
	TP53	Frameshift (deletion)	p.S260Qfs3	Deletion
17	KRAS	Missense	p.Q61L	SNV
	BRCA2	Missense	p.S3147Y	SNV
	TP53	Missense	p.R249G	SNV
18	KRAS	Missense	p.G12C	SNV
	ARID1A	Frameshift (deletion)	p.Q611Hfs7	Deletion
19	TP53	Missense	p.Y220C	SNV
20	KRAS	Missense	p.A59G	SNV
	KRAS	Missense	p.G12D	SNV
	TP53	Missense	p.H214R	SNV

21	NRAS	Missense	p.Q61R	SNV
	ARID1A	Frameshift (deletion)	p.K1072Nfs21	SNV
22	TP53	Missense	p.R273C	SNV
23	TP53	Nonsense	p.E51	SNV
	ARID1A	Frameshift (deletion)	p.Q372Sfs19	SNV
24	TP53	Missense	p.R248W	SNV
	PIK3CA	Missense	p.E545K	SNV
25	PTEN	Nonsense	p.Q149	SNV
	KRAS	Missense	p.G13D	SNV
	TSC2	Missense	p.R1713C	SNV
	TP53	Missense	p.R273C	SNV
	TP53	Missense	p.R158H	SNV
	ARID1A	Nonsense	p.R1335	SNV
26	BRCA2	Missense	p.E3002K	SNV
	TP53	Missense	p.C176Y	SNV

SNV: Single nucleotide variant.



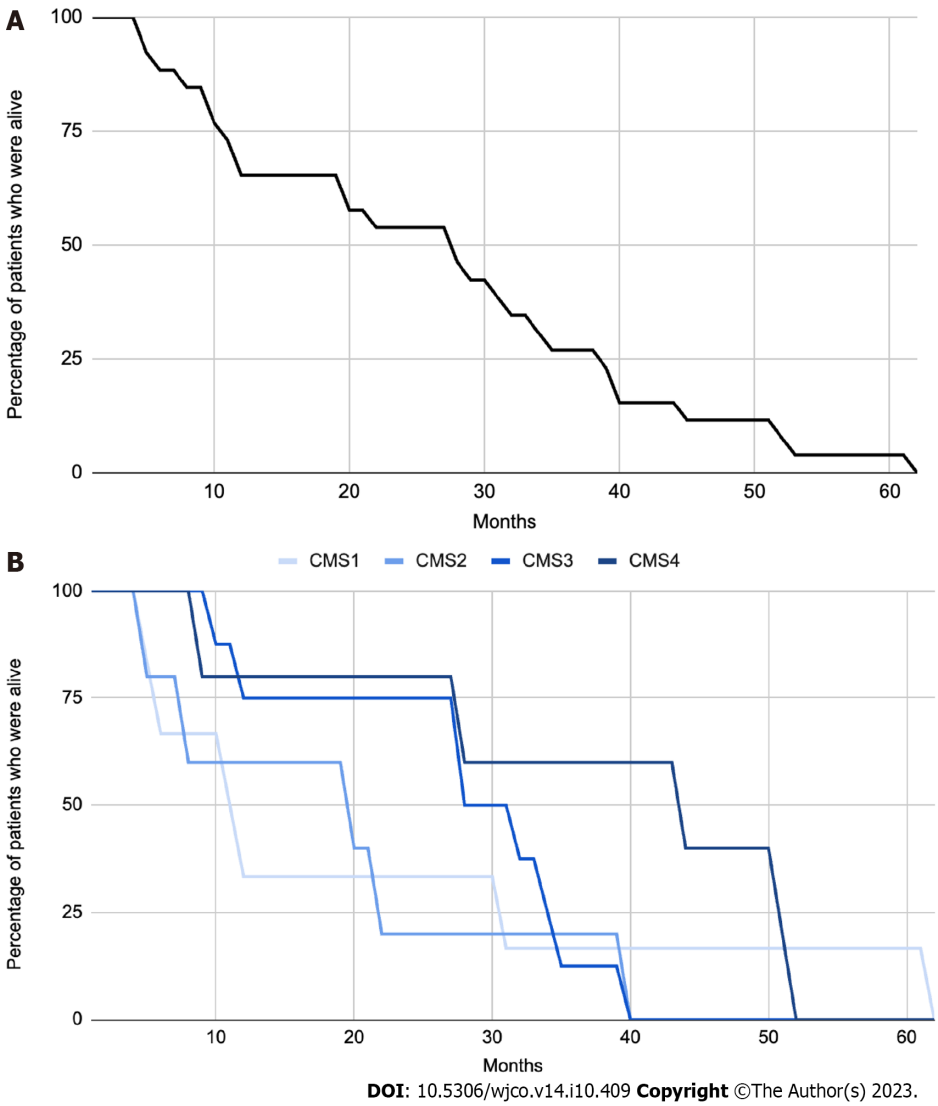
DOI: 10.5306/wjco.v14.i10.409 **Copyright** ©The Author(s) 2023.

Figure 1 Proportion of patients in each consensus molecular subtype after analysis by the Tumour Board among the 26 patients included on the final analysis. A specific consensus molecular subtype (CMS) was successfully identified in 24 out of the 26 patients. CMS1 *n* = 6. CMS2 *n* = 5. CMS3 *n* = 8. CMS4 *n* = 5. Not classifiable *n* = 2. Each patient underwent an individual assessment by the Tumour Board, and a consensus was reached to determine their molecular subtype. Classification was based on clinical and histological characteristics, as well as the results of RT-qPCR (β-catenin, c-MYC and TGF- β) and NGS (TumorSec panel). CMS: Consensus molecular subtypes.

the four CMS categories. Specifically, 23% (*n* = 6), 19% (*n* = 5), 31% (*n* = 8), and 19% (*n* = 5) were classified as CMS1, CMS2, CMS3 and CMS4, respectively. Remarkably, the percentage of patients classified into each CMS category closely aligns with findings reported by Guinney *et al*[4]. The median overall survival of the total sample was 28 mo (Figure 2A), and for CMS1, CMS2, CMS3 and CMS4 it was 11, 20, 30 and 45 mo respectively, with no statistically significant differences between groups (Figure 2B).

DISCUSSION

The objective of the workflow outlined in this manuscript was to develop an RT-qPCR- and NGS-based method by which to classify mCRC patients into CMS categories. Our results demonstrate that it is possible to classify mCRC patients into a



DOI: 10.5306/wjco.v14.i10.409 **Copyright** ©The Author(s) 2023.

Figure 2 Classification of patients into consensus molecular subtype categories. A: Kaplan-Meier Curve with overall survival (OS) of the *n* = 26 patients included on the final analysis. mOS = 28 mo; B: Kaplan-Meier curve which shows OS of patients based on their molecular subtype classification. The median overall survival times were 11, 20, 30, and 45 mo for CMS1, CMS2, CMS3, and CMS4, respectively. There were no statistically significant differences observed among the studied groups (*P* = 0.0968).

specific CMS in approximately 90% of the cases.

To date, there are no validated tools from prospective studies for classifying patients into the four CMS categories. Although genomic platforms such as ColotypeR[17] and CMSCaller[18] have been utilized, they have not significantly impacted clinical practice. Our findings present an alternative protocol for patient classification, leveraging a 25-gene panel (TumorSec) and a three-gene RT-qPCR panel (TGF-β, β-catenin, and c-MYC). The selected genes play vital roles in the epithelial-mesenchymal transition, particularly TGF-β and β-catenin, which are specific to CMS4 (fibrotic)[19]. Additionally, c-MYC was chosen due to its utility for identifying CMS2 (metabolic)[20]. However, distinguishing between CMS2 and CMS3 remains challenging as they share genetic signatures and patterns of gene expression.

The relevance of classifying mCRC patients into CMS categories must be contextualized. Thus far, the selection of targeted therapies and the design of clinical studies have primarily relied on the identification of *KRAS*, *NRAS*, and *BRAF* mutations and MMR expression analyses[7-8]. However, incorporating knowledge of the CMS categories can offer significant advantages in both aspects. First, it can enhance the selection of targeted therapies, enabling a more personalized approach. Additionally, a better understanding of the CMS categories can lead to improved clinical study design, allowing for more tailored and effective treatments for patients with specific CMS profiles[6]. For instance, CMS1, characterized by high lymphocytic infiltration and a worse prognosis, may benefit from aggressive therapeutic strategies such as combination triplet chemotherapy (FOLFOXIRI) and anti-angiogenic agents[21]. Monodrug immunotherapy could also be beneficial for these patients given their high frequency of microsatellite instability-high tumours as demonstrated in the KEYNOTE177 study[22]. Considering the high prevalence of *BRAF* mutations, future studies should examine the efficacy of BRAF inhibitors for these patients[23]. CMS2 and CMS3 share significant features and may respond to similar agents. For example, they may show sensitivity to anti-EGFR therapy, especially in CMS2 cases[24]. However, CMS3 patients frequently develop *KRAS* mutations, primarily in exon 2, leading to constitutive activation of the mitogen-associated protein kinase pathway, associated with a poorer prognosis and response to standard treatment[25]. CMS4,

which carries the worst prognosis, calls for the development of new strategies targeting the epithelial-mesenchymal transition or the TGF-β pathway. CMS4 tumours also show better response to irinotecan-based treatments or anti-angiogenic agents such as bevacizumab[26].

It is important to note that the classification of CMS can also predict the prognosis of patients with mCRC[4]. While this study documented the overall survival of patients, there were no significant differences between groups, likely due to the low number of patients in each CMS category. Therefore, it cannot be established whether patients with different CMSs have different prognoses.

The principal innovation of this exploratory study lies in the establishment of a protocol for the classification of mCRC patients into CMS through RT-qPCR (TGF-β, β-catenin, and c-MYC) and a 25-gene NGS panel (TumorSec). Our results demonstrates that this combined approach has the potential to classify patients with mCRC into one of the four CMS categories in over 90% of cases. As there is currently no gold-standard for conducting this clinical-molecular classification, this approach may represent a significant advancement in the development of an optimal technique that could become the standard for these purposes. In the future, it is important to further explore CMS categories and incorporate this knowledge into clinical practice. While this protocol proposes a CMS classification scheme, prospective and large-scale studies are imperative to assessing whether this methodology truly influences therapeutic decisions for patients[5] and for validating the clinical utility of CMS categories[6].

CONCLUSION

In conclusion, we successfully classified mCRC patients into CMS categories using an RT-qPCR and NGS-based workflow. This approach opens avenues for tailoring therapies according to CMS subtypes, potentially leading to improved patient outcomes.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer is a heterogeneous disease; therefore, it is crucial to progress towards a molecular consensus classification in order to predict prognosis and therapy response.

Research motivation

The primary motivation is to progress towards a consensus molecular classification of metastatic colorectal cancer patients, to better guide targeted therapy.

Research objectives

The aim of this study is to classify a sample of metastatic colorectal cancer patients into consensus molecular subtypes using a reverse transcription -quantitative polymerase chain reaction polymerase chain reaction (RT-qPCR) and next-generation genomic sequencing (NGS) protocol.

Research methods

Patients with unresectable metastatic colorectal cancer who were undergoing systemic treatment with chemotherapy and/or targeted therapy. Molecular biology techniques were employed to analyse primary tumour samples from these patients. RT-qPCR was utilized to assess the expression of genes associated with fibrosis (TGF-β and β-catenin) and cell growth pathways. NGS using a 25-gene panel (TumorSec) was performed to identify specific genomic mutations. The patients were then classified into one of the four CMS categories according to the clinical consensus of a Tumour Board.

Research results

n = 26 metastatic colorectal cancer patients analyzed. 23% (n = 6), 19% (n = 5), 31% (n = 8), and 19% (n = 5) were classified as CMS1, CMS2, CMS3, and CMS4, respectively. Additionally, 8% of patients (n = 2) could not be classified into any of the four CMS categories.

Research conclusions

It is possible to classify patients with metastatic colorectal cancer into consensus molecular subtypes through RT-qPCR and NGS techniques.

Research perspectives

Prospective studies are needed to determine if this classification is useful and if it has an impact on predicting the survival of patients with metastatic colorectal cancer.

FOOTNOTES

Author contributions: González-Montero J led the study, wrote the manuscript, and created the figures and tables; González-Montero J, Burotto M, and Barajas O led the molecular classification of the patients; Valenzuela G, Toro J, and Marcelain K performed molecular biology procedures; Barajas O, Mateluna D, and Buen-Abad F recruited the patients.

Supported by Agencia Nacional de Investigación y Desarrollo de Chile, Fondo Nacional de Investigación en Salud (FONIS), No. SA20I0059.

Institutional review board statement: The study was reviewed and approved by the Medical Oncology Supervisor of Bradford Hill Clinical Research Center.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All the authors declare have not conflict of interest to declare.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Chile

ORCID number: Jaime González-Montero 0000-0003-0324-2948; Mauricio Burotto 0000-0002-7150-4033; Guillermo Valenzuela 0000-0002-8711-729X; Debora Mateluna 0009-0006-9030-7146; Florencia Buen-Abad 0009-0003-5914-6725; Jessica Toro 0000-0002-7813-1380; Olga Barajas 0000-0002-3013-2106; Katherine Marcelain 0000-0003-4018-6623.

S-Editor: Liu JH
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Alese OB**, Wu C, Chapin WJ, Ulanja MB, Zheng-Lin B, Amankwah M, Eads J. Update on Emerging Therapies for Advanced Colorectal Cancer. *Am Soc Clin Oncol Educ Book* 2023; **43**: e389574 [PMID: 37155942 DOI: 10.1200/EDBK_389574]

3 **Sagaert X**, Vanstapel A, Verbeek S. Tumor Heterogeneity in Colorectal Cancer: What Do We Know So Far? *Pathobiology* 2018; **85**: 72-84 [PMID: 29414818 DOI: 10.1159/000486721]

4 **Guinney J**, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, De Sousa E Melo F, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L, Delorenzi M, Kopetz S, Vermeulen L, Tejpar S. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; **21**: 1350-1356 [PMID: 26457759 DOI: 10.1038/nm.3967]

5 **Ten Hoorn S**, de Back TR, Sommeijer DW, Vermeulen L. Clinical Value of Consensus Molecular Subtypes in Colorectal Cancer: A Systematic Review and Meta-Analysis. *J Natl Cancer Inst* 2022; **114**: 503-516 [PMID: 34077519 DOI: 10.1093/jnci/djab106]

6 **Valenzuela G**, Canepa J, Simonetti C, Solo de Zaldívar L, Marcelain K, González-Montero J. Consensus molecular subtypes of colorectal cancer in clinical practice: A translational approach. *World J Clin Oncol* 2021; **12**: 1000-1008 [PMID: 34909395 DOI: 10.5306/wjco.v12.i11.1000]

7 **Benson AB**, Venook AP, Al-Hawary MM, Cederquist L, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Engstrom PF, Garrido-Laguna I, Grem JL, Grothey A, Hochster HS, Hoffe S, Hunt S, Kamel A, Kirilcuk N, Krishnamurthi S, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Murphy JD, Nurkin S, Saltz L, Sharma S, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Wuthrick E, Gregory KM, Freedman-Cass DA. NCCN Guidelines Insights: Colon Cancer, Version 2.2018. *J Natl Compr Canc Netw* 2018; **16**: 359-369 [PMID: 29632055 DOI: 10.6004/jnccn.2018.0021]

8 **Van Cutsem E**, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, Aranda Aguilar E, Bardelli A, Benson A, Bodoky G, Ciardiello F, D'Hoore A, Diaz-Rubio E, Douillard JY, Ducreux M, Falcone A, Grothey A, Gruenberger T, Haustermans K, Heinemann V, Hoff P, Köhne CH, Labianca R, Laurent-Puig P, Ma B, Maughan T, Muro K, Normanno N, Österlund P, Oyen WJ, Papamichael D, Pentheroudakis G, Pfeiffer P, Price TJ, Punt C, Ricke J, Roth A, Salazar R, Scheithauer W, Schmoll HJ, Tabernero J, Taïeb J, Tejpar S, Wasan H, Yoshino T, Zaanan A, Arnold D. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol* 2016; **27**: 1386-1422 [PMID: 27380959 DOI: 10.1093/annonc/mdw235]

9 **Livak KJ**, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**: 402-408 [PMID: 11846609 DOI: 10.1006/meth.2001.1262]

10 **Salmani A**, Mohammadi M, Farid Hosseini R, Tavakol Afshari J, Fouladvand A, Dehnavi S, Khoshkhooi M, Jabbari Azad F. A significant increase in expression of FOXP3 and IL-17 genes in patients with allergic rhinitis underwent accelerated rush immunotherapy. *Iran J Basic Med Sci* 2019; **22**: 989-996 [PMID: 31807241 DOI: 10.22038/ijbms.2019.32979.7878]

11 **Fan L**, Shen H, Huang H, Yang R, Yao L. Impairment of Wnt/β-catenin signaling in blood cells of patients with severe cavitary pulmonary tuberculosis. *PLoS One* 2017; **12**: e0172549 [PMID: 28333932 DOI: 10.1371/journal.pone.0172549]

12 **Yu S**, Yang Q, Yang JH, Du Z, Zhang G. Identification of suitable reference genes for investigating gene expression in human gallbladder carcinoma using reverse transcription quantitative polymerase chain reaction. *Mol Med Rep* 2015; **11**: 2967-2974 [PMID: 25434674 DOI: 10.3892/mmr.2014.3008]

13 **Kheirelseid EA**, Chang KH, Newell J, Kerin MJ, Miller N. Identification of endogenous control genes for normalisation of real-time quantitative PCR data in colorectal cancer. *BMC Mol Biol* 2010; **11**: 12 [PMID: 20122155 DOI: 10.1186/1471-2199-11-12]

14 **Dydensborg AB**, Herring E, Auclair J, Tremblay E, Beaulieu JF. Normalizing genes for quantitative RT-PCR in differentiating human intestinal epithelial cells and adenocarcinomas of the colon. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G1067-G1074 [PMID: 16399877 DOI: 10.1152/ajpgi.00234.2005]

15 **Varela T**, Laizé V, Conceição N, Caldeira P, Marreiros A, Guerreiro H, Cancela ML. Expression of DUSP4 transcript variants as a potential biomarker for colorectal cancer. *Biomark Med* 2020; **14**: 639-650 [PMID: 32613839 DOI: 10.2217/bmm-2019-0369]

16 **Salvo M**, González-Feliú E, Toro J, Gallegos I, Maureira I, Miranda-González N, Barajas O, Bustamante E, Ahumada M, Colombo A, Armisén R, Villamán C, Ibañez C, Bravo ML, Sanhueza V, Spencer ML, de Toro G, Morales E, Bizama C, García P, Carrasco AM, Gutiérrez L, Bermejo JL, Verdugo RA, Marcelain K. Validation of an NGS Panel Designed for Detection of Actionable Mutations in Tumors Common in Latin America. *J Pers Med* 2021; **11** [PMID: 34575676 DOI: 10.3390/jpm11090899]

17 **Buechler SA**, Badve SS, Gokmen-Polar Y, Herring E, Ludwig K, Hummon A. ColotypeR: A tool to classify colon cancers by consensus molecular subtype and subtype-specific risk of recurrence. *J Clin Oncol* 2018; **36** Suppl 4: 632-632

18 **Eide PW**, Bruun J, Lothe RA, Sveen A. CMScaller: an R package for consensus molecular subtyping of colorectal cancer pre-clinical models. *Sci Rep* 2017; **7**: 16618 [PMID: 29192179 DOI: 10.1038/s41598-017-16747-x]

19 **Najdi R**, Holcombe RF, Waterman ML. Wnt signaling and colon carcinogenesis: beyond APC. *J Carcinog* 2011; **10**: 5 [PMID: 21483657 DOI: 10.4103/1477-3163.78111]

20 **Sadanandam A**, Wang X, de Sousa E Melo F, Gray JW, Vermeulen L, Hanahan D, Medema JP. Reconciliation of classification systems defining molecular subtypes of colorectal cancer: interrelationships and clinical implications. *Cell Cycle* 2014; **13**: 353-357 [PMID: 24406433 DOI: 10.4161/cc.27769]

21 **Cremolini C**, Antoniotti C, Lonardi S, Bergamo F, Cortesi E, Tomasello G, Moretto R, Ronzoni M, Racca P, Loupakis F, Zaniboni A, Tonini G, Buonadonna A, Marmorino F, Allegrini G, Granetto C, Masi G, Zagonel V, Sensi E, Fontanini G, Boni L, Falcone A. Primary tumor sidedness and benefit from FOLFOXIRI plus bevacizumab as initial therapy for metastatic colorectal cancer. Retrospective analysis of the TRIBE trial by GONO. *Ann Oncol* 2018; **29**: 1528-1534 [PMID: 29873679 DOI: 10.1093/annonc/mdy140]

22 **André T**, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, Smith D, Garcia-Carbonero R, Benavides M, Gibbs P, de la Fouchardiere C, Rivera F, Elez E, Bendell J, Le DT, Yoshino T, Van Cutsem E, Yang P, Farooqui MZH, Marinello P, Diaz LA Jr; KEYNOTE-177 Investigators. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. *N Engl J Med* 2020; **383**: 2207-2218 [PMID: 33264544 DOI: 10.1056/NEJMoa2017699]

23 **Molina-Cerrillo J**, San Román M, Pozas J, Alonso-Gordoa T, Pozas M, Conde E, Rosas M, Grande E, García-Bermejo ML, Carrato A. BRAF Mutated Colorectal Cancer: New Treatment Approaches. *Cancers (Basel)* 2020; **12** [PMID: 32545884 DOI: 10.3390/cancers12061571]

24 **Ten Hoorn S**, Sommeijer DW, Elliott F, Fisher D, de Back TR, Trinh A, Koens L, Maughan T, Seligmann J, Seymour MT, Quirke P, Adams R, Richman SD, Punt CJA, Vermeulen L. Molecular subtype-specific efficacy of anti-EGFR therapy in colorectal cancer is dependent on the chemotherapy backbone. *Br J Cancer* 2021; **125**: 1080-1088 [PMID: 34253874 DOI: 10.1038/s41416-021-01477-9]

25 **Lal N**, White BS, Goussous G, Pickles O, Mason MJ, Beggs AD, Taniere P, Willcox BE, Guinney J, Middleton GW. KRAS Mutation and Consensus Molecular Subtypes 2 and 3 Are Independently Associated with Reduced Immune Infiltration and Reactivity in Colorectal Cancer. *Clin Cancer Res* 2018; **24**: 224-233 [PMID: 29061646 DOI: 10.1158/1078-0432.CCR-17-1090]

26 **Peters NA**, Constantinides A, Ubink I, van Kuik J, Bloemendal HJ, van Dodewaard JM, Brink MA, Schwartz TP, Lolkema MPJK, Lacle MM, Moons LM, Geesing J, van Grevenstein WMU, Roodhart JML, Koopman M, Elias SG, Borel Rinkes IHM, Kranenburg O. Consensus molecular subtype 4 (CMS4)-targeted therapy in primary colon cancer: A proof-of-concept study. *Front Oncol* 2022; **12**: 969855 [PMID: 36147916 DOI: 10.3389/fonc.2022.969855]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 October 24; 14(10): 420-439

DOI: 10.5306/wjco.v14.i10.420

ISSN 2218-4333 (online)

SCIENTOMETRICS

What should be the future direction of development in the field of prostate cancer with lung metastasis?

Zhi-Guang Huang, Yi Chen, Tong Wu, Bin-Tong Yin, Xiao Feng, Sheng-Hua Li, Dong-Ming Li, Gang Chen, Ji-Wen Cheng, Juan He

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Fu L, China; Shalaby MN, Egypt

Received: August 14, 2023
Peer-review started: August 14, 2023
First decision: August 24, 2023
Revised: September 12, 2023
Accepted: September 25, 2023
Article in press: September 25, 2023
Published online: October 24, 2023

Zhi-Guang Huang, Yi Chen, Tong Wu, Bin-Tong Yin, Dong-Ming Li, Gang Chen, Juan He, Department of Pathology, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Xiao Feng, Department of Radiology, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Sheng-Hua Li, Ji-Wen Cheng, Department of Urology, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Corresponding author: Juan He, MM, Doctor, Department of Pathology, First Affiliated Hospital of Guangxi Medical University, No. 6 Shuangyong Road, Nanning 530021, Guangxi Zhuang Autonomous Region, China. hejuan@stu.gxmu.edu.cn

Abstract

BACKGROUND

Since the start of the 21st century, prostate cancer with lung metastasis (PCLM) has accumulated significant scientific research output. However, a systematic knowledge framework for PCLM is still lacking.

AIM

To reconstruct the global knowledge system in the field of PCLM, sort out hot research directions, and provide reference for the clinical and mechanism research of PCLM.

METHODS

We retrieved 280 high-quality papers from the Web of Science Core Collection and conducted a bibliometric analysis of keywords, publication volume, and citation frequency. Additionally, we selected differentially expressed genes from global high-throughput datasets and performed enrichment analysis and protein-protein interaction analysis to further summarize and explore the mechanisms of PCLM.

RESULTS

PCLM has received extensive attention over the past 22 years, but there is an uneven spatial distribution in PCLM research. In the clinical aspect, the treatment of PCLM is mainly based on chemotherapy and immunotherapy, while diagnosis relies on methods such as prostate-specific membrane antigen positron emission

tomography/computed tomography. In the basic research aspect, the focus is on cell adhesion molecules and signal transducer and activator of transcription 3, among others. Traditional treatments, such as chemotherapy, remain the mainstay of PCLM treatment, while novel approaches such as immunotherapy have limited effectiveness in PCLM. This study reveals for the first time that pathways related to coronavirus disease 2019, cytokine-cytokine receptor interaction, and ribosome are closely associated with PCLM.

CONCLUSION

Future research should focus on exploring and enhancing mechanisms such as cytokine-cytokine receptor interaction and ribosome and improve existing mechanisms like cadherin binding and cell adhesion molecules.

Key Words: Prostate cancer; Lung metastasis; Chemotherapy; Immunotherapy; Bibliometric analysis; Enrichment analysis

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Discovering new insights into prostate cancer with lung metastasis (PCLM), this study presents a systematic analysis of 280 high-quality papers and global datasets. The uneven distribution of PCLM research is highlighted. Notably, this study uncovers the association of PCLM with pathways related to coronavirus disease 2019, cytokine-cytokine receptor interaction, and ribosomes. While traditional treatments remain crucial, novel approaches like immunotherapy show limited effectiveness. Future research should prioritize exploring mechanisms such as cytokine-cytokine receptor interaction and ribosomes while enhancing existing mechanisms like cell adhesion molecules. This study's innovative findings contribute to the advancement of PCLM research, stimulating further exploration and potential improvements in diagnosis and treatment strategies.

Citation: Huang ZG, Chen Y, Wu T, Yin BT, Feng X, Li SH, Li DM, Chen G, Cheng JW, He J. What should be the future direction of development in the field of prostate cancer with lung metastasis? *World J Clin Oncol* 2023; 14(10): 420-439
URL: https://www.wjgnet.com/2218-4333/full/v14/i10/420.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i10.420

INTRODUCTION

Prostate cancer (PC) is the second most common cause of cancer-related fatalities[1]. Globally, there are more than 1.4 million new cases of PC and over 370000 deaths related to PC each year[2]. Due to the prostate's unique location and function in the male anatomy, the early diagnosis and treatment of PC face numerous challenges[3]. Consequently, many PC patients develop metastasis. Lung metastasis (LM) is a relatively common occurrence in PC, with over 10% of PC patients experiencing LM[4]. Patients with PC with LM (PCLM) often present symptoms such as difficulty breathing, persistent dry cough, chest tightness, hemoptysis, and pain, which significantly impact their overall health[5]. Moreover, PCLM often accompanies metastasis to other organs or tissues[6,7], which complicates the treatment process and increases patients' suffering, further reducing the chances of a cure. Currently, treatment options for PCLM such as radiation therapy, chemotherapy, and surgical resection impose significant physiological, psychological, and economic burdens on patients due to their complex treatment procedures and high-risk operations, and these treatment strategies have a limited ability to achieve a complete cure for PCLM[8-10]. Therefore, PCLM is a very harmful disease, regardless of the clinical characteristics of PCLM or the base number of patients.

Over the past 22 years, researchers have increasingly focused on the field of PCLM. With the development of PCLM, researchers have generated significant scientific output. However, as scientific output on PCLM has accumulated over the years, the knowledge structure of PCLM has become both disorganized and a hindrance to research efficiency[11,12]. Bibliometrics, a method that quantitatively analyzes and measures literature information using statistical methods and information technology has been widely applied in medical research with promising results[13-17]. Therefore, bibliometric analysis may provide a partial solution to the aforementioned challenges.

To comprehensively analyze and summarize the field of PCLM, this study retrieved relevant papers on PCLM from the Web of Science Core Collection (WOSCC) and conducted a bibliometric analysis of the citation references and keywords. Additionally, we conducted a preliminary exploration of potential biological behavior in the field of PCLM. This article aims to help researchers interested in the field of PCLM grasp the research trends in this field more accurately and quickly, and to deeply understand the related fields and technology development trends. We hope that this study can provide inspiration and assistance in the development and promotion of the research field of PCLM on a global scale.

MATERIALS AND METHODS

Collection of PCLM paper data

The data for PCLM papers were collected from the WOSCC (https://www.webofscience.com/). The search strategy used in this study was TS = (("prostat* cancer") OR ("prostat* carcinoma")) AND (("pulmonary metastas*") OR ("lung metastas*") OR ("metasta* tumor of lung") OR ("metasta* carcinoma of lung") OR ("metasta* lung carcinoma")).

The inclusion and exclusion criteria for PCLM papers in this study were as follows: (1) To avoid the impact of data fluctuation due to WOSCC updates and restrictions, only papers published between 2000 and 2022 were included; (2) To ensure analytical rigor, only research articles, review articles, and early access papers were included; (3) Due to restrictions of the relevant software, only English-language papers were included; and (4) Finally, after manual screening, papers that were not relevant to the topic were excluded. Therefore, 280 articles were included in this study (see Figure 1).

All the data for this study were downloaded from WOSCC in BibTeX format on May 2, 2023, with the recorded content being "full record" and "cited reference". The data collection work was conducted separately by two authors. Any discrepancies that arose between the two authors during this process were resolved through in-depth discussions involving both authors and other collaborators to reach a consensus.

Bibliometric analysis of PCLM paper data

We utilized R software (version 4.2.2) for advanced statistical calculations, visualization, and comprehensive bibliometric analysis. This included creating topic evolution maps and keyword temporal heat maps. Additionally, we employed VOSviewer software (version 1.6.18) to handle large amounts of data and create keyword clustering visualizations.

Exploration of molecular mechanisms in PCLM

We searched the Gene Expression Omnibus, the Cancer Genome Atlas, Sequence Read Archive, and ArrayExpress databases, to identify suitable human tissue datasets that included both PC and PCLM tissues. One dataset, GSE 74367, met our inclusion criteria, and we downloaded the corresponding data. Using R software, we extracted expression matrices from the dataset and identified differentially expressed genes (DEGs) specific to PCLM. The criteria for DEG selection were $|\log FC| > 1$ and P-value < 0.05. Subsequently, we performed gene ontology and Kyoto Encyclopedia of Genes and Genomes analyses of the selected DEGs to gain preliminary insights into the potential molecular mechanisms of PCLM. Furthermore, we utilized STING (version 11.5) and Cytoscape (version 3.9.1) to construct protein-protein interaction networks for further analysis of PCLM mechanisms.

RESULTS

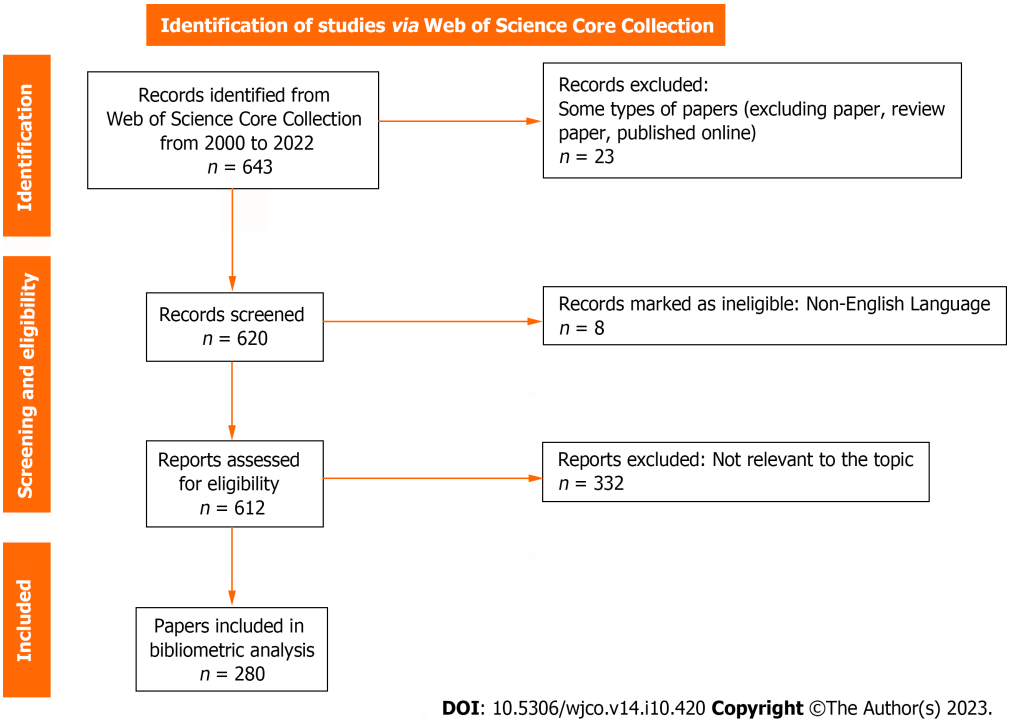
Spatial and temporal distribution and changes in PCLM knowledge volume

From a spatial dimension, Figure 2A illustrates the overall increasing trend in the publication and citation count of PCLM papers since 2000. However, the annual publication trends appear to be less stable. This does not indicate that the PCLM field has not received enough attention, but may be related to some bottlenecks encountered in the PCLM field. The steady increase in citation counts over the years further supports this statement. In terms of spatial distribution, Figure 2B reveals that developed countries have made significant contributions to the PCLM field, including the United States (130 papers), Japan (41 papers), Germany (27 papers), and Canada (20 papers), among others. This reflects the imbalance in the development of PCLM research across different regions. Encouragingly, emerging economies such as China and India are gaining importance and playing an increasingly significant role in the field.

Transition of hot topics in the PCLM field

Major hot directions in the PCLM field: Figure 3 illustrates that "expression", "metastasis", and "E-cadherin" are popular keywords in the PCLM field. We conducted a co-occurrence analysis using VOSviewer to identify the main hot directions in the PCLM field and provide an in-depth understanding of its knowledge composition. We selected 111 keywords with a frequency of occurrence greater than four times from the PCLM papers to construct a co-occurrence network. Based on Figure 4 and Supplementary Table 1, the network can be primarily divided into four clusters. Cluster 1: Basic research on tumor metastasis mechanisms (red portion in Figure 4A) includes keywords such as epithelial-mesenchymal transition (EMT), E-cadherin, adhesion, and migration. Cluster 2: Clinical treatment and related research (green portion in Figure 4A) includes keywords such as therapy, surgery, radiotherapy, radical prostatectomy, gene therapy, immunotherapy, and chemotherapy. Cluster 3: Clinical diagnosis-related research [blue portion in Figure 4A includes keywords such as diagnosis, prostate-specific membrane antigen (PSMA), and positron emission tomography/computed tomography (PET/CT)]. Cluster 4: Other basic research on PCLM (yellow portion in Figure 4A) includes keywords such as signal transducer and activator of transcription 3 (STAT3), microenvironment, androgen receptor (AR), mouse model, and angiogenesis. Surprisingly, recent hot topics, such as immunotherapy, are not emerging trends in this field, while phrases related to chemotherapy and targeted therapy, such as abiraterone acetate, docetaxel, cabazitaxel, and enzalutamide, are emerging keywords in this field (Figure 4B).

Evolution of hot topics in the PCLM field: Figures 5 and 6 demonstrate the evolution of hot topics in the PCLM field. In recent years, themes such as interleukin (IL)-12, gene therapy, and ganciclovir therapy have experienced a significant



DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 1 Flowchart of data collection from papers on prostate cancer with lung metastasis.

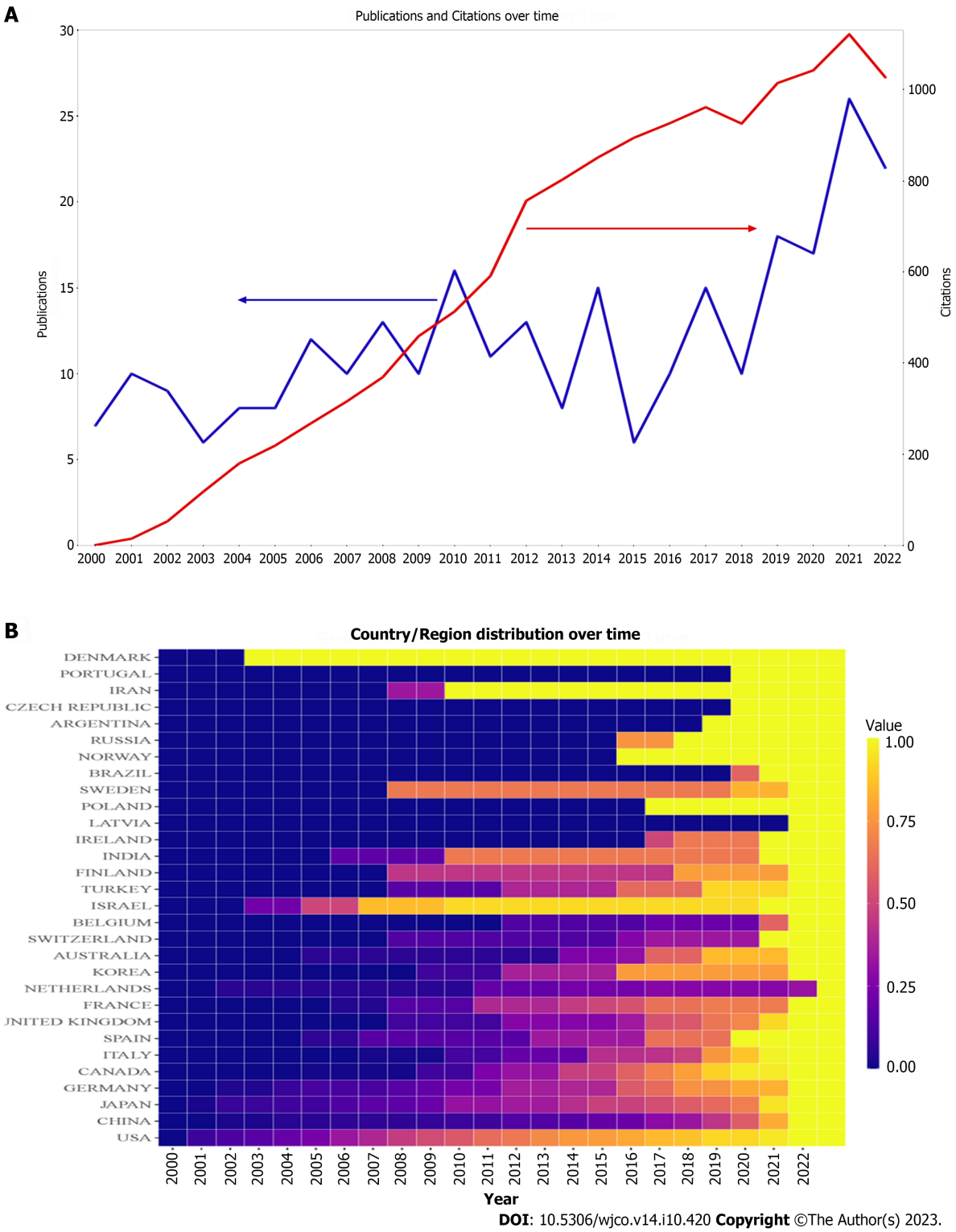
decrease in attention. On the other hand, PET/CT and PSMA in the diagnostic domain, enzalutamide, abiraterone acetate, and cabazitaxel in the clinical treatment domain, and metabolism and BReast-CAncer susceptibility gene 2 (*BRCA2*) in the basic research domain have emerged as new hot topics. Meanwhile, immunohistochemistry, immunotherapy, radiotherapy, migration, and angiogenesis have remained long-standing hot topics in the PCLM field. Additionally, it is surprising that terms related to bone metastasis, such as bone, bone metastasis, and bone scintigraphy, have appeared with a relatively high frequency in the PCLM field.

Development status of major research topics in PCLM

We constructed a thematic strategic coordinate map based on Keyword Plus (ID) and Author Keywords (DE) in the PCLM literature to determine the development status of major research topics. Figure 7 reflects the following themes in the field of PCLM: Motor themes, including chemotherapy, docetaxel, migration, and mitoxantrone, which are important and well-developed topics; niche themes, including *Ga-68-PSMA*, *STAT3*, and tumor-associated macrophages, which currently have low impact but need further strengthening; emerging or declining themes, including cisplatin, immunotherapy, gene therapy, and *IL-12*; and basic themes, including PET/CT, radiotherapy, and radical prostatectomy, which are important but have not yet received significant development in the field.

Exploration of the biological behavior of PCLM

We collected 12729 DEGs from a global dataset and compared PC patients without LM (*i.e.*, locally metastatic) and PC patients with LM. Among these DEGs, 6138 genes were upregulated, and 6591 genes were downregulated. Figure 8A, which presents the gene ontology functional annotations, shows that, in the biological process category, there are pathways such as regulation of the immune effector process and lymphocyte proliferation. The molecular function category has pathways such as focal adhesion, while in the cellular component category, cytokine activity and cadherin binding are prominent (Supplementary Table 2). Figure 8B, representing the Kyoto Encyclopedia of Genes and Genomes' functional annotations, reveals pathways such as cell adhesion molecules, neuroactive ligand-receptor interaction, salmonella infection, cytokine-cytokine receptor interaction, and the cAMP signaling pathway (Supplementary Table 3). It is worth noting that the findings related to cadherin binding and cell adhesion molecules align with the previous discussions, further confirming their promotional role in the development of LM in PC patients. To further investigate and explore the relevant pathways of PCLM, we applied the maximal clique centrality method to identify the top 20 key proteins from the cadherin binding and cell adhesion molecule pathways and construct a protein-protein interaction network. We found that cell adhesion molecules are closely associated with the immunoglobulin superfamily, such as *CD8A*, *CD86*, and *ICAM1*, as well as integrin family proteins, including *ITGB1*, *ITGB2*, and *ITGAM* (Figure 9A and Supplementary Table 4). On the other hand, cadherin binding shows close correlations with calcium-binding proteins from the cadherin family, such as *CDH1*, *CDH5*, and *CDH11*, as well as with catenin family proteins, such as *CTNNA1* and *CTNNB1* (Figure 9B and Supplementary Table 5).

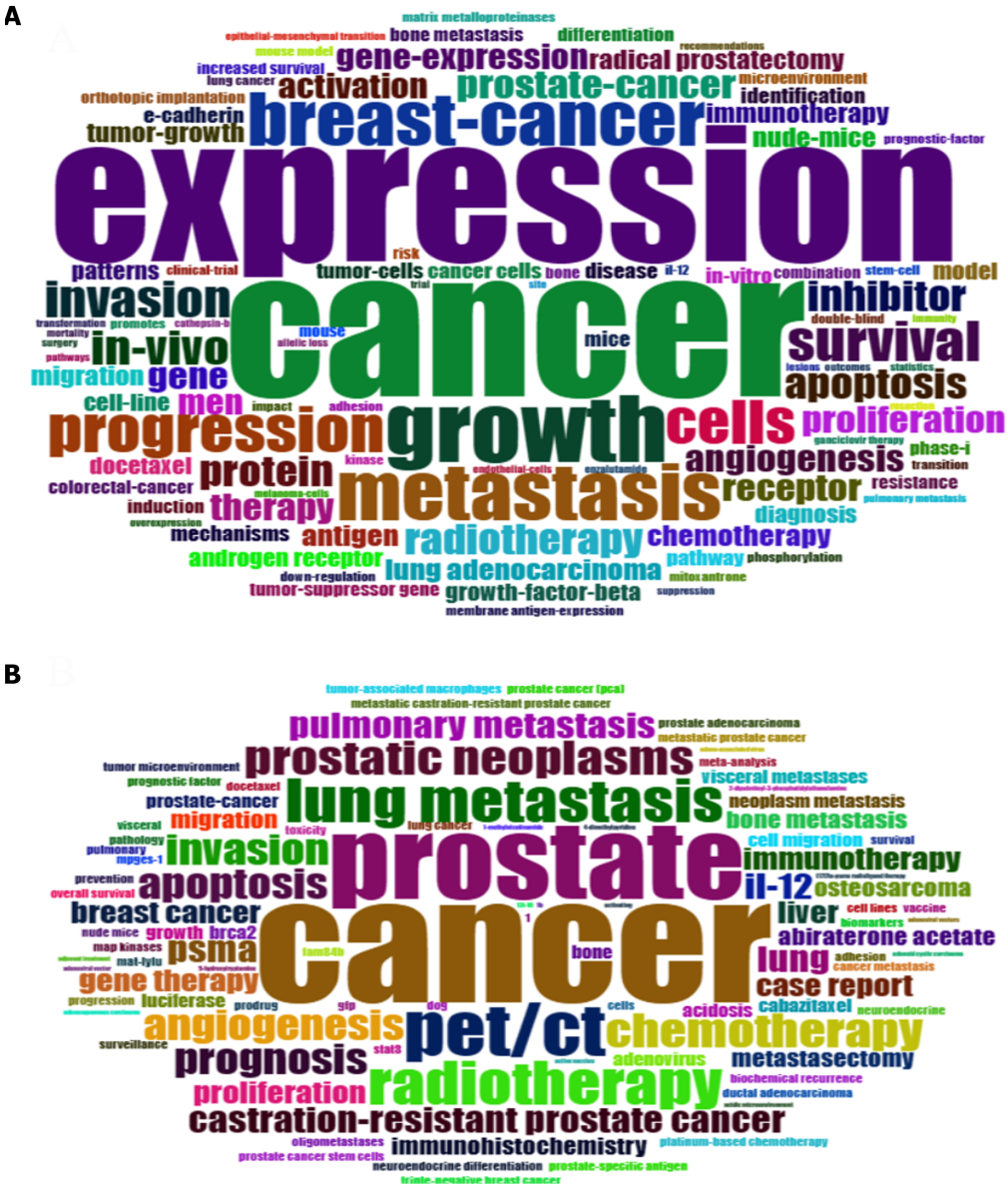


DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 2 Distribution and change in time and space of knowledge volume in the prostate cancer with lung metastasis field. A: Annual publications and citations of papers on prostate cancer with lung metastasis (2000-2022); B: Thermal diagram of the time distribution of national/regional papers. The *b* values represent the ratio of the total number of papers published in a country from 2000 to a certain year to the total number of papers published in a country.

DISCUSSION

PCLM is typically characterized by the presence of multiple nodules or areas of increased density in the lungs[18,19]. Metastatic lesions in the lungs can affect respiratory function and cause symptoms such as shortness of breath and chest tightness[5,20]. They can also exacerbate pre-existing lung diseases in patients, leading to poor prognoses. Extensive research efforts have been dedicated to understanding the biological behavior of PCLM, which has contributed to the continuous development of clinical treatment strategies. In recent years, the explosive growth and widespread adoption of bioinformatics, particularly next-generation sequencing technologies and single-cell sequencing, have enabled



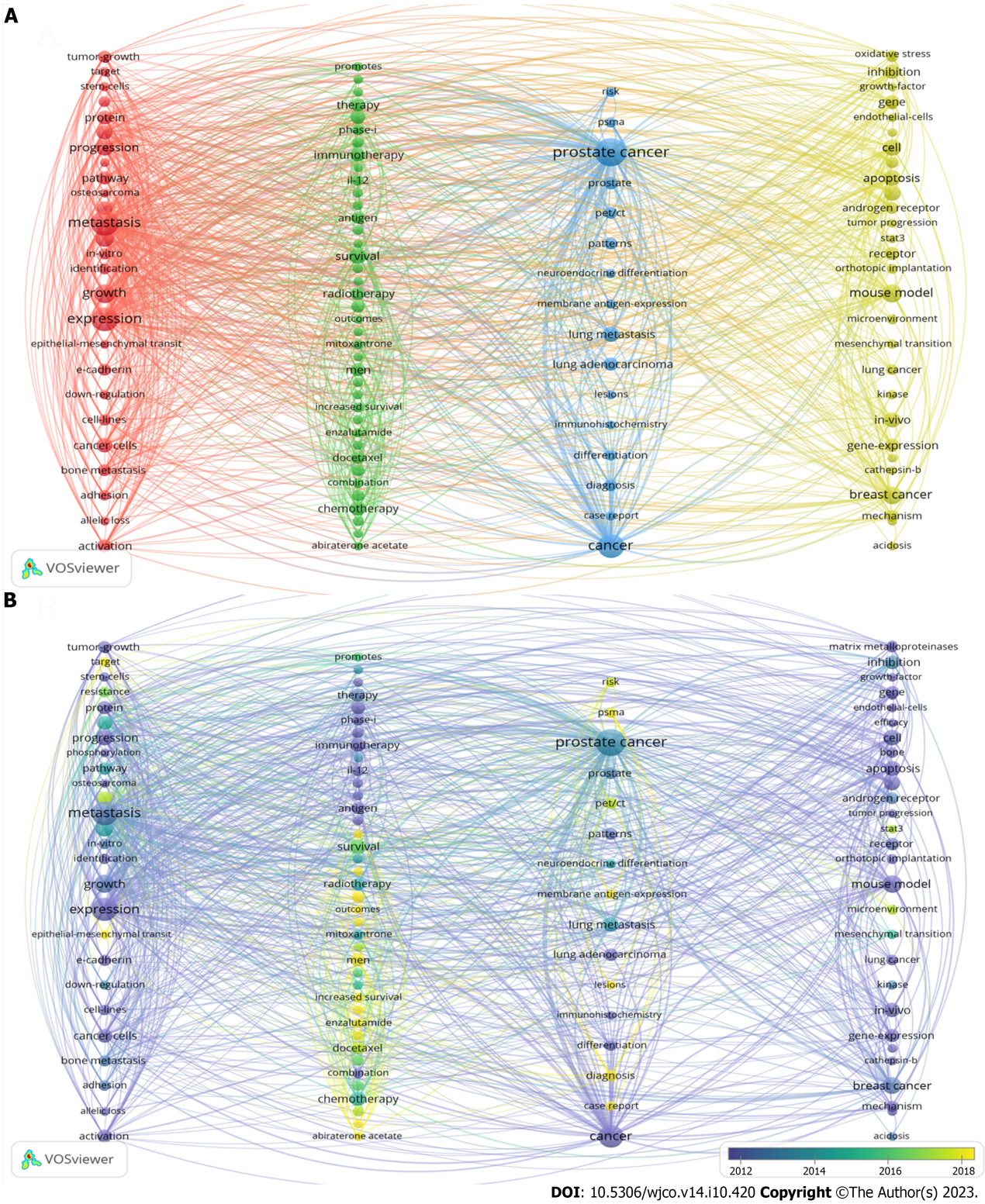
DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 3 Word clouds of high-frequency keywords in the papers on prostate cancer with lung metastasis. A: Keywords plus; B: Author's keywords.

researchers to delve into the molecular mechanisms of PCLM in depth, leading to unprecedented progress in the field. However, the accumulation of scientific output over the years has resulted in a chaotic knowledge landscape in the field of PCLM. Therefore, this study aimed to systematically reconstruct the global knowledge system of PCLM, providing a reference for the future development of PCLM.

Spatial and temporal distribution and changes in scientific output in the PCLM field

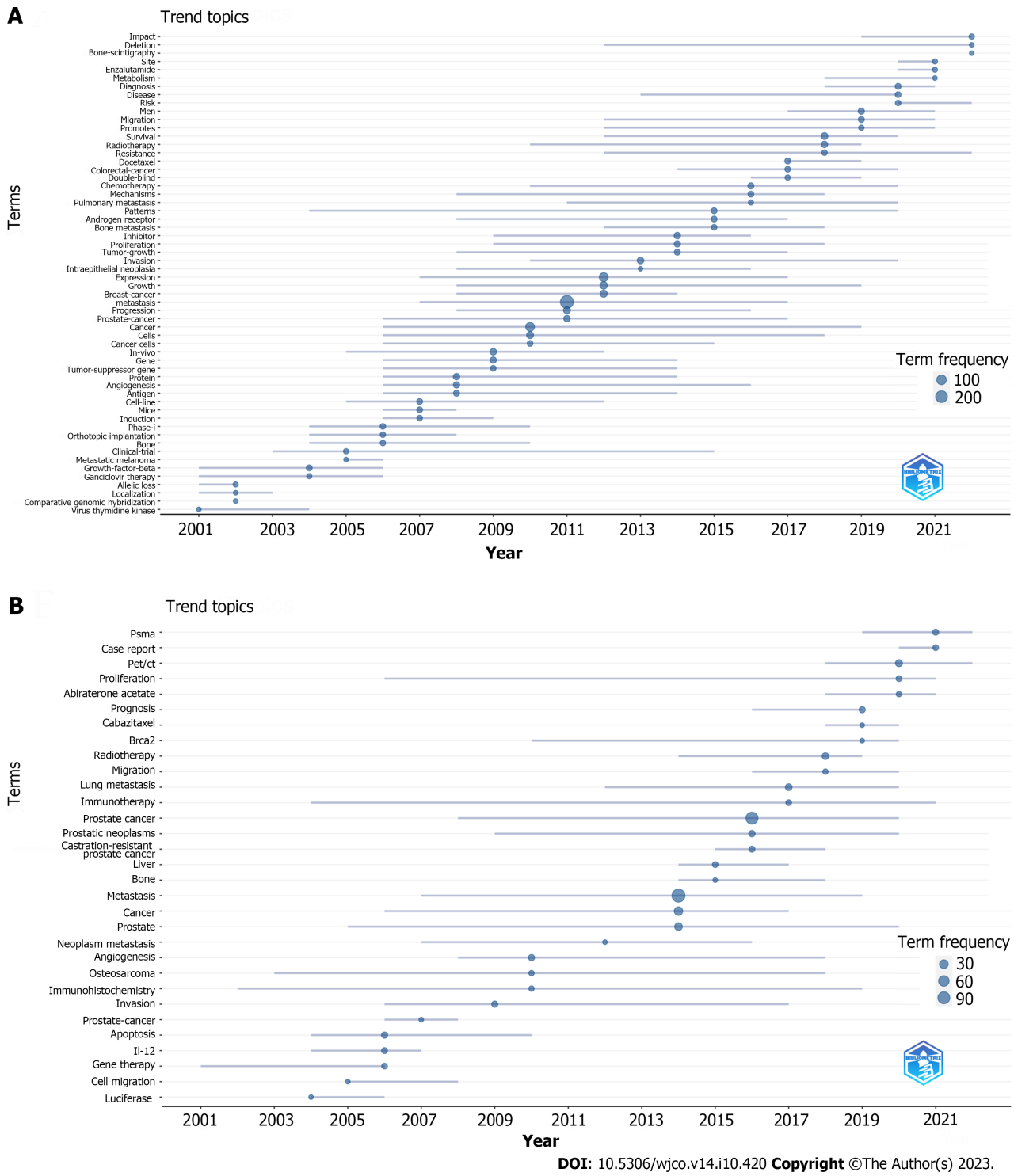
In recent years, more systematic and precise screening and treatment have significantly improved the prognosis of PC up to a point[21,22]. However, effective treatment of PCLM still faces significant challenges and requires further exploration and breakthroughs[23]. Moreover, the number of PCLM patients is very large globally[1,2,4], which is further driving the exploration of and research into PCLM by scholars worldwide. This is consistent with the expanding volume of PCLM knowledge over the years. However, the uneven distribution of scientific output in the field of PCLM across regions in the spatial dimension may be related to the social and scientific development capabilities of those regions[24]. This implies that the uneven country/region distribution of scientific output about PCLM in the spatial dimension may be related to two factors. First, developed countries and regions have invested more in healthcare resources and scientific research infrastructure. Second, they have a higher number of research institutes, laboratories, and researchers. In contrast, some developing countries or poor regions may face the challenges of limited funding and inadequate research conditions, resulting in a relative lag in scientific research. In this way, a contradiction has arisen between developing countries with limited medical technology but high PC morbidity and mortality and developed countries with advanced medical technology but reduced PC morbidity and mortality[25,26]. Therefore, developed countries should proactively



DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 4 Analysis of the co-occurrence of all keywords in the papers on prostate cancer with lung metastasis. A: Network visualization map; B: Overlay visualization map. The small circle represents the keyword. The area of the small circle represents the frequency of the keyword. The colors of the different areas represent their categories. The lines of the connecting circles represent keywords that appear in an article simultaneously.

conduct international exchanges and cooperation in the field of PCLM to promote the sharing of data, funds and equipment, technology and methods, and the establishment of international cooperation networks. Developing countries should increase their investment in PCLM-related research and actively seek transnational cooperation in the future. This will not only benefit the lives and health of the world's people but will also benefit the development of the field of PCLM by making full use of clinical resources and research due to the international cooperation network and the improvement of the technological level of developing countries. Additionally, it is exciting that, in recent years, some developing countries have been contributing more to research in the field of PCLM, which should further narrow the uneven spatial



DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 5 Topic trend graph. A: Keywords plus; B: Author's keywords.

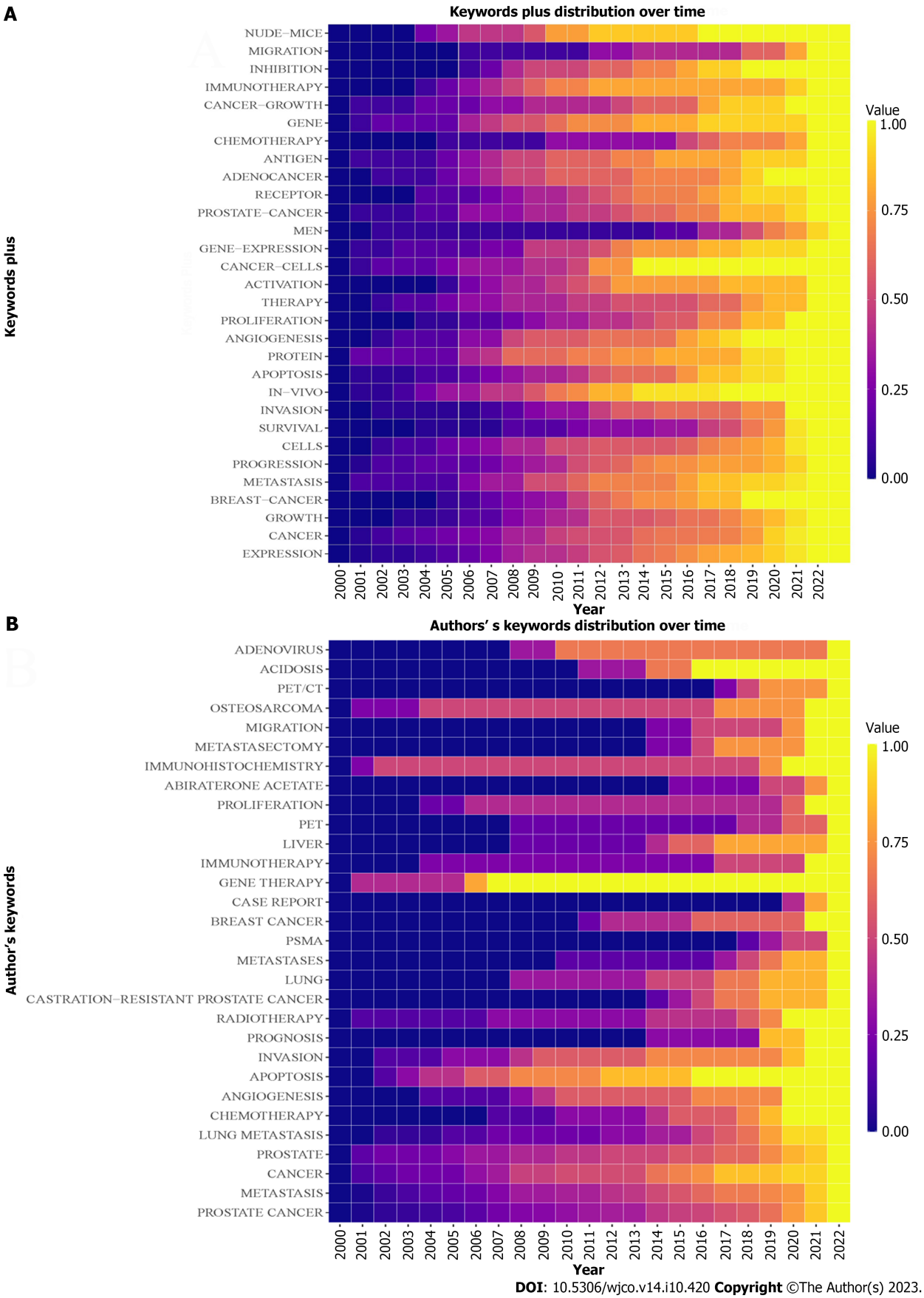
distribution of scientific output in PCLM.

Evaluation of hot research directions in the PCLM field

Researchers' continuous exploration and attention worldwide have propelled ongoing iterations and updates in the field of PCLM knowledge. These changes are primarily reflected in the aspects described next.

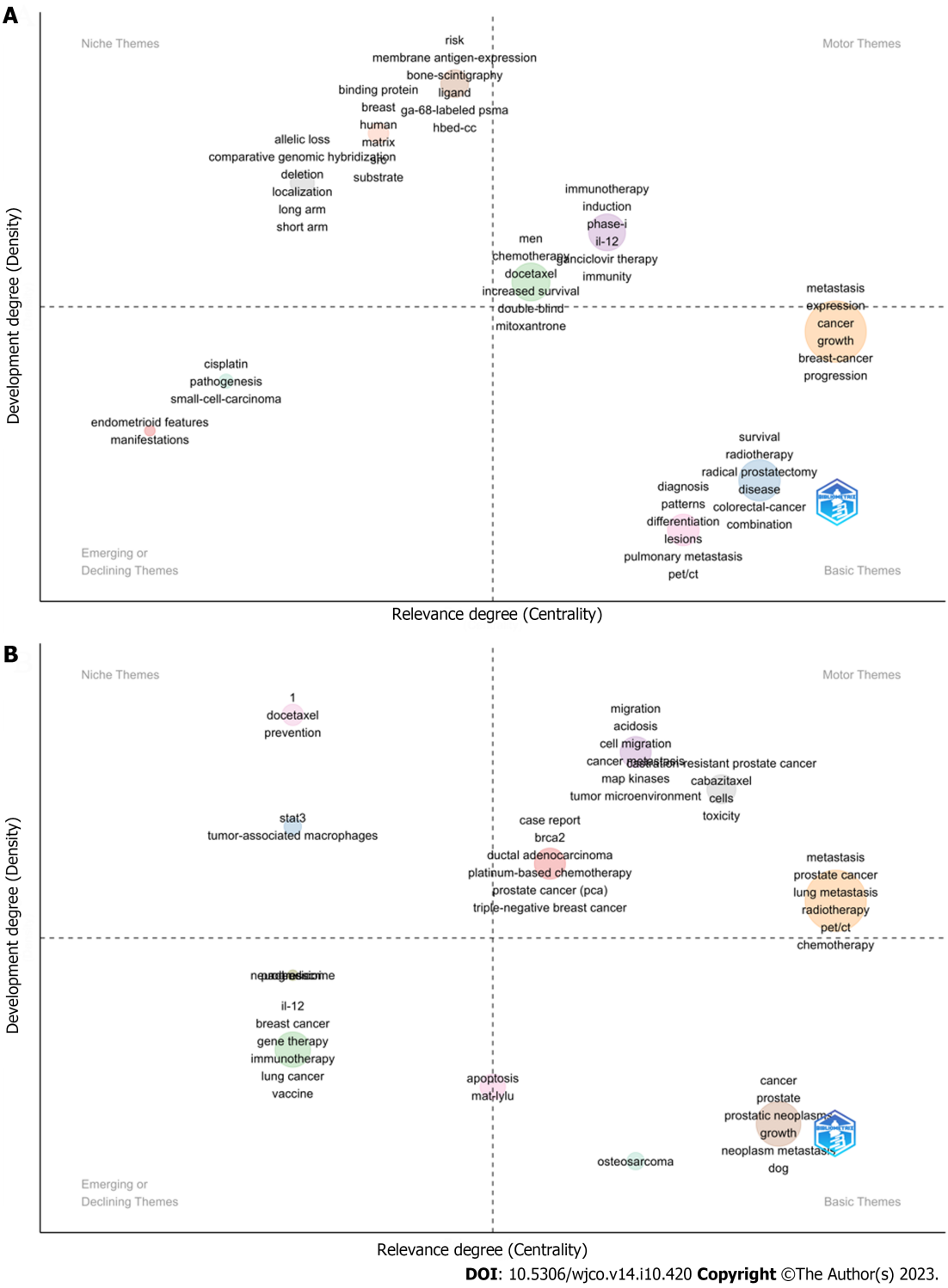
Clinical treatment directions in the PCLM field

In the early years, researchers such as Ren *et al*[27] utilized techniques like gene modification to enhance the expression of interferon-beta in mesenchymal stem cells in a mouse model of PCLM and found that tumor cell apoptosis increased and that natural killer cell activity, which is associated with anti-tumor activity, significantly increased. In addition, the invasion and metastasis suppressor gene *RhoGDI2* was identified by DNA microarray technology, and after the reconsti-



DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 6 Keyword time heat map. A: Keywords plus; B: Author's keywords. The values represent the ratio of the total frequency of the keyword from 2000 to a certain year to its total frequency; from top to bottom, the number of papers published by the country increased in turn.

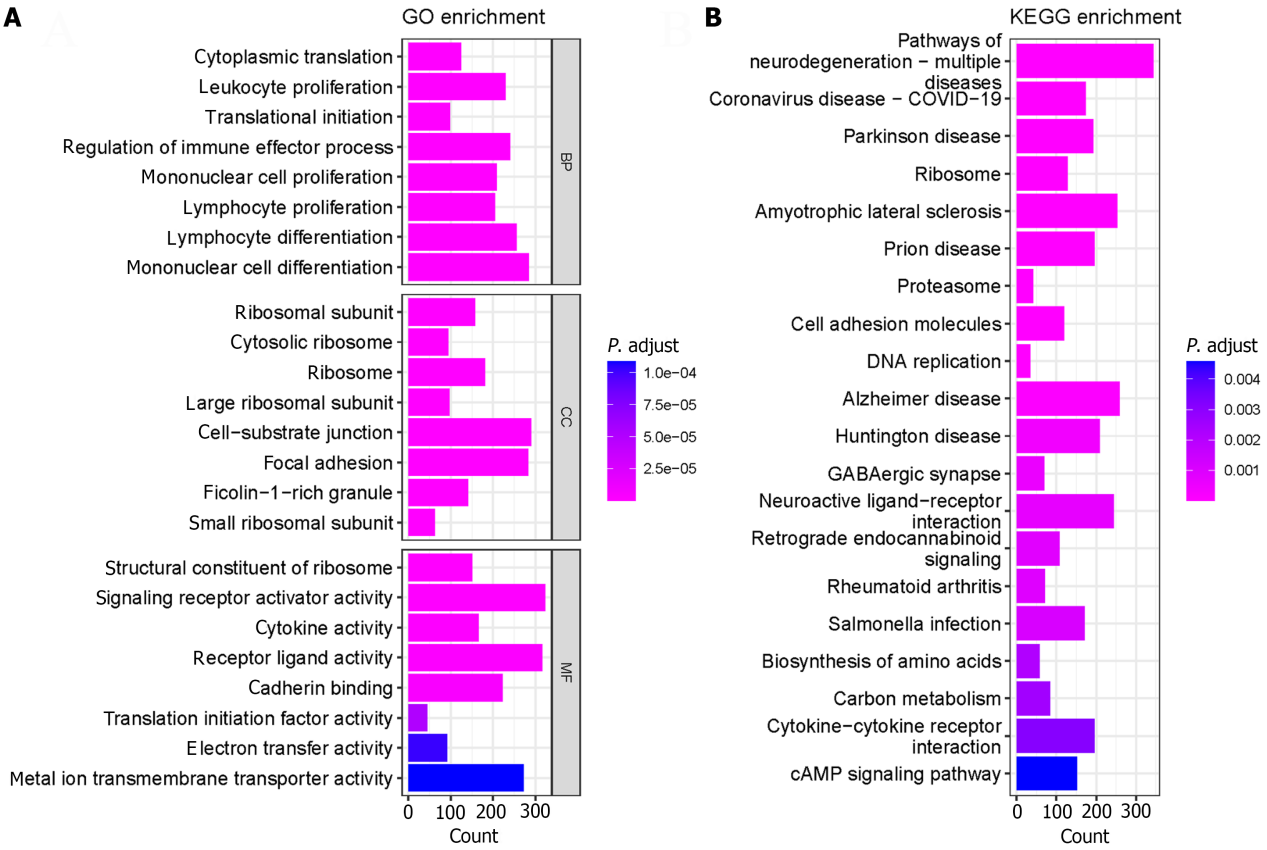


DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 7 Theme strategic coordinate map. A: Keywords plus; B: Author's keywords. PET/CT: Positron emission tomography/computed tomography; IL-12: Interleukin-12.

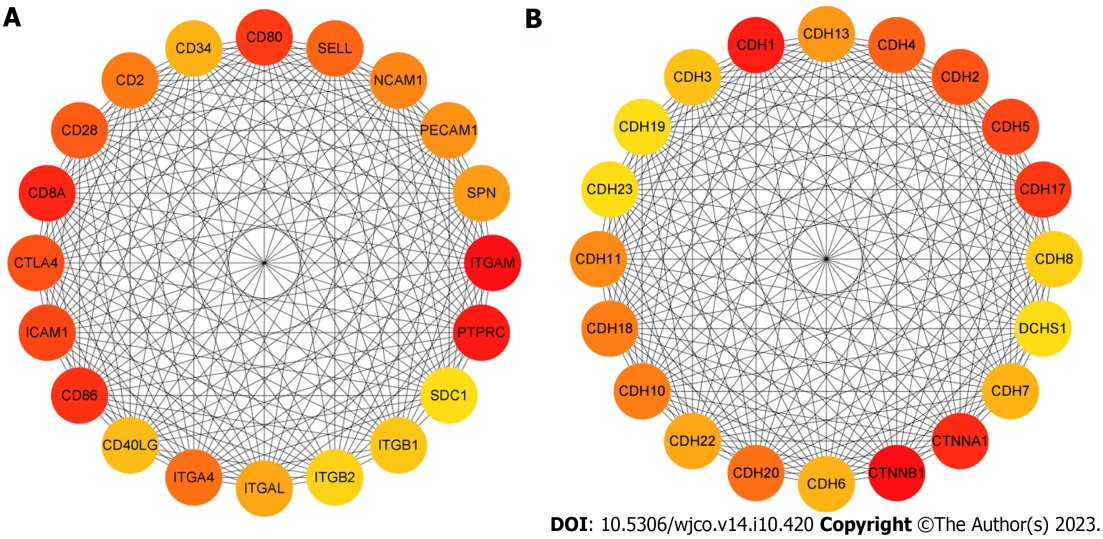
tution of *RhoGDI2* in metastatic cancer cells, it was found that LM was inhibited and the motility of cancer cells *in vitro* was reduced[28]. Therefore, interferon-beta and *RhoGDI2* are considered effective potential targets for gene therapy. Additionally, researchers combined *AdV-tk* gene therapy with radiotherapy and chemotherapy in a mouse model of PCLM and found a significant reduction in lung nodules and cancer cell colonization in the lungs[29]. However, studies have indicated that these gene therapies are challenging to deliver effectively to the tumor site, leading to inadequate gene delivery and resulting in various adverse outcomes[30]. Currently, no effective clinical trials have successfully addressed this challenge. As a result, gene therapy is currently considered a peripheral topic, and it is not surprising that its popularity in the PCLM field has declined significantly in recent years.

In this field, radiation therapy has always been an important and highly regarded topic. In recent years, some reports have shown promising results in the treatment of PCLM patients using 177Lu-PSMA radioligand therapy (Lu-PRLT)[31,



DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 8 Molecular pathway map of prostate cancer with lung metastasis. A: Bubble map of differentially expressed genes (DEGs) based on the gene ontology enrichment analysis; B: Bubble map of DEGs based on the Kyoto Encyclopedia of Genes and Genomes enrichment analysis. GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.



DOI: 10.5306/wjco.v14.i10.420 **Copyright** ©The Author(s) 2023.

Figure 9 Protein–protein interaction network graph of the top 20 key proteins ranked by the maximal clique centrality method. A: Cell adhesion molecules; B: Cadherin binding.

32]. However, PCLM patients who are PSMA(-)/fluorescein Di-β-D-galactopyranoside (FDG)(+) may not benefit from Lu-PRLT[33]. Therefore, some researchers have combined biologically guided radiation therapy with Lu-PRLT for PSMA(-)/FDG(+) PCLM patients and found this combination therapy to be beneficial[33]. Moreover, there have been case reports suggesting that the combination of stereotactic body radiation therapy and androgen deprivation therapy (ADT) can confer benefits in terms of biochemical response and disease-free survival in PCLM patients[34]. However, recurrences after radiation therapy in the treatment of PCLM frequently occur[35]. Furthermore, guidelines define radiation therapy for PCLM as palliative treatment and do not recommend it as part of curative approaches[35].

Therefore, although radiation therapy has broad prospects, it is considered a significant but underdeveloped topic in the PCLM field due to the many challenges it currently faces.

Radical surgery, as a traditional topic, has been widely applied in clinical practice for PC. However, there is currently insufficient evidence from evidence-based medicine and international guidelines to clearly define the role of radical surgery in PCLM patients[36]. Thus, radical surgery is a significant but underdeveloped topic in the PCLM field. Studies have shown that performing radical prostatectomy in animal models of PCLM can significantly reduce the number of lung metastases[37]. Furthermore, research reports have indicated that when the criteria for resection are met, LM resection as the preferred choice for PCLM patients can avoid or delay the use of ADT and its adverse effects, significantly improving patient prognosis[35]. This also demonstrates the promising development prospects of radical surgery in the PCLM field.

Immunotherapy, as an emerging direction, has been a topic of long-standing interest in this field. In the field of immunotherapy for PC, treatment plans have limitations. One example is sipuleucel-T, the only United States Food and Drug Administration-approved immunotherapeutic agent for metastatic desmoplasia-resistant PC, but it is indicated for asymptomatic or minimally symptomatic patients only[38]. Immune resistance poses another challenge in PC treatment. Factors like low tumor mutation loads and the presence of immunosuppressive cells can disrupt the immune system and create an immunosuppressive tumor microenvironment, leading to reduced therapeutic efficacy[39]. Additionally, there can be adverse effects associated with immunosuppressant therapy. For instance, patients may experience immune-related adverse events, such as ulceration of the lower lip[40]. Furthermore, the clinical utility of certain treatments has yet to be validated. For example, a study by Komaru *et al*[41] that is currently in the animal experimentation stage has a long way to go before its potential in clinical practice can be determined. In addition, there have been fewer studies on relatively well-established immunotherapies in the field of PCLM relative to other treatments. These may be due to the unclear mechanisms currently available, which do not provide a solid theoretical basis for the development of more effective immunotherapies. These are significant barriers to the widespread clinical application of immunotherapy in PCLM. However, in recent years, targeted and less toxic immunotherapies have shown better and sustained response rates compared to conventional therapies. Immunotherapy has the potential to cure malignant tumors, including metastatic melanoma, lung cancer, and others[42-45]. This also explains the broad prospects of immunotherapy, as it emerges as a relatively new and promising hotspot in the strategic landscape (Figure 7). For example, recent research reports have made clinical applications of oncolytic viruses, which can specifically replicate, proliferate, and destroy PCLM cells through the nanodrug packaging approach[46]. Additionally, researchers have designed a spatially drug-loaded M1 macrophage system in which M1 macrophage accumulates significantly in LM lesions, effectively enhancing the infiltration of cytotoxic T cells into lung metastases and boosting local anti-tumor immunity[47]. If these approaches could be widely implemented in clinical practice, a complete cure for PCLM might be within reach. In summary, the exploration of immunotherapy in this field has been long and challenging. However, breakthroughs in new technologies and a deeper understanding of molecular mechanisms in recent years have accelerated the progress of PCLM immunotherapy.

Contrary to immunotherapy, chemotherapy is a relatively new topic in the field of PCLM, despite being a traditional subject. Currently, there are several main directions for chemotherapy, including docetaxel, cabazitaxel, and combination therapy. Docetaxel is a well-established chemotherapy drug that has been proven to significantly prolong the survival of PCLM patients[48-50]. However, most PCLM patients develop resistance to docetaxel, leading to disease progression[50]. As for cabazitaxel, a phase 2 clinical trial has shown that it can significantly alleviate or stabilize the condition of metastatic castration-resistant PC and has the advantages of better tolerance and lower toxicity[51]. Furthermore, one study designed a cabazitaxel nanoparticle carrier that can be inhaled by M2 macrophage vesicles and that, in experimental models, was able to more effectively enter tumor tissue and inhibit over 93% of LM occurrences[52]. Additionally, combining chemotherapy with targeted therapy or immunotherapy has shown promising efficacy against LM[53-55]. Chemotherapy is utilized in PCLM treatment, but it has limitations and challenges. One issue is resistance, such as the enhancement of doxorubicin resistance in PC by the TrkB protein[50]. Additionally, PC cells display inherent and acquired resistance to cisplatin, making it ineffective as a first-line chemotherapeutic agent for PC[56]. Most PC patients who undergo ADT eventually develop castration-resistant disease[57]. Chemotherapy also has adverse effects. For instance, potentially life-threatening events like neutropenia and febrile neutropenia can occur in patients with metastatic PC who receive doxorubicin-related chemotherapy[58]. Furthermore, ADT for PC increases the risk of cardiovascular and metabolic syndrome, which can lead to fatal outcomes[59]. Despite these treatment efforts, chemotherapy alone cannot fully cure PCLM. However, in the context of the limitations of other non-traditional treatments, chemotherapy has been widely adopted in clinical practice, and its efficacy has been clearly demonstrated, whether applied alone or in combination with other therapeutic means. Hence, it is not surprising that chemotherapy is recognized as a mature and important topic in this field.

In addition, in the field of targeted therapy, enzalutamide, a next-generation AR inhibitor, has been proven to significantly prolong the survival of patients with metastatic PC, despite the inevitable resistance mediated by SPP1 through the phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (Akt) and extracellular regulated kinase 1/2 pathways or the reactivation and splice variants of the AR[60-62]. MiR-33b-3p inhibits metastasis by targeting DOCK4 in PC[63]. We could enhance miR-33b-3p expression to overcome the poor efficacy of proteasome inhibitors in metastatic PC in the future. It has also been reported that treatment with Lu-177-PSMA radioligand showed significant efficacy in PC patients and responded favorably to the treatment and regression of lung metastases after PSMA radioligand therapy (Lu-PRLT)[31]. High expression of C-C motif ligand 2 induced the production of carbon catabolite repression 4 (CCR4) in PC cells, which promotes migration and invasion of PC cells through enhanced Akt phosphorylation[64]. This study reveals CCR4 as a potential target for the treatment of PCLM. Putz *et al*[65] found that the cytokine signaling checkpoint CIS plays an important role in the occurrence of PC with LM and has a promising future in the treatment of PCLM.

Furthermore, in recent years, the emergence of abiraterone acetate has been confirmed by numerous studies to alleviate lung metastases and significantly prolong the survival of PCLM patients, and it has been regarded as a safe and effective treatment for many advanced PCLM patients[8,54,66,67].

In recent years, precision medicine has played an important role in a variety of diseases. In particular, tumors involve alterations in the biological behavior of multiple genes. The biological behaviors of various tumors are complex and diverse. Therefore, precision medicine with personalized treatment characteristics is a solution to the difficult problem of PCLM, which is hard to cure completely. One study reported that AR plays dual and opposite roles in vasculature encapsulating tumor clusters, emphasizing the complex function of AR and its importance in individualized cancer therapy[68]. This study provides new insights into the complex regulatory network of AR in metastatic tumors and lays the foundation for relevant precision medicine. It has also been reported that AuNSs@PDA-Ce6 nanoprobes significantly reduced tumor growth and inhibited LM, which has considerable potential for precise therapeutic diagnosis and metastasis inhibition[69]. In addition, Hlavac *et al*[70] revealed the characterization of prognostically distinct subgroups with precision medicine value by targeted sequencing of blood and archival samples from LM patients. However, regrettably, no mature precision medicine or personalized treatment for PCLM has been reported. In the future, precision medicine will also be an important endeavor in the field of PCLM.

Clinical diagnostic approaches in the field of PCLM

PSMA has been widely utilized in the PC screening. Many researchers have combined PSMA with PET/CT for clinical diagnosis. This includes the use of [99mTc]PSMA-T4 and 68Ga-PSMA-11, which have shown high efficacy in the diagnosis and detection of metastatic PC and recurrence, outperforming traditional imaging techniques[71-75]. However, there is still a notable false-negative rate in some patients[75]. Additionally, there are cases where lung metastases in PC patients are PSMA-negative, rendering PSMA-PET/CT unsuitable for detecting such patients[76]. Furthermore, 18F-fluorocholine PET/CT has shown higher specificity compared to traditional methods for staging PCLM patients, but its sensitivity still needs improvement[77]. Therefore, although this approach has some influence in the field of PCLM, several issues still need to be further addressed and developed in the future.

Exploratory mechanisms in the field of PCLM

In recent years, with the advancement and widespread application of bioinformatics, particularly the progress in second-generation DNA sequencing and single-cell sequencing technologies, researchers have been able to identify key molecules in PCLM more thoroughly and comprehensively, elucidating additional pathway mechanisms. This has led to the emergence of new research hotspots in basic research.

In terms of organism metabolism, studies have found that *Camkk2* not only mediates the metastasis and colonization of PC cells in the lungs, but also disrupts normal metabolism, such as glucose and lipids, leading to the occurrence of metabolic syndrome and other complications[78]. It has also been reported that the regulation of glutamine metabolism can upregulate *ARPC1A* in PC cells, resulting in changes in the PC cell cytoskeleton and the cells' migration and invasion of the lungs[79]. Furthermore, the regulatory role of the positive feedback loop between tryptophan hydroxylase 1 and β-catenin/ZBP-89 signaling, as well as the modulation of microribonucleic acids in acidosis mediated by the Warburg effect, can enhance the metastatic ability of PC cells[80,81]. These findings indicate a close relationship between organism metabolism and the metastatic behavior of PC cells. In recent years, numerous studies have shown that mutations in *BRCA2*, which possesses DNA repair functions, enhance the ability of PC cells to develop LM and other types of metastases[8,82,83]. However, these studies are based on sporadic cases, and it is necessary to conduct more comprehensive and systematic research for supplementary validation. Regarding *STAT3*, *CCL5* secreted by M2 macrophages enriched in the PC tissue microenvironment can promote *STAT3*-dependent EMT, enhancing the resistance and metastatic ability of PC cells toward the lungs[84]. In addition, immune checkpoints can inhibit T lymphocyte immune responses through the EGFR/JAK1/STAT3 pathway, promoting PC progression and the occurrence of LM[85,86]. Encouragingly, based on the related mechanisms of STAT3, research has found that the traditional Chinese medicine CFF-1 can effectively inhibit LM, prolong survival, and improve the quality of life for patients[85]. In terms of AR, PC cell growth is androgen-dependent *in vitro*, and the level of androgens in the body is positively correlated with tumor size *in vivo*[87]. Studies have also revealed that cell cycle proteins interact with AR, regulate the promoters of vascular endothelial-derived growth factor and matrix metalloproteinase 2, and enhance their expression, thereby promoting PC progression and increasing metastatic capacity[88]. These findings regarding AR indirectly provide theoretical evidence for the development and improvement of new-generation targeted drugs, such as enzalutamide, an AR inhibitor. These examples highlight the importance of translating basic research findings to clinical applications and improving PCLM treatment.

In addition, some mechanisms of PC metastasis have become independent clusters (Figure 4), indicating that this direction is relatively mature and independent as a hotspot. Studies have reported that the downregulation of E-cadherin, a result of certain inducing factors, promotes the migration and invasion of PC cells[89]. It has also been found that silencing *AKT1* downregulates epithelial-associated E-cadherin and upregulates mesenchymal-associated N-cadherin, promoting the occurrence of EMT closely related to PCLM[90]. Furthermore, some studies have indicated that decreased cell adhesion caused by C-terminal binding protein or metabolic acidosis-induced abnormal expression of microribonucleic acids enhances the metastatic ability of PC cells[81,91]. These findings suggest that abnormalities in cell-cell connections can enhance the likelihood of PC cell metastasis.

Finally, the presence of phrases such as "rats" suggests that many research results are still in the cellular, animal, and *in vitro* stages of experimentation and are still some distance from clinical translation. For example, the studies by Komaru *et al*[41], Pan *et al*[89], and Azhati *et al*[92] are still in the cellular, animal, and *in vitro* experimental stages and a long way from clinical practice. As mentioned above, PCLM scientific outputs represent countries/regions with a high

level of PCLM research but with fewer clinical case data due to the small number of PCLM patients, while countries/regions with high PCLM morbidity and mortality have a relatively weak level of research on PCLM. This may also be a major obstacle to the translation of basic research results into clinical practice. For this reason, international collaboration and knowledge sharing are particularly important. In addition, basic research often involves complex cellular, molecular, and biological processes, which may lead to problems of instability and reproducibility of results. One strategy to address this challenge is to increase the reliability and reproducibility of results through multicenter studies, validation experiments, and mutual evaluation. Clinical translation requires significant financial and resource support. However, research funding is often limited, and industry needs to consider commercial viability. Strategies to address this challenge include seeking support from public and private funding, building partnerships, and exploring new sustainable financing models. Thus, the translation of basic research findings into clinical applications is urgent in the context of the limited effectiveness of contemporary treatment options. In conclusion, basic research on PCLM is important but underdeveloped at the present time.

In addition, bone metastasis is a prominent point in the field of PCLM. This is mainly because LM often coexists with bone metastasis and other metastatic lesions, while isolated PCLM is less common, accounting for approximately 20.4% of all PCLM cases[6,7]. This highlights the complexity and refractoriness of PCLM. Therefore, further exploration of the relevant mechanisms is necessary.

Summary and exploration of mechanisms in PCLM

The global state-of-the-art PCLM pathway map we have constructed suggests that LM in PC patients is likely closely related to abnormalities in pathways, such as cadherin binding and cell adhesion molecules. This is in line with existing reports and the information discussed herein. However, most of these studies have only associated adhesion or cadherin abnormalities with PC cell migration and invasion, and there is still a lack of mature research revealing their specific roles in *in vivo* metastasis. Nevertheless, the specific interactions between the immunoglobulin superfamily and the integrin family, as well as the mechanisms leading to abnormal cell adhesion, have been elucidated in other tumors[93]. The mechanisms by which members of the cadherin gene family regulate EMT and promote breast cancer metastasis have also been identified[94]. Therefore, the interactions we have identified among the immunoglobulin superfamily and the integrin, cadherin, and calcium-binding protein families in the cadherin binding and cell adhesion molecule pathways in PCLM may be directions that merit further exploration in the field of PCLM.

Additionally, mutual interactions between coronavirus [coronavirus disease 2019 (COVID-19)] and LM of other tumors have been reported[95-97]. Cytokine-cytokine receptor interaction and cytokine activity have been shown to be closely associated with enhanced invasion in distant metastasis of thyroid cancer, lymph node metastasis of gastric adenocarcinoma, and liver metastasis of colon cancer[98-100]. Furthermore, the ribosomal protein S6 kinase, which is closely related to EMT, invasion, and metastasis of tumor cells, has been proven to be an effective target for anticancer therapy [101]. These findings indicate that COVID-19, cytokine-cytokine receptor interaction, and ribosomal pathways are closely related to tumor metastasis and have broad clinical application value. However, the detailed roles of these pathways in PCLM have not been reported. Therefore, these directions are also among worthy future explorations required in the field of PCLM.

Limitations of this study and future work plans

Several limitations of this study deserve attention. First, although most data in this study were analyzed using computer-based analysis methods that are objective, efficient, and relatively accurate, occasional errors that are difficult to avoid and detect may have occurred. In the future, we should strengthen manual interventions to address this issue. Second, due to the limitations of the analysis tools, our bibliometric analysis included only detailed data from English papers that are available globally. Some high-quality, non-English papers may have been overlooked. In the future, we should improve our analytical methods to further analyze these papers. Third, the paper data in this study came only from WOSCC. In future, we should analyze data from multiple databases to complement and validate our results. Fourth, due to the poor timeliness of the data, some emerging hotspots may have been overlooked. In the future, we should update the data in a timely manner and improve the analysis methods to better capture emerging hotspots. Fifth, while we have gained new insights into the pathways involved in the biological behaviors of PCLM, they still lack *in vivo* and *in vitro* experimental verification. In the future, we should conduct further experimental validations related to these pathways.

CONCLUSION

In conclusion, with the continuous advancement of scientific technology in recent years, PCLM has received widespread attention. In this study, we conducted a bibliometric analysis to summarize the global knowledge system of PCLM over the past 22 years. This included clinical aspects based on chemotherapy and immunotherapy, diagnostic aspects based on PSMA-PET/CT, and basic aspects based on cell adhesion molecules and STAT3. Although current treatment approaches can improve the prognosis of PCLM patients to some extent, resistance to traditional therapies and the limitations of novel therapies still prevent the complete cure of PCLM. Furthermore, we identified the close association of COVID-19, cytokine-cytokine receptor interaction, and ribosome-related pathways with PCLM for the first time. Therefore, future research in the field of PCLM should focus on exploring and enhancing mechanisms such as cytokine-cytokine receptor interaction and ribosome-related pathways, and further improving existing mechanisms such as cadherin binding and cell adhesion molecules. This study establishes a robust theoretical foundation for the advancement and enhancement of novel therapeutic approaches with the potential to facilitate the full remission of PCLM as soon as possible.

ARTICLE HIGHLIGHTS

Research background

Over the past 22 years, researchers have increasingly focused on prostate cancer (PC) with lung metastasis (LM), generating significant scientific output, but the accumulated knowledge has become disorganized and hindered research efficiency.

Research motivation

With the increase of researchers' research enthusiasm in the field of PCLM over the years, scientific output has continued to increase, but there is no complete PCLM knowledge structure system. The purpose of this article is to establish a complete structural knowledge system and future development direction.

Research objectives

In order to further clarify the future development direction of PCLM, we reconstruct the global knowledge system in the field of PCLM. This research aims to help researchers interested in the field of PCLM grasp the research trends in this field more accurately and quickly, and to deeply understand the related fields and technology development trends. This study can provide inspiration and assistance in the development and promotion of the research field of PCLM on a global scale.

Research methods

The research gathered data on PCLM papers from Web of Science Core Collection (https://www.webofscience.com/) using a specific search strategy, resulting in 280 high-quality articles published between 2000 and 2022. Data was downloaded on May 2, 2023. We conducted a bibliometric analysis of keywords, publication volume, and citation frequency. Additionally, we selected differentially expressed genes from global high-throughput datasets and performed enrichment analysis and protein-protein interaction analysis to further summarize and explore the mechanisms of PCLM.

Research results

Over the past 22 years, PCLM has gained attention, with uneven research distribution. Clinically, chemotherapy and immunotherapy are primary treatments, while diagnosis relies on prostate-specific membrane antigen and positron emission tomography/computed tomography. Basic research focuses on cell adhesion molecules and signal transducer and activator of transcription 3. Traditional treatments like chemotherapy dominate, but novel approaches like immunotherapy show limited effectiveness. This research unveils the coronavirus disease 2019 (COVID-19)-related pathway's newfound associations with PCLM.

Research conclusions

Recent scientific advancements have drawn attention to PCLM. This 22-year bibliometric analysis covered clinical diagnostic, and basic aspects. Current treatment improves prognosis, but resistance and limitations persist. The study identified novel associations with COVID-19 and pathways, suggesting future research should explore these mechanisms. This research provides a foundation for advancing novel PCLM therapies.

Research perspectives

Future research should prioritize enhancing cytokine-cytokine receptor interactions and ribosomal mechanisms while improving existing cadherin binding and cell adhesion molecules.

ACKNOWLEDGEMENTS

We would like to thank "Guangxi Zhuang Autonomous Region Clinicopathology Diagnosis and Research Center" for providing technical support.

FOOTNOTES

Author contributions: Huang ZG, Chen Y, Feng X, Li SH, Li DM, Chen G, Cheng JW, and He J conceived and designed the research; Chen Y, Wu T, and Yin BT did the experiments, analyzed the data and made all the graphs; Huang ZG, Feng X, Li SH, Li DM, Chen G, Cheng JW, and He J revised the manuscript; and all authors have read and approve the final manuscript.

Supported by the Natural Science Foundation of Guangxi, China, No. 2020GXNSFBA238017; Guangxi Zhuang Autonomous Region Health Commission Self-financed Scientific Research Project, No. Z20200963.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Gang Chen 0000-0003-2402-2987; Juan He 0000-0002-6485-1336.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Ragavi R**, Muthukumaran P, Nandagopal S, Ahirwar DK, Tomo S, Misra S, Guerriero G, Shukla KK. Epigenetics regulation of prostate cancer: Biomarker and therapeutic potential. *Urol Oncol* 2023; **41**: 340-353 [PMID: 37032230 DOI: 10.1016/j.urolonc.2023.03.005]
2 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]
3 **Fan H**, Li J, Manuel AM, Zhao Z. Enzalutamide-induced signatures revealed by epigenetic plasticity using single-cell multi-omics sequencing in prostate cancer. *Mol Ther Nucleic Acids* 2023; **31**: 648-661 [PMID: 36910711 DOI: 10.1016/j.omtn.2023.02.022]
4 **Shiota M**, Terada N, Saito T, Yokomizo A, Kohei N, Goto T, Kawamura S, Hashimoto Y, Takahashi A, Kimura T, Tabata KI, Tomida R, Hashimoto K, Sakurai T, Shimazui T, Sakamoto S, Kamiyama M, Tanaka N, Mitsuzuka K, Kato T, Narita S, Yasumoto H, Teraoka S, Kato M, Osawa T, Nagumo Y, Matsumoto H, Enokida H, Sugiyama T, Kuroiwa K, Inoue T, Mizowaki T, Kamoto T, Kojima T, Kitamura H, Sugimoto M, Nishiyama H, Eto M; Japanese Urological Oncology Group (JUOG). Differential prognostic factors in low- and high-burden de novo metastatic hormone-sensitive prostate cancer patients. *Cancer Sci* 2021; **112**: 1524-1533 [PMID: 33159829 DOI: 10.1111/cas.14722]
5 **Yamazaki H**, Iwasaki H, Masudo K, Toda S, Matsui A, Rino Y. Prognostic significance of lung metastasis-related finding in lenvatinib treatment for differentiated thyroid cancer. *Endocrine* 2022; **78**: 543-551 [PMID: 36070050 DOI: 10.1007/s12020-022-03183-9]
6 **Guo Y**, Mao S, Zhang A, Wang R, Zhang Z, Zhang J, Wang L, Zhang W, Wu Y, Ye L, Yang B, Yao X. Prognostic Significance of Young Age and Non-Bone Metastasis at Diagnosis in Patients with Metastatic Prostate Cancer: a SEER Population-Based Data Analysis. *J Cancer* 2019; **10**: 556-567 [PMID: 30719152 DOI: 10.7150/jca.29481]
7 **Tarabaih M**, Degheili JA, Nasser M. Isolated Solitary Lung Nodule in a Patient With Idiopathic Pulmonary Fibrosis and Concomitant Prostate Cancer: A Challenging Diagnosis. *Cureus* 2021; **13**: e14218 [PMID: 33948408 DOI: 10.7759/cureus.14218]
8 **Izawa M**, Kosaka T, Nakamura K, Oba J, Hishida T, Hongo H, Mikami S, Nishihara H, Oya M. Pulmonary metastasis secondary to abiraterone-resistant prostate cancer with homozygous deletions of BRCA2: First Japanese case. *IJU Case Rep* 2021; **4**: 14-17 [PMID: 33426488 DOI: 10.1002/iju5.12224]
9 **Nakashima H**, Takatsu T, Imai R. Radiation-induced osteosarcoma in the pubic bone after proton radiotherapy for prostate cancer: a case report. *J Rural Med* 2022; **17**: 94-100 [PMID: 35432636 DOI: 10.2185/jrm.2021-047]
10 **Ceylan KC**, Batıhan G, Kaya SO. Pulmonary metastases in urogenital cancers: Surgical treatment and outcomes. *Cir Esp (Engl Ed)* 2023; **101**: 116-122 [PMID: 36774001 DOI: 10.1016/j.cireng.2021.11.025]
11 **Hess W**, Marggraf G, Reidemeister C. [Pulmonary artery rupture caused by a Swan-Ganz catheter during heart surgery. A successful therapeutic procedure]. *Anaesthesist* 1988; **37**: 446-449 [PMID: 3414956 DOI: 10.3389/fpsyg.2021.669000]
12 **Wu H**, Cheng K, Guo Q, Yang W, Tong L, Wang Y, Sun Z. Mapping Knowledge Structure and Themes Trends of Osteoporosis in Rheumatoid Arthritis: A Bibliometric Analysis. *Front Med (Lausanne)* 2021; **8**: 787228 [PMID: 34888333 DOI: 10.3389/fmed.2021.787228]
13 **Sholklapper TN**, Ballon J, Sayegh AS, La Riva A, Perez LC, Huang S, Eppler M, Nelson G, Marchegiani G, Hinchliffe R, Gordini L, Furrer M, Brenner MJ, Dell-Kuster S, Biyani CS, Francis N, Kaafarani HMA, Siepe M, Winter D, Sosa JA, Bandello F, Siemens R, Walz J, Briganti A, Gratzke C, Abreu AL, Desai MM, Sotelo R, Agha R, Lillemoe KD, Wexner S, Collins GS, Gill I, Cacciamani GE. Bibliometric analysis of academic journal recommendations and requirements for surgical and anesthesiologic adverse events reporting. *Int J Surg* 2023; **109**: 1489-1496 [PMID: 37132189 DOI: 10.1097/JS9.0000000000000323]
14 **Porwal A**, Al Moaleem MM, Adawi HA, Nandalur KR, Satpathy A, Mehta V, Cicciù M, Minervini G. Bibliographic analysis and evaluation of the mesh keywords in the journal of prosthodontics: Implant, esthetic, and reconstructive dentistry. *Technol Health Care* 2023 [PMID: 37125591 DOI: 10.3233/THC-230204]
15 **Xiao H**, Tang J, Zhang F, Liu L, Zhou J, Chen M, Li M, Wu X, Nie Y, Duan J. Global trends and performances in diabetic retinopathy studies: A bibliometric analysis. *Front Public Health* 2023; **11**: 1128008 [PMID: 37124794 DOI: 10.3389/fpubh.2023.1128008]
16 **Ninkov A**, Frank JR, Maggio LA. Bibliometrics: Methods for studying academic publishing. *Perspect Med Educ* 2022; **11**: 173-176 [PMID: 34914027 DOI: 10.1007/s40037-021-00695-4]
17 **Brown R**. Health care for the indigent. *J Natl Med Assoc* 1986; **78**: 359-360 [PMID: 3712474 DOI: 10.3389/fendo.2023.1109456]
18 **Große Hokamp N**, Kobe C, Linzenich E, Maintz D, Drzezga A. Solitary PSMA-Positive Pulmonary Metastasis in Biochemical Relapse of Prostate Cancer. *Clin Nucl Med* 2017; **42**: 406-407 [PMID: 28195907 DOI: 10.1097/RLU.0000000000001582]
19 **Lubin DJ**, Holden SB, Rettig MB, Reiter RE, King CR, Lee JM, Wallace DW, Calais J. Prostate Cancer Pulmonary Metastasis Presenting as a Ground-Glass Pulmonary Nodule on 68Ga-PSMA-11 PET/CT. *Clin Nucl Med* 2019; **44**: e353-e356 [PMID: 30789399 DOI: 10.1097/RLU.0000000000002499]
20 **Liu H**, Zhang GN, Luo M, Zhang XD, Fan Y, Peng CR. [Clinicopathological features and prognostic factors of patients with lung metastasis of stage Ia~IIIb cervical cancer]. *Zhonghua Zhong Liu Za Zhi* 2023; **45**: 340-347 [PMID: 37078216 DOI:

10.3760/cma.j.cn112152-20211230-00984]
21 **Schade AE**, Kuzmickas R, Rodriguez CL, Mattioli K, Enos M, Gardner A, Cichowski K. Combating castration-resistant prostate cancer by co-targeting the epigenetic regulators EZH2 and HDAC. *PLoS Biol* 2023; **21**: e3002038 [PMID: 37104245 DOI: 10.1371/journal.pbio.3002038]
22 **Castro-Espin C**, Agudo A. The Role of Diet in Prognosis among Cancer Survivors: A Systematic Review and Meta-Analysis of Dietary Patterns and Diet Interventions. *Nutrients* 2022; **14** [PMID: 35057525 DOI: 10.3390/nu14020348]
23 **Chung I**, Zhou K, Barrows C, Banyard J, Wilson A, Rummel N, Mizokami A, Basu S, Sengupta P, Shaikh B, Sengupta S, Bielenberg DR, Zetter BR. Unbiased Phenotype-Based Screen Identifies Therapeutic Agents Selective for Metastatic Prostate Cancer. *Front Oncol* 2020; **10**: 594141 [PMID: 33738243 DOI: 10.3389/fonc.2020.594141]
24 **Shi Y**, Wei W, Li L, Wei Q, Jiang F, Xia G, Yu H. The global status of research in breast cancer liver metastasis: a bibliometric and visualized analysis. *Bioengineered* 2021; **12**: 12246-12262 [PMID: 34783637 DOI: 10.1080/21655979.2021.2006552]
25 **Lin J**, Zhuo Y, Zhang Y, Liu R, Zhong W. Molecular predictors of metastasis in patients with prostate cancer. *Expert Rev Mol Diagn* 2023; **23**: 199-215 [PMID: 36860119 DOI: 10.1080/14737159.2023.2187289]
26 **Bosland MC**, Shittu OB, Ikpi EE, Akinloye O. Potential New Approaches for Prostate Cancer Management in Resource-Limited Countries in Africa. *Ann Glob Health* 2023; **89**: 14 [PMID: 36843668 DOI: 10.5334/aogh.3994]
27 **Ren C**, Kumar S, Chanda D, Kallman L, Chen J, Mountz JD, Ponnazhagan S. Cancer gene therapy using mesenchymal stem cells expressing interferon-beta in a mouse prostate cancer lung metastasis model. *Gene Ther* 2008; **15**: 1446-1453 [PMID: 18596829 DOI: 10.1038/gt.2008.101]
28 **Gildea JJ**, Seraj MJ, Oxford G, Harding MA, Hampton GM, Moskaluk CA, Frierson HF, Conaway MR, Theodorescu D. RhoGDI2 is an invasion and metastasis suppressor gene in human cancer. *Cancer Res* 2002; **62**: 6418-6423 [PMID: 12438227]
29 **Chhikara M**, Huang H, Vlachaki MT, Zhu X, Teh B, Chiu KJ, Woo S, Berner B, Smith EO, Oberg KC, Aguilar LK, Thompson TC, Butler EB, Aguilar-Cordova E. Enhanced therapeutic effect of HSV-tk+GCV gene therapy and ionizing radiation for prostate cancer. *Mol Ther* 2001; **3**: 536-542 [PMID: 11319915 DOI: 10.1006/mthe.2001.0298]
30 **Gao Y**, Yao A, Zhang W, Lu S, Yu Y, Deng L, Yin A, Xia Y, Sun B, Wang X. Human mesenchymal stem cells overexpressing pigment epithelium-derived factor inhibit hepatocellular carcinoma in nude mice. *Oncogene* 2010; **29**: 2784-2794 [PMID: 20190814 DOI: 10.1038/onc.2010.38]
31 **Zhang J**, Kulkarni HR, Singh A, Baum RP. Complete Regression of Lung Metastases in a Patient With Metastatic Castration-Resistant Prostate Cancer Using 177Lu-PSMA Radioligand Therapy. *Clin Nucl Med* 2020; **45**: e48-e50 [PMID: 31162261 DOI: 10.1097/RLU.0000000000002655]
32 **Kairemo K**, Joensuu T. Lu-177-PSMA treatment for metastatic prostate cancer: case examples of major responses. *Clin Transl Imaging* 2018; **6**: 223-237 [DOI: 10.1007/s40336-018-0274-y]
33 **Gaudreault M**, Chang D, Hardcastle N, Jackson P, Kron T, Hofman MS, Siva S. Combined biology-guided radiotherapy and Lutetium PSMA theranostics treatment in metastatic castrate-resistant prostate cancer. *Front Oncol* 2023; **13**: 1134884 [PMID: 36994211 DOI: 10.3389/fonc.2023.1134884]
34 **Mosca A**, Mantica G, Giavarra M, Perrone V, De Marchi L, Gennari A, Toncini C, Terrone C. Curative Lung Metastasectomy Without Concomitant Androgen Deprivation Therapy in Oligometastatic Castration-resistant Prostate Cancer: A Case Report and Review of the Literature. *Clin Genitourin Cancer* 2020; **18**: e295-e299 [PMID: 31917170 DOI: 10.1016/j.clgc.2019.11.018]
35 **Raveglia F**, Rosso L, Nosotti M, Cardillo G, Maffeis G, Scarci M. Pulmonary metastasectomy in germ cell tumors and prostate cancer. *J Thorac Dis* 2021; **13**: 2661-2668 [PMID: 34012615 DOI: 10.21037/jtd.2020.04.51]
36 **Caristo JM**, Tian DH, Yan TD. Pulmonary metastasectomy: a cross sectional survey. *J Thorac Dis* 2018; **10**: 3757-3766 [PMID: 30069374 DOI: 10.21037/jtd.2018.05.45]
37 **Rebello RJ**, Oing C, Knudsen KE, Loeb S, Johnson DC, Reiter RE, Gillessen S, Van der Kwast T, Bristow RG. Prostate cancer. *Nat Rev Dis Primers* 2021; **7**: 9 [PMID: 33542230 DOI: 10.1038/s41572-020-00243-0]
38 **McNeel DG**, Bander NH, Beer TM, Drake CG, Fong L, Harrelson S, Kantoff PW, Madan RA, Oh WK, Peace DJ, Petrylak DP, Porterfield H, Sartor O, Shore ND, Slovin SF, Stein MN, Vieweg J, Gulley JL. The Society for Immunotherapy of Cancer consensus statement on immunotherapy for the treatment of prostate carcinoma. *J Immunother Cancer* 2016; **4**: 92 [PMID: 28031820 DOI: 10.1186/s40425-016-0198-x]
39 **Movassaghi M**, Chung R, Anderson CB, Stein M, Saenger Y, Faiena I. Overcoming Immune Resistance in Prostate Cancer: Challenges and Advances. *Cancers (Basel)* 2021; **13** [PMID: 34638243 DOI: 10.3390/cancers13194757]
40 **Kan S**, Ren H, Gao Z, Dai E, Liu Y, Yang L, Cai Q. Lichenoid drug eruption on the lower lip caused by anti-PD-1 monoclonal antibody: a case report and literature review. *Immunotherapy* 2021; **13**: 1373-1378 [PMID: 34632814 DOI: 10.2217/imt-2021-0234]
41 **Komaru A**, Ueda Y, Furuya A, Tanaka S, Yoshida K, Kato T, Kinoh H, Harada Y, Suzuki H, Inoue M, Hasegawa M, Ichikawa T, Yonemitsu Y. Sustained and NK/CD4+ T cell-dependent efficient prevention of lung metastasis induced by dendritic cells harboring recombinant Sendai virus. *J Immunol* 2009; **183**: 4211-4219 [PMID: 19734206 DOI: 10.4049/jimmunol.0803845]
42 **Ostrowski SM**, Fisher DE. Biology of Melanoma. *Hematol Oncol Clin North Am* 2021; **35**: 29-56 [PMID: 33759772 DOI: 10.1016/j.hoc.2020.08.010]
43 **Kumar P**, Brazel D, DeRogatis J, Valerin JBG, Whiteson K, Chow WA, Tinoco R, Moyers JT. The cure from within? a review of the microbiome and diet in melanoma. *Cancer Metastasis Rev* 2022; **41**: 261-280 [PMID: 35474500 DOI: 10.1007/s10555-022-10029-3]
44 **Chaft JE**, Rimner A, Weder W, Azzoli CG, Kris MG, Cascone T. Evolution of systemic therapy for stages I-III non-metastatic non-small-cell lung cancer. *Nat Rev Clin Oncol* 2021; **18**: 547-557 [PMID: 33911215 DOI: 10.1038/s41571-021-00501-4]
45 **de Scordilli M**, Michelotti A, Bertoli E, De Carlo E, Del Conte A, Bearz A. Targeted Therapy and Immunotherapy in Early-Stage Non-Small Cell Lung Cancer: Current Evidence and Ongoing Trials. *Int J Mol Sci* 2022; **23** [PMID: 35806230 DOI: 10.3390/ijms23137222]
46 **Iscaro A**, Jones C, Forbes N, Mughal A, Howard FN, Janabi HA, Demiral S, Perrie Y, Essand M, Weglarz A, Cruz LJ, Lewis CE, Muthana M. Targeting circulating monocytes with CCL2-loaded liposomes armed with an oncolytic adenovirus. *Nanomedicine* 2022; **40**: 102506 [PMID: 34875352 DOI: 10.1016/j.nano.2021.102506]
47 **Xu X**, Wang Q, Qian X, Wu Y, Wang J, Li J, Li Y, Zhang Z. Spatial-Drug-Laden Protease-Activatable M1 Macrophage System Targets Lung Metastasis and Potentiates Antitumor Immunity. *ACS Nano* 2023; **17**: 5354-5372 [PMID: 36877635 DOI: 10.1021/acsnano.2c08834]
48 **Halabi S**, Kelly WK, Ma H, Zhou H, Solomon NC, Fizazi K, Tangen CM, Rosenthal M, Petrylak DP, Hussain M, Vogelzang NJ, Thompson IM, Chi KN, de Bono J, Armstrong AJ, Eisenberger MA, Fandi A, Li S, Araujo JC, Logothetis CJ, Quinn DI, Morris MJ, Higano CS, Tannock IF, Small EJ. Meta-Analysis Evaluating the Impact of Site of Metastasis on Overall Survival in Men With Castration-Resistant Prostate Cancer.

J Clin Oncol 2016; **34**: 1652-1659 [PMID: 26951312 DOI: 10.1200/JCO.2015.65.7270]

49 **Pond GR**, Sonpavde G, de Wit R, Eisenberger MA, Tannock IF, Armstrong AJ. The prognostic importance of metastatic site in men with metastatic castration-resistant prostate cancer. *Eur Urol* 2014; **65**: 3-6 [PMID: 24120464 DOI: 10.1016/j.eururo.2013.09.024]

50 **Xing Z**, Li S, Xing J, Yu G, Wang G, Liu Z. Silencing of LINC01963 enhances the chemosensitivity of prostate cancer cells to docetaxel by targeting the miR-216b-5p/TrkB axis. *Lab Invest* 2022; **102**: 602-612 [PMID: 35152275 DOI: 10.1038/s41374-022-00736-4]

51 **Climent MÁ**, Pérez-Valderrama B, Mellado B, Fernández Parra EM, Fernández Calvo O, Ochoa de Olza M, Muinelo Romay L, Anido U, Domenech M, Hernando Polo S, Arranz Arija JÁ, Caballero C, Juan Fita MJ, Castellano D. Weekly cabazitaxel plus prednisone is effective and less toxic for 'unfit' metastatic castration-resistant prostate cancer: Phase II Spanish Oncology Genitourinary Group (SOGUG) trial. *Eur J Cancer* 2017; **87**: 30-37 [PMID: 29102858 DOI: 10.1016/j.ejca.2017.09.028]

52 **Wang Y**, Gong X, Li J, Wang H, Xu X, Wu Y, Wang J, Wang S, Li Y, Zhang Z. M2 macrophage microvesicle-inspired nanovehicles improve accessibility to cancer cells and cancer stem cells in tumors. *J Nanobiotechnology* 2021; **19**: 397 [PMID: 34838042 DOI: 10.1186/s12951-021-01143-5]

53 **Ochi T**, Wada H, Nakajima T, Tanaka K, Yamamoto T, Sakairi Y, Suzuki H, Yonekura S, Hanazawa T, Yoshino I. Surgical outcomes of pulmonary metastasectomy for head and neck cancer in the current era of advances in chemotherapy and immunotherapy. *Gen Thorac Cardiovasc Surg* 2021; **69**: 1214-1221 [PMID: 33754238 DOI: 10.1007/s11748-021-01611-7]

54 **Facchini G**, Cavaliere C, D'Aniello C, Iovane G, Rossetti S. Abiraterone acetate treatment in patients with castration-resistant prostate cancer with visceral metastases: a real-world experience. *Anticancer Drugs* 2019; **30**: 179-185 [PMID: 30320608 DOI: 10.1097/CAD.0000000000000703]

55 **Bolzacchini E**, Patriarca C, Giordano M. Abiraterone acetate and acute leukemia: a casual association? *Anticancer Drugs* 2021; **32**: 102-104 [PMID: 32932280 DOI: 10.1097/CAD.0000000000000988]

56 **Li QQ**, Wang G, Reed E, Huang L, Cuff CF. Evaluation of cisplatin in combination with β-elemene as a regimen for prostate cancer chemotherapy. *Basic Clin Pharmacol Toxicol* 2010; **107**: 868-876 [PMID: 22545969 DOI: 10.1111/j.1742-7843.2010.00592.x]

57 **Baciarello G**, Sternberg CN. Treatment of metastatic castration-resistant prostate cancer (mCRPC) with enzalutamide. *Crit Rev Oncol Hematol* 2016; **106**: 14-24 [PMID: 27637350 DOI: 10.1016/j.critrevonc.2016.07.005]

58 **Yanagisawa T**, Kimura T, Hata K, Narita S, Hatakeyama S, Enei Y, Atsuta M, Mori K, Obayashi K, Yoshihara K, Kondo Y, Oguchi T, Sadakane I, Habuchi T, Ohyama C, Shariat SF, Egawa S. Does castration status affect docetaxel-related adverse events? :Identification of risk factors for docetaxel-related adverse events in metastatic prostate cancer. *Prostate* 2022; **82**: 1322-1330 [PMID: 35767376 DOI: 10.1002/pros.24406]

59 **Kakkat S**, Pramanik P, Singh S, Singh AP, Sarkar C, Chakroborty D. Cardiovascular Complications in Patients with Prostate Cancer: Potential Molecular Connections. *Int J Mol Sci* 2023; **24** [PMID: 37108147 DOI: 10.3390/ijms24086984]

60 **Wang Y**, Chen J, Wu Z, Ding W, Gao S, Gao Y, Xu C. Mechanisms of enzalutamide resistance in castration-resistant prostate cancer and therapeutic strategies to overcome it. *Br J Pharmacol* 2021; **178**: 239-261 [PMID: 33150960 DOI: 10.1111/bph.15300]

61 **Gao L**, Zhang W, Zhang J, Liu J, Sun F, Liu H, Hu J, Wang X, Su P, Chen S, Qu S, Shi B, Xiong X, Chen W, Dong X, Han B. KIF15-Mediated Stabilization of AR and AR-V7 Contributes to Enzalutamide Resistance in Prostate Cancer. *Cancer Res* 2021; **81**: 1026-1039 [PMID: 33277366 DOI: 10.1158/0008-5472.CAN-20-1965]

62 **Pang X**, Zhang J, He X, Gu Y, Qian BZ, Xie R, Yu W, Zhang X, Li T, Shi X, Zhou Y, Cui Y. SPP1 Promotes Enzalutamide Resistance and Epithelial-Mesenchymal-Transition Activation in Castration-Resistant Prostate Cancer *via* PI3K/AKT and ERK1/2 Pathways. *Oxid Med Cell Longev* 2021; **2021**: 5806602 [PMID: 34721759 DOI: 10.1155/2021/5806602]

63 **Mei Y**, Li K, Zhang Z, Li M, Yang H, Wang H, Huang X, Li X, Shi S. miR-33b-3p Acts as a Tumor Suppressor by Targeting DOCK4 in Prostate Cancer. *Front Oncol* 2021; **11**: 740452 [PMID: 34804930 DOI: 10.3389/fonc.2021.740452]

64 **Maolake A**, Izumi K, Shigehara K, Natsagdorj A, Iwamoto H, Kadomoto S, Takezawa Y, Machioka K, Narimoto K, Namiki M, Lin WJ, Wufuer G, Mizokami A. Tumor-associated macrophages promote prostate cancer migration through activation of the CCL22-CCR4 axis. *Oncotarget* 2017; **8**: 9739-9751 [PMID: 28039457 DOI: 10.18632/oncotarget.14185]

65 **Putz EM**, Guillerey C, Kos K, Stannard K, Miles K, Delconte RB, Takeda K, Nicholson SE, Huntington ND, Smyth MJ. Targeting cytokine signaling checkpoint CIS activates NK cells to protect from tumor initiation and metastasis. *Oncoimmunology* 2017; **6**: e1267892 [PMID: 28344878 DOI: 10.1080/2162402X.2016.1267892]

66 **Perez PM**, Hope TA, Behr SC, van Zante A, Small EJ, Flavell RR. Intertumoral Heterogeneity of 18F-FDG and 68Ga-PSMA Uptake in Prostate Cancer Pulmonary Metastases. *Clin Nucl Med* 2019; **44**: e28-e32 [PMID: 30394930 DOI: 10.1097/RLU.0000000000002367]

67 **Baciarello G**, Özgüroğlu M, Mundle S, Leitz G, Richarz U, Hu P, Feyerabend S, Matsubara N, Chi KN, Fizazi K. Impact of abiraterone acetate plus prednisone in patients with castration-sensitive prostate cancer and visceral metastases over four years of follow-up: A post-hoc exploratory analysis of the LATITUDE study. *Eur J Cancer* 2022; **162**: 56-64 [PMID: 34953443 DOI: 10.1016/j.ejca.2021.11.026]

68 **Zhou HC**, Liu CX, Pan WD, Shang LR, Zheng JL, Huang BY, Chen JY, Zheng L, Fang JH, Zhuang SM. Dual and opposing roles of the androgen receptor in VETC-dependent and invasion-dependent metastasis of hepatocellular carcinoma. *J Hepatol* 2021; **75**: 900-911 [PMID: 34004215 DOI: 10.1016/j.jhep.2021.04.053]

69 **Li Z**, Yang F, Wu D, Liu Y, Gao Y, Lian H, Zhang H, Yin Z, Wu A, Zeng L. Ce6-Conjugated and polydopamine-coated gold nanostars with enhanced photoacoustic imaging and photothermal/photodynamic therapy to inhibit lung metastasis of breast cancer. *Nanoscale* 2020; **12**: 22173-22184 [PMID: 33135699 DOI: 10.1039/d0nr05386d]

70 **Hlavac V**, Mohelnikova-Duchonova B, Lovecek M, Ehrmann J, Brynychova V, Kolarova K, Soucek P. Targeted Sequencing of Pancreatic Adenocarcinomas from Patients with Metachronous Pulmonary Metastases. *Genes (Basel)* 2020; **11** [PMID: 33255265 DOI: 10.3390/genes11121391]

71 **Sergieva S**, Mangaldgiev R, Dimcheva M, Nedev K, Zahariev Z, Robev B. SPECT-CT Imaging with [99mTc]PSMA-T4 in patients with Recurrent Prostate Cancer. *Nucl Med Rev Cent East Eur* 2021; **24**: 70-81 [PMID: 34382671 DOI: 10.5603/NMR.2021.0018]

72 **Williams IS**, McVey A, Perera S, O'Brien JS, Kostos L, Chen K, Siva S, Azad AA, Murphy DG, Kasivisvanathan V, Lawrentschuk N, Frydenberg M. Modern paradigms for prostate cancer detection and management. *Med J Aust* 2022; **217**: 424-433 [PMID: 36183329 DOI: 10.5694/mja2.51722]

73 **Farolfi A**, Calderoni L, Mattana F, Mei R, Telo S, Fanti S, Castellucci P. Current and Emerging Clinical Applications of PSMA PET Diagnostic Imaging for Prostate Cancer. *J Nucl Med* 2021; **62**: 596-604 [PMID: 33712536 DOI: 10.2967/jnumed.120.257238]

74 **Morawitz J**, Kirchner J, Hertelendy J, Loberg C, Schimmöller L, Dabir M, Häberle L, Mamlins E, Antke C, Arsov C, Antoch G, Sawicki LM. Is there a diagnostic benefit of late-phase abdomino-pelvic PET/CT after urination as part of whole-body (68) Ga-PSMA-11 PET/CT for

restaging patients with biochemical recurrence of prostate cancer after radical prostatectomy? *EJNMMI Res* 2022; **12**: 12 [PMID: 35244791 DOI: 10.1186/s13550-022-00885-z]

75 **Zhou J**, Wu R, Wang W, Zhao Y, Liu X. (68)Ga-PSMA PET/CT for the evaluation of metastasis in patients with prostate cancer: A systematic review and meta-analysis. *Hell J Nucl Med* 2022; **25**: 297-311 [PMID: 36576728 DOI: 10.1967/s002449912525]

76 **Damjanovic J**, Janssen JC, Furth C, Diederichs G, Walter T, Amthauer H, Makowski MR. (68) Ga-PSMA-PET/CT for the evaluation of pulmonary metastases and opacities in patients with prostate cancer. *Cancer Imaging* 2018; **18**: 20 [PMID: 29769114 DOI: 10.1186/s40644-018-0154-8]

77 **Gauvin S**, Rompré-Brodeur A, Chaussé G, Anidjar M, Bladou F, Probst S. (18)F-fluorocholine positron emission tomography-computed tomography ((18)F-FCH PET/CT) for staging of high-risk prostate cancer patients. *Can Urol Assoc J* 2019; **13**: 84-91 [PMID: 30273114 DOI: 10.5489/cuaj.5142]

78 **Pulliam TL**, Awad D, Han JJ, Murray MM, Ackroyd JJ, Goli P, Oakhill JS, Scott JW, Ittmann MM, Frigo DE. Systemic Ablation of Camkk2 Impairs Metastatic Colonization and Improves Insulin Sensitivity in TRAMP Mice: Evidence for Cancer Cell-Extrinsic CAMKK2 Functions in Prostate Cancer. *Cells* 2022; **11** [PMID: 35741020 DOI: 10.3390/cells11121890]

79 **Chen YH**, Chen H, Lin TT, Zhu JM, Chen JY, Dong RN, Chen SH, Lin F, Ke ZB, Huang JB, Wei Y, Zheng QS, Xue XY, Xu N. ARPC1A correlates with poor prognosis in prostate cancer and is up-regulated by glutamine metabolism to promote tumor cell migration, invasion and cytoskeletal changes. *Cell Biosci* 2023; **13**: 38 [PMID: 36814338 DOI: 10.1186/s13578-023-00985-w]

80 **Ge C**, Yan J, Yuan X, Xu G. A positive feedback loop between tryptophan hydroxylase 1 and β-Catenin/ZBP-89 signaling promotes prostate cancer progression. *Front Oncol* 2022; **12**: 923307 [PMID: 36172162 DOI: 10.3389/fonc.2022.923307]

81 **Hüsing T**, Lange L, Rauschner M, Riemann A, Thews O. Functional Impact of Acidosis-Regulated MicroRNAs on the Migration and Adhesion of Tumor Cells. *Adv Exp Med Biol* 2021; **1269**: 151-155 [PMID: 33966210 DOI: 10.1007/978-3-030-48238-1_24]

82 **Tang T**, Wang LA, Wang P, Tong D, Liu G, Zhang J, Dai N, Zhang Y, Yuan G, Geary K, Zhang D, Liu Q, Jiang J. Case Report: Co-Existence of BRCA2 and PALB2 Germline Mutations in Familial Prostate Cancer With Solitary Lung Metastasis. *Front Oncol* 2020; **10**: 564694 [PMID: 33194641 DOI: 10.3389/fonc.2020.564694]

83 **Kosaka T**, Hongo H, Aimono E, Matsumoto K, Hayashida T, Mikami S, Nishihara H, Oya M. A first Japanese case of neuroendocrine prostate cancer accompanied by lung and brain metastasis with somatic and germline BRCA2 mutation. *Pathol Int* 2019; **69**: 715-720 [PMID: 31631483 DOI: 10.1111/pin.12860]

84 **Esposito M**, Ganesan S, Kang Y. Emerging strategies for treating metastasis. *Nat Cancer* 2021; **2**: 258-270 [PMID: 33899000 DOI: 10.1038/s43018-021-00181-0]

85 **Zhang Y**, Wei Y, Jiang S, Dang Y, Yang Y, Zuo W, Zhu Q, Liu P, Gao Y, Lu S. Traditional Chinese medicine CFF-1 exerts a potent anti-tumor immunity to hinder tumor growth and metastasis in prostate cancer through EGFR/JAK1/STAT3 pathway to inhibit PD-1/PD-L1 checkpoint signaling. *Phytomedicine* 2022; **99**: 153939 [PMID: 35172257 DOI: 10.1016/j.phymed.2022.153939]

86 **Lu X**, Wu X, Jing L, Tao L, Zhang Y, Huang R, Zhang G, Ren J. Network Pharmacology Analysis and Experiments Validation of the Inhibitory Effect of JianPi Fu Recipe on Colorectal Cancer LoVo Cells Metastasis and Growth. *Evid Based Complement Alternat Med* 2020; **2020**: 4517483 [PMID: 32774415 DOI: 10.1155/2020/4517483]

87 **Abou-Kheir W**, Hynes PG, Martin P, Yin JJ, Liu YN, Seng V, Lake R, Spurrier J, Kelly K. Self-renewing Pten-/- TP53-/- protospheres produce metastatic adenocarcinoma cell lines with multipotent progenitor activity. *PLoS One* 2011; **6**: e26112 [PMID: 22022528 DOI: 10.1371/journal.pone.0026112]

88 **Wegiel B**, Bjartell A, Tuomela J, Dizeyi N, Tinzl M, Helczynski L, Nilsson E, Otterbein LE, Härkönen P, Persson JL. Multiple cellular mechanisms related to cyclin A1 in prostate cancer invasion and metastasis. *J Natl Cancer Inst* 2008; **100**: 1022-1036 [PMID: 18612129 DOI: 10.1093/jnci/djn214]

89 **Pan C**, Qin H, Yan M, Qiu X, Gong W, Luo W, Guo H, Han X. Environmental microcystin exposure triggers the poor prognosis of prostate cancer: Evidence from case-control, animal, and *in vitro* studies. *J Environ Sci (China)* 2023; **127**: 69-81 [PMID: 36522098 DOI: 10.1016/j.jes.2022.05.051]

90 **Yang M**, Liu H, Qiu GP, Gao F. Silencing Akt1 enhances the resistance of prostate cancer cells to starvation and inhibits starvation-induced lung metastasis through epithelial-mesenchymal transition in prostate cancer. *Med Oncol* 2021; **39**: 8 [PMID: 34761338 DOI: 10.1007/s12032-021-01600-z]

91 **Dalton GN**, Massillo C, Scalise GD, Duca R, Porretti J, Farré PL, Gardner K, Paez A, Gueron G, De Luca P, De Siervi A. CTBP1 depletion on prostate tumors deregulates miRNA/mRNA expression and impairs cancer progression in metabolic syndrome mice. *Cell Death Dis* 2019; **10**: 299 [PMID: 30931931 DOI: 10.1038/s41419-019-1535-z]

92 **Azhati B**, Reheman A, Dilixiati D, Rexiati M. FTO-stabilized miR-139-5p targets ZNF217 to suppress prostate cancer cell malignancies by inactivating the PI3K/Akt/mTOR signal pathway. *Arch Biochem Biophys* 2023; **741**: 109604 [PMID: 37080415 DOI: 10.1016/j.abb.2023.109604]

93 **Lu S**, Lu T, Zhang J, Gan L, Wu X, Han D, Zhang K, Xu C, Liu S, Qin W, Yang F, Wen W. CD248 promotes migration and metastasis of osteosarcoma through ITGB1-mediated FAK-paxillin pathway activation. *BMC Cancer* 2023; **23**: 290 [PMID: 36997926 DOI: 10.1186/s12885-023-10731-7]

94 **Ku SC**, Liu HL, Su CY, Yeh IJ, Yen MC, Anuraga G, Ta HDK, Chiao CC, Xuan DTM, Prayugo FB, Wang WJ, Wang CY. Comprehensive analysis of prognostic significance of cadherin (CDH) gene family in breast cancer. *Aging (Albany NY)* 2022; **14**: 8498-8567 [PMID: 36315446 DOI: 10.18632/aging.204357]

95 **Pooladanda V**, Thatikonda S, Priya Muvvala S, Godugu C. Acute respiratory distress syndrome enhances tumor metastasis into lungs: Role of BRD4 in the tumor microenvironment. *Int Immunopharmacol* 2023; **115**: 109701 [PMID: 36641892 DOI: 10.1016/j.intimp.2023.109701]

96 **Sousa LG**, McGrail DJ, Li K, Marques-Piubelli ML, Gonzalez C, Dai H, Ferri-Borgogno S, Godoy M, Burks J, Lin SY, Bell D, Ferrarotto R. Spontaneous tumor regression following COVID-19 vaccination. *J Immunother Cancer* 2022; **10** [PMID: 35241495 DOI: 10.1136/jitc-2021-004371]

97 **He C**, Hua X, Sun S, Li S, Wang J, Huang X. Integrated Bioinformatic Analysis of SARS-CoV-2 Infection Related Genes ACE2, BSG and TMPRSS2 in Aerodigestive Cancers. *J Inflamm Res* 2021; **14**: 791-802 [PMID: 33732005 DOI: 10.2147/JIR.S300127]

98 **Tong J**, Jiang W, Zhang X, Wang R, Qiao T, Song Y, Gao D, Yu X, Lv Z, Li D. CCL22 and CCL26 are potential biomarkers for predicting distant metastasis in thyroid carcinoma. *J Int Med Res* 2022; **50**: 3000605221139555 [PMID: 36495170 DOI: 10.1177/03000605221139555]

99 **Wang X**, Zhang W, Guo Y, Zhang Y, Bai X, Xie Y. Identification of critical prognosis signature associated with lymph node metastasis of stomach adenocarcinomas. *World J Surg Oncol* 2023; **21**: 61 [PMID: 36823639 DOI: 10.1186/s12957-023-02940-y]

100 **Fang S**, Cheng X, Shen T, Dong J, Li Y, Li Z, Tian L, Zhang Y, Pan X, Yin Z, Yang Z. CXCL8 Up-Regulated LSECtin through AKT Signal and Correlates with the Immune Microenvironment Modulation in Colon Cancer. *Cancers (Basel)* 2022; **14** [PMID: 36358719 DOI: 10.3390/cancers14215300]

101 **Koutsougianni F**, Alexopoulou D, Uvez A, Lamprianidou A, Sereti E, Tsimplouli C, Ilkay Armutak E, Dimas K. P90 ribosomal S6 kinases: A bona fide target for novel targeted anticancer therapies? *Biochem Pharmacol* 2023; **210**: 115488 [PMID: 36889445 DOI: 10.1016/j.bcp.2023.115488]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 October 24; 14(10): 440-444

DOI: 10.5306/wjco.v14.i10.440

ISSN 2218-4333 (online)

CASE REPORT

Splenic lymphangioma masquerading as splenic abscess managed by laparoscopic splenectomy: A case report

Santosh Thorat, Febin Mohammed Shaji

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gad EH, Egypt; Luo W, China; Tenreiro N, Portugal

Received: May 26, 2023
Peer-review started: May 26, 2023
First decision: July 6, 2023
Revised: July 19, 2023
Accepted: September 19, 2023
Article in press: September 19, 2023
Published online: October 24, 2023

Santosh Thorat, Febin Mohammed Shaji, Department of General Surgery, Pimpri Chinchwad Municipal Corporation's Postgraduate Institute and Yashwantrao Chavan Memorial Hospital, Pune 411018, Maharashtra, India

Corresponding author: Febin Mohammed Shaji, MBBS, Surgeon, Department of General Surgery, Pimpri Chinchwad Municipal Corporation's Postgraduate Institute and Yashwantrao Chavan Memorial Hospital, Sant Tukaram Nagar, Pimpri, Pune 411018, Maharashtra, India. febinmshaji@gmail.com

Abstract

BACKGROUND

Primary benign splenic tumours are unique and account for < 0.007% of all tumours identified during surgery and autopsy. Splenic lymphangiomas are rarely seen in adults. Splenic lymphangiomas may be asymptomatic, or may present with upper left abdominal pain, splenomegaly, hypersplenism, or splenic rupture with haemorrhagic shock. The clinical and radiological features of these lesions are not specific. This case report serves to remind the clinician to consider the rare but important differential diagnosis of splenic lymphangioma while treating splenic lesions.

CASE SUMMARY

We report a case of splenic lymphangioma in a 22-year-old woman who presented with left upper quadrant abdominal pain for three months. Initial investigations were unremarkable; however, computed tomography later revealed multiple splenic micro-abscesses. The patient underwent laparoscopic splenectomy, and histopathological examination revealed splenic lymphangioma. The patient was discharged on postoperative day three. One month after surgery, the abdominal pain resolved completely, with no new complaints. Splenic lymphangiomas present clinically as splenomegaly or left upper quadrant abdominal pain; prompt intervention is necessary for avoiding complications.

CONCLUSION

This case report concludes that splenic lymphangiomas should be considered in the differential diagnosis of splenomegaly or left upper quadrant pain, even in adults, because they are amenable to curative treatment. Delays in surgical intervention may lead to severe complications, such as infection, rupture, and hemorrhage. Such lesions can be safely managed with laparoscopy, involving less postoperative pain and early patient discharge with excellent cosmetic outcomes.

Key Words: Spleen; Lymphangioma; Oncology; Rare; Laparoscopic splenectomy; Hamartomatous process; Case report

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: We present the rare case report of splenic lymphangioma in an adult female. This is a very rare entity with only around 200 cases reported between 1970 to 2017. Isolated splenic lymphangioma is very rare and should be considered in the differential diagnosis of splenomegaly, for early intervention and prevention of potential complications.

Citation: Thorat S, Shaji FM. Splenic lymphangioma masquerading as splenic abscess managed by laparoscopic splenectomy: A case report. *World J Clin Oncol* 2023; 14(10): 440-444
URL: https://www.wjgnet.com/2218-4333/full/v14/i10/440.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i10.440

INTRODUCTION

Lymphangiomas are benign congenital malformations of the lymphatic vessels, commonly localised to the head, neck, and axillary regions. Intra-abdominal localisation is rare and occurs preferentially in the mesentery. Splenic lymphangiomas occur mainly in children and rarely in adults. Between 1939 and 2017, only 209 cases of splenic lymphangiomas in adults have been reported in the literature[1]. The rarity of lymphangiomas and their uncommon localisation pose a challenge for clinicians in making accurate preoperative diagnosis.

CASE PRESENTATION

Chief complaints

Complaints of intermittent left upper quadrant abdominal pain and fever for the three months.

History of present illness

Complaints of intermittent left upper quadrant abdominal pain and fever for the past three months, for which she had consulted multiple medical practitioners with no relief from her agony.

History of past illness

No history of any significant illness in the past.

Personal and family history

No significant personal or family history.

Physical examination

Physical examination results were unremarkable, except for mild tenderness on deep palpation in the left upper abdomen.

Laboratory examinations

Initial investigations, including blood tests, were unremarkable, except for a slight elevated white blood cell count.

The patient was investigated for possible sources of infection; blood cultures, urine cultures, sputum cultures were performed. Additionally, an infection panel screening was performed, and the result was negative. Cultures were also negative for the presence of any infection.

Imaging examinations

Abdominal ultrasonography revealed multiple splenic micro-abscesses. Further workup with contrast-enhanced computed tomography of the abdomen revealed splenomegaly and multiple hypodense lesions in the splenic parenchyma (the largest measuring 15 mm × 15 mm), suggestive of multiple splenic abscesses/cysts (Figure 1). The splenic vein and distal Superior Mesenteric Artery were dilated, indicating portal hypertension. Other findings included a right-sided hemorrhagic ovarian cyst. Upper gastrointestinal endoscopy was performed for portal hypertension, which showed mild gastritis and a Hill's grade 1 hiatal hernia.

DOI: 10.5306/wjco.v14.i10.440 **Copyright** ©The Author(s) 2023.

Figure 1 Contrast-enhanced computed tomography film showing splenic cyst.

FINAL DIAGNOSIS

Splenic lymphangioma.

TREATMENT

Laparoscopic splenectomy.

OUTCOME AND FOLLOW-UP

The patient was admitted to our hospital and prepared for laparoscopic splenectomy (Figure 2). She was started on intravenous third generation cephalosporins and metronidazole to provide aerobic and anaerobic coverage, respectively, and administered vaccinations (including meningococcal, pneumococcal, and *H. Influenza* vaccines) 14 d before the surgery.

The patient underwent laparoscopic splenectomy. She was placed in the supine position, and the surgeon was positioned at the right lower side.

The spleen was removed using a Pfannenstiel incision, which was closed cosmetically with subcuticular sutures.

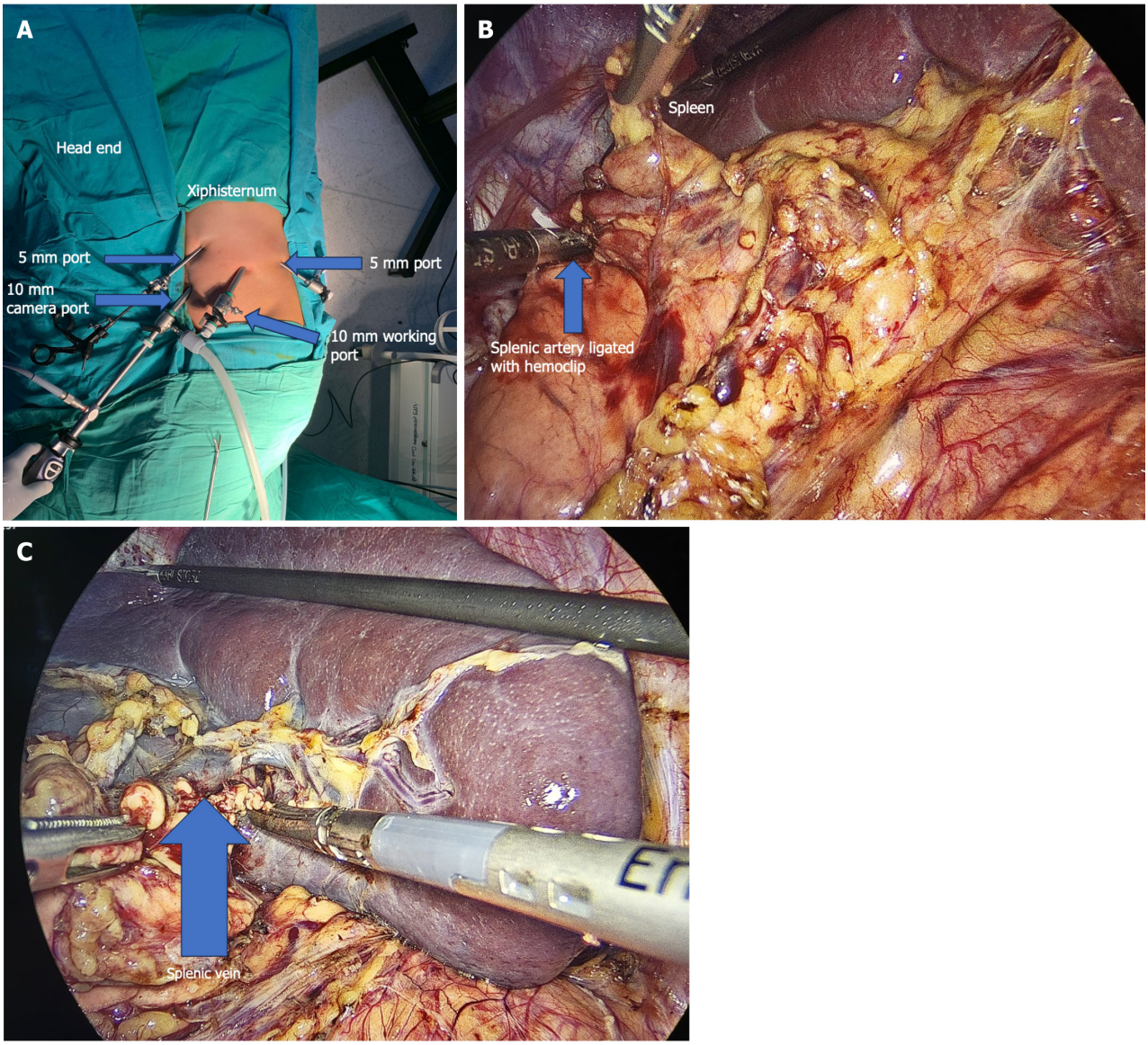
The total operative time was 160 min, with an estimated blood loss of 110 mL. Drains were placed at the postoperative site because the spleen formed adhesions with the pancreas. The drains were kept in place to check for any leakage, and they were removed on postoperative day (POD) two. The postoperative period was uneventful, and the patient was discharged on POD three.

The splenectomy specimen was sent for histopathological examination (Figure 3A). The spleen weighed 247 g. Histological examination findings revealed lymphangioma of the spleen, with areas of congestive splenomegaly (Figure 3B).

Postoperatively, all precautions were taken to prevent hospital acquired infections. The patient was advised to immediately present to the hospital in case fever develops after hospital discharge. One month after surgery, the abdominal pain resolved completely, with no new complaints.

DISCUSSION

Splenic lymphangioma is an uncommon malformation of the lymphatics of spleen mainly seen in children and rarely in



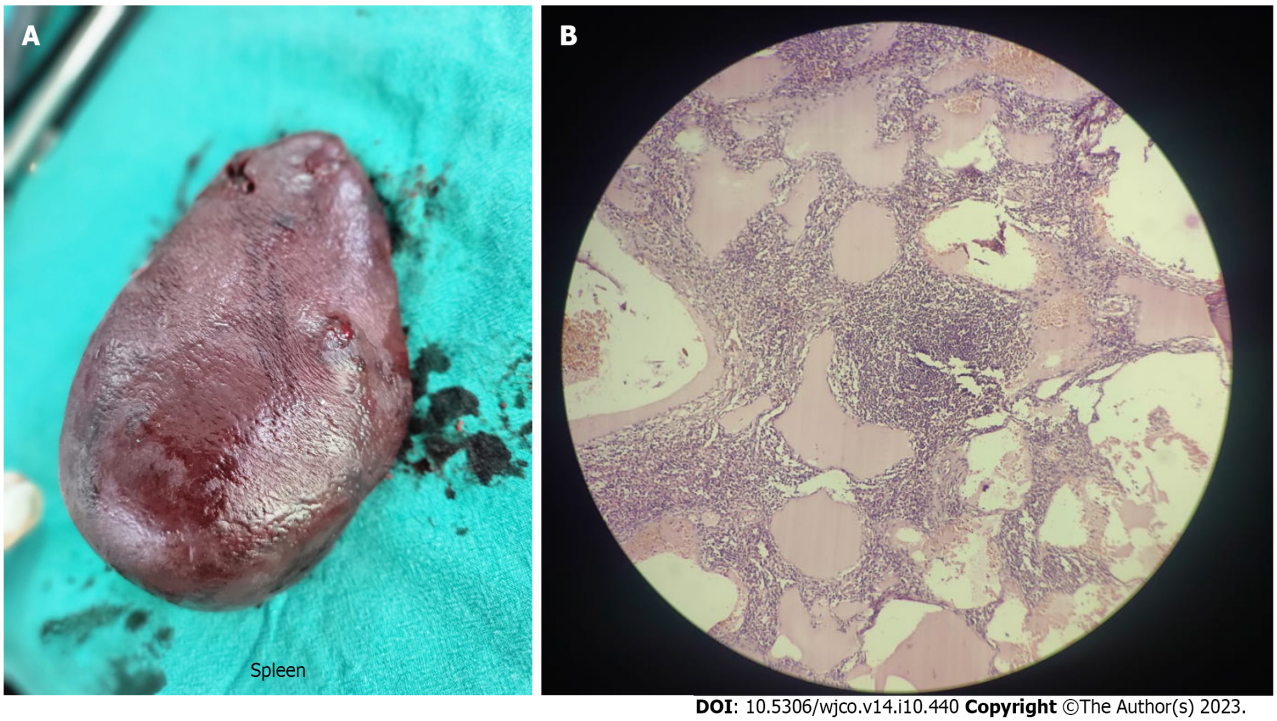
DOI: 10.5306/wjco.v14.i10.440 **Copyright** ©The Author(s) 2023.

Figure 2 Laparoscopic splenectomy. A: Port placement for laparoscopic splenectomy; B: Intraoperative view of the splenic artery ligated with a haemoclip at the upper border of the pancreas; C: Intraoperative view of the spleen.

adults. Although its aetiology is unclear, it is widely regarded as a hamartomatous change rather than a neoplasm[2]. Histologically, splenic lymphangiomas are characterised by cystic spaces lined by attenuated endothelial cells[3]. This condition may present involving only the spleen. However, in most cases, it is part of a systemic involvement of the lymphatic channels affecting multiple organs (systemic lymphangiomatosis)[4]. Most lesions are detected in imaging studies incidentally, whereas larger lesions can cause compression symptoms due to pressure on adjacent organs.

CONCLUSION

This case report concludes that in patients presenting with splenomegaly and left upper abdominal pain, splenic lymphangioma should be considered as an important differential diagnosis. Missed diagnosis and delayed treatment can lead to serious complications such as rupture and hemorrhage[5]. Such lesions can be safely managed with laparoscopy, involving less postoperative pain and early patient discharge with excellent cosmetic outcomes.



DOI: 10.5306/wjco.v14.i10.440 **Copyright** ©The Author(s) 2023.

Figure 3 Splenectomy specimen and histopathological slide. A: Splenectomy specimen; B: Histopathological slide showing multiple cystic spaces filled with eosinophilic proteinaceous material.

FOOTNOTES

Author contributions: Thorat S performed the surgery and was the chief consultant surgeon; Shaji FM assisted the case and compiled the information and created the manuscript.

Informed consent statement: The study participant, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: No conflicts of interest declared.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: India

ORCID number: Santosh Thorat 0009-0004-4801-4847; Febin Mohammed Shaji 0009-0008-2802-0384.

S-Editor: Fan JR
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Duvvada S**, Senapati D, Challa SR, Kalluri T. Cystic lymphangioma of spleen in adults. *BMJ Case Rep* 2017; **2017** [PMID: 28122799 DOI: 10.1136/bcr-2016-216267]
2 **Jindal R**, Sullivan R, Rodda B, Arun D, Hamady M, Cheshire NJ. Splenic malformation in a patient with Klippel-Trenaunay syndrome: a case report. *J Vasc Surg* 2006; **43**: 848-850 [PMID: 16616249 DOI: 10.1016/j.jvs.2005.11.059]
3 **Perez A**, Perez MEC, Yuga AC, Viray BAG. Splenic lymphangioma in adulthood: A case report. *Int J Surg Case Rep* 2020; **67**: 250-253 [PMID: 32086147 DOI: 10.1016/j.ijscr.2020.01.061]
4 **Chang WC**, Liou CH, Kao HW, Hsu CC, Chen CY, Yu CY. Solitary lymphangioma of the spleen: dynamic MR findings with pathological correlation. *Br J Radiol* 2007; **80**: e4-e6 [PMID: 17267469 DOI: 10.1259/bjr/93530580]
5 **Avigad S**, Jaffe R, Frand M, Izhak Y, Rotem Y. Lymphangiomatosis with splenic involvement. *JAMA* 1976; **236**: 2315-2317 [PMID: 989835]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 November 24; 14(11): 445-548

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 11 November 24, 2023

ORIGINAL ARTICLE

Retrospective Study

445 Analysis of clinicopathological features and prognostic factors of breast cancer brain metastasis

Chen YR, Xu ZX, Jiang LX, Dong ZW, Yu PF, Zhang Z, Gu GL

459 Clinical study of standard residual liver volume and transient elastography in predicting poor prognosis of patients after hemihepatectomy

Yue ZQ, Zhang P, Yan S, Ju LL, Wang HX, Yuan LX, Chen L, Wu JZ, Cao YL

Observational Study

471 System describing surgical field extension associated with flap reconstruction after resection of a superficial malignant soft tissue tumor

Sakamoto A, Noguchi T, Matsuda S

Basic Study

479 Computational exploration of the significance of *COPS6* in cancer: Functional and clinical relevance across tumor types

Wang SL, Zhuo GZ, Wang LP, Jiang XH, Liu GH, Pan YB, Li YR

META-ANALYSIS

504 Circulating tumor cells as potential prognostic biomarkers for early-stage pancreatic cancer: A systematic review and meta-analysis

Zhang ZH, Bao YW, Zhao YJ, Wang JQ, Guo JT, Sun SY

SCIENTOMETRICS

518 Bibliometric analysis of the global research status and trends of mechanotransduction in cancer

Zhang YZ, Li MZ, Wang GX, Wang DW

CASE REPORT

535 Autoimmune diabetes from pembrolizumab: A case report and review of literature

Bhanderi H, Khalid F, Bodla ZH, Muhammad T, Du D, Meghal T

544 Calcitriol induced hypercalcemia - a rare phenomenon in lung cancer: A case report

Prakash A, Khalid F, Alalwan A, Bader H, Du D, Meghal T

ABOUT COVER

Peer Reviewer of *World Journal of Clinical Oncology*, Bene Ekine-Afolabi, PhD, Research Associate, Department of Biomedical Sciences, School of Health, Sport and Biosciences, University of East London, Stratford-London E15 4LZ, Universal Scientific Education & Research Network (USERN), London, United Kingdom.
dcr@zeabtherapeutic.com

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJCO* as 2.8; IF without journal self cites: 2.8; 5-year IF: 3.0; Journal Citation Indicator: 0.36.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Xu Guo*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
November 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 November 24; 14(11): 445-458

DOI: 10.5306/wjco.v14.i11.445

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

Analysis of clinicopathological features and prognostic factors of breast cancer brain metastasis

Yu-Rui Chen, Zu-Xin Xu, Li-Xin Jiang, Zhi-Wei Dong, Peng-Fei Yu, Zhi Zhang, Guo-Li Gu

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: King MC, United States; Skarping I, Sweden

Received: September 7, 2023
Peer-review started: September 7, 2023
First decision: September 20, 2023
Revised: September 29, 2023
Accepted: October 16, 2023
Article in press: October 16, 2023
Published online: November 24, 2023

Yu-Rui Chen, Li-Xin Jiang, Department of General Surgery, Air Force Clinical College, China Medical University, Beijing 100142, China

Zu-Xin Xu, Guo-Li Gu, Department of General Surgery, Fifth Clinical College (Air Force Clinical College) of Anhui Medical University, Beijing 100142, China

Zhi-Wei Dong, Peng-Fei Yu, Zhi Zhang, Guo-Li Gu, Department of General Surgery, Air Force Medical Center, Air Force Clinical College of China Medical University, Beijing 100142, China

Corresponding author: Guo-Li Gu, Doctor, MD, Chief Doctor, Department of General Surgery, Air Force Medical Center, Air Force Clinical College of China Medical University, No. 30 Fucheng Road, Haidian District, Beijing 100142, China. kzggl@163.com

Abstract

BACKGROUND

Breast cancer (BC) has become the most common malignancy in women. The incidence and detection rates of BC brain metastasis (BCBM) have increased with the progress of imaging, multidisciplinary treatment techniques and the extension of survival time of BC patients. BM seriously affects the quality of life and survival prognosis of BC patients. Therefore, clinical research on the clinicopathological features and prognostic factors of BCBM is valuable. By analyzing the clinicopathological parameters of BCBM patients, and assessing the risk factors and prognostic indicators, we can perform hierarchical diagnosis and treatment on the high-risk population of BCBM, and achieve clinical benefits of early diagnosis and treatment.

AIM

To explore the clinicopathological features and prognostic factors of BCBM, and provide references for diagnosis, treatment and management of BCBM.

METHODS

The clinicopathological data of 68 BCBM patients admitted to the Air Force Medical Center, Chinese People's Liberation Army (formerly Air Force General Hospital) from 2000 to 2022 were collected. Another 136 BC patients without BM were matched at a ratio of 1:2 based on the age and site of onset for retrospective analysis. Categorical data were subjected to χ^2 test or Fisher's exact probability test, and the variables with $P < 0.05$ in the univariate Cox proportional hazards

model were incorporated into the multivariate model to identify high-risk factors and independent prognostic factors of BCBM, with a hazard ratio (HR) > 1 suggesting poor prognostic factors. The survival time of patients was estimated by the Kaplan–Meier method, and overall survival was compared between groups by log-rank test.

RESULTS

Multivariate Cox regression analysis showed that patients with stage III/IV tumor at initial diagnosis [HR: 5.58, 95% confidence interval (CI): 1.99–15.68], lung metastasis (HR: 24.18, 95%CI: 6.40–91.43), human epidermal growth factor receptor 2 (HER2)-overexpressing BC and triple-negative BC were more prone to BM. As can be seen from the prognostic data, 52 of the 68 BCBM patients had died by the end of follow-up, and the median time from diagnosis of BC to the occurrence of BM and from the occurrence of BM to death or last follow-up was 33.5 and 14 mo, respectively. It was confirmed by multivariate Cox regression analysis that patients with neurological symptoms (HR: 1.923, 95%CI: 1.005–3.680), with bone metastasis (HR: 2.011, 95%CI: 1.056-3.831), and BM of HER2-overexpressing and triple-negative BC had shorter survival time.

CONCLUSION

HER2-overexpressing, triple-negative BC, late tumor stage and lung metastasis are risk factors of BM. The presence of neurological symptoms, bone metastasis, and molecular type are influencing prognosis factors of BCBM.

Key Words: Breast cancer; Brain metastasis; Clinicopathological features; High-risk factors; Prognostic analysis

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: We aimed to identify the high-risk factors of breast cancer brain metastasis (BCBM) and conducted prognostic analyses. Sixty-eight BCBM patients diagnosed and treated in the Air Force Medical Center in 2000–2022 were enrolled. Patients with human epidermal growth factor receptor 2 overexpressing and triple-negative breast cancer were more prone to BM and had shorter survival time. Late tumor stage and lung metastasis were independent risk factors for BM. The presence or absence of neurological symptoms and bone metastasis, and molecular type were independent prognostic factors for BCBM. Early screening of high-risk patients for BM helps improve survival rate.

Citation: Chen YR, Xu ZX, Jiang LX, Dong ZW, Yu PF, Zhang Z, Gu GL. Analysis of clinicopathological features and prognostic factors of breast cancer brain metastasis. *World J Clin Oncol* 2023; 14(11): 445-458
URL: https://www.wjgnet.com/2218-4333/full/v14/i11/445.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i11.445

INTRODUCTION

Breast cancer (BC) has become the malignancy with the highest morbidity rate in women[1]. The overall survival (OS) of BC patients has been prolonged with the popularization of universal screening and advances in treatment and management. The proportion of patients with BC brain metastasis (BM) (BCBM) is about 15%[2], which increases with the extension of OS[3]. BM seriously threatens the life expectancy and quality of life of BC patients, and leads to poor prognosis, with a median OS of only 7.4 mo[4]. At present, medical surveillance of the brain is not regarded as a routine follow-up item for BC patients in China and globally. BM has an insidious onset, and most patients are not given targeted diagnosis and treatment of the brain until clinical symptoms emerge, thus losing the best opportunity of diagnosis and treatment and, affecting the survival rate. Therefore, clinical research on the clinicopathological features and prognostic factors of BCBM is required. To identify the clinicopathological features and prognostic factors of BCBM, guide targeted medical monitoring and intervention and raise the survival rate of BCBM patients, 68 BCBM patients were screened from 2238 BC patients admitted to our center from 2000 to 2022, and their clinical data were retrospectively studied.

MATERIALS AND METHODS

Inclusion and exclusion criteria

Inclusion criteria: patients pathologically diagnosed with BC in the Air Force Medical Center (formerly Air Force General Hospital) from 2000 to 2022 were retrospectively collected. Patients with BM (including brain parenchymal metastasis and meningeal metastasis) identified by imaging, cytology or histology were selected.

Exclusion criteria: (1) Patients with concomitant malignancy or with a history of malignancy of other origin; (2) patients with incomplete clinical data; (3) patients diagnosed with other neurological diseases; (4) patients complicated with serious fatal clinical diseases; and (5) male patients with BC.

Enrolled cases and follow-up: 2238 BC patients were admitted to the Air Force Medical Center between 2000 to 2022, of whom, 101 (4.5%) developed BM. After ineligible cases were excluded, 68 patients were enrolled. Another 136 BC patients without BM (control group) were matched at a ratio of 1:2 based on the age and tumor site at initial diagnosis. Follow-up was performed by telephone interview, outpatient re-examination and inpatient examination until April 2023.

Indicators and parameters

Clinicopathological parameters of BC patients were statistically analyzed. Univariate Cox proportional hazards model analysis was performed on age (at diagnosis of BC; menstrual status (at diagnosis of BC); family history of tumors; lymph node stage; primary tumor size; tumor stage at initial diagnosis; estrogen receptor (ER) and progesterone receptor (PR) status; molecular and pathological type; presence or absence of liver, lung and bone metastasis; number of liver, lung and bone metastases; presence or absence of metastasis to other sites; number of metastases to other sites; and whether or not surgical treatment was performed. Covariates with statistical significance were further incorporated into a multivariate Cox proportional hazards model for analysis.

Prognostic indicators: age at diagnosis of BM; time from diagnosis of BC to occurrence of BM; menstrual status at diagnosis of BM; molecular type; presence or absence of liver, lung and bone metastasis; number of liver, lung and bone metastases; presence or absence of metastasis to other sites; number of metastases to other sites; tumor stage at initial diagnosis; lymph node stage; pathological type; presence or absence of symptoms at diagnosis of BM; size and number of BMs; and treatment means for BM (systemic or local therapy).

Parameters of local therapy: surgical resection and radiotherapy [mainly including whole brain radiation therapy (WBRT) and stereotactic radiotherapy (SRT) of brain tumor].

Multidisciplinary treatment (MDT) was defined as systemic therapy combined with radiotherapy or surgery. The presence or absence of symptoms was determined according to whether abnormal vision, ataxia, symptoms of intracranial hypertension (headache, nausea and vomiting, lethargy, *etc*.), motor dysfunction, and paresthesia occurred in patients diagnosed previously with BC.

Parameters of hormone receptor: hormone receptor and human epidermal growth factor receptor 2 (HER2) status was determined by immunohistochemistry. HER2 positive was defined as HER2(3+), or HER2(2+) was positive in *in situ* hybridization test, and HER2 negative was defined as HER2(-), HER2(+), or HER2(2+) was negative in *in situ* hybridization test. The positive threshold of ER and PR in immunohistochemistry was ≥ 1%. Molecular typing of BC was performed according to hormone receptor status and HER2 expression: luminal A type (ER- and/or PR-positive, and HER2-negative); luminal B type (ER- and/or PR-positive, and HER2-positive); HER2-overexpressing type (ER- and PR-negative, and HER2-positive); and triple-negative type (ER-, PR- and HER2-negative). Tumor–node–metastasis (TNM) staging was carried out based on the American Joint Committee on Cancer 8th Edition Staging System.

Statistical analysis

Numerical data were compared between the two groups by χ^2 test or Fisher's exact probability test. The risk factors associated with BM at initial diagnosis of BC were first subjected to univariate Cox proportional hazards model analysis, and then covariates with $P < 0.05$ (selected by the backward conditional method) were incorporated into the multivariate Cox proportional hazards model. OS, defined as the time from the initial diagnosis of BM to death from any cause, or last follow-up, was compared between the groups using the log-rank test, and the survival time of patients was estimated by the Kaplan–Meier method. In the prognostic analysis of BCBM patients, univariate Cox proportional hazards model analysis was first performed, and then covariates with $P < 0.05$ (selected by the forward conditional method) were incorporated into the multivariate Cox proportional hazards model to identify the covariates related to survival outcomes, with a 95% confidence interval (95%CI). $P < 0.05$ was considered statistically significant. All statistical analyses were conducted with SPSS version 27.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Tumor characteristics of BCBM patients

Among the 2238 patients with BC, BM was found at the initial diagnosis in two cases (0.089%) and during follow-up in 99 cases (4.42%). In the BCBM group, the median age at diagnosis of BC was 47 (29–69) years; 35 cases (51.5%) were postmenopausal, eight (11.8%) had a family history of malignancy, and most patients (51.5%) had stage III/IV tumors. In terms of molecular type, there were 13 cases (19.1%) of luminal A BC, 22 (32.4%) of luminal B BC, 14 (20.6%) of HER2-overexpressing BC, and 14 (20.6%) of triple-negative BC. Fifty-one cases (75.0%) were pathologically classified as invasive ductal carcinoma. Bone metastasis was the most common (55.9%), followed by lung and liver metastasis. Ten cases (14.7%) developed liver, lung and bone metastasis and BM during the course of disease. Modified radical mastectomy dominated in both groups, and the proportion of patients undergoing neoadjuvant chemotherapy, radiotherapy and HER2-targeted therapy in the BCBM group was higher than that in the non-BCBM group (Table 1).

Risk factors of BM

Sixty-eight BCBM patients were matched at a ratio of 1:2 with 136 BC patients of the same age and tumor site at initial diagnosis. The median time from the diagnosis of BC to the occurrence of BM was 33.5 (0–181) mo in BCBM patients. The risk factors of BCBM are shown in Table 2. In univariate Cox analysis, lymph node stage; tumor stage at the initial diagnosis; ER status; PR status; molecular type; presence or absence of bone metastasis, liver metastasis and lung

Table 1 Clinicopathological features and tumor characteristics of patients

Item	Non-BCBM group (*n* = 136) [*n* (%)]	BCBM group (*n* = 68) [*n* (%)]	*P*
Age at diagnosis of BC (yr)			0.009
≤ 35	9 (6.6)	12 (17.6)	
35-55	79 (58.1)	43 (63.2)	
> 55	48 (35.3)	13 (19.2)	
Menstrual status at diagnosis of BC			0.77
Premenopause	69 (50.7)	33 (48.5)	
Menopause	67 (49.3)	35 (51.5)	
Family history of cancer			0.12
None	112 (82.4)	60 (88.2)	
Other malignancies	16 (11.8)	8 (11.8)	
BC	8 (5.9)	0 (0.0)	
Lymph node stage			0.01
N0	56 (41.2)	19 (27.9)	
N1	43 (31.6)	14 (20.6)	
N2	25 (18.4)	17 (25.0)	
N3	12 (8.8)	15 (22.1)	
Missing	0 (0.0)	3 (4.4)	
Tumor size			0.17
T1	53 (39.0)	18 (26.5)	
T2	67 (49.3)	37 (54.4)	
T3	13 (9.6)	11 (16.2)	
T4	1 (0.7)	0 (0.0)	
Missing	2 (1.5)	2 (2.9)	
Tumor stage at the initial diagnosis			< 0.001
IA	29 (21.3)	3 (4.4)	
IIA	39 (28.7)	19 (27.9)	
IIB	29 (21.3)	7 (10.3)	
IIIA	22 (16.2)	17 (25.0)	
IIIB	2 (1.5)	0 (0.0)	
IIIC	11 (8.1)	11 (16.2)	
IV	3 (2.2)	7 (10.3)	
Missing	1 (0.7)	4 (5.9)	
ER			0.009
Negative	42 (30.9)	32 (47.1)	
Positive	94 (69.1)	32 (47.1)	
Missing	0 (0.0)	4 (5.9)	
PR			0.003
Negative	52 (38.2)	39 (57.4)	
Positive	84 (61.8)	25 (36.8)	
Missing	0 (0.0)	4 (5.9)	
Molecular type			< 0.001

Luminal A type	71 (52.2)	13 (19.1)	
Luminal B type	28 (20.6)	22 (32.4)	
HER2-overexpressing type	18 (13.2)	14 (20.6)	
Triple-negative type	19 (14.0)	14 (20.6)	
Missing	0 (0.0)	5 (7.4)	
Pathological type			0.42
Noninvasive carcinoma	10 (7.4)	2 (2.9)	
Invasive ductal carcinoma	95 (69.9)	51 (75.0)	
Invasive lobular carcinoma	5 (3.7)	2 (2.9)	
Others	26 (19.1)	8 (11.8)	
Missing	0 (0.0)	5 (7.4)	
Metastasis			
Bone metastasis			< 0.001
Yes	12 (8.8)	38 (55.9)	
No	124 (91.2)	30 (44.1)	
Liver metastasis			< 0.001
Yes	7 (5.1)	20 (29.4)	
No	129 (94.9)	48 (70.6)	
Lung metastasis			< 0.001
Yes	8 (5.9)	35 (51.5)	
No	128 (94.1)	33 (48.5)	
Number of liver, lung and bone metastases			< 0.001
0	123 (90.4)	21 (30.9)	
1	8 (5.9)	19 (27.9)	
2	3 (2.2)	18 (26.5)	
3	2 (1.5)	10 (14.7)	
Number of metastases to other sites			< 0.001
0	118 (86.8)	33 (48.5)	
1	15 (11.0)	24 (35.3)	
2	2 (1.5)	8 (11.8)	
3	1 (0.7)	3 (4.4)	
Surgical treatment			< 0.001
No	3 (2.2)	7 (10.3)	
Breast-conserving surgery	20 (14.7)	5 (7.4)	
Modified radical mastectomy	106 (77.9)	43 (63.2)	
Others	7 (5.1)	13 (19.1)	
Radiotherapy			0.006
No	76 (55.9)	24 (35.3)	
Yes	60 (44.1)	44 (64.7)	
Chemotherapy			
Neoadjuvant chemotherapy			0.21
No	119 (87.5)	55 (80.9)	
Yes	17 (12.5)	13 (19.1)	

Anthracyclines			< 0.001
No	28 (20.6)	35 (51.5)	
Yes	107 (78.7)	32 (47.1)	
Missing	1 (0.7)	1 (1.5)	
Taxane			0.35
No	35 (25.7)	22 (32.4)	
Yes	101 (74.3)	46 (67.6)	
HER2 targeted therapy			< 0.001
No	118 (86.8)	36 (52.9)	
Yes	18 (13.2)	32 (47.1)	

BC: Breast cancer; BCBM: Breast cancer brain metastasis; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2.

metastasis; number of bone, liver and lung metastases; number of metastases to other sites; and surgical mode were statistically significant. The above factors were incorporated into multivariate Cox analysis, and it was found that patients with stage III/IV tumor at initial diagnosis [hazard ratio (HR): 5.58, 95%CI: 1.99–15.68], lung metastasis (HR: 24.18, 95%CI: 6.40–91.43), and HER2-overexpressing and triple-negative BC were more prone to BM.

Prognostic analysis

The median age in 68 BCBM patients at diagnosis of BM was 50.5 (30–70) years. Presence or absence of bone metastasis, molecular type, and presence or absence of neurological symptoms at initial diagnosis of BCBM were significant covariates in multivariate Cox analysis. The median time from initial diagnosis of BM to death from any cause or last follow-up was 14 (2–138) mo in the 68 BCBM patients. The survival time of BCBM patients with different molecular types is shown in Figure 1. Of the 68 BCBM patients, 44 (64.7%) were diagnosed with BM due to neurological symptoms; mainly dizziness, which was the initial symptom of 23 (52.3%) patients with BCBM. In addition, typical symptoms of BM included headache (19 cases), nausea and vomiting (10 cases), walking instability (7 cases), blurred vision (5 cases), memory loss (4 cases) and slow response (3 cases), and they often occurred simultaneously. The median survival time was 12 mo among BCBM patients with neurological symptoms, 30 mo among asymptomatic patients (Figure 2), 14 mo among BCBM patients with bone metastasis, and 23 mo among those without bone metastasis (Figure 3). The relevant results are presented in Table 3.

Of the 68 BCBM patients, four (5.9%) underwent no treatment, 27 (39.7%) underwent MDT with local therapy plus systemic medication, 30 (44.1%) were given local therapy only, and seven (10.3%) were given medication only. The median survival time of patients receiving MDT was 21 mo, which was superior to that of patients receiving medication or local therapy alone. Tumor resection was performed in 11 cases and all of them were treated with postoperative radiotherapy. After BM, 16 patients received HER2-targeted therapy, including trastuzumab single targeted therapy for five cases, trastuzumab plus tyrosine kinase inhibitors (TKIs) for two cases, and capecitabine plus TKI for seven cases, and their median survival time was 17, 23 and 54 mo, respectively. The remaining two patients received trastuzumab + pertuzumab dual-targeted therapy, and they were still alive as of the follow-up endpoint (Table 4).

DISCUSSION

In this study, the median time from the diagnosis of BC to the occurrence of BCBM was 33.5 (0–181) mo. The risk of BM varied among patients with different molecular subtypes of BC. HER2-overexpressing and triple-negative BC had a high tendency to BM, consistent with previous reports[5]. Patients with advanced stage and lung metastasis were also at high risk of BM. Due to the specificity of the physiological structure of the brain (such as the presence of the blood–brain barrier), there is still a lack of effective intervention means, and BM predicts poor survival outcomes. The results of descriptive statistics, univariate and multivariate Cox proportional hazards model analysis showed that the molecular type, and presence or absence of neurological symptoms and bone metastasis at diagnosis of BM were independent prognosis factors of patients with BM.

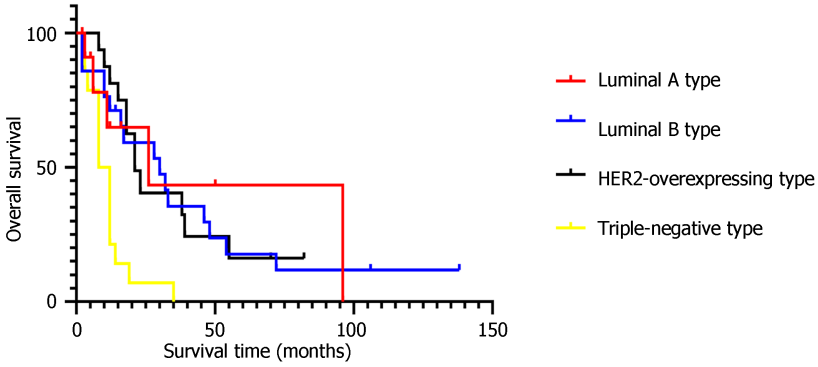
In this study, it was found that HER2-overexpressing and triple-negative types were high-risk molecular types of BCBM. Patients with HER2-overexpressing and triple-negative BC accounted for 20.6% of BM patients, in line with research findings that the incidence rate of BM in HER2-overexpressing type and triple-negative BC is 20%–30%[6]. Sixty-nine studies involving 28 countries on risk factors of BCBM concluded that young age, ER-negative, HER2 overexpression, later tumor stage, histological grade, tumor size, and lymph node metastasis are all independent risk factors of BCBM[7]. Univariate analysis of this study showed that lung, liver and bone metastasis, the number of liver, lung and bone metastases, and the number of metastases to other sites were associated with an increased risk of BM in BC patients. In multivariate analysis, only lung metastasis was statistically significant (HR: 24.18, 95%CI: 6.40–91.43). As shown in

Table 2 High-risk factors of breast cancer brain metastasis

Item	Univariate Cox		Multivariate Cox	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age at diagnosis of BC (yr)				
≤ 35	Reference			
> 35	0.008 (0-1.89)	0.083		
Menstrual status at diagnosis of BC				
Premenopause	Reference			
Menopause	1.29 (0.52-3.20)	0.59		
Family history of cancer				
None	Reference			
Yes	0.62 (0.26-1.44)	0.26		
Lymph node stage				
N0-N1	Reference			
N2-N3	2.77 (1.40-5.48)	0.004	NS	
Tumor size				
T1-T2	Reference			
T3-T4	1.44 (0.65-3.15)	0.37		
Tumor stage at the initial diagnosis				
I-II	Reference			
III-IV	3.84 (1.89-7.78)	< 0.001	5.58 (1.99-15.68)	0.001
ER				
Negative	Reference			
Positive	0.49 (0.27-0.87)	0.015	NS	
PR				
Negative	Reference			
Positive	0.36 (0.18-0.69)	0.002	NS	
Molecular type				
Luminal A type	Reference			
Luminal B type	3.95 (1.71-9.14)	0.001	5.36 (1.61-17.76)	0.006
HER2-overexpression type	4.01 (1.61-9.96)	0.003	5.0 (1.30-19.25)	0.019
Triple-negative type	4.34 (1.55-12.11)	0.005	NS	NS
Pathological type				
Others	Reference			
Invasive ductal carcinoma	1.83 (0.83-4.05)	0.14		
Invasive lobular carcinoma	1.31 (0.22-7.69)	0. 77		
Surgical treatment				
Others	Reference			
Modified radical mastectomy	0.48 (0.26-0.90)	0.021	NS	
Bone metastasis				
No	Reference			
Yes	7.19 (3.13-16.52)	< 0.001	NS	
Liver metastasis				

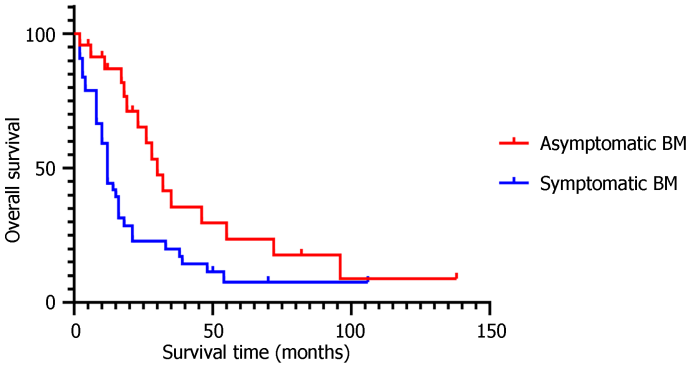
No	Reference			
Yes	7.44 (2.77-20.01)	< 0.001	NS	
Lung metastasis				
No	Reference			
Yes	15.87 (5.61-44.89)	< 0.001	24.18 (6.40-91.43)	< 0.001
Number of liver, lung and bone metastases				
< 2	Reference			
≥ 2	17.78 (5.38-58.73)	< 0.001	NS	
Number of metastases to other sites				
< 2	Reference			
≥ 2	7.33 (2.05-26.27)	0.002	NS	

95%CI: 95% confidence interval; NS: Not statistically significant; HR: Hazard ratio; ER: Estrogen receptor; PR: Progesterone receptor; BC: Breast Cancer; HER2: Human epidermal growth factor receptor 2.



DOI: 10.5306/wjco.v14.i11.445 **Copyright** ©The Author(s) 2023.

Figure 1 Survival analysis of patients with different molecular types.



DOI: 10.5306/wjco.v14.i11.445 **Copyright** ©The Author(s) 2023.

Figure 2 Survival analysis of patients with or without symptoms.

previous studies, cyclooxygenase 2 and epidermal growth factor receptor ligand can serve as mediators of cancer cells passing through the blood–brain barrier[8], and they are associated with lung cancer infiltration, which may account for the predisposition of patients with lung metastases to BM[9]. Lymph node status and age at diagnosis of BC have been verified to be associated with the risk of BM[10], but no clear association was observed in this study. The later tumor stage often corresponds to later seeking of treatment, greater tumor burden, greater lymph node infiltration, and increased risk of metastasis and recurrence, including BM[11].

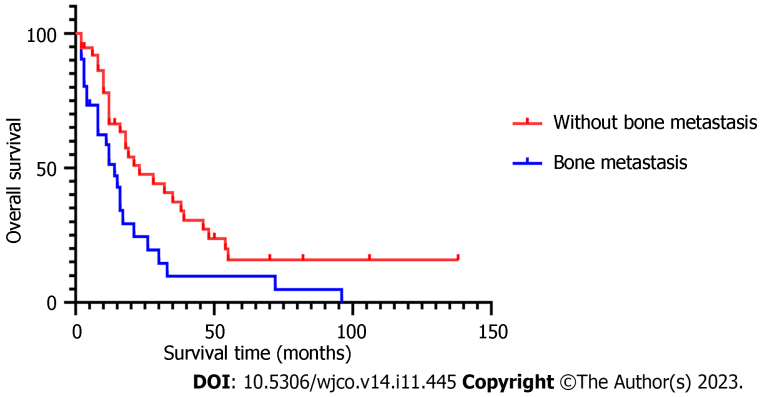
Clinically, the treatment regimen is often selected based on the number, location and size of BMs, the patient's physical condition, extracranial metastasis, and the possible benefits of treatment. SRT is mainly applied to BM patients with < 4

Table 3 Prognostic factors of breast cancer brain metastasis

Item	Univariate Cox		Multivariate Cox	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age at diagnosis of BM (yr)				
≤ 35	Reference			
> 35	1.033 (0.315-3.385)	0.957		
Time from BC to BM (mo)				
≤ 24	Reference			
> 24	0.896 (0.482-1.664)	0.727		
Menstrual status at diagnosis of BM				
Premenopause	Reference			
Menopause	0.685 (0.331-1.416)	0.307		
Molecular type				
Luminal A type	0.253 (0.087-0.730)	0.011	0.227 (0.074-0.693)	0.009
Luminal B type	0.279 (0.128-0.607)	0.001	0.293 (0.129-0.663)	0.003
HER2-overexpressing type	0.274 (0.121-0.618)	0.002	0.319 (0.135-0.754)	0.009
Triple-negative type	Reference			
Bone metastasis				
No	Reference			
Yes	1.980 (1.135-3.453)	0.016	2.011 (1.056-3.831)	0.034
Liver metastasis				
No	Reference			
Yes	1.121 (0.626-2.007)	0.701		
Lung metastasis				
No	Reference			
Yes	1.616 (0.929-2.810)	0.089		
Number of liver, lung and bone metastases				
≤ 2	Reference			
> 2	1.548 (0.894-2.682)	0.119		
Number of metastases to other sites				
< 2	Reference			
≥ 2	1.425 (0.711-2.858)	0.318		
Tumor stage				
I-II	Reference			
III-IV	0.813 (0.466-1.419)	0.466		
Lymph node stage				
N0-N1	Reference			
N2-N3	0.843 (0.486-1.464)	0.545		
Pathological type				
Others	0.287 (0.073-1.133)	0.075		
Invasive lobular carcinoma	Reference			
Invasive ductal carcinoma	0.208 (0.060-0.725)	0.014	NS	
Symptoms				

No	Reference			
Yes	2.171 (1.191-3.959)	0.011	1.923 (1.005-3.680)	0.048
Size of BM (cm)				
≤ 3	Reference			
> 3	0.803 (0.449-1.439)	0.462		
Number of BM				
≤ 3	Reference			
> 3	1.248 (0.721-2.160)	0.428		
Surgery for BM				
No	Reference			
Yes	0.744 (0.348-1.591)	0.446		
Treatment after BM				
Local therapy	Reference			
Medication alone	1.531 (0.638-3.674)	0.064		
Systemic therapy	0.748 (0.317-1.765)	0.507		
Local therapy				
SRT	1.350 (0.513-3.549)	0.543		
WBRT	1.600 (0.633-4.043)	0.320		
Both	1.935 (0.680-5.506)	0.216		
Neither	Reference			

95%CI: 95% confidence interval; NS: Not statistically significant; HR: Hazard ratio; BC: Breast Cancer; BM□Brain Metastasis; WBRT: Whole brain radiation therapy; SRT: Stereotactic radiotherapy; HER2: Human epidermal growth factor receptor 2.



DOI: 10.5306/wjco.v14.i11.445 **Copyright** ©The Author(s) 2023.

Figure 3 Survival analysis of patients with or without bone metastasis.

tumor lesions and brain tumor < 3 cm. In this study, the median survival time was 12 mo among patients treated with WBRT alone, 16 mo among patients treated with SRT alone, 18 mo among patients treated with WBRT + SRT, and 18 mo among patients undergoing surgery for brain tumors. Consistent with this study, a study showed that WBRT produces no OS benefit but significant neurocognitive decline[12]. Of the 68 patients, 24 had BMs ≤ 3 cm, and 22 patients (91.7%) underwent SRT. SRT has become the first-line treatment for BC patients with small brain metastases[13].

Despite advances in early diagnosis and effective treatment, distant metastasis remains an important factor threatening the survival of BC patients[14]. In this study, the median survival time of patients with luminal A, luminal B, HER2-overexpressing and triple-negative BC was 26, 30, 21 and 8 mo, respectively. The prognosis of HER2-overexpressing and triple-negative BC patients was poor. It is difficult for most drugs to reach effective blood concentration in the brain due to the presence of the blood–brain barrier. With the progress made in novel targeted drugs in the past decade, breakthroughs have been made in the treatment of HER2-positive BC. Trastuzumab, pertuzumab, antibody-drug conjugate and TKIs (lapatinib, pyrotinib, *etc*.) have been marketed successively, extending the OS of HER2-positive patients. Studies have revealed that lapatinib + capecitabine can delay the time of WBRT[15]. The PERMEATE study

Table 4 Treatment methods of brain metastasis

Item	Patients with BM (*n* = 68)	Median survival time (mo)
Medication	7	12
MDT	27	21
Local therapy alone	30	15
Neither	4	
Local therapy	*n* = 57	
SRT alone	19	16
Surgery + SRT	6	19
SRT + WBRT	10	18
Surgery + WBRT	3	30
WBRT	17	12
Surgery + SRT + WBRT	2	21
HER2 targeted therapy	*n* = 16	
Trastuzumab	5	17
Trastuzumab + pertuzumab	2	
Trastuzumab + TKI	2	23
Capecitabine + TKI	7	54

MDT: Multidisciplinary treatment; WBRT: Whole brain radiation therapy; SRT: Stereotactic radiotherapy; TKI: Trastuzumab plus tyrosine kinase inhibitors; BM: Brain Metastasis; HER2: Human epidermal growth factor receptor 2.

explored the efficacy of lapatinib plus capecitabine in the treatment of patients with HER2-positive BM, and found that the objective response rate of brain can reach 74.6% in patients undergoing the initial neurological radiotherapy[16]. In this study, the median survival time of patients given capecitabine plus TKIs was 54 mo. As of April 2023, the median survival time of HER2-positive BCBM patients enrolled in PERMEATE is up to 31.5 mo[16]. In this study, the patients treated from 2000 to 2022 were enrolled, and trastuzumab was marketed in China since 2002, so some patients did not undergo targeted therapy due to early drug shortage and high treatment cost, which may be one of the reasons for poor prognosis of HER2-overexpressing BC patients. In view of the current effective treatment, HER2-positive patients should be more active in undergoing brain examination and timely treatment.

The results of multivariate Cox regression analysis showed that the HR value of BM patients with neurological symptoms was 1.923 times (95%CI: 1.005–3.680) that of asymptomatic patients (P = 0.048), suggesting that the presence of neurological symptoms at diagnosis is associated with a poor prognosis. The median survival time was 30 mo for asymptomatic patients and 12 mo for symptomatic patients. A study involving long-term survivors of BCBM showed that asymptomatic BCBM patients are more likely to achieve long-term survival of > 15 mo[17]. In this study, asymptomatic patients with BM had a smaller diameter of brain tumor (≤ 3 cm: 95.8% *vs* 75.0%, P = 0.031), fewer brain metastases (≤ 3: 58.3% *vs* 43.2%, P = 0.232), and younger age at diagnosis of BC (≤ 35 years: 95.8% *vs* 75%, P = 0.031) than symptomatic patients, consistent with the characteristics (young age, small diameter of brain tumor, small number of brain tumors, and good physical status without neurological burden) of asymptomatic patients[18]. Due to smaller size and number of brain tumors of asymptomatic patients, a wider range of treatment options is available, and SRT is preferred, which is associated with milder neurological impairment[17]. A prospective study on 1196 asymptomatic patients with BCBM treated with SRT also confirmed that compared with symptomatic patients, asymptomatic BM patients have good neurological status and reduced neurological mortality[18]. A previous study showed that early detection of BM is associated with longer OS as compared to symptomatic BM[19]. Considering the health economic benefits, however, brain screening has not been utilized as routine follow-up for BC patients in China and globally. According to an American study, regular head magnetic resonance imaging screening can save an average of USD 1326 in treatment costs for each BCBM patient. Although there are differences in the medical system between the USA and China, some references are still provided for the formulation of follow-up plans for BC patients in China[20].

The presence of extracranial metastases was identified as an independent prognostic factor in the 2020 version of the Breast Graded Prognostic Assessment. Bone metastasis is the most common mode of metastasis, accounting for 60%–70% of metastatic BC[21]. In this study, 38 (55.9%) patients with BM had bone metastasis. The prognosis of BC bone metastases is better than that of other distant metastases, with a median survival time of 36 mo[22], and the survival rate significantly declines when complicated with metastasis to other sites[23]. In a cohort of 1330 triple-negative BC patients with BM, the median OS was 13 mo (95%CI: 11.5–14.5 mo) for bone metastasis alone and 8 mo (95%CI: 6.3–9.7 mo) for bone metastasis + metastasis to other sites[24]. In this study, the median survival time was 14 mo for patients with BM + bone metastasis

and 23 mo for patients without bone metastasis, and the HR value in multivariate analysis was 2.011 times that of patients without bone metastasis (95%CI: 1.056–3.831) (P = 0.034). Common skeletal-related events in patients with bone metastasis include pathological fracture, spinal cord compression, hypercalcemia, and bone pain[21], resulting in limited daily activity and reduced quality of life. In this study, the median age of 68 BCBM patients was 50.5 (30–70) years. The mean menopausal age of Chinese women is 49.5 years, and menopausal women are prone to osteoporosis as well as an increased risk of pathological fracture when complicated with bone metastasis. Fracture-induced immobilization, decrease of physical performance score, and long-term complications related to immobilization (thromboembolism, respiratory tract infection, *etc*.) are all reasons for the decrease in survival rate. In the case of paralysis caused by spinal cord compression, the survival rate declines further, with a 1-year survival rate of only 17.6%[25].

There were some limitations to this study, such as its small sample size and single-center, retrospective nature, which inevitably introduced selection and recall bias. The clinical data collected from 2000 to 2022 may have had missing follow-up data. Notably, the absence of pathological results and imaging data for some patients diagnosed before 2010 may have influenced the analytical outcomes. Moreover, shifts in clinical guidelines, the introduction of new medications, and advances in the healthcare economy during this period have altered the diagnostic criteria, treatment modalities, and patient management approaches. Other potential confounding factors like the genotype of BC patients were not extensively analyzed due to their low detection rate. The lack of prospective studies introduced uncertainty in pinpointing the precise onset time for patients with asymptomatic BM. Currently, the efficacy of early screening for BCBM, its potential for early intervention, and whether early detection enhances survival rate, necessitate validation through multicenter prospective studies.

CONCLUSION

Stage III/IV, lung metastasis, and HER2-overexpressing and triple-negative types are high-risk factors of BCBM, and aggressive monitoring of BM is required. It is recommended that BC patients undergo regular brain examinations since detecting and treating BC before neurological symptoms emerge may produce better outcomes. Patients with central nervous system symptoms, HER2-overexpressing and triple-negative BC, and bone metastasis have poor prognosis.

ARTICLE HIGHLIGHTS

Research background

Breast cancer (BC) brain metastasis (BCBM) is an important influencing factor of the long-term prognosis of BC patients. Triple-negative type is a known risk factor of BCBM, suggesting that patients with different clinicopathological types have differences in survival time.

Research motivation

To explore the influencing factors of the occurrence, development, and prognostic survival of BCBM to provide references for the diagnosis, treatment and management of patients with BM.

Research objectives

To perform more aggressive screening of high-risk patients of BCBM, benefiting patients from early diagnosis and treatment, and producing better outcomes.

Research methods

Clinicopathological data of 68 BCBM patients admitted to the Air Force Medical Center (formerly Air Force General Hospital) between 2000 and 2022 and another 136 matched BC patients were retrospectively analyzed. The high-risk factors and prognostic factors of BCBM patients were analyzed by univariate and multivariate Cox regression analyses, the survival time of patients was estimated by the Kaplan–Meier method, and the overall survival was compared between two groups by log-rank test.

Research results

Stage III/IV, lung metastasis, and human epidermal growth factor receptor 2 (HER2)-overexpressing and triple-negative types were high-risk factors of BCBM. Patients with neurological symptoms, bone metastasis, and HER2-overexpressing and triple-negative BC had poor prognosis, requiring more effective treatment to improve the survival rate of these patients.

Research conclusions

The prognosis of BCBM is poor. Active follow-up and screening of the brain should be performed for patients with late stage at initial treatment, lung metastasis, and HER2-overexpressing and triple-negative BC. The median survival time of patients with neurological symptoms, bone metastasis, and HER2-overexpressing and triple-negative BC significantly decreases.

Research perspectives

More multicenter large studies on BCBM are required to provide references for the management of high-risk patients, and more effective treatment is needed to raise the survival rate of patients with poor prognosis.

ACKNOWLEDGEMENTS

We thank all medical staff and technicians of dialysis centers who agreed to participate in this study.

FOOTNOTES

Co-first authors: Yu-Rui Chen and Zu-Xin Xu.

Author contributions: Chen YR and Xu ZX contributed equally to this study, and share joint first authorship; Gu GL designed the research; Chen YR and Xu ZX conceived the study, developed the methodology, collected data, analyzed and interpreted data, and wrote the manuscript; Jiang LX, Zhang Z, Yu PF and Dong ZW collected and analyzed the clinical data; Gu GL and Dong ZW provided the material support, and revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Supported by Outstanding Young Talents Program of Air Force Medical Center, PLA, No. 22BJQN004; Clinical Program of Air Force Medical University, No. Xiaoke2022-07.

Institutional review board statement: The study was approved by the ethics committee of the Air Force Medical Center, Chinese People's Liberation Army (Beijing, China).

Informed consent statement: Consent was obtained from patients or their relatives for publication of this report.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author kzggl@163.com.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Yu-Rui Chen 0000-0002-2101-9435; Zu-Xin Xu 0000-0002-5068-9024; Li-Xin Jiang 0000-0002-8361-4280; Zhi-Wei Dong 0000-0001-7009-9331; Peng-Fei Yu 0000-0002-0528-1839; Zhi Zhang 0000-0001-5870-1940; Guo-Li Gu 0000-0002-9998-047X.

S-Editor: Lin C
L-Editor: Kerr C
P-Editor: Chen YX

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **DeSantis CE**, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL. Breast cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 438-451 [PMID: 31577379 DOI: 10.3322/caac.21583]

3 **Bollig-Fischer A**, Michelhaugh S, Ali-Fehmi R, Mittal S. The molecular genomics of metastatic brain tumours. *OA Mol Oncol* 2013; **1** [PMID: 25400938 DOI: 10.13172/2052-9635-1-1-759]

4 **Witzel I**, Laakmann E, Weide R, Neunhöffer T, Park-Simon TJ, Schmidt M, Fasching PA, Hesse T, Polasik A, Mohrmann S, Würschmidt F, Schem C, Bechtner C, Würstlein R, Fehm T, Möbus V, Burchardi N, Loibl S, Müller V. Treatment and outcomes of patients in the Brain Metastases in Breast Cancer Network Registry. *Eur J Cancer* 2018; **102**: 1-9 [PMID: 30099223 DOI: 10.1016/j.ejca.2018.07.004]

5 **Kennecke H**, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, Nielsen TO, Gelmon K. Metastatic behavior of breast cancer subtypes. *J Clin Oncol* 2010; **28**: 3271-3277 [PMID: 20498394 DOI: 10.1200/JCO.2009.25.9820]

6 **Kuksis M**, Gao Y, Tran W, Hoey C, Kiss A, Komorowski AS, Dhaliwal AJ, Sahgal A, Das S, Chan KK, Jerzak KJ. The incidence of brain metastases among patients with metastatic breast cancer: a systematic review and meta-analysis. *Neuro Oncol* 2021; **23**: 894-904 [PMID: 33367836 DOI: 10.1093/neuonc/noaa285]

7 **Koniali L**, Hadjisavvas A, Constantinidou A, Christodoulou K, Christou Y, Demetriou C, Panayides AS, Pitris C, Pattichis CS, Zamba-Papanicolaou E, Kyriacou K. Risk factors for breast cancer brain metastases: a systematic review. *Oncotarget* 2020; **11**: 650-669 [PMID: 32110283 DOI: 10.18632/oncotarget.27453]

8 **Bos PD**, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, Minn AJ, van de Vijver MJ, Gerald WL, Foekens JA, Massagué J. Genes that mediate breast cancer metastasis to the brain. *Nature* 2009; **459**: 1005-1009 [PMID: 19421193 DOI: 10.1038/nature08021]

9 **Gupta GP**, Nguyen DX, Chiang AC, Bos PD, Kim JY, Nadal C, Gomis RR, Manova-Todorova K, Massagué J. Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature* 2007; **446**: 765-770 [PMID: 17429393 DOI: 10.1038/nature05760]

10 **Aversa C**, Rossi V, Geuna E, Martinello R, Milani A, Redana S, Valabrega G, Aglietta M, Montemurro F. Metastatic breast cancer subtypes and central nervous system metastases. *Breast* 2014; **23**: 623-628 [PMID: 24993072 DOI: 10.1016/j.breast.2014.06.009]

11 **Azim HA**, Abdel-Malek R, Kassem L. Predicting Brain Metastasis in Breast Cancer Patients: Stage Versus Biology. *Clin Breast Cancer* 2018; **18**: e187-e195 [PMID: 28888580 DOI: 10.1016/j.clbc.2017.08.004]

12 **Yamamoto M**, Serizawa T, Shuto T, Akabane A, Higuchi Y, Kawagishi J, Yamanaka K, Sato Y, Jokura H, Yomo S, Nagano O, Kenai H, Moriki A, Suzuki S, Kida Y, Iwai Y, Hayashi M, Onishi H, Gondo M, Sato M, Akimitsu T, Kubo K, Kikuchi Y, Shibasaki T, Goto T, Takanashi M, Mori Y, Takakura K, Saeki N, Kunieda E, Aoyama H, Momoshima S, Tsuchiya K. Stereotactic radiosurgery for patients with multiple brain metastases (JLGK0901): a multi-institutional prospective observational study. *Lancet Oncol* 2014; **15**: 387-395 [PMID: 24621620 DOI: 10.1016/S1470-2045(14)70061-0]

13 **Churilla TM**, Ballman KV, Brown PD, Twohy EL, Jaeckle K, Farace E, Cerhan JH, Anderson SK, Carrero XW, Garces YI, Barker FG 2nd, Deming R, Dixon JG, Burri SH, Chung C, Ménard C, Stieber VW, Pollock BE, Galanis E, Buckner JC, Asher AL. Stereotactic Radiosurgery With or Without Whole-Brain Radiation Therapy for Limited Brain Metastases: A Secondary Analysis of the North Central Cancer Treatment Group N0574 (Alliance) Randomized Controlled Trial. *Int J Radiat Oncol Biol Phys* 2017; **99**: 1173-1178 [PMID: 28939223 DOI: 10.1016/j.ijrobp.2017.07.045]

14 **Liang Y**, Zhang H, Song X, Yang Q. Metastatic heterogeneity of breast cancer: Molecular mechanism and potential therapeutic targets. *Semin Cancer Biol* 2020; **60**: 14-27 [PMID: 31421262 DOI: 10.1016/j.semcancer.2019.08.012]

15 **Petrelli F**, Ghidini M, Lonati V, Tomasello G, Borgonovo K, Ghilardi M, Cabiddu M, Barni S. The efficacy of lapatinib and capecitabine in HER-2 positive breast cancer with brain metastases: A systematic review and pooled analysis. *Eur J Cancer* 2017; **84**: 141-148 [PMID: 28810186 DOI: 10.1016/j.ejca.2017.07.024]

16 **Yan M**, Ouyang Q, Sun T, Niu L, Yang J, Li L, Song Y, Hao C, Chen Z, Orlandi A, Ishii N, Takabe K, Franceschini G, Ricci F, Verschraegen C, Liu Z, Zhang M, Lv H, Liu L, Yang X, Xiao H, Gao Z, Li X, Dong F, Chen X, Qiao J, Zhang G. Pyrotinib plus capecitabine for patients with human epidermal growth factor receptor 2-positive breast cancer and brain metastases (PERMEATE): a multicentre, single-arm, two-cohort, phase 2 trial. *Lancet Oncol* 2022; **23**: 353-361 [PMID: 35085506 DOI: 10.1016/S1470-2045(21)00716-6]

17 **Riecke K**, Müller V, Neunhöffer T, Park-Simon TW, Weide R, Polasik A, Schmidt M, Puppe J, Mundhenke C, Lübbe K, Hesse T, Thill M, Wuerstlein R, Denkert C, Decker T, Fehm T, Nekljudova V, Rey J, Loibl S, Laakmann E, Witzel I. Long-term survival of breast cancer patients with brain metastases: subanalysis of the BMBC registry. *ESMO Open* 2023; **8**: 101213 [PMID: 37075697 DOI: 10.1016/j.esmoop.2023.101213]

18 **Gao YK**, Kuksis M, Id Said B, Chehade R, Kiss A, Tran W, Sickandar F, Sahgal A, Warner E, Soliman H, Jerzak KJ. Treatment Patterns and Outcomes of Women with Symptomatic and Asymptomatic Breast Cancer Brain Metastases: A Single-Center Retrospective Study. *Oncologist* 2021; **26**: e1951-e1961 [PMID: 34506676 DOI: 10.1002/onco.13965]

19 **Laakmann E**, Witzel I, Neunhöffer T, Weide R, Schmidt M, Park-Simon TW, Möbus V, Mundhenke C, Polasik A, Lübbe K, Hesse T, Riecke K, Thill M, Fasching PA, Denkert C, Fehm T, Nekljudova V, Rey J, Loibl S, Müller V. Characteristics and Clinical Outcome of Breast Cancer Patients with Asymptomatic Brain Metastases. *Cancers (Basel)* 2020; **12** [PMID: 32998430 DOI: 10.3390/cancers12102787]

20 **Lester SC**, Taksler GB, Kuremsky JG, Lucas JT Jr, Ayala-Peacock DN, Randolph DM 2nd, Bourland JD, Laxton AW, Tatter SB, Chan MD. Clinical and economic outcomes of patients with brain metastases based on symptoms: an argument for routine brain screening of those treated with upfront radiosurgery. *Cancer* 2014; **120**: 433-441 [PMID: 24452675 DOI: 10.1002/cncr.28422]

21 **Xiong Z**, Deng G, Huang X, Li X, Xie X, Wang J, Shuang Z, Wang X. Bone metastasis pattern in initial metastatic breast cancer: a population-based study. *Cancer Manag Res* 2018; **10**: 287-295 [PMID: 29467583 DOI: 10.2147/CMAR.S155524]

22 **Wang R**, Zhu Y, Liu X, Liao X, He J, Niu L. The Clinicopathological features and survival outcomes of patients with different metastatic sites in stage IV breast cancer. *BMC Cancer* 2019; **19**: 1091 [PMID: 31718602 DOI: 10.1186/s12885-019-6311-z]

23 **Plunkett TA**, Smith P, Rubens RD. Risk of complications from bone metastases in breast cancer. implications for management. *Eur J Cancer* 2000; **36**: 476-482 [PMID: 10717523 DOI: 10.1016/s0959-8049(99)00331-7]

24 **Yao Y**, Chu Y, Xu B, Hu Q, Song Q. Risk factors for distant metastasis of patients with primary triple-negative breast cancer. *Biosci Rep* 2019; **39** [PMID: 31113872 DOI: 10.1042/BSR20190288]

25 **Oka H**, Kondoh T, Seichi A, Hozumi T, Nakamura K. Incidence and prognostic factors of Japanese breast cancer patients with bone metastasis. *J Orthop Sci* 2006; **11**: 13-19 [PMID: 16437343 DOI: 10.1007/s00776-005-0966-9]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 November 24; 14(11): 459-470

DOI: 10.5306/wjco.v14.i11.459

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

Clinical study of standard residual liver volume and transient elastography in predicting poor prognosis of patients after hemihepatectomy

Zhi-Qiang Yue, Peng Zhang, Shuai Yan, Lin-Ling Ju, Hui-Xuan Wang, Liu-Xia Yuan, Lin Chen, Jin-Zhu Wu, Ya-Li Cao

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Sahin TT, Turkey

Received: September 4, 2023
Peer-review started: September 4, 2023
First decision: September 19, 2023
Revised: September 28, 2023
Accepted: October 16, 2023
Article in press: October 16, 2023
Published online: November 24, 2023

Zhi-Qiang Yue, Shuai Yan, Medical School of Nantong University, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, Nantong 226000, Jiangsu Province, China

Peng Zhang, Jin-Zhu Wu, Department of Hepatobiliary Surgery, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, Nantong 226000, Jiangsu Province, China

Lin-Ling Ju, Hui-Xuan Wang, Liu-Xia Yuan, Lin Chen, Nantong Institute of Liver Disease, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, Nantong 226000, Jiangsu Province, China

Ya-Li Cao, Preventive Health Department, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, Nantong 226000, Jiangsu Province, China

Corresponding author: Jin-Zhu Wu, MMed, Associate Chief Physician, Department of Hepatobiliary Surgery, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, No. 60 Qingnian Middle Road, Chongchuan District, Nantong 226000, Jiangsu Province, China. 39235978@qq.com

Abstract

BACKGROUND

Liver cancer resection, especially in patients with hemihepatectomy or extended hemihepatectomy, often leads to poor prognosis, such as liver insufficiency and even liver failure and death, because the standard residual liver volume (SRLV) cannot be fully compensated after surgery.

AIM

To explore the risk factors of poor prognosis after hemihepatectomy for hepatocellular carcinoma and evaluate the application value of related prognostic approaches.

METHODS

The clinical data of 35 patients with primary liver cancer in Nantong Third People's Hospital from February 2016 to July 2020 were retrospectively analyzed.

The receiver operating characteristic curve was created using medcac19.0.4 to compare the critical values of the SRLV in different stages of liver fibrosis after hemihepatectomy with those of liver dysfunction after hemihepatectomy. It was constructed by combining the Child-Pugh score to evaluate its application value in predicting liver function compensation.

RESULTS

The liver stiffness measure (LSM) value and SRLV were associated with liver dysfunction after hemihepatectomy. Logistic regression analysis showed that an LSM value ≥ 25 kPa [odds ratio (OR) = 6.254, $P < 0.05$] and SRLV ≤ 0.290 L/m^2 (OR = 5.686, $P < 0.05$) were independent risk factors for postoperative liver dysfunction. The accuracy of the new liver reserve evaluation model for predicting postoperative liver function was higher than that of the Child-Pugh score ($P < 0.05$).

CONCLUSION

SRLV and LSM values can be used to evaluate the safety of hemihepatectomy. The new liver reserve evaluation model has good application potential in the evaluation of liver reserve function after hemihepatectomy.

Key Words: Hepatocellular carcinoma; Hemihepatectomy; Prognosis; Standard residual liver volume; Liver stiffness measure value

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: To explore the risk factors and predictive methods of poor prognosis after hemihepatectomy for hepatocellular carcinoma and evaluate its application value. The clinical data of 35 patients with primary liver cancer were retrospectively analyzed. The critical values of standard residual liver volume (SRLV) in different stages of liver fibrosis after hemihepatectomy were compared with those of liver dysfunction after hemihepatectomy. We found that SRLV and liver stiffness measure values can be used to evaluate the safety of hemihepatectomy.

Citation: Yue ZQ, Zhang P, Yan S, Ju LL, Wang HX, Yuan LX, Chen L, Wu JZ, Cao YL. Clinical study of standard residual liver volume and transient elastography in predicting poor prognosis of patients after hemihepatectomy. *World J Clin Oncol* 2023; 14(11): 459-470

URL: https://www.wjgnet.com/2218-4333/full/v14/i11/459.htm

DOI: https://dx.doi.org/10.5306/wjco.v14.i11.459

INTRODUCTION

Liver cancer is a malignant tumor associated with high mortality worldwide[1-3]. Hepatocellular carcinoma (HCC), one of the main types of liver cancer, is often found in advanced stages and cannot be cured[4-6]. As a highly heterogeneous disease, HCC mostly develops as a result of hepatitis B cirrhosis[7]. China has the largest number of hepatitis B virus infections in the world; therefore, the number of HCC patients accounts for more than half of the total number of HCCs worldwide[8]. To date, surgical resection and liver transplantation are still effective treatments for HCC; however, due to the shortage of liver sources, the main treatment for HCC is surgery[9]. Liver cancer resection, especially in patients with hemihepatectomy or extended hemihepatectomy, often leads to poor prognosis, such as liver insufficiency and even liver failure and death, because the standard residual liver volume (SRLV) cannot be fully compensated after surgery[10]. Research suggests that preoperative liver fibrosis and cirrhosis are the main causes[11]. In recent years, an increasing number of studies have shown that the liver stiffness measure (LSM) value is significantly related to the degree of cirrhosis, which can reflect the degree of liver inflammation and fibrosis[12,13]. Accordingly, the purpose of this study was to investigate the risk factors and predictive methods of poor prognosis after hemihepatectomy for HCC and verify whether the changes in liver structure can be reflected by the LSM value and SRLV to assess the liver's compensatory capacity. Finally, we established a liver reserve function evaluation model by combining the LSM value and Child-Pugh scores and evaluated its application value.

MATERIALS AND METHODS

Patient characteristics

The clinical case data were obtained from 35 HCC patients undergoing hemihepatectomy in the Nantong Third People's Hospital between February 2016 and July 2020, and all patients met the inclusion criteria for this study. The study was approved by the Ethics Committee of the Nantong Third People's Hospital Affiliated with Nantong University. Written informed consent was obtained from all patients before being enrolled in the study. The inclusion criteria were as follows:

(1) According to the China liver cancer staging for the diagnosis and treatment standard of primary liver cancer (2019 edition), the stage of liver cancer was stage Ia, Ib or IIa, the tumor was located only in the left or right half of the liver, and hemihepatectomy was needed; (2) all patients were positive for HBsAg before the operation, and HCC was confirmed by pathology after surgery; (3) liver enhancement computed tomography (CT) was performed before the operation; (4) the LSM value was detected by transient elastography (Fibro Touch) before the operation; and (5) patients had more complete clinical case data. The exclusion criteria were as follows: (1) The patient did not have a standard hemihepatectomy; (2) the postoperative pathology of the patients was confirmed as cholangiocarcinoma or metastatic carcinoma; (3) the patient had a preoperative intervention, ablation, or chemoradiotherapy; (4) secondary operation; (5) other complications affecting liver function before the operation, such as hepatic encephalopathy, abdominal dropsy, and other conditions; and (6) the presence of other malignant tumors or serious diseases.

Surgical procedure

The patient was placed in a supine position with a soft pad on the high right lumbar back (no pad height was required for left hemihepatectomy), an oblique incision was made at the right abdominal costal margin, approximately 30 cm in length, layer by layer into the abdominal cavity; adhesions were separated, and each connective tissue and ligament of the liver were cut to fully expose the liver. The texture and morphology of the liver and spleen were observed, the completely free left or right lobe of the liver was selected according to the location of the tumor, the lesions that had not been detected before the operation were examined by intraoperative ultrasound, and the abdominal cavity was explored for the presence or absence of tumor implantation and metastasis. Subsequently, the liver hilum was selectively blocked, and the left or right hemiliver was resected, along with the gallbladder removal and extended hemihepatectomy according to the preoperative conditions, with surgical margins generally larger than 1 cm from the tumor margin. The operation area was carefully checked for the presence or absence of bleeding and biliary fistula, an abdominal drainage tube was placed, and each layer of the abdominal wall was closed layer by layer.

Determination of SRLV

Preoperatively, Philips brilliance CT was used to perform a routine double-phase scan of the patient's liver with a thickness of 1.25 mm. Then, portal vein stage tomography was selected, and rapid liver volume measurement software was used to draw the liver boundary layer-by-layer (the inferior vena cava and gallbladder were avoided) and calculate the total liver volume (TLV). The volume of half of the liver was measured by drainage after the liver was isolated. The body surface area (BSA) was calculated according to the literature[14,15]. Finally, postoperative remnant liver volume (RLV) = TLV-the volume of half of the liver, and SRLV = RLV/BSA.

Determination of LSM values

According to the measurement requirements of the American Association for the Study of Liver Diseases[16], we measured the LSM value using a liver FibroTouch (FT) device developed by Haskell Medical Technology Company. In detail, the LSM value was measured 10 consecutive times for each patient, the quartile spacing was specified to be less than 30% as the effective measurement, and the median was chosen as the LSM value. All operations were performed by the same physician with extensive experience in the diagnosis of hepatobiliary diseases using ultrasound. A schematic illustration of the measurement results is shown in Figure 1. Then, a new model of liver reserve assessment was constructed according to the combination of the Child-Pugh score and the LSM value (Table 1).

Evaluation of postoperative hepatic fibrosis

By observing the paraffin sections of the liver under the microscope, we phased fibrosis according to the Scheuer scoring system as follows[17]: S0 stage, no liver fibrosis; S1 stage, liver fibrosis limited to the portal region; S2 stage, liver fibrosis extending to the portal region or portal interval, but the vascular relationship was normal; S3 stage, liver fibrosis with structural changes but not obvious cirrhosis; and S4 stage, cirrhosis (Figure 2).

Evaluation of postoperative liver function

According to the definition of liver dysfunction after hepatectomy from the International Study Group of Liver Surgery [18], we defined liver dysfunction as the results of a 5-d laboratory examination after hepatectomy that showed elevated international normalized ratio (INR) and total bilirubin (INR > 1.5; total bilirubin > 20.5 mmol/L); additionally, the patient was assessed for liver function, kidney function, respiratory function and the need for special assessment and special clinical treatment.

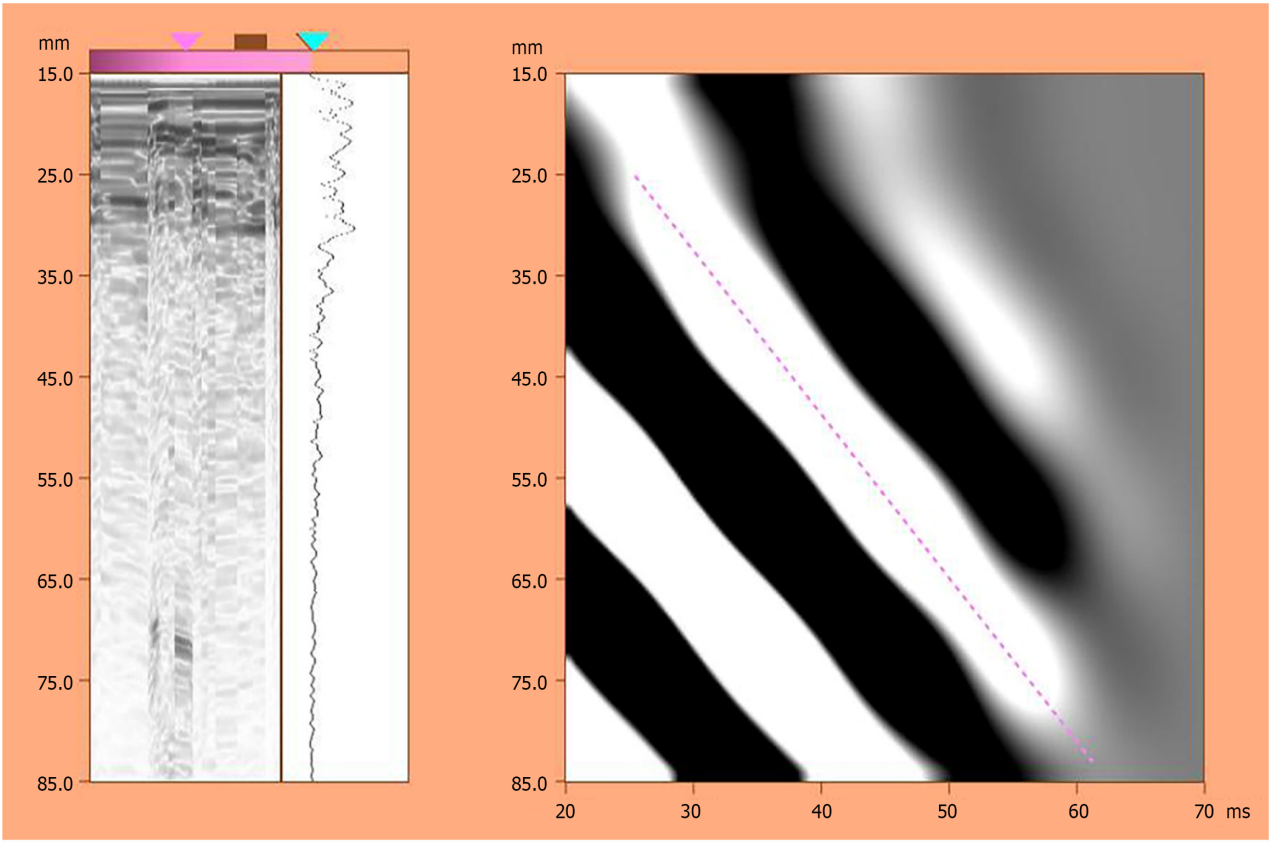
Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 25.0, and the measurement data were compared using the *t* test or single-factor analysis of variance (ANOVA). The Wilcoxon rank-sum test was used when the variance was uneven, and the Chi square test was used for counting data. Analysis of independent risk factors was completed using unconditional logistic regression. We used medcalc19.0.4 to draw the receiver operating characteristic (ROC) curve of the subjects and analyzed the area under the ROC curve under different factors. $P < 0.05$ was considered statistically significant.

Table 1 The new liver reserve assessment model

Score	1	2	3
HE	No	1-2	3-4
ABD	No	Mild	Moderate to severe
TIBL (μmol/L)	< 34	34-51	> 51
ALB (g/L)	> 35	28-35	> 28
Prothrombin Time (secprolonged)	< 15	15-17	> 17
LSM (kPa)	< 15	15-25	> 25

Note-scoring criteria: A score less than 9 = grade I; a score between 9 and 12 = grade II; a score greater than 12 = grade III. HE: Hepatic encephalopathy; ABD: Abdominal dropsy; TIBL: Total bilirubin; ALB: Albumin; LSM: Liver stiffness measure.



DOI: 10.5306/wjco.v14.i11.459 **Copyright** ©The Author(s) 2023.

Figure 1 Schematic diagram of transient elastography results of the liver.

RESULTS

Risk factors for liverdysfunction after hemihepatectomy

The 35 patients in this study were grouped according to the presence or absence of liver dysfunction after surgery as follows: 12 patients had postoperative liver dysfunction, and 23 patients had no liver dysfunction. Then, the following 25 factors were analyzed. The results showed that the preoperative LSM value and SRLV were correlated with liver dysfunction after hemihepatectomy in HCC patients ($P < 0.05$, Table 2).

LSM value and SRLV are independent risk factors for liver dysfunction after hemihepatectomy

The preoperative LSM value and SRLV were selected as independent variables, and regardless of whether liver dysfunction was selected as the dependent variable, a logistic regression model was developed for analysis. The results showed that the preoperative LSM value and SRLV were independent risk factors for liver dysfunction after hemihepatectomy ($P < 0.05$, Table 3).

Table 2 Comparison of the clinical features of the surgical safety group and liver dysfunction group (mean ± SD)

Variables	Total	Postoperative liver function		*P* value
		No liver dysfunction	Liver dysfunction	
Sex				
Male	19	12	7	1.000
Female	16	11	5	
Age (yr)				
< 60	20	14	6	0.721
≥ 60	15	9	6	
BMI				
< 24 kg/m^2	21	13	8	0.721
> 24 kg/m^2	14	10	4	
BSA (m^2)		1.71 ± 0.18	1.75 ± 0.16	0.514
WBC (× 10^9/L)		5.51 ± 2.39	5.19 ± 1.55	0.672
RBC (10^{12}/L)		4.30 ± 0.65	4.61 ± 0.44	0.146
PLT (× 10^9/L)		126.17 ± 53.74	149.33 ± 79.83	0.314
ALB (g/L)		40.71 ± 4.23	39.63 ± 4.18	0.478
Scr (μmol/L)		76.98 ± 39.07	64.87 ± 10.63	0.303
ALT [U/L, M (QR)]		67.26 ± 94.41	46.58 ± 30.40	0.468
AST [U/L, M (QR)]		45.61 ± 23.63	58.00 ± 68.68	0.436
TB [umol/L, M (QR)]		15.63 ± 7.20	20.99 ± 8.36	0.056
GGT [U/L, M (QR)]		115.17 ± 112.78	124.00 ± 145.78	0.844
AFP [ng/mL, M (QR)]		9999.23 ± 25773.40	8545.66 ± 15372.77	0.859
PIVKA-II [μg/L, M (QR)]		1799.54 ± 5017.78	3574.73 ± 6543.79	0.378
PT (s)		12.59 ± 1.46	12.39 ± 1.04	0.682
INR (s)		1.10 ± 0.12	1.05 ± 0.11	0.227
LSM value (kPa)		20.34 ± 4.89	25.78 ± 5.38	0.005[a]
ICG R15 (%)		7.99 ± 5.13	11.96 ± 6.43	0.055
SRLV (L/m^2)		0.349 ± 0.075	0.276 ± 0.036	0.003[a]
Tumor-localizing				
Left half liver	15	10	5	1.000
Right half liver	20	13	7	
Tumor diameter [cm, M (QR)]		6.63 ± 3.86	7.81 ± 4.91	0.436
Time of hepatic portal occlusion [min, M (QR)]		14.65 ± 19.42	15.75 ± 14.10	0.864
Intraoperative bleeding [mL, M (QR)]		908.70 ± 818.76	1541.67 ± 1612.57	0.130
Operation time (min)		176.30 ± 49.98	185.83 ± 72.89	0.651

[a]*P* < 0.01 *vs* group of safe operation (no liver dysfunction) and liver dysfunction. PIVKA-II was abnormal prothrombin. BMI: Body mass index; BSA: Body surface area; WBC: White blood cell; RBC: Red blood cell; PLT: Platelet; ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TB: Total bilirubin; GGT: γ-glutamyl transpeptidase; AFP: Alpha fetoprotein; PT: Prothrombin time; INR: International normalized ratio; LSM: Liver stiffness measure; ICG: Indocyanine green; SRLV: Standard residual liver volume.

The critical value of SRLV for different stages of postoperative fibrosis

The staging results of postoperative liver fibrosis showed 0 cases in the S0 stage, 6 cases in the S1 stage, 14 cases in the S2-S3 stage, and 15 cases in the S4 stage. Then, we compared and analyzed the critical values of SRLV for different stages, and the results showed that the difference in SRLV among the three phases was statistically significant ($P < 0.05$, Table 4). ROC curve analysis showed that the area under the curve for the S2-S3 stage was 0.743, the sensitivity was 0.467, the specificity was 0.100, and the critical value of SRLV was 0.257 L/m^2; the area under the curve for the S4 phase was 0.861, the sensitivity was 0.857, the specificity was 0.762, and the critical value of SRLV was 0.311 L/m^2 (Figure 3).

The critical value of SRLV for postoperative liver dysfunction

In 12 patients with postoperative liver dysfunction, the staging results of postoperative liver fibrosis showed 0 cases in the S0 stage, 1 case in the S1 stage, 7 cases in the S2-S3 stage, and 4 cases in the S4 stage. Additionally, the corresponding SRLVs were compared and analyzed, and the results showed that the difference in SRLV among the three phases was statistically significant ($P < 0.05$, Table 5). ROC curve analysis showed that the area under the curve for stage S2-S3 was 0.943, the sensitivity was 0.857, the specificity was 0.100, and the safety-critical value for SRLV was 0.285 L/m^2; the area under the curve for stage S4 was 0.938, the sensitivity was 0.100, the specificity was 0.750, and the safety-critical value of SRLV was 0.285 L/m^2 (Figure 4).

Application of a new assessment model in predicting liver dysfunction after hemihepatectomy

We reviewed and analyzed the clinical data of 35 patients in this study and followed up with the patients. The results showed that there were no postoperative deaths, and all patients were discharged within 3 wk after the operation. Statistical analysis showed that with Child-Pugh score was grade A, the accuracy rate of predicting postoperative liver function compensation was 54.8%; the accuracy rate of grade B was 25.0%. The new model was classified as grade I, and the accuracy rate of predicting postoperative liver function compensation was 100.0%, which was higher than that of the Child-Pugh score ($\chi^2 = 7.452$, $P = 0.007$). Similarly, that of grade II was 91.3%, which was higher than the Child-Pugh score ($\chi^2 = 9.928$, $P = 0.013$). There was a significant difference between the two models in evaluating the prognosis after hemihepatectomy ($P < 0.05$, Table 6).

DISCUSSION

HCC is one of the most common malignant tumors. With the improvement of the technical level of hepatectomy, the mortality rate after HCC resection has decreased significantly[19-21]. However, the mortality rate is still 5%-8%, especially in patients with hemihepatectomy[21]. The main cause of death after hemihepatectomy is liver failure[22]. The surgical resection range is so large such that the postoperative remnant liver cannot meet the needs of the body; more importantly, doctors lack a comprehensive understanding of the liver reserve function of patients before surgery. As a single evaluation indicator, indocyanine green (ICG) is better than many biochemical indicators. When many conventional liver function indicators have not yet become abnormal in value, the ICG retention rate at 15 min (ICG R15) can reflect liver function damage or occult liver disease in a timely manner[23]. However, ICG has certain limitations and is easily interfered with by factors such as the patient's cooperation ability, liver cell uptake capacity, liver blood flow, bile duct obstruction, bilirubin, *etc*[24,25]. SRLV is a reliable index of preoperative liver reserve function at home and abroad [26,27]. However, considering that HCC patients often have varying degrees of liver fibrosis before the operation, the liver reserve and regeneration function in such patients may vary depending on the extent of liver fibrosis, even if the SRLV is the same; therefore, it is not satisfactory to evaluate liver reserve function only in terms of liver volume. The diagnosis of preoperative liver fibrosis mainly depends on liver histopathological examination; however, because of invasive examination, a low positive rate, difficulty in follow-up, and dynamic detection, the need to consider the wishes of patients and other factors, scholars at home and abroad have explored the use of elastic techniques instead of liver biopsy to assess the extent of liver fibrosis or cirrhosis by measuring the LSM value[17]. Therefore, it is very important to evaluate the safety of hemihepatectomy by correctly staging the degree of liver fibrosis before surgery.

First, in this study, the factors that may be related to liver dysfunction in HCC patients after hemihepatectomy were statistically analyzed. The results showed that preoperative LSM and SRLV were associated with liver dysfunction in HCC patients after hemihepatectomy ($P < 0.05$). Multivariate logistic regression analysis showed that preoperative LSM and SRLV were independent risk factors for liver dysfunction in HCC patients after hemihepatectomy.

Then, according to the Scheuer score standard, we observed the degree of liver fibrosis using microscopy and analyzed the SRLV critical value of different stages of liver fibrosis in all patients and the SRLV critical value of different stages of liver fibrosis in postoperative liver insufficiency cases by ROC curve analysis. The results showed that the critical values of SRLV were 0.257 L/m^2 and 0.310 L/m^2 in patients with liver fibrosis in stages S2-S3 and S4, respectively, and 0.285 L/m^2 in patients with postoperative liver dysfunction. SRLV critical values were similar in both cases, suggesting that it is safe and feasible to predict the SRLV threshold of HCC patients undergoing hemihepatectomy by pathological stages of liver fibrosis. It is suggested that the operation is safe if SRLV > 0.310 L/m^2.

At present, the elastic technique has been used to evaluate the degree of liver fibrosis or cirrhosis. It has been widely accepted because of its simplicity, repeatability, noninvasiveness, low cost, and other factors. At present, studies have reported that the sensitivity and specificity of the LSM value to predict the degree of hepatitis cirrhosis are high, and the LSM value is confirmed to be related to complications after partial hepatectomy in patients[28]. However, there is no uniform standard for the patient's disease background, and the operation is limited to only partial or segmental hepatectomy. There is no study on the application of transient elastography to predict the degree of liver fibrosis and

Table 3 Logistic regression analysis based on preoperative the liver stiffness measure value and standard residual liver volume

Independent variables	*P* value	OR	95%CI
LSM ≥ 25 kPa	0.032	6.254	1.172-33.374
SRLV ≤ 290 ML/m^2	0.048	5.686	1.017-31.793

OR: Odds ratio; CI: Confidence interval; LSM: Liver stiffness measure; SRLV: Standard residual liver volume.

Table 4 The standard residual liver volume of different stages of liver fibrosis in 35 patients after hemihepatectomy (mean ± SD)

Liver fibrosis stage	Number	SRLV (L/m^2)	*F* value	*P* value
S1	6	289.43 ± 22.36	8.164	0.001
S2-S3	15	290.33 ± 56.70		
S4	14	375.53 ± 72.24		

SRLV: Standard residual liver volume.

Table 5 The standard residual liver volume of different liver fibrosis stages in 12 patients with liver insufficiency after hemihepatectomy

Liver fibrosis stage	Number	SRLV (L/m^2)	*F* value	*P* value
S1	1	234.20	4.768	0.039
S2-S3	7	263.14 ± 31.28		
S4	4	308.98 ± 18.02		

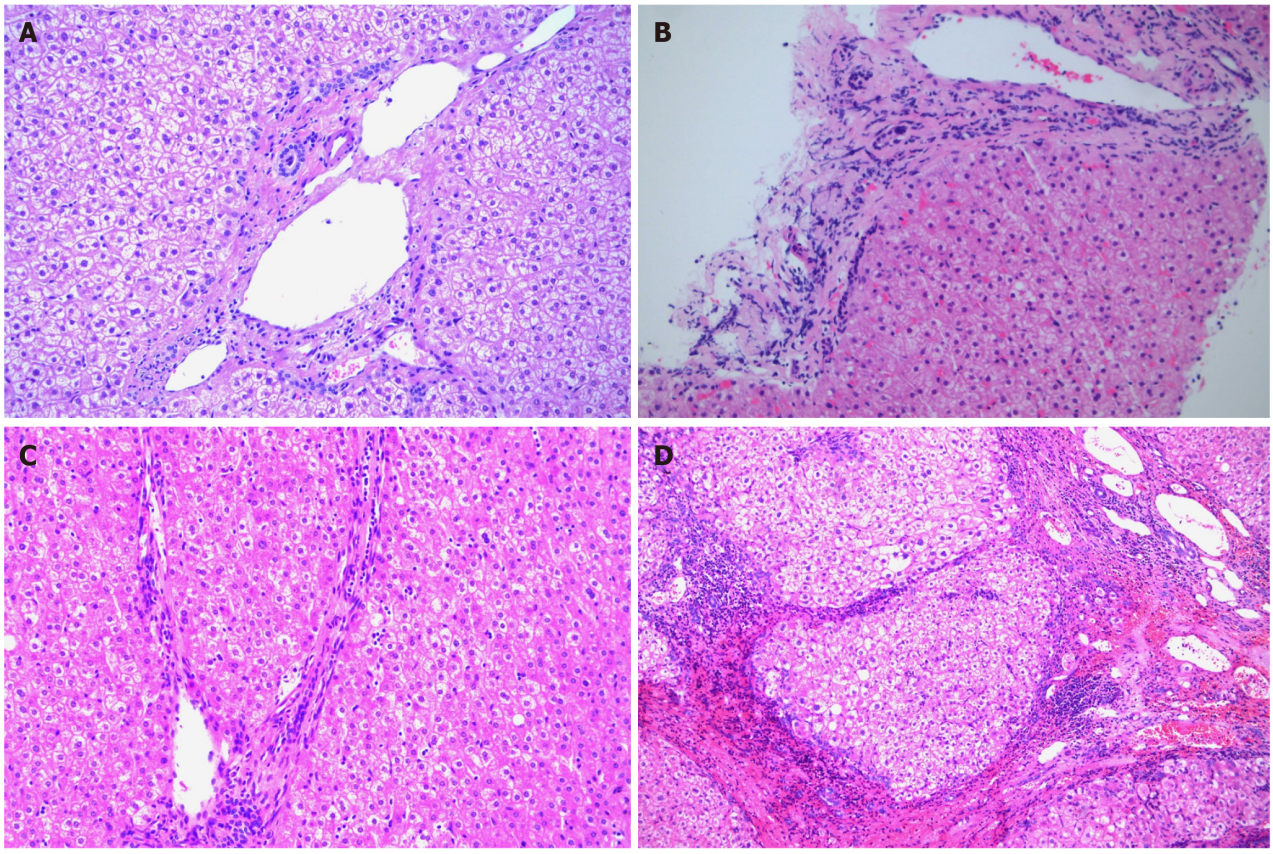
SRLV: Standard residual liver volume.

Table 6 The comparison of two assessment methods, *n* (%)

Model	Total	Grade	Number	Grade 3 wk after surgery (cases)		
				A (I)	B (II)	C (III)
Child-Pugh score	35	A	31	17 (54.8)	14 (45.2)	0
		B	4	0	1 (25)	3 (75)
		C	0	0	0	0
The new evaluation model	35	I	11	11 (100)[a]	0	0
		II	23	0	21 (91.3)[b]	2 (18.7)
		III	1	0	0	1

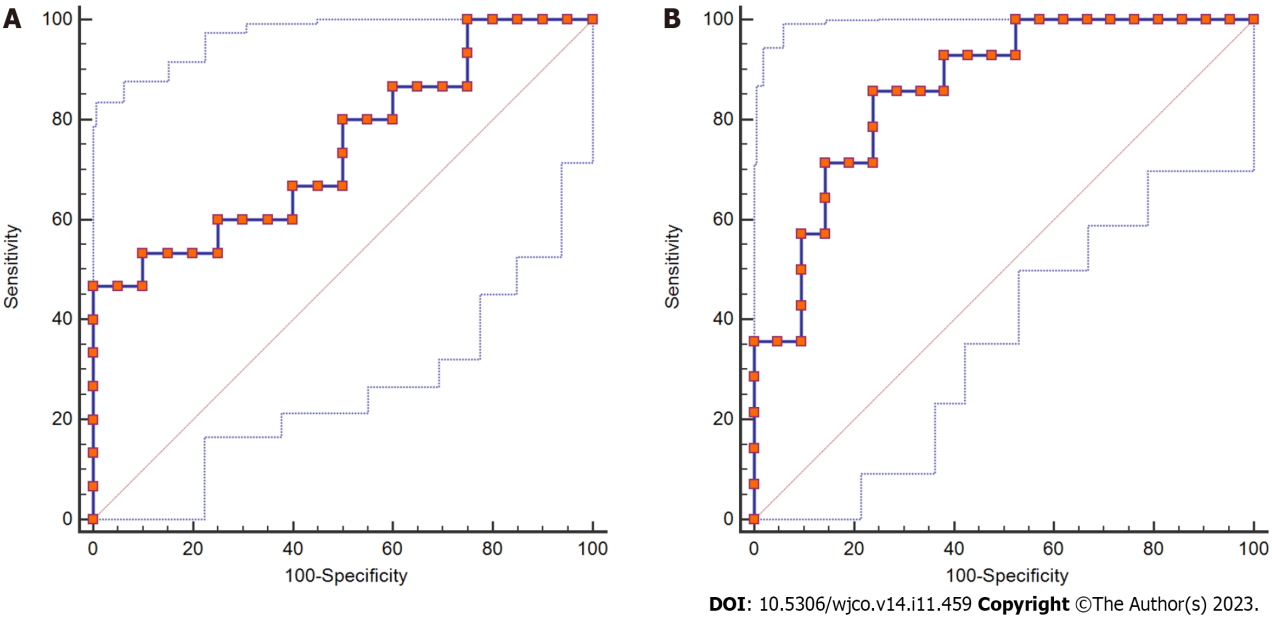
[a]$P < 0.05$ *vs* new evaluation model grade I and Child-Pugh grade A.
[b]$P < 0.05$ *vs* new evaluation model grade II and Child-Pugh grade B.

cirrhosis in hemihepatectomy, and there is no study on the LSM value in evaluating liver function reserve before hemihepatectomy. In addition, recent studies have shown that transient elastography cannot be used to accurately assess patients with obstructive jaundice. Therefore, more rigorous inclusion and exclusion criteria were adopted in this study. We used Fibro Touch elastic imaging equipment (FT-3.5R50) developed by Haskell Medical Technology Company and a two-dimensional ultrasonic probe to avoid the influence of liver tumors and large blood vessels inside and outside the liver on the measurement results. The measured LSM value was 22.20 ± 5.63 kPa, which is similar to that reported at home and abroad[12]. We established a new liver reserve assessment model based on the Child-Pugh score combined with the LSM value and observed its application in the evaluation of liver reserve function in patients with HCC undergoing hemihepatectomy. The results showed that the accuracy of the new evaluation model in predicting postoperative liver function compensation was 100.0% ($P < 0.05$), and the accuracy rate of predicting mildly poor liver function compensation after the operation was 91.3% ($P < 0.05$), which was higher than that of the Child-Pugh score.



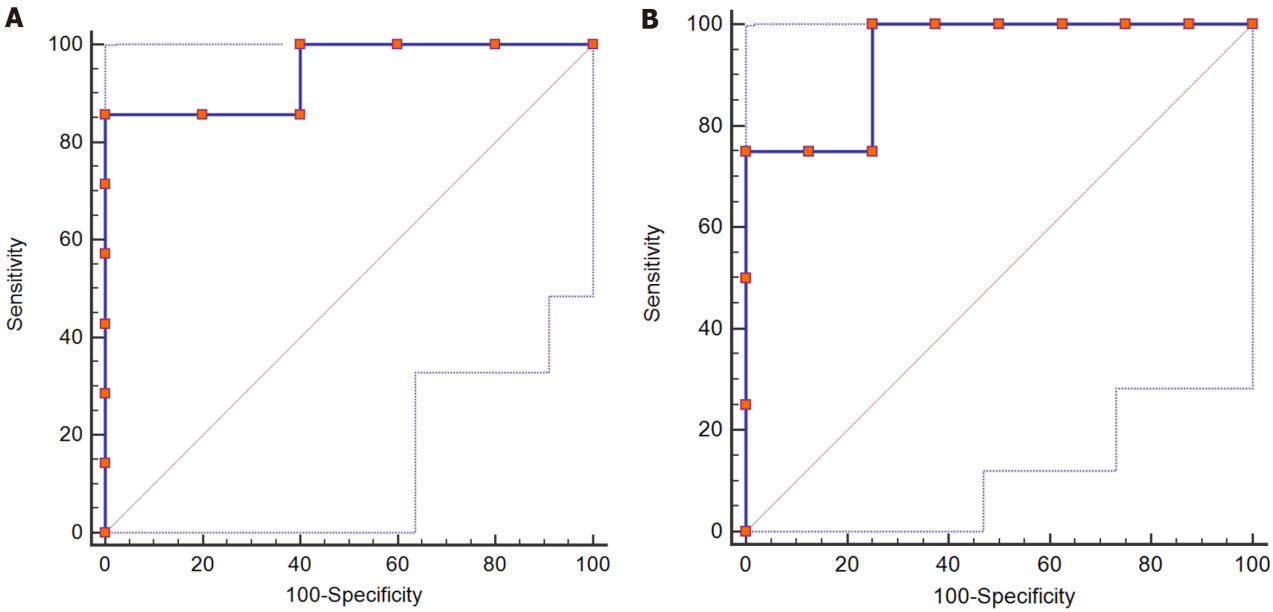
DOI: 10.5306/wjco.v14.i11.459 **Copyright** ©The Author(s) 2023.

Figure 2 The different stages of liver fibrosis in 35 cases of hemihepatectomy (hepatic encephalopathy × 200). A: Stage S1; B: Stage S2; C: Stage S3; D: Stage S4.



DOI: 10.5306/wjco.v14.i11.459 **Copyright** ©The Author(s) 2023.

Figure 3 The receiver operating characteristic curve of the standard residual liver volume in different stages of liver fibrosis. A: Stages S2-S3; B: Stage S4.



DOI: 10.5306/wjco.v14.i11.459 **Copyright** ©The Author(s) 2023.

Figure 4 The receiver operating characteristic curve of the standard residual liver volume in different stages of liver fibrosis in patients with liver dysfunction. A: Stage S2-S3; B: Stage S4.

Therefore, we believe that the new liver reserve assessment model can provide a reference for preoperative safety assessment of patients with liver cancer undergoing hemihepatectomy, which can increase patient safety during the perioperative period and reduce the incidence of liver failure after the operation. Additionally, it can provide a reference for patients with liver cancer who are expected to receive hemihepatectomy or extended hemihepatectomy.

CONCLUSION

In summary, through this study, we found that for patients with moderate or severe liver fibrosis, when the predicted SRLV is greater than 0.310 L/m^2, the new evaluation model of liver function reserve predicts that the postoperative liver function compensation is good before the operation, and hemihepatectomy is safe; when the predicted SRLV is less than 0.285 L/m^2, the new liver reserve assessment model predicts poor liver function compensation after hepatectomy, and the probability of liver dysfunction after hemihepatectomy is higher. A blind operation should be avoided, and the operation should be evaluated after full liver protection. Patients in whom severe liver dysfunction is expected after surgery need to undergo antiviral treatment and undergo portal vein embolization or associated life partition and portal vein ligation for staged hepatectomy, and the values of SRLV and LSM should be reevaluated after liver regeneration. After contralateral liver regeneration, the SRLV and LSM values are reevaluated. It is expected that hemihepatectomy is still feasible for patients with well-compensated liver function. The LSM value combined with SRLV is safe and reliable.

However, the sample size involved in this study is too small and has no statistical significance in theory; nevertheless, the author believes that the LSM value and SRLV are useful safety indices for the evaluation of HCC hemihepatectomy. The new liver reserve evaluation model based on the Child-Pugh score combined with the LSM value can improve on the Child-Pugh score; it has important clinical guiding importance for the evaluation of liver reserve function in HCC patients with hemihepatectomy and provides a theoretical basis for further investigations conducted by our research group.

ARTICLE HIGHLIGHTS

Research background

Liver cancer resection often leads to poor prognosis, because the standard residual liver volume (SRLV) cannot be fully compensated after surgery.

Research motivation

Hemihepatectomy or extended hemihepatectomy often leads to liver insufficiency and even liver failure.

Research objectives
This study aimed to explore the risk factors of poor prognosis after hemihepatectomy for hepatocellular carcinoma and evaluate the application value of related prognostic approaches.

Research methods
The clinical data of 35 patients with primary liver cancer were retrospectively analyzed. The critical values of SRLV in different stages of liver fibrosis after hemihepatectomy were compared with those of liver dysfunction after hemihepatectomy.

Research results
Logistic regression analysis showed that the liver stiffness measure (LSM) value ≥ 25 kPa [odds ratio (OR) = 6.254, $P < 0.05$)] and SRLV ≤ 0.290 L/m^2 (OR = 5.686, $P < 0.05$) were independent risk factors for postoperative liver dysfunction. The accuracy of the new liver reserve evaluation model for predicting postoperative liver function was higher than that of the Child-Pugh score ($P < 0.05$).

Research conclusions
LSM values and SRLV can be used to evaluate the safety of hemihepatectomy.

Research perspectives
The new liver reserve evaluation model has good application potential in the evaluation of liver reserve function after hemihepatectomy.

ACKNOWLEDGEMENTS

We appreciate teachers and classmates support and guidance through this process. Express respect and gratitude to patients who provide medical records.

FOOTNOTES

Co-first authors: Zhi-Qiang Yue and Peng Zhang.

Co-corresponding authors: Jin-Zhu Wu and Ya-Li Cao.

Author contributions: Yue ZQ, Zhang P, Yan S, Yuan LX, Ju LL, Wang HX, Chen L, Cao YL and Wu JZ contributed equally to this work; Yue ZQ, Zhang P, Yan S, Yuan LX and Wu JZ were hepatobiliary surgeons; Ju LL, Wang HX, Yuan LX, Chen L and Cao YL were researchers in Nantong Institute of Liver Disease; Wu JZ, Chen L, Cao YL designed the research study; Yue ZQ, Zhang P, Yan S, Yuan LX, Ju LL and Wang HX performed the primary literature and data extraction; Yue ZQ, Zhang P analyzed the data and wrote the manuscript; Chen L, Cao YL and Wu JZ were responsible for revising the manuscript for important intellectual content; and all authors read and approved the final version. Yue ZQ and Zhang P have made equally significant contributions to this thesis. In accordance with the principles of rigorous and objective research and respect for the contributions of all team members, Zhang P is co-designated as the first author. Wu JZ (corresponding author) and Cao YL (co-corresponding author) provided financial support for this research. Wu JZ (corresponding author), as the research advisor of Yue ZQ (first author), was primarily responsible for the design and guidance of the paper. Meanwhile, Cao YL (co-corresponding author), as an authoritative expert, significantly contributed to the advancement of this study. Therefore, Cao YL is co-designated as the co-corresponding author.

Supported by Nantong Municipal Health Commission, No. MSZ2022036 and No. QN2022041; Nantong Science and Technology Bureau, No. JCZ2022036.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Nantong Third People's Hospital (approval NO. EK201628).

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at 39235978@qq.com.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Zhi-Qiang Yue 0009-0001-0837-7414; Lin-Ling Ju 0000-0002-9541-0533; Lin Chen 0000-0002-1108-4735; Jin-Zhu Wu 0000-0002-7295-2030.

S-Editor: Qu XL
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Marrero JA**, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [PMID: 29624699 DOI: 10.1002/hep.29913]

2 **Liu Z**, Suo C, Mao X, Jiang Y, Jin L, Zhang T, Chen X. Global incidence trends in primary liver cancer by age at diagnosis, sex, region, and etiology, 1990-2017. *Cancer* 2020; **126**: 2267-2278 [PMID: 32201944 DOI: 10.1002/cncr.32789]

3 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

4 **Shen S**, Qiu Y, Yang X, Wang W. Remnant Liver-to-Standard Liver Volume Ratio Below 40% is Safe in Ex Vivo Liver Resection and Autotransplantation. *J Gastrointest Surg* 2019; **23**: 1964-1972 [PMID: 30374819 DOI: 10.1007/s11605-018-4022-4]

5 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 7-34 [PMID: 30620402 DOI: 10.3322/caac.21551]

6 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

7 **Liu PJ**, Harris JM, Marchi E, D'Arienzo V, Michler T, Wing PAC, Magri A, Ortega-Prieto AM, van de Klundert M, Wettengel J, Durantel D, Dorner M, Klenerman P, Protzer U, Giotis ES, McKeating JA. Hypoxic gene expression in chronic hepatitis B virus infected patients is not observed in state-of-the-art in vitro and mouse infection models. *Sci Rep* 2020; **10**: 14101 [PMID: 32839523 DOI: 10.1038/s41598-020-70865-7]

8 **Zheng Y**, Wu J, Ding C, Xu K, Yang S, Li L. Disease burden of chronic hepatitis B and complications in China from 2006 to 2050: an individual-based modeling study. *Virol J* 2020; **17**: 132 [PMID: 32859216 DOI: 10.1186/s12985-020-01393-z]

9 **Salimi S**, Pandya K, Sastry V, West C, Virtue S, Wells M, Crawford M, Pulitano C, McCaughan GW, Majumdar A, Strasser SI, Liu K. Impact of Having a Planned Additional Operation at Time of Liver Transplant on Graft and Patient Outcomes. *J Clin Med* 2020; **9** [PMID: 32102393 DOI: 10.3390/jcm9020608]

10 **Mai RY**, Lu HZ, Bai T, Liang R, Lin Y, Ma L, Xiang BD, Wu GB, Li LQ, Ye JZ. Artificial neural network model for preoperative prediction of severe liver failure after hemihepatectomy in patients with hepatocellular carcinoma. *Surgery* 2020; **168**: 643-652 [PMID: 32792098 DOI: 10.1016/j.surg.2020.06.031]

11 **Möller K**, Safai Zadeh E, Görg C, Dong Y, Cui X, Lim A, de Molo C, Serra C, Martín Algíbez A, Berzigotti A, Piscaglia F, Faiss S, Dietrich CF. Focal Liver Lesions other than Hepatocellular Carcinoma in Cirrhosis: Diagnostic Challenges. *J Transl Int Med* 2022; **10**: 308-327 [PMID: 36860624 DOI: 10.2478/jtim-2022-0068]

12 **Hwang JY**, Yoon HM, Kim JR, Lee JS, Jung AY, Kim KM, Cho YA. Diagnostic Performance of Transient Elastography for Liver Fibrosis in Children: A Systematic Review and Meta-Analysis. *AJR Am J Roentgenol* 2018; **211**: W257-W266 [PMID: 30106615 DOI: 10.2214/AJR.18.19535]

13 **Zhang Y**, Wang C, Li H, Ding Y. Decreased liver stiffness by transient elastography indicates lower incidence of hepatocellular carcinoma in patients with chronic hepatitis B. *Medicine (Baltimore)* 2019; **98**: e13929 [PMID: 30653099 DOI: 10.1097/MD.0000000000013929]

14 **Feng LM**, Wang PQ, Yu H, Chen RT, Wang J, Sheng X, Yuan ZL, Shi PM, Xie WF, Zeng X. New formula for predicting standard liver volume in Chinese adults. *World J Gastroenterol* 2017; **23**: 4968-4977 [PMID: 28785151 DOI: 10.3748/wjg.v23.i27.4968]

15 **van Wissen J**, Bakker N, Doodeman HJ, Jansma EP, Bonjer HJ, Houdijk AP. Preoperative Methods to Reduce Liver Volume in Bariatric Surgery: a Systematic Review. *Obes Surg* 2016; **26**: 251-256 [PMID: 26123526 DOI: 10.1007/s11695-015-1769-5]

16 **Furlan A**, Tublin ME, Yu L, Chopra KB, Lippello A, Behari J. Comparison of 2D Shear Wave Elastography, Transient Elastography, and MR Elastography for the Diagnosis of Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *AJR Am J Roentgenol* 2020; **214**: W20-W26 [PMID: 31714842 DOI: 10.2214/AJR.19.21267]

17 **Taneja S**, Tohra S, Duseja A, Dhiman RK, Chawla YK. Noninvasive Assessment of Liver Fibrosis By Transient Elastography and FIB4/APRI for Prediction of Treatment Response in Chronic Hepatitis C-An Experience from a Tertiary Care Hospital. *J Clin Exp Hepatol* 2016; **6**: 282-290 [PMID: 28003717 DOI: 10.1016/j.jceh.2016.08.001]

18 **Maruyama M**, Yoshizako T, Araki H, Yoshida R, Ando S, Nakamura M, Kitagaki H. Future Liver Remnant Indocyanine Green Plasma Clearance Rate as a Predictor of Post-hepatectomy Liver Failure After Portal Vein Embolization. *Cardiovasc Intervent Radiol* 2018; **41**: 1877-1884 [PMID: 30132102 DOI: 10.1007/s00270-018-2065-2]

19 **Bozkurt B**, Emek E, Arikan T, Ceyhan O, Yazici P, Sahin T, Mammadov E, Serin A, Gurcan NI, Yuzer Y, Tokat Y. Liver Graft Volume Estimation by Manual Volumetry and Software-Aided Interactive Volumetry: Which is Better? *Transplant Proc* 2019; **51**: 2387-2390 [PMID: 31324483 DOI: 10.1016/j.transproceed.2019.01.152]

20 **Endo Y**, Ohta M, Tada K, Nakanuma H, Saga K, Masuda T, Hirashita T, Iwashita Y, Ozeki Y, Masaki T, Inomata M. Improvement of non-alcoholic fatty liver disease after laparoscopic sleeve gastrectomy in Japanese obese patients. *Ann Gastroenterol Surg* 2019; **3**: 285-290 [PMID: 31131357 DOI: 10.1002/ags3.12234]

21 **Gong WF**, Zhong JH, Lu Z, Zhang QM, Zhang ZY, Chen CZ, Liu X, Ma L, Zhang ZM, Xiang BD, Li LQ. Evaluation of liver regeneration and post-hepatectomy liver failure after hemihepatectomy in patients with hepatocellular carcinoma. *Biosci Rep* 2019; **39** [PMID: 31383787 DOI: 10.1042/BSR20190088]

22 **Xue C**, Chu Q, Li L. Research Progress on the Role of Probiotics in Acute Liver Failure. *J Transl Int Med* 2022; **10**: 83-85 [PMID: 35959453 DOI: 10.2478/jtim-2021-0052]

23 **Zhang D**, Pan Y, Yang Z, Zeng H, Wang X, Chen J, Wang J, Zhang Y, Zhou Z, Chen M, Hu D. A Nomogram Based on Preoperative Lab Tests, BMI, ICG-R15, and EHBF for the Prediction of Post-Hepatectomy Liver Failure in Patients with Hepatocellular Carcinoma. *J Clin Med* 2022; **12** [PMID: 36615125 DOI: 10.3390/jcm12010324]

24 **Allaire M**, Goumard C, Lim C, Le Cleach A, Wagner M, Scatton O. New frontiers in liver resection for hepatocellular carcinoma. *JHEP Rep* 2020; **2**: 100134 [PMID: 32695968 DOI: 10.1016/j.jhepr.2020.100134]

25 **Kokudo T**, Hasegawa K, Shirata C, Tanimoto M, Ishizawa T, Kaneko J, Akamatsu N, Arita J, Demartines N, Uldry E, Kokudo N, Halkic N. Assessment of Preoperative Liver Function for Surgical Decision Making in Patients with Hepatocellular Carcinoma. *Liver Cancer* 2019; **8**: 447-456 [PMID: 31799202 DOI: 10.1159/000501368]

26 **Fitzpatrick JA**, Kim JU, Cobbold JF, McPhail MJ, Crossey MM, Bak-Bol AA, Zaky A, Taylor-Robinson SD. Changes in Liver Volume in Patients with Chronic Hepatitis C Undergoing Antiviral Therapy. *J Clin Exp Hepatol* 2016; **6**: 15-20 [PMID: 27194891 DOI: 10.1016/j.jceh.2015.11.004]

27 **Yang X**, Yang JD, Lee S, Hwang HP, Ahn S, Yu HC, You H. Estimation of Standard Liver Volume Using CT Volume, Body Composition, and Abdominal Geometry Measurements. *Yonsei Med J* 2018; **59**: 546-553 [PMID: 29749138 DOI: 10.3349/ymj.2018.59.4.546]

28 **Serra-Burriel M**, Graupera I, Torán P, Thiele M, Roulot D, Wai-Sun Wong V, Neil Guha I, Fabrellas N, Arslanow A, Expósito C, Hernández R, Lai-Hung Wong G, Harman D, Darwish Murad S, Krag A, Pera G, Angeli P, Galle P, Aithal GP, Caballeria L, Castera L, Ginès P, Lammert F; investigators of the LiverScreen Consortium. Transient elastography for screening of liver fibrosis: Cost-effectiveness analysis from six prospective cohorts in Europe and Asia. *J Hepatol* 2019; **71**: 1141-1151 [PMID: 31470067 DOI: 10.1016/j.jhep.2019.08.019]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 November 24; 14(11): 471-478

DOI: 10.5306/wjco.v14.i11.471

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Observational Study

System describing surgical field extension associated with flap reconstruction after resection of a superficial malignant soft tissue tumor

Akio Sakamoto, Takashi Noguchi, Shuichi Matsuda

Specialty type: Orthopedics

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gupta R, India

Received: August 29, 2023
Peer-review started: August 29, 2023
First decision: September 14, 2023
Revised: September 24, 2023
Accepted: October 13, 2023
Article in press: October 13, 2023
Published online: November 24, 2023

Akio Sakamoto, Takashi Noguchi, Shuichi Matsuda, Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan

Corresponding author: Akio Sakamoto, MD, PhD, Adjunct Professor, Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Shogoin, Kawahara-cho 54, Sakyo-ku, Kyoto 606-8507, Japan. akiosaka@kuhp.kyoto-u.ac.jp

Abstract

BACKGROUND

Flap reconstruction after resection of a superficial malignant soft tissue tumor extends the surgical field and is an indicator for potential recurrence sites.

AIM

To describe a grading system for surgical field extension of soft tissue sarcomas.

METHODS

Grading system: CD-grading is a description system consisting of C and D values in the surgical field extension, which are related to the compartmental position of the flap beyond the nearby large joint and deeper extension for the pedicle, respectively. C1/D1 are positive values and C0/D0 are negative. With a known location, 1/0 values can be "p" (proximal), "d" (distal), and "b" (in the tumor bed), and the description method is as follows: flap type, CxDx [x = 0, 1, p, d or b].

RESULTS

Four representative patients with subcutaneous sarcomas who underwent reconstruction using fasciocutaneous flaps are presented. The cases involved a distal upper arm (elbow) synovial sarcoma reconstructed using a pedicled latissimus dorsi (pedicled flap: CpDp); a distal upper arm (elbow) pleomorphic rhabdomyosarcoma reconstructed using a transpositional flap from the forearm (transpositional flap: CdD0); an undifferentiated pleomorphic sarcoma in the buttocks reconstructed using a transpositional flap (transpositional flap: C0D0); and a myxofibrosarcoma in the buttocks reconstructed using a propeller flap from the thigh (pedicled flap: CdDd).

CONCLUSION

The reconstruction method is chosen by the surgeon based on size, location, and

other tumor characteristics; however, the final surgical field cannot be determined based on preoperative images alone. CD-grading is a description system consisting of C and D values in the surgical field extension that are related to the compartmental position of the flap beyond the nearby large joint and deeper extension for the pedicle, respectively. The CD-grading system gives a new perspective to the flap reconstruction classification. The CD-grading system also provides important information for follow-up imaging of a possible recurrence.

Key Words: Soft tissue; Sarcoma; Surgery; Sarcoma; Grading system; Surgical flap

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Flap reconstruction after resection of a superficial malignant soft tissue tumor extends the surgical field and is an indicator for potential recurrence sites. CD-grading is a description system consisting of C and D values in the surgical field extension that are related to the compartmental position of the flap beyond the nearby large joint and deeper extension for the pedicle, respectively. C1/D1 and C0/D0 are positive and negative values, respectively. The CD-grading system gives a new perspective to flap reconstruction classification. The CD-grading system also provides important information for follow-up imaging of a possible recurrence.

Citation: Sakamoto A, Noguchi T, Matsuda S. System describing surgical field extension associated with flap reconstruction after resection of a superficial malignant soft tissue tumor. *World J Clin Oncol* 2023; 14(11): 471-478
URL: https://www.wjgnet.com/2218-4333/full/v14/i11/471.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i11.471

INTRODUCTION

Soft tissue sarcomas comprise a group of rare heterogeneous neoplasms that account for < 1% of all cancers[1]. Soft tissue sarcomas can occur in any soft tissue, but are most common in the extremities. Wide resection of the sarcoma and the surrounding normal tissue is necessary to reduce the recurrence rate[2]. Flap reconstruction is used to repair soft tissue defects after resection of a soft tissue sarcoma, especially a superficial soft tissue sarcoma[3,4].

The term "tumor bed" refers to the area of tissue remaining after a malignant tumor is removed. The tumor bed includes the tumor and surrounding healthy tissues where cancer cells may exist[5]. Use of a reconstruction flap following soft tissue sarcoma resection can extend the surgical field or tumor bed because of flap elevation or dissection of recipient vessels.

The current Cancer Staging Manual of the American Joint Committee on Cancer (AJCC) of soft tissue sarcoma is widely used based upon tumor size, histologic grade, and the presence of metastasis[6]. AJCC supports the *R classification*, which categorizes surgical margins as negative (R0), microscopically positive (R1), or grossly positive (R2)[7,8]. Flaps can be classified based on several factors (pedicled, free, or the tissue type from which the flap is made). Classification of flaps according to clinical complications has also been reported[9]; however, there is no system describing surgical field extension related to flap reconstruction.

In the current report we propose a grading classification, the CD-grading system, to describe extension of the surgical field related to flap reconstruction after superficial soft tissue sarcoma resection. Representative cases are also presented.

MATERIALS AND METHODS

Classification: CD-grading system for a superficial sarcoma in the extremities

The new grading system (CD-grading system) was used herein for superficial soft tissue sarcomas with extremity resection reconstructed by fascio-(musculo)-cutaneous flaps. Upper extremity tumors are defined as lesions arising distal to the acromioclavicular joint and include tumors of the shoulder girdle and axilla. Lower extremity tumors are defined as lesions arising distal to the iliac crest, including tumors of the gluteal region[9]. Additional skin grafting does not affect the grade; the skin grafting cases were not excluded.

The CD-grading system consists of C- and D-values. The C-value indicates the "compartmental position of the flap beyond the nearby large joint " and when the flap crosses a nearby large joint, the C-value is positive (C1). When the flap is within the compartment, the C-value is negative (C0). Large joints include the shoulders, elbows, wrists, hips, knees, and ankles. If the location of a flap crossing the joint location is proximal, the C-value is Cp (p = proximal) and when crossing a distal large joint the C-value is Cd (d = distal).

D-value means "deeper extension for the pedicle." The pedicle is already exposed, and the negative D-value is D0. If dissection of the pedicle is necessary, the positive D-value is D1. When the dissected pedicle is located proximal to the surgical field, the D-value is Dp (p = proximal), when the dissected pedicle is located distally, the D-value is Dd (d = distal), and when the pedicle dissection is within the surgical bed, the D-value is Db (b = surgical bed; Tables 1 and 2).

Table 1 C-value for flap location beyond the nearby large joint

	C-value	Description
Within the compartment	0	C0
Extra compartment	1: any	C1
	p: proximal	Cp
	d: distal	Cd

Table 2 D-value for surgical field extension for the pedicle dissection

Pedicle	D-value	Description
Already exposed	0	D0
Dissection of the pedicle	1: Any	D1
	p: Proximal	Dp
	d: Distal	Dd
	b: Within tumor bed	Db

The flap type is described before the CD-values as "flap type, CxDx," in which x can be 0, 1, p, d, or b. There is no strict rule in the description of the flap type; however, an easy and understandable description, such as distinguishing between a local or free flap, would be required.

Transpositional fascial flap/propeller flap

In cases involving transpositional fasciocutaneous or propeller flaps[10], the flap is located within the compartment, the C-value is C0, the D-value is D0, and the CD-grade is C0D0. When the flap is from the extra compartment across the large joint, the C-value is C1, the D-value is D0, and the CD-grade is C1D0. When the flap is obtained proximally and crosses a large joint, the CD-grade is CpD0, and when the flap is derived distally and crosses a large joint, the CD-grade is CdD0 (Tables 3 and 4).

Pedicled flap

The C-value in the pedicle flap is the same as the transpositional flap. The flap is located within the compartment and the C-value is C0. When the flap comes from the extra compartment across the joint, the C-value is C1. C1 can be Cp or Cd depending on the flap location (proximal or distal). The D-value reflects the location of the pedicle. The pedicle flap needs extension of the surgical field to deeper tissues, therefore the D-value is always D1. When the pedicle is located proximal to the surgical field, D1 can be Dp (p = proximal), and when the pedicle is located distal to the surgical field, the D1 can be Dd (d = distal; Tables 3 and 5).

Free flap

The donated area of the flap does not affect the surgical field in terms of tumor contamination, and the C-value in the free flap is always C0. When the pedicle is already exposed at the surgical field, the D-value is D0. When the pedicle is not exposed, and the pedicle needs to be exposed, then the D-value is D1. When the pedicle is located proximal to the surgical field, D1 can be Dp (p = proximal), and when the pedicle is located distal to the surgical field, the D1 can be Dd (d = distal). When the pedicle is exposed at the deeper tissues within the surgical field, the D-value is Db (Tables 3 and 6).

RESULTS

Herein we present four cases of superficial soft tissue sarcomas. Two elbow soft tissue sarcomas and two buttock soft-tissue sarcomas are presented. One elbow soft tissue sarcoma patient was a 47-year-old female with a synovial sarcoma at the elbow (distal upper arm) reconstructed with a pedicled latissimus dorsi; the CD-grade was CpDp (pedicled flap, CpDp; Figure 1). The second elbow soft tissue sarcoma patient was an 85-year-old male with a pleomorphic rhabdomyosarcoma at the elbow (distal upper arm) reconstructed using a transpositional flap from the forearm; the CD-grade was CdD0 (transpositional flap, CdD0; Figure 2). The first patient with a buttock soft tissue sarcoma was a 65-year-old female with an undifferentiated pleomorphic sarcoma at the buttock reconstructed using a transpositional flap; the CD-grade was C0D0 (transpositional flap, C0D0[11]; Figure 3). The second patient with a buttock sarcoma was a 46-year-old male with a myxofibrosarcoma that was reconstructed using a propeller flap from the thigh; the CD-grade was CdDd (pedicled flap, CdDd; Figure 4).

Table 3 Possible classification of flap reconstruction

CD-grade	Transpositional flap	Pedicled flap	Free flap
C0D0	C0D0	Not applied	C0D0
C0D1	NA	C0Dp, C0Dd	C0Dp, C0Dd, C0Db
C1D0	CpD0, CdD0	NA	NA
C1D1	NA	CpDp, CdDd	NA

NA: Not available.

Table 4 Transpositional fasciocutaneous flap/propeller flap

Flap location	C-value	D-value	CD-grade
Within the compartment	0	0	C0D0
Extra compartment: from any	1	0	C1D0
From proximal	p	0	CpD0
From distal	d	0	CdD0

Table 5 Pedicled flap

Flap location	C-value	D-value (pedicle location)	CD-grade
Within the compartment	0	1 (any)	C0D1
From proximal	0	p (proximal)	C0Dp
From distal	0	d (distal)	C0Dd
Extra compartment	1	1 (any)	C1D1
From proximal	p	p (proximal)	CpDp
From distal	d	d (distal)	CdDd

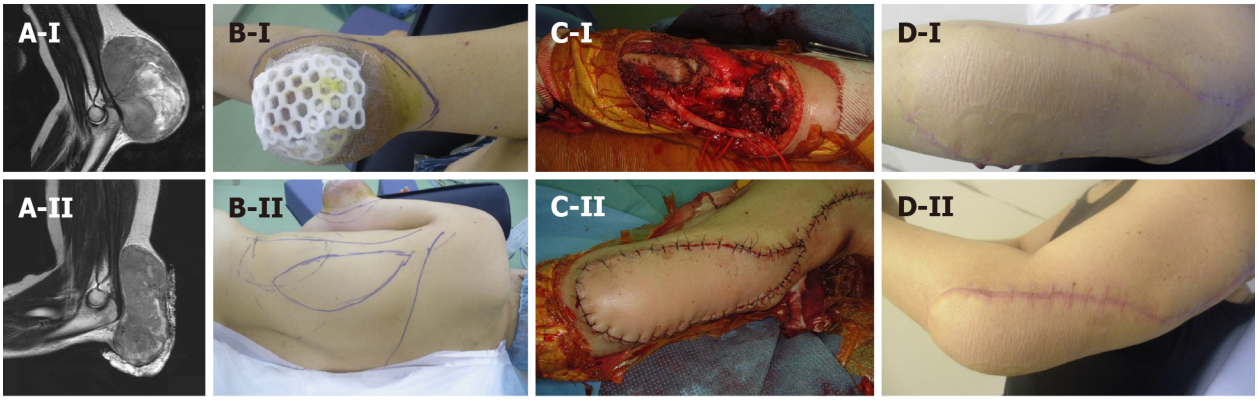
Table 6 Free flap

Pedicle location	C-value	D-value (pedicle location)	CD-grade
Already exposed	0	0	C0D0
Necessary for dissection	0	1 (any)	C0D1
From proximal	0	p (proximal)	C0Dp
From distal	0	d (distal)	C0Dd
Within tumor bed	0	b (within tumor bed)	C0Db

DISCUSSION

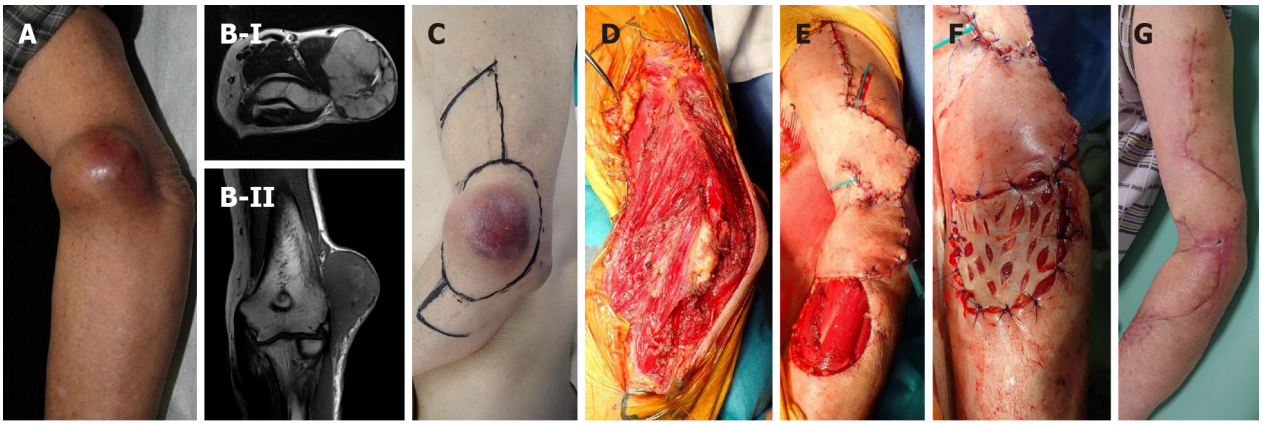
Soft tissue sarcomas require wide resection with healthy tissue margins[12,13]. Thus, the surgical field is wider than the tumor size. The extension of the tumor bed has the possibility of tumor contamination. Therefore, recognition of tumor bed extension is necessary. Discrepancies between the preoperative tumor burden and postoperative tumor bed contour have been identified after tumor burden replacement with a latissimus dorsi flap[14]. Flap reconstruction increases the surgical field during superficial soft tissue sarcoma resection[15].

A flap is applied to the defect after resection of a soft tissue sarcoma, especially a superficial soft tissue sarcoma. The choice of flap is often determined by the surgeon's preference, as well as the location of the tumor. The tumor bed after resection of soft tissue sarcomas cannot be predicted solely based on preoperative imaging. If amputation is necessary in the case of a re-occurrence, the level of amputation is important. Extension of the tumor bed due to flap reconstruction carries the risk of tumor contamination and may require more proximal amputation. The C-value gives information that indicates the likelihood of tumor contamination across the greater joint.



DOI: 10.5306/wjco.v14.i11.471 **Copyright** ©The Author(s) 2023.

Figure 1 Synovial sarcoma at the distal upper arm (elbow) reconstructed by a pedicled latissimus dorsi (pedicled flap, CpDp). A: A 47-year-old female with a synovial sarcoma at the elbow (distal upper arm). Magnetic resonance imaging showed a tumor with heterogenous low-to-high signal intensity on the T2-weighted image. Before (A-I) and after (A-II) chemotherapy of doxorubicin and ifosfamide, the tumor size was reduced. B-D: A wide surgical resection was performed with a pedicled latissimus dorsi. The CD-grade was CpDp (pedicled flap, CpDp).

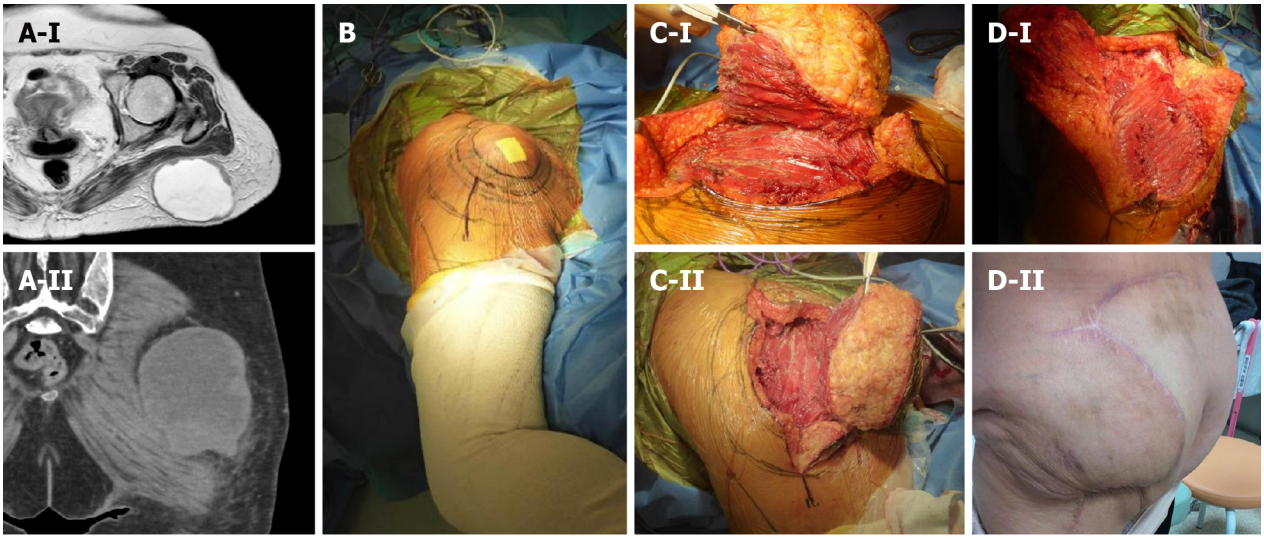


DOI: 10.5306/wjco.v14.i11.471 **Copyright** ©The Author(s) 2023.

Figure 2 Pleomorphic rhabdomyosarcoma at the distal upper arm (elbow) reconstructed by transpositional flap (transpositional flap, CdD0). A: An 85-year-old male with a pleomorphic rhabdomyosarcoma at the elbow (distal upper arm); B: Magnetic resonance imaging showed a tumor with homogenous high-signal intensity on T2-weighted images (B-I) and low-signal intensity on T1-weighted images (B-II). A wide surgical resection was performed. The transpositional flap was obtained from the upper arm and forearm; C-G: Skin grafting was performed at the forearm. The CD-grade was CdD0 (transpositional flap, CdD0).

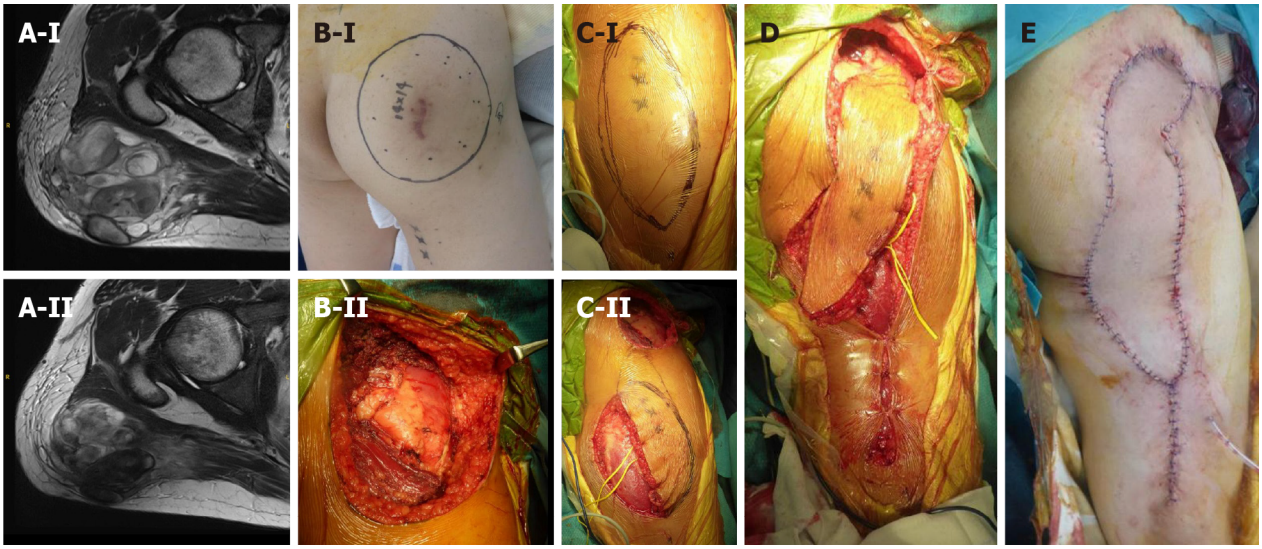
There is a risk of tumor contamination if deep tissues are created in the surgical field. The D-value represents the location of the pedicle. Dissection of the pedicle and recipient vessels requires dissection to the deeper layers, resulting in extension of the surgical field, with a D-value of D1. Transposition flaps in the compartment do not require exposure of the donor vessels, therefore less deep tissue exposure is advantageous in terms of reducing the potential for tumor recurrence, with a D-value of D0. Similarly, even in cases of a free flap requiring microsurgery, if the recipient vessels are already exposed, the D-value is D0 because deeper tissue dissection is not necessary.

The AJCC Staging of Soft Tissue Sarcomas (eighth edition) is based upon the tumor size, histologic grade, and the presence of metastasis. Tumor size is classified into four categories with border values of 5, 10, and 15 cm. The notation regarding tumor depth (superficial or deep from the superficial fascia) has been eliminated from the seventh edition of the AJCC Staging of Soft Tissue Sarcomas[6]. The surgical staging of musculoskeletal sarcomas has 4 types of surgical margins [intralesional, marginal, wide, and radical (compartmental)], as proposed by Enneking *et al*[16]. A 2-3 cm surgical margin provides reasonable local control of soft tissue sarcomas[17]. The AJCC supports the *R classification,* which categorizes margins as negative (R0), microscopically positive (R1), or grossly positive (R2)[7,8]. Furthermore, the Union Against Cancer (UICC) proposed a *R + 1 mm classification* that requires 1 mm of healthy tissue between the tumor and margin to define a negative margin (R0)[18,19], thus resulting in more resections being considered microscopically positive (R1). Radiation therapy can be performed as adjuvant therapy, especially if cancer cells remain after the resection. Radiation has a role in reducing the risk of recurrence in soft tissue sarcoma resection[5]. The term, tumor bed, refers to the area of tissue remaining after a malignant tumor is removed. The tumor bed may have cancer cells[5]. Recognition of tumor bed extension is necessary for postoperative radiation. Without flap reconstruction following soft tissue sarcoma resection, the tumor bed can largely be predicted with the preoperative staging based upon the images



DOI: 10.5306/wjco.v14.i11.471 **Copyright** ©The Author(s) 2023.

Figure 3 Undifferentiated pleomorphic sarcoma at the buttock reconstructed by a transpositional flap (transpositional flap, C0D0). A: A 65-year-old female with an undifferentiated pleomorphic sarcoma at the buttock. Magnetic resonance imaging revealed a subcutaneous tumor. The tumor had a cystic appearance and contained liquid with slightly high signal intensity on the T2-weighted image. The periphery of the cystic wall was thick with a solid neoplastic lesion and intermediate signal intensity on T2-weighted images (A-I). Computed tomography showed that the lesion is located at the buttock (A-II); B: A resection of the tumor was designed; C and D: The tumor was resected and the defect was reconstructed with a transpositional flap donated from the lateral abdomen. The CD-grade was C0D0 (transpositional flap, C0D0).



DOI: 10.5306/wjco.v14.i11.471 **Copyright** ©The Author(s) 2023.

Figure 4 Myxofibrosarcoma at the buttock reconstructed by a propeller flap (pedicled flap, CdDd). A: A 46-year-old male with a myxofibrosarcoma at the buttock. Magnetic resonance imaging revealed that the tumor showed heterogenous low-to-high signal intensity on the T2-weighted image. Before (A-I) and after (A-II) chemotherapy of doxorubicin and ifosfamide, the tumor size was reduced; B: The resection of the tumor was designed (B-I) and performed (B-II); C and E: A propeller flap from the thigh was designed (C-I) and the pedicle was preserved (C-II) and performed. The CD-grade was CdDd (pedicled flap, CdDd).

and the histologic findings. With flap reconstruction, tumor bed prediction is difficult without the surgical method information. Indeed, the new grading system can give information of surgical field extension associated with flap reconstruction.

There are several limitations in the new grading system. First, this classification is completely new and still theoretical. Clinical use of the assembled clinical data would be necessary, and some modification may be required for improvement. Second, the new grading system was used for superficial soft tissue sarcomas resected in the extremities and fascio-(musculo)-cutaneous flaps, in which hands and feet were not included. The new grading system might be modified for any part of bones and soft tissue sarcomas. Third, flap type description is not strictly defined in the new grading system, which may result in ambiguity; however, according to the flap technique improvement, description of the flap would be diverse. Therefore, no flap description restrictions were used in the new grading system. Finally, the new grading system cannot describe the length or area required for postoperative radiation. Excessive information in the grading system,

however, would make the new grading system difficult for clinical use.

CONCLUSION

We have proposed a method to describe extension of the surgical field in reconstruction after superficial soft tissue sarcoma resection. The method described can give values for tumor bed extension after flap reconstruction. The description of whether or not the operative field has been extended due to reconstruction is considered to be important information for image evaluation of recurrence.

ARTICLE HIGHLIGHTS

Research background

Flap reconstruction can extend the surgical field or tumor bed because of flap elevation or dissection of recipient vessels during resection of superficial soft tissue sarcomas. There is currently no method describing extension of the surgical field.

Research motivation

Extension of the surgical field cannot be predicted based on preoperative images for flap reconstruction after superficial soft tissue sarcoma resection. Knowledge of the surgical field extension is important information for evaluation of recurrence images or possible postoperative radiation.

Research objectives

A theoretical CD-grading system was developed consisting of C and D values in the surgical field extension. The C-value represents the flap beyond the nearby large joint and the D-value pertains to a deeper extension.

Research methods

C1/D1 and C0/D0 are positive and negative values, respectively. With a known location, C values are "p" (proximal), "d" (distal), and "b" (in the tumor bed). The description method is as follows: flap type, CxDx [x = 0, 1, p, d or b].

Research results

Classification and possible values are shown in the tables (transpositional fascial flap/propeller, pedicled, and free flaps). Four representative patients with subcutaneous sarcomas who underwent reconstruction using fasciocutaneous flaps are presented.

Research conclusions

The new grading system can give values for tumor bed extension after flap reconstruction following superficial soft tissue sarcoma resection. The description of whether or not the operative field has been extended due to reconstruction is thought to be important information for evaluation of recurrence images.

Research perspectives

Clinical use of assembled clinical data would be necessary and some modification may be required for improvement, especially if the new grading system is modified for any part of bone and soft tissue sarcomas.

FOOTNOTES

Author contributions: Sakamoto A developed the classification and drafted the manuscript; Noguchi T and Matsuda S participated in the study design; All authors read and approved the final manuscript.

Institutional review board statement: Analysis of clinical data accumulation in patients with bone and soft tissue tumors. Retrospective study.

Informed consent statement: The patients represented in this study were informed that the data from the case would be de-identified and used in a journal publication. There is a specific signed document because the analysis used anonymous clinical data that were obtained after each patient had been notified at the Kyoto University home page that the data could be used for a clinical study.

Conflict-of-interest statement: We, the authors, declare no conflicts of interest regarding our manuscript entitled "A system describing surgical field extension associated with flap reconstruction after resection of a malignant soft tissue tumor."

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Japan

ORCID number: Akio Sakamoto 0000-0002-7518-1606; Takashi Noguchi 0000-0003-2965-4772; Shuichi Matsuda 0000-0003-0802-1255.

S-Editor: Liu JH
L-Editor: A
P-Editor: Liu JH

REFERENCES

1 **Nijhuis PH**, Schaapveld M, Otter R, Molenaar WM, van der Graaf WT, Hoekstra HJ. Epidemiological aspects of soft tissue sarcomas (STS)--consequences for the design of clinical STS trials. *Eur J Cancer* 1999; **35**: 1705-1710 [PMID: 10674017 DOI: 10.1016/s0959-8049(99)00152-5]
2 **Samà L**, Binder JP, Darrigues L, Couturaud B, Boura B, Helfre S, Chiche L, Nicolas N, Tzanis D, Bouhadiba T, Gentile D, Perlbarg-Samson J, Bonvalot S. Safe-margin surgery by plastic reconstruction in extremities or parietal trunk soft tissue sarcoma: A tertiary single centre experience. *Eur J Surg Oncol* 2022; **48**: 526-532 [PMID: 34702592 DOI: 10.1016/j.ejso.2021.10.010]
3 **Slump J**, Hofer SOP, Ferguson PC, Wunder JS, Griffin AM, Hoekstra HJ, Bastiaannet E, O'Neill AC. Flap choice does not affect complication rates or functional outcomes following extremity soft tissue sarcoma reconstruction. *J Plast Reconstr Aesthet Surg* 2018; **71**: 989-996 [PMID: 29706552 DOI: 10.1016/j.bjps.2018.04.002]
4 **Koulaxouzidis G**, Schlagnitweit P, Anderl C, Braig D, Märdian S. Microsurgical Reconstruction in Orthopedic Tumor Resections as Part of a Multidisciplinary Surgical Approach for Sarcomas of the Extremities. *Life (Basel)* 2022; **12** [PMID: 36362956 DOI: 10.3390/life12111801]
5 **Brinkmann EJ**, Ahmed SK, Houdek MT. Extremity Soft Tissue Sarcoma: Role of Local Control. *Curr Treat Options Oncol* 2020; **21**: 13 [PMID: 32025823 DOI: 10.1007/s11864-020-0703-9]
6 **Tanaka K**, Ozaki T. New TNM classification (AJCC eighth edition) of bone and soft tissue sarcomas: JCOG Bone and Soft Tissue Tumor Study Group. *Jpn J Clin Oncol* 2019; **49**: 103-107 [PMID: 30423153 DOI: 10.1093/jjco/hyy157]
7 **Hermanek P**, Wittekind C. The pathologist and the residual tumor (R) classification. *Pathol Res Pract* 1994; **190**: 115-123 [PMID: 8058567 DOI: 10.1016/S0344-0338(11)80700-4]
8 **Hoang K**, Gao Y, Miller BJ. The Variability in Surgical Margin Reporting in Limb Salvage Surgery for Sarcoma. *Iowa Orthop J* 2015; **35**: 181-186 [PMID: 26361463]
9 **O'Neill AC**, Roy M, Boucher A, Fitzpatrick AM, Griffin AM, Tsoi K, Ferguson PC, Wunder JS, Hofer SOP. The Toronto Sarcoma Flap Score: A Validated Wound Complication Classification System for Extremity Soft Tissue Sarcoma Flap Reconstruction. *Ann Surg Oncol* 2021; **28**: 3345-3353 [PMID: 33005992 DOI: 10.1245/s10434-020-09166-2]
10 **Pignatti M**, Ogawa R, Mateev M, Georgescu AV, Balakrishnan G, Ono S, Cubison T, Pinto V, D'Arpa S, Koshima I, Hyakusoku H, Hallock GG. Our Definition of Propeller Flaps and Their Classification. *Semin Plast Surg* 2020; **34**: 139-144 [PMID: 33041682 DOI: 10.1055/s-0040-1715158]
11 **Sakamoto A**, Noguchi T, Matsuda S. A Transposition Flap Reconstruction after Resection of a Soft-Tissue Sarcoma in the Buttock. *J Orthop Case Rep* 2021; **11**: 38-40 [PMID: 35415100 DOI: 10.13107/jocr.2021.v11.i10.2458]
12 **Garreffa E**, Hughes-Davies L, Russell S, Lightowlers S, Agrawal A. Definition of Tumor Bed Boost in Oncoplastic Breast Surgery: An Understanding and Approach. *Clin Breast Cancer* 2020; **20**: e510-e515 [PMID: 32284305 DOI: 10.1016/j.clbc.2020.03.003]
13 **Tse T**, Knowles S, Bélec J, Caudrelier JM, Lock M, Brackstone M, Arnaout A. Consensus statement on tumour bed localization for radiation after oncoplastic breast surgery. *Curr Oncol* 2020; **27**: e326-e331 [PMID: 32669940 DOI: 10.3747/co.27.5977]
14 **Alço G**, Igdem S, Okkan S, Dincer M, Sarsenov D, Ilgun AS, Agacayak F, Elbüken F, Ercan T, Selamoglu D, Ozmen V. Replacement of the tumor bed following oncoplastic breast-conserving surgery with immediate latissimus dorsi mini-flap. *Mol Clin Oncol* 2016; **5**: 365-371 [PMID: 27699027 DOI: 10.3892/mco.2016.984]
15 **Pu LL**. Reconstruction of a large gluteal soft-tissue defect with the double-opposing V-Y fasciocutaneous advancement flap. *Plast Reconstr Surg* 2007; **119**: 599-603 [PMID: 17230096 DOI: 10.1097/01.prs.0000246508.21562.f1]
16 **Enneking WF**, Spanier SS, Goodman MA. A system for the surgical staging of musculoskeletal sarcoma. *Clin Orthop Relat Res* 1980; 106-120 [PMID: 7449206]
17 **Sampo M**, Tarkkanen M, Huuhtanen R, Tukiainen E, Böhling T, Blomqvist C. Impact of the smallest surgical margin on local control in soft tissue sarcoma. *Br J Surg* 2008; **95**: 237-243 [PMID: 17703500 DOI: 10.1002/bjs.5906]
18 **Gronchi A**, Miceli R, Fiore M, Collini P, Lozza L, Grosso F, Mariani L, Casali PG. Extremity soft tissue sarcoma: adding to the prognostic meaning of local failure. *Ann Surg Oncol* 2007; **14**: 1583-1590 [PMID: 17260106 DOI: 10.1245/s10434-006-9325-0]
19 **Wittekind C**, Compton CC, Greene FL, Sobin LH. TNM residual tumor classification revisited. *Cancer* 2002; **94**: 2511-2516 [PMID: 12015777 DOI: 10.1002/cncr.10492]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 November 24; 14(11): 479-503

DOI: 10.5306/wjco.v14.i11.479

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

Computational exploration of the significance of *COPS6* in cancer: Functional and clinical relevance across tumor types

Shi-Lin Wang, Guang-Zheng Zhuo, Li-Ping Wang, Xiang-Hu Jiang, Guo-Hong Liu, Yun-Bao Pan, Yi-Rong Li

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Martinou E, United Kingdom

Received: August 28, 2023
Peer-review started: August 28, 2023
First decision: September 14, 2023
Revised: September 20, 2023
Accepted: October 23, 2023
Article in press: October 23, 2023
Published online: November 24, 2023

Shi-Lin Wang, Guang-Zheng Zhuo, Li-Ping Wang, Xiang-Hu Jiang, Yun-Bao Pan, Yi-Rong Li, Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, Hubei Province, China

Guo-Hong Liu, Department of Radiology, Zhongnan Hospital of Wuhan University, Wuhan 430071, Hubei Province, China

Corresponding author: Yi-Rong Li, Professor, Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, No. 169 Donghu Road, Wuchang District, Wuhan 430071, Hubei Province, China. liyirong2021@126.com

Abstract

BACKGROUND

The COP9 signalosome subunit 6 (*COPS6*) has been implicated in cancer progression, while its precise role in most types of cancer remains elusive.

AIM

To investigate the functional and clinical relevance of *COPS6* across various tumor types using publicly available databases.

METHODS

We used R software and online analysis databases to analyze the differential expression, prognosis, mutation and related functions of *COPS6* in pan-cancer.

RESULTS

Differential expression analysis and survival analysis demonstrated that *COPS6* was highly expressed and associated with high-risk profiles in the majority of cancer types. Possible associations between *COPS6* expression level and prognostic outcomes were found using data from public databases. Mutational analysis revealed that missense mutations were the predominant type of *COPS6* mutation. Additionally, positive correlations were identified between *COPS6* expression level and tumor mutational burden and microsatellite instability in most types of cancer. Immune infiltration analysis demonstrated a negative correlation between *COPS6* expression level and CD8+ T cell infiltration in certain types of cancer. The correlation between *COPS6* expression level and cancer-associated fibroblast infiltration exhibited heterogeneity, in which a positive correlation was found in head and neck squamous cell carcinoma and tenosynovial giant cell tumor, and a negative correlation was identified in diffuse large

B-cell lymphoma and thymoma. The correlation between *COPS6* expression level and macrophage infiltration was closely related to macrophage type. Gene co-expression and enrichment analysis highlighted transcription elongation factor B polypeptide 2 and G protein pathway suppressor 1 were significantly and positively associated with *COPS6* expression level. These genes were predominantly involved in processes, such as ubiquitin-mediated proteolysis and human immunodeficiency virus 1 infection.

CONCLUSION

In conclusion, this study systematically explored the significance of *COPS6* across different tumor types, providing a solid foundation for considering *COPS6* as a novel biomarker in cancer research.

Key Words: *COPS6*; Biomarker; Tumor mutational burden; Immune infiltration; Prognostic analysis

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The COP9 signalosome subunit 6 *COPS6* has been implicated in several cancer types. However, its precise role in most cancer types remains poorly understood. Therefore, we aimed to investigate the function of *COPS6* in various tumor types. Through our analysis, we discovered that *COPS6* is highly expressed and associated with high-risk profiles in most cancers. Meanwhile, *COPS6* expression was positively correlated with tumor mutation burden, microsatellite instability, and immune infiltration of the tumor microenvironment. Our findings suggest that *COPS6* could be a potential biomarker for cancer research. Our study contributes to the understanding of the role of *COPS6* in cancer progression and highlights the clinical applications.

Citation: Wang SL, Zhuo GZ, Wang LP, Jiang XH, Liu GH, Pan YB, Li YR. Computational exploration of the significance of *COPS6* in cancer: Functional and clinical relevance across tumor types. *World J Clin Oncol* 2023; 14(11): 479-503
URL: https://www.wjgnet.com/2218-4333/full/v14/i11/479.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i11.479

INTRODUCTION

According to recent data from the American Cancer Society, it is projected that the United States will witness 1958310 new cancer cases and 609820 cancer-related deaths by 2023[1]. While there has been a 1.5% decrease in cancer mortality rates between 2019 and 2020, with an overall decline of 33% since 1991, the incidence of breast cancer, prostate cancer, and uterine cancer is on the rise, imposing potential challenges to the future progress. Furthermore, these types of cancer demonstrate significant disparities in mortality rates among different ethnic groups. Conversely, gastric cancer, esophageal cancer, and cervical cancer have shown downward mortality rates, while lung cancer, colorectal cancer (CRC), and female breast cancer continue to exhibit gradually upward mortality rates[2,3]. These trends underscore the persisting challenges caused by cancer. Hence, it is imperative to explore new targets for early diagnosis and personalized treatment, as emphasized by previous studies[4,5]. The identification and analysis of novel pan-cancer genes can provide valuable insights into the intricate process of tumorigenesis. Public databases and online analysis tools, such as The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) provide convenient access to comprehensive cancer-related functional genomics datasets across diverse cancer types, enabling in-depth pan-cancer analysis[6,7].

The COP9 signalosome (COPS) is a multiprotein complex involved in protein degradation, transcriptional activation, signal transduction, and tumor progression[8,9]. *COPS6*, together with its dimerization partner *COPS5*, plays a crucial role in the activation process of deneddylase activity by embedding into the core of the helical bundle[10]. While the literature has reported the mechanisms of *COPS6* in human malignancies, such as cervical cancer, papillary thyroid carcinoma (THCA), CRC, breast cancer, lung adenocarcinoma (LUAD), and glioblastoma, the available information is incomplete and the underlying mechanisms remain to be fully elucidated[11].

The present study aimed to comprehensively elucidate the involvement and clinical implications of *COPS6* in many diverse types of cancer. This involved an in-depth analysis of differential expression patterns, prognostic values, gene mutations, immune infiltration, correlation analysis, and functional enrichment assessment, utilizing publicly available databases.

MATERIALS AND METHODS

Differential expression analysis

GEPIA2 (http://gepia2.cancer-pku.cn/#degenes) was utilized for analysis of RNA-seq expression data collected from TCGA and Genotype-Tissue Expression (GTEx) projects, enabling differential analysis, correlation analysis, and survival analysis[12]. Genetic difference analysis was conducted using TIMER2 (http://timer.cistrome.org/)[13]. *COPS6*

expression level in tumor and normal samples was compared using R (ver. 4.0.3), TIMER2, and GEPIA2. Box plots were generated using the ggpubr R (ver. 4.0.3) package, and differential expression of *COPS6* in TCGA samples was determined using the Wilcoxon test. For cancers lacking normal controls in the TCGA database, the TIMER2 website was employed for differential analysis of the *COPS6* gene.

For protein analysis, UALCAN online portal (http://ualcan.path.uab.edu/analysis-prot.html) was utilized to examine gene, protein, methylation, and phosphorylation differences[14]. UALCAN facilitated the comparison of differential expression of *COPS6* protein between tumor and normal tissues. Age-differential expression data was obtained using the limma and ggpubr R packages, while clinical stage differential expression of *COPS6* was obtained from the GEPIA2 website.

Survival analysis

To perform survival analysis for *COPS6*, the "Survival Map" feature of GEPIA2 was utilized. This facilitated plotting heatmaps representing overall survival (OS) and disease-free survival (DFS) using data from TCGA database. Forest plots, encompassing OS, progression-free interval (PFI), disease-specific survival (DSS), and disease-free interval (DFI), were generated using the survival and forestplot R packages in association with Cox analysis.

In March 2022, the pan-cancer data were downloaded from the TCGA database, including tumor stage, tumor grade, survival time, and mutation information. The raw data were preprocessed by the R programming language. The survminer R package was utilized to generate Kaplan-Meier survival curves for OS, PFI, DSS, and DFI, with a significance level set at $P < 0.05$.

Genetic alteration analysis

For mutation analysis, cBioPortal (https://www.cbioportal.org/) was utilized[15]. In this study, the "Quick Search" feature of cBioPortal was employed to examine the mutation frequency, type, copy number alteration (CNA), and structural variants of TCGA tumors involving *COPS6*. Furthermore, information related to the specific mutation sites and three-dimensional (3D) structure of the *COPS6* protein was collected. To assess the impact of *COPS6* alterations on patient survival, "TCGA, PanCancer Atlas" and "Compassion/Survival" modes were utilized to plot OS, DSS, DFS, and progression-free survival curves for TCGA cases with and without *COPS6* alterations, respectively, using the log-rank test.

Immune infiltration analysis

To investigate immune infiltration, TIMER2 web server was used. In the present study, the presence of CD8+ T cells, cancer-associated fibroblasts, natural killer (NK) cells, and macrophages was assessed using the "Immune" module. To explore the relationship between immune inflammatory cells and *COPS6* expression, multiple algorithms were utilized, including XCELL, EPIC, TIMER, MCPCOUNTER, CIBERSORT-ABS, TIDE, CIBERSORT, and QUANTISEQ. *P* values and partial correlation values were obtained using the purity-adjusted Spearman's rank correlation test to quantify the strength and significance of the observed correlations.

Enrichment analysis and correlation analysis

The "Similar Gene Detection" module on the GEPIA2 website was utilized to identify the top 100 genes correlated with *COPS6*. Further analysis using the "correlation analysis" module on the same website narrowed down the selection to the top 5 genes with the highest correlation coefficients. Heatmap analysis of these genes was conducted using the "Gene_Corr" module available on the TIMER2 website. The purity-adjusted Spearman's rank correlation test was applied to obtain P-values and partial correlation values.

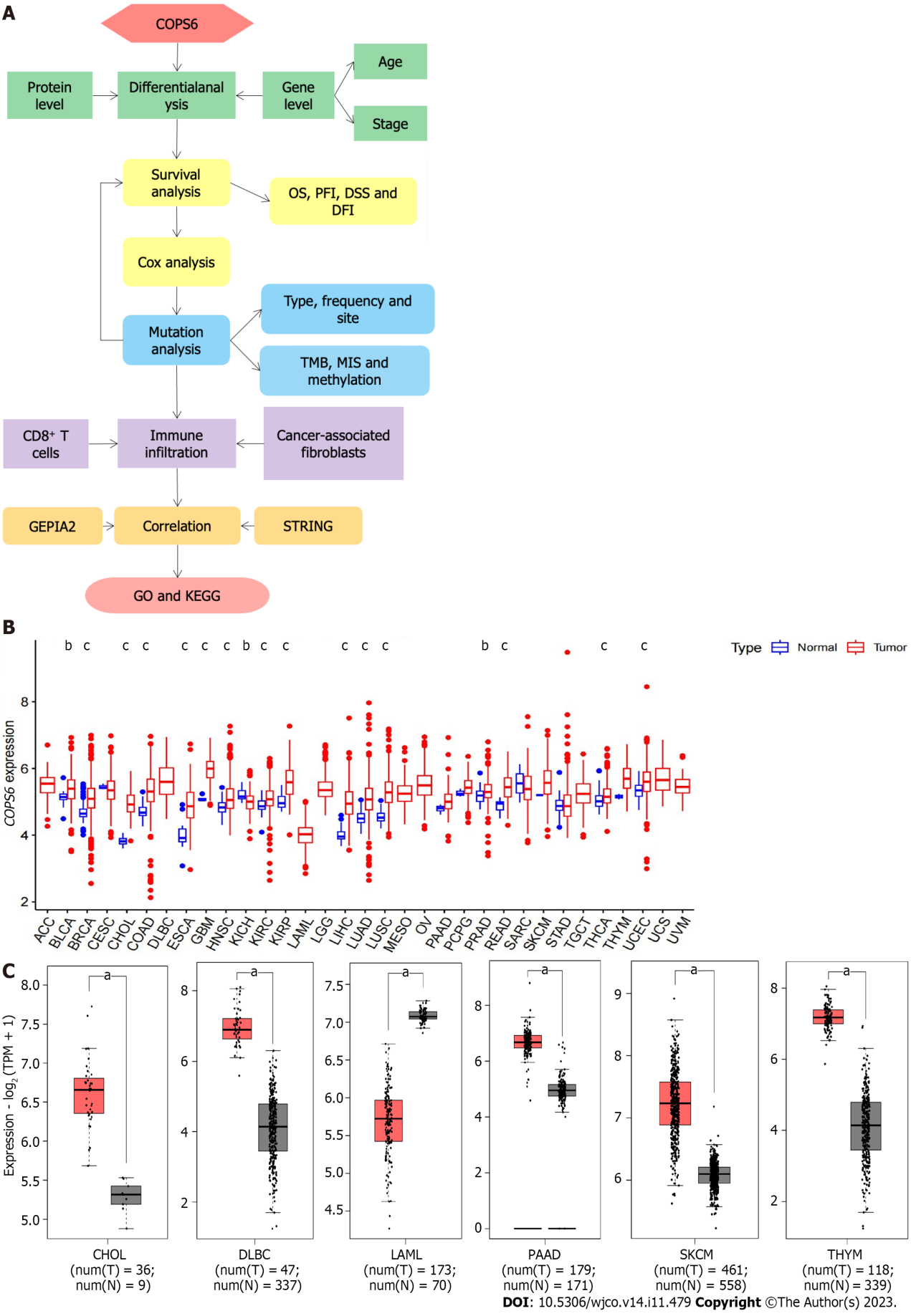
For protein-protein interaction (PPI) analysis, the STRING database (https://string-db.org/) was employed[16]. *COPS6* was submitted to the database to generate a PPI network with *Homo sapiens* as the reference organism. The network settings included a full network type, evidence-based network edges, experiments as active interaction sources, a minimum required interaction score of high confidence (0.7), and a maximum number of interactors shown in the 1st and 2nd shells.

To identify overlapping genes between the *COPS6*-related genes obtained from the GEPIA2 and STRING, a Venn diagram was generated using GraphPad Prism 9.0.0 software (GraphPad Software Inc., San Diego, CA, United States). The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the DAVID database (https://david.ncifcrf.gov/home.jsp) with involvement of parameters, such as "OFFICIAL_GENE_SYMBOL", "Homo sapiens", and "functional annotation chart".

RESULTS

COPS6 expression level varied in various tumors

The overview of the pan-cancer analysis workflow is shown in Figure 1A. Differential expression analysis of *COPS6* was conducted on TCGA data using R programming language. Significant differential expression ($P < 0.05$) of *COPS6* was found between normal and tumor tissues in several cancer types, including bladder cancer (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC),



DOI: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

Figure 1 Differential expression analysis of *COP9 signalosome subunit 6* in pan-cancer. A: Overview of the pan-cancer analysis workflow; B:

Differential expression analysis of *COP9 signalosome subunit 6 (COPS6)* in the The Cancer Genome Atlas (TCGA) database using the Wilcoxon test in R software; C: Differential expression analysis of *COPS6* in matched TCGA normal and Genotype-Tissue Expression data using the GEPIA2 website with specific cutoff criteria. [b] *P* 0.01 *P* 0.001. CHOL: Cholangiocarcinoma; DFI: Disease-free interval; DLBC: Diffuse large B-cell; DSS: Disease-specific survival; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; LAML: Acute myeloid leukemia; MIS: Microsatellite instability; OS: Overall survival; PAAD: Pancreatic adenocarcinoma; PFI: Progression-free interval; SKCM: Skin cutaneous melanoma; THYM: Thymoma; TMB: Tumor mutational burden.

LUAD, lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), THCA, and uterine corpus endometrial carcinoma (UCEC) (Figure 1B). Differential expression analysis results of *COPS6* in these tumors and normal samples were obtained from the GEPIA2 website. Furthermore, *COPS6* expression level exhibited significant differences in skin cutaneous melanoma (SKCM), pancreatic adenocarcinoma (PAAD), thymoma (THYM), diffuse large B-cell lymphoma (DLBCL), acute myeloid leukemia (LAML), and CHOL (Figure 1C) compared with TCGA normal samples using GTEx data.

To compare protein expression level of *COPS6* among multiple types of cancer, protein expression differences between tumor and normal tissues from the CPTAC database on the UALCAN website were compared. The results revealed the elevated expression level of *COPS6* in hepatocellular carcinoma and clear cell renal cell carcinoma tissues (Figure 2A). Additionally, the relationship between *COPS6* expression level and clinical parameters was investigated using the GEPIA2 website, indicating the presence of association between *COPS6* expression level and clinical stages of LUAD, KICH, KIRP, and LIHC ($P < 0.05$) (Figure 2B). Furthermore, significantly upregulated *COPS6* expression level in ESCA, LUAD, and LUSC in cases who aged < 65-years-old in TCGA database was found, whereas *COPS6* expression level was reduced in KIRC ($P < 0.05$) (Figure 2C).

COPS6 expression level was associated with the prognosis of patients with diverse types of cancer

To assess the relationship between *COPS6* expression level and patient prognosis across various tumors, patients were divided into high and low *COPS6* expression groups based on the median *COPS6* expression level. Utilizing the GEPIA2 website, it was revealed that high expression level of *COPS6* was significantly associated with poor OS in GBM ($P = 0.017$), KICH ($P = 0.031$), mesothelioma (MESO) ($P = 0.0026$), lower grade glioma (LGG) ($P = 0.007$), LIHC ($P = 0.011$), and LUAD ($P = 0.018$) (Figure 3A). Conversely, low expression level of *COPS6* was correlated with poor DFS in KIRP ($P = 0.042$), while high expression level of *COPS6* was associated with poor DFS in LGG ($P = 0.0013$), LIHC ($P = 0.024$), adrenocortical carcinoma (ACC) ($P = 0.034$), KIRC ($P = 0.009$), MESO ($P = 0.0027$), and stomach adenocarcinoma (STAD) ($P = 0.049$) (Figure 3B). Cox regression analysis indicated that *COPS6* was a high-risk gene for OS in HNSC, KICH, KIRC, LGG, LIHC, and MESO ($P < 0.05$), while it was appeared as a low-risk gene for OS in BRCA ($P < 0.05$). Additionally, *COPS6* was identified as a high-risk gene for DSS in KICH, KIRC, LGG, MESO, and READ ($P < 0.05$), as well as a low-risk gene for DSS in BRCA ($P < 0.05$). Moreover, *COPS6* was found as a high-risk gene for DFI in ACC, LGG, LIHC, and STAD ($P < 0.001$), as well as a high-risk gene for PFI in KICH, KIRC, LGG, MESO, and STAD ($P < 0.05$), while a low-risk gene for PFI in BRCA ($P < 0.05$). These findings were derived from TCGA database using the survival and forestplot R package (Figure 3C). The association between *COPS6* expression level and OS (Figure 4A), PFI (Figure 4B), DSS (Figure 4C), and DFI (Figure 4D) was further confirmed through Kaplan-Meier survival analysis in pan-cancer patients from TCGA database.

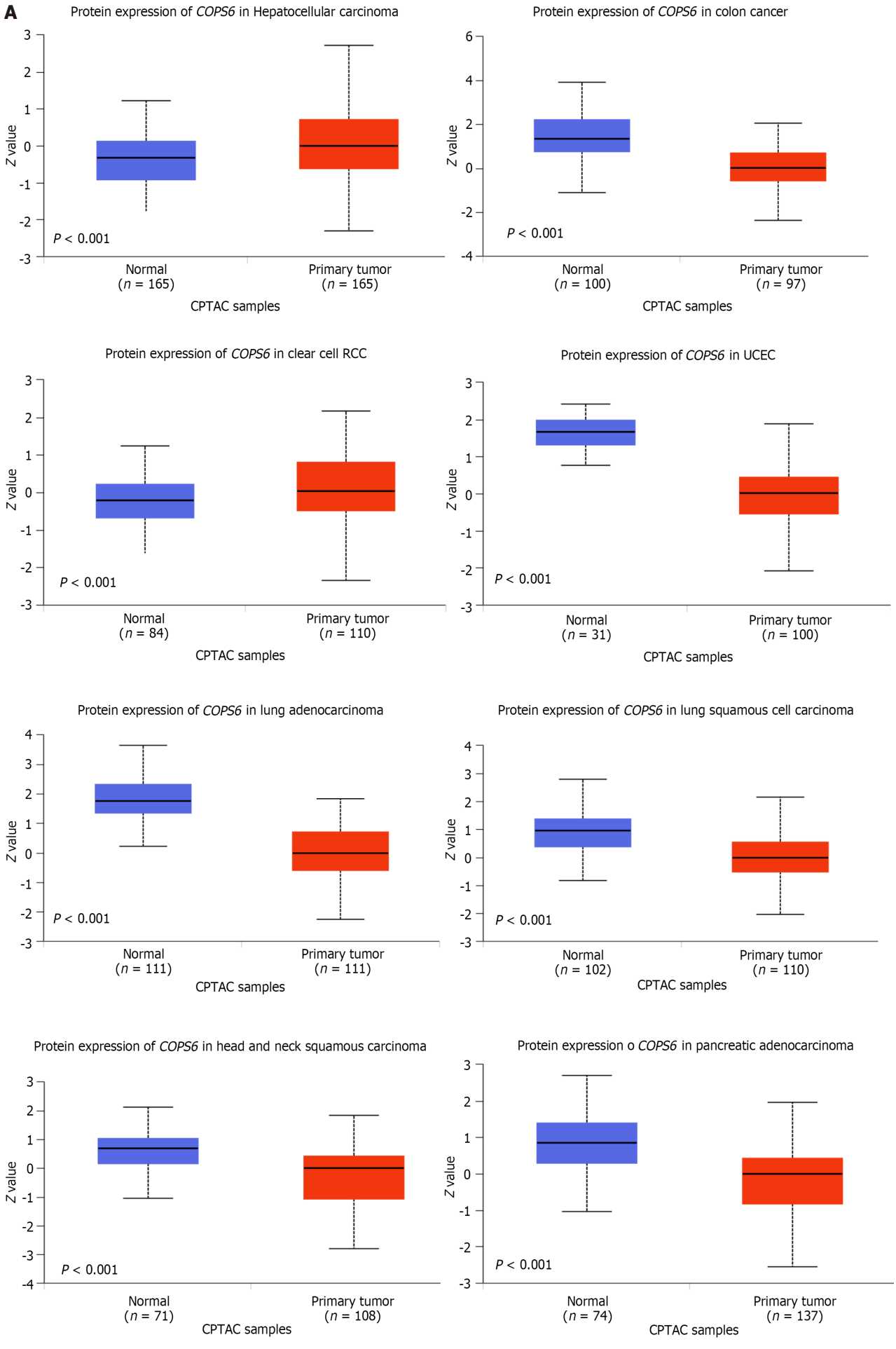
Correlation between COPS6 mutation and tumor progression

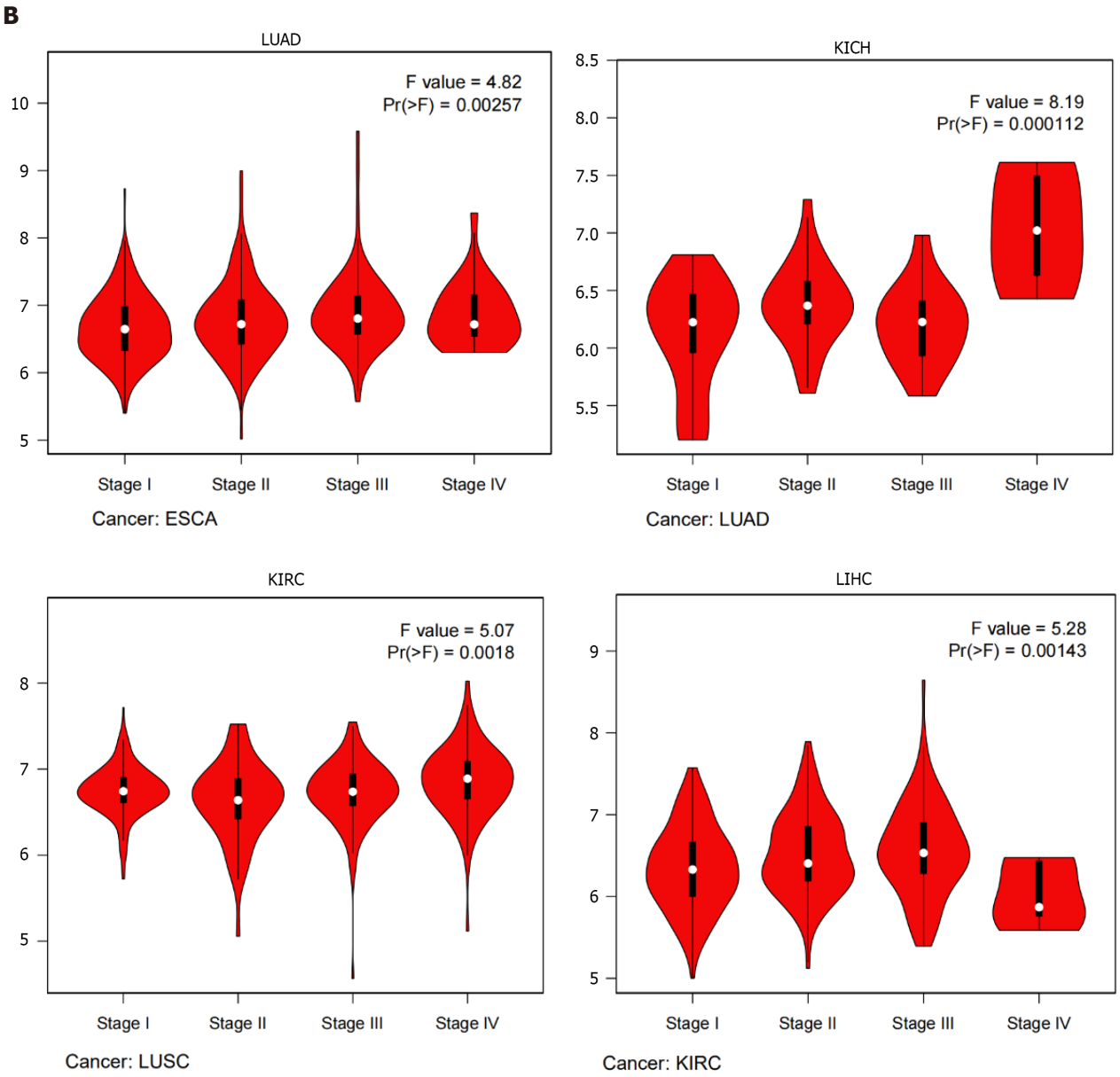
Using the cBioPortal website, comprehensive information was obtained regarding the mutation types, frequency, CNAs, and structural variants of *COPS6* across all TCGA tumors. Missense mutations were identified as the predominant mutation type. Among all TCGA tumors, the highest frequency of variations was found in esophageal adenocarcinoma (9.89%), with amplification being the most frequent alteration (9.34%) (Figure 5A). A 3D representation of the *COPS6* protein (Figure 5B) was constructed, revealing a notable mutation site, R197C/H, observed in one case each of adrenocortical carcinoma and endometrioid carcinoma (Figure 5C). Investigation of the relationship between *COPS6* mutations and prognosis in TCGA cases revealed no significant impact of mutation status on the prognosis of all types of cancer (Figure 5D).

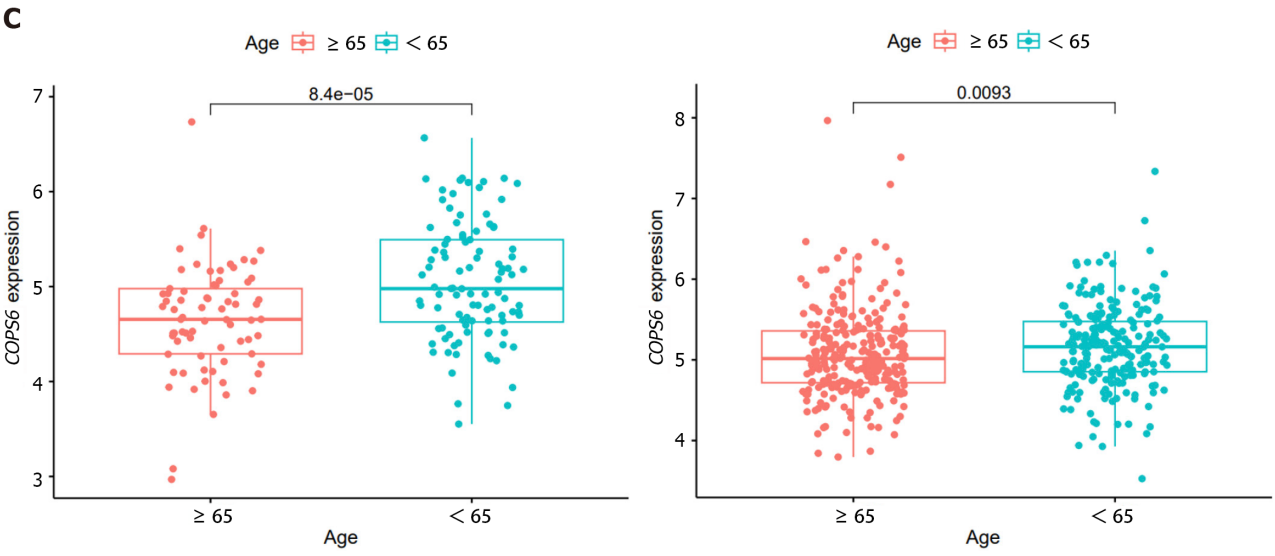
Furthermore, the correlations between *COPS6* expression level and tumor mutational burden (TMB) and microsatellite instability (MSI) were analyzed. Positive correlations were identified between *COPS6* expression level and TMB in LUAD, KIRP, LUSC, HNSC, PAAD, KICH, LIHC, KIRC, UCEC, LGG, BRCA, and PRAD ($P < 0.05$), while negative correlations were found in THYM, COAD, ESCA, and LAML ($P < 0.05$) (Figure 5E). Positive associations between *COPS6* expression level and MSI were observed in BRCA, USC, THCA, SKCM, SARC, PRAD, PAAD, KIRP, KIRC, HNSC, DLBC, and LIHC ($P < 0.05$), with a positive association observed in COAD ($P < 0.05$) (Figure 5F). Additionally, comparison of the *COPS6* promoter methylation level between normal and tumor samples revealed a higher methylation level in the tumor group in PRAD, LUSC, HNSC, BRCA, and KIRC, whereas a lower methylation level in BLCA (Figure 5G).

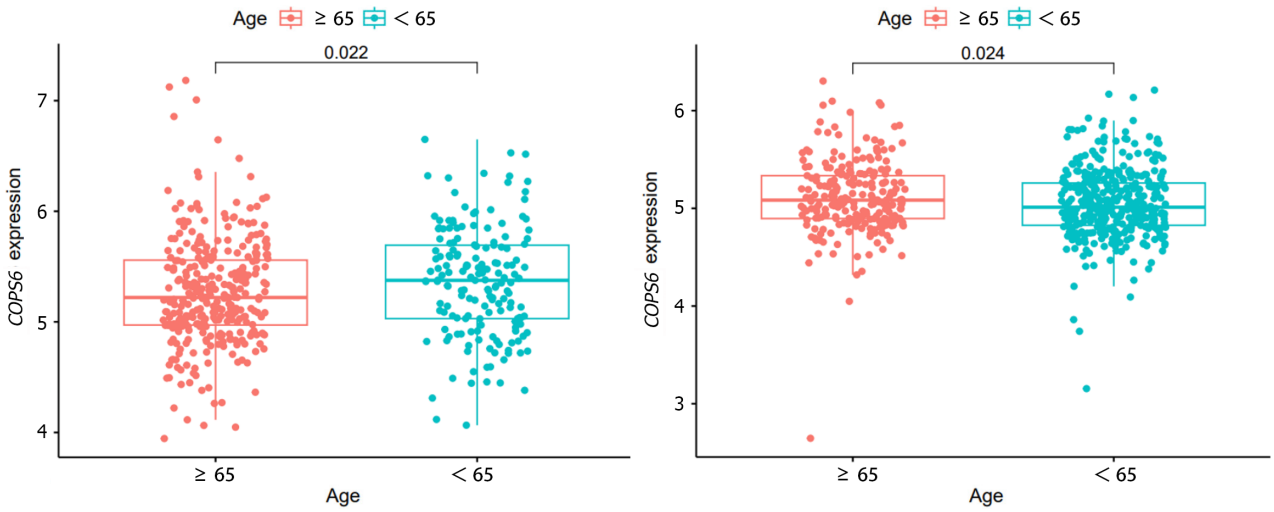
Correlation between COPS6 expression level and immune infiltration

The relationship between *COPS6* expression level and immune-infiltrating cells in TCGA tumors was examined using the TIMER2 website. Across multiple algorithms, a negative correlation was found between *COPS6* expression level and CD8+ T cell infiltration in BRCA-LumA, HNSC, HNSC-HPV-, SKCM, SKCM-metastasis, and tenosynovial giant cell tumor (TGCT) (Figure 6A and B). Conversely, the correlation between cancer-associated fibroblast infiltration and *COPS6* expression level exhibited heterogeneity. Negative correlations were identified in DLBCL, OV, SARC, THYM, and THCA,


A
Protein expression of COPS6 in Hepatocellular carcinoma
Z value
P < 0.001
Normal (n = 165)
Primary tumor (n = 165)
CPTAC samples
Protein expression of COPS6 in colon cancer
Z value
P < 0.001
Normal (n = 100)
Primary tumor (n = 97)
CPTAC samples
Protein expression of COPS6 in clear cell RCC
Z value
P < 0.001
Normal (n = 84)
Primary tumor (n = 110)
CPTAC samples
Protein expression of COPS6 in UCEC
Z value
P < 0.001
Normal (n = 31)
Primary tumor (n = 100)
CPTAC samples
Protein expression of COPS6 in lung adenocarcinoma
Z value
P < 0.001
Normal (n = 111)
Primary tumor (n = 111)
CPTAC samples
Protein expression of COPS6 in lung squamous cell carcinoma
Z value
P < 0.001
Normal (n = 102)
Primary tumor (n = 110)
CPTAC samples
Protein expression of COPS6 in head and neck squamous carcinoma
Z value
P < 0.001
Normal (n = 71)
Primary tumor (n = 108)
CPTAC samples
Protein expression o COPS6 in pancreatic adenocarcinoma
Z value
P < 0.001
Normal (n = 74)
Primary tumor (n = 137)
CPTAC samples


B
LUAD
F value = 4.82
Pr(>F) = 0.00257
Stage I
Stage II
Stage III
Stage IV
Cancer: ESCA
KICH
F value = 8.19
Pr(>F) = 0.000112
Cancer: LUAD
KIRC
F value = 5.07
Pr(>F) = 0.0018
Cancer: LUSC
LIHC
F value = 5.28
Pr(>F) = 0.00143
Cancer: KIRC


C
Age ≥ 65 < 65
8.4e-05
COPS6 expression
≥ 65
< 65
Age
0.0093



DOI: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

Figure 2 Association between *COP9 signalosome subunit 6* and clinical parameters in pan-cancer. A: Differential analysis of *COP9 signalosome subunit 6 (COPS6)* protein expression in pan-cancers using the CPTAC database accessed through the UALCAN website; B: Relationship between *COPS6* expression and clinical stage analyzed with the GEPIA2 website; C: Correlation between *COPS6* expression and age using R software. KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma.

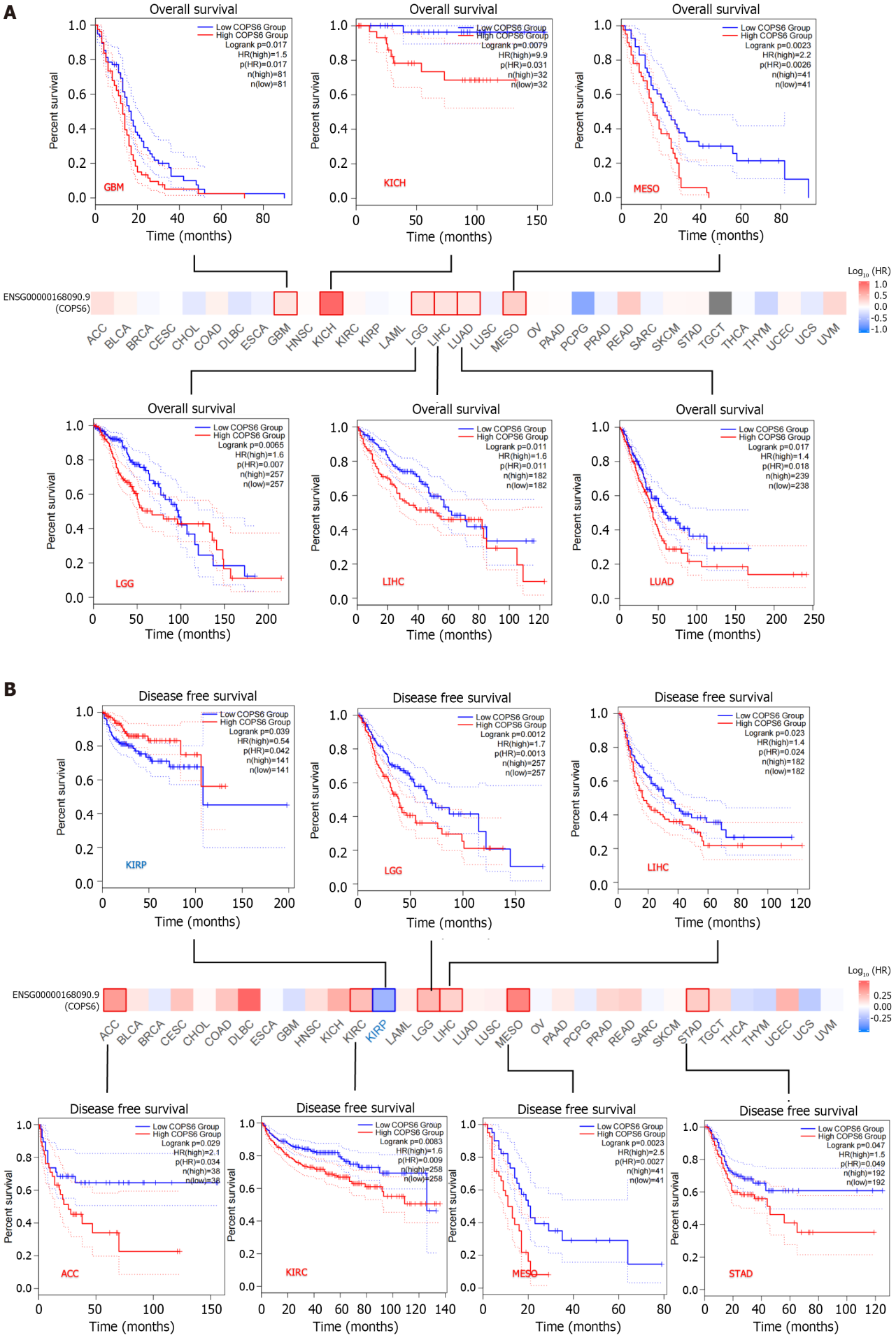
while positive correlations were found in HNSC, HNSC-HPV-, and TGCT (Figure 6C and D). The relationship between *COPS6* expression level and macrophage infiltration varied depending on macrophage subtype. An inverse association was detected between *COPS6* expression level and M1 macrophages, along with a positive association between *COPS6* expression level and M2 macrophages in certain tumors (Figure 6E and F). For instance, in DLBCL, four algorithms demonstrated a negative association between *COPS6* expression level and M1 macrophages, while TIDE algorithm revealed a positive association between *COPS6* expression level and M2 macrophages. Moreover, in BRCA, KIRC, THCA, and THYM, the TIDE algorithm indicated a positive correlation between *COPS6* expression level and M2 macrophages. In most of the tumors, NK cell infiltration exhibited a weak correlation with *COPS6* expression level, and clear associations were found only in a few tumors (Figure 6G and H). For instance, in THCA and THYM, *COPS6* expression level showed a negative correlation with NK cell infiltration. Further analysis of NK cell subtypes revealed a negative correlation between *COPS6* expression level and activated NK cell infiltration, as well as a positive correlation between *COPS6* expression level and resting NK cell infiltration.

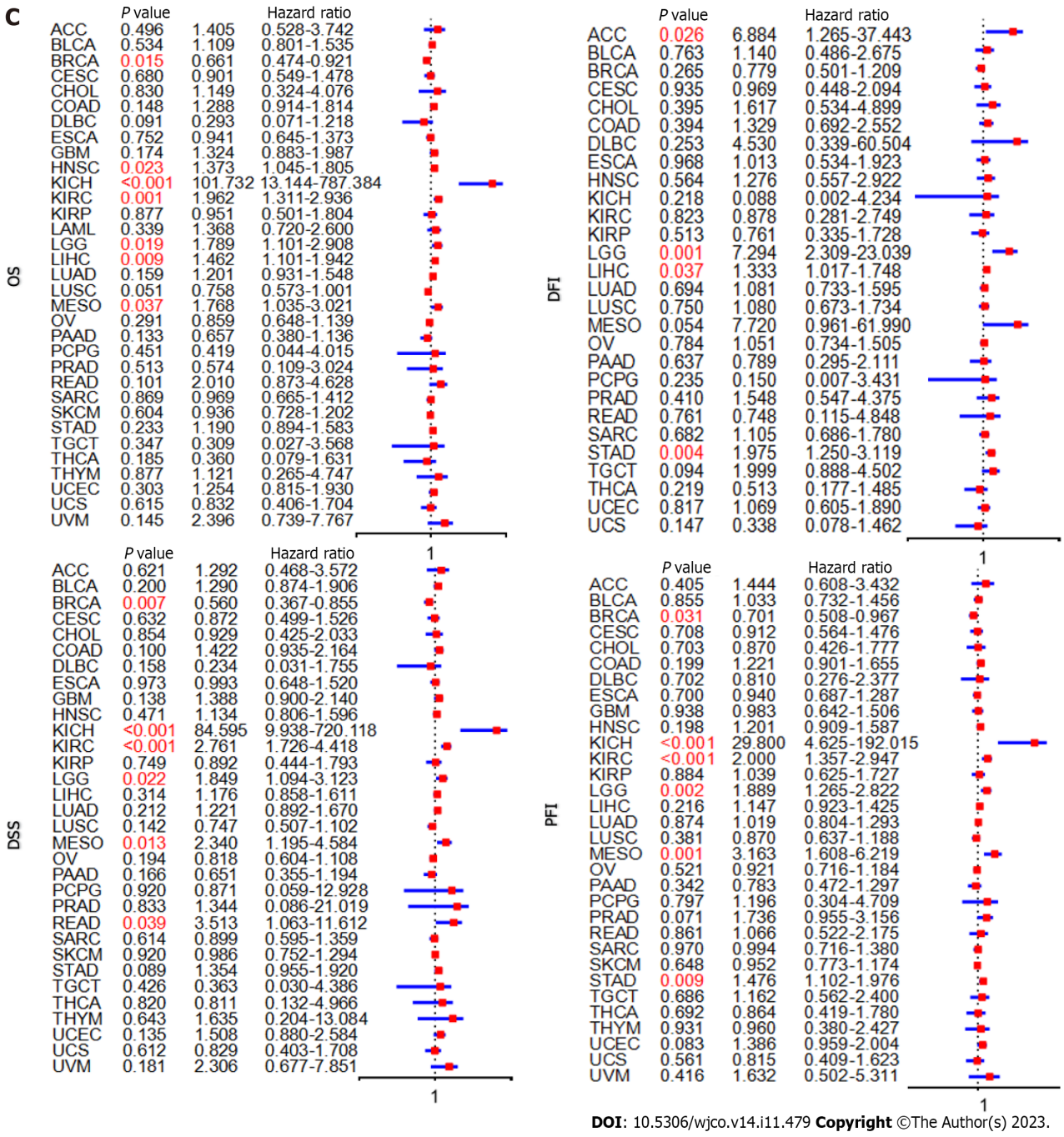
Enrichment analysis and correlation analysis of COPS6-associated genes

To gain deeper insights into the molecular mechanisms involving *COPS6* in growth and progression of tumors, the GEPIA2 website was employed to screen the top 100 *COPS6*-associated genes. Subsequently, the top 5 genes with the highest correlation coefficients were identified and summarized as follows: POLR2J ($r = 0.69$, $P < 0.001$), BUD31 ($r = 0.65$, $P < 0.001$), TAF6 ($r = 0.66$, $P < 0.001$), ALKBH4 ($r = 0.62$, $P < 0.001$), and POP7 ($r = 0.61$, $P < 0.001$) (Figure 7A and B). The PPI network analysis was performed using the STRING website, resulting in the establishment of a network of 35 node genes (Figure 7C). The intersection of *COPS6*-associated genes obtained from the GEPIA2 and STRING led to the identification of *GPS1* and *TCEB2* (Figure 7D). Furthermore, the genes derived from both databases were merged, resulting in the detection of a total of 135 *COPS6*-related genes. Subsequently, GO and KEGG pathway enrichment analyses were conducted (Figure 7E and F). The KEGG pathway analysis revealed that *COPS6*-associated genes were enriched in pathways, such as ubiquitin-mediated proteolysis, nucleotide excision repair, human immunodeficiency virus 1 infection, Parkinson's disease, and circadian rhythm. The GO enrichment analysis indicated enrichment in the proteasomal protein catabolic process, proteasome-mediated ubiquitin-dependent protein catabolic process, protein modification by small protein removal, intrinsic apoptotic signaling pathway, protein deneddylation, COPS, Cullin-RING ubiquitin ligase (CRL) complex, SCF ubiquitin ligase complex, Cul4A-RING E3 ubiquitin ligase complex, Cullin family protein binding, ubiquitin-protein transferase activity, and ubiquitin-like protein transferase activity.

DISCUSSION

The COP9 signalosome (CSN) is a complex protein composed of eight subunits (CSN1-CSN8), participating in various physiological processes. The CSN1, 2, 3, 4, 7, and 8 subunits contain a percutaneous coronary intervention domain, which acts as a scaffold in CSN assembly, while the *COPS6* and *COPS5* subunits possess an Mpr1-Pad 1-N-terminal (MPN) domain[17]. *COPS5* primarily exerts catalytic enzymatic activity, whereas *COPS6*, as an essential component of CSN, lacks the metal-binding site and isopeptidase activity associated with the *COPS5* MPN domain. The precise function of *COPS6* remains has still remained elusive[18]. *COPS6* is involved in various processes, including the ubiquitin proteasome system, signal transduction, DNA damage response, and tumor progression. It exhibits a high expression


A
Overall survival
Low COPS6 Group
High COPS6 Group
Logrank p=0.017
HR(high)=1.5
p(HR)=0.017
n(high)=81
n(low)=81
GBM
Overall survival
Logrank p=0.0079
HR(high)=9.9
p(HR)=0.031
n(high)=32
n(low)=32
KICH
Overall survival
Logrank p=0.0023
HR(high)=2.2
p(HR)=0.0026
n(high)=41
n(low)=41
MESO
Percent survival
Time (months)
ENSG00000168090.9
(COPS6)
Log10 (HR)
1.0
0.5
0.0
-0.5
-1.0
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KICH KIRC KIRP LAML LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA THYM UCEC UCS UVM
Overall survival
Logrank p=0.0065
HR(high)=1.6
p(HR)=0.007
n(high)=257
n(low)=257
LGG
Overall survival
Logrank p=0.011
HR(high)=1.6
p(HR)=0.011
n(high)=182
n(low)=182
LIHC
Overall survival
Logrank p=0.017
HR(high)=1.4
p(HR)=0.018
n(high)=239
n(low)=238
LUAD
B
Disease free survival
Logrank p=0.039
HR(high)=0.54
p(HR)=0.042
n(high)=141
n(low)=141
KIRP
Disease free survival
Logrank p=0.0012
HR(high)=1.7
p(HR)=0.0013
n(high)=257
n(low)=257
LGG
Disease free survival
Logrank p=0.023
HR(high)=1.4
p(HR)=0.024
n(high)=182
n(low)=182
LIHC
0.25
0.00
-0.25
Disease free survival
Logrank p=0.029
HR(high)=2.1
p(HR)=0.034
n(high)=38
n(low)=38
ACC
Disease free survival
Logrank p=0.0083
HR(high)=1.6
p(HR)=0.009
n(high)=258
n(low)=258
KIRC
Disease free survival
Logrank p=0.0023
HR(high)=2.5
p(HR)=0.0027
n(high)=41
n(low)=41
MESO
Disease free survival
Logrank p=0.047
HR(high)=1.5
p(HR)=0.049
n(high)=192
n(low)=192
STAD

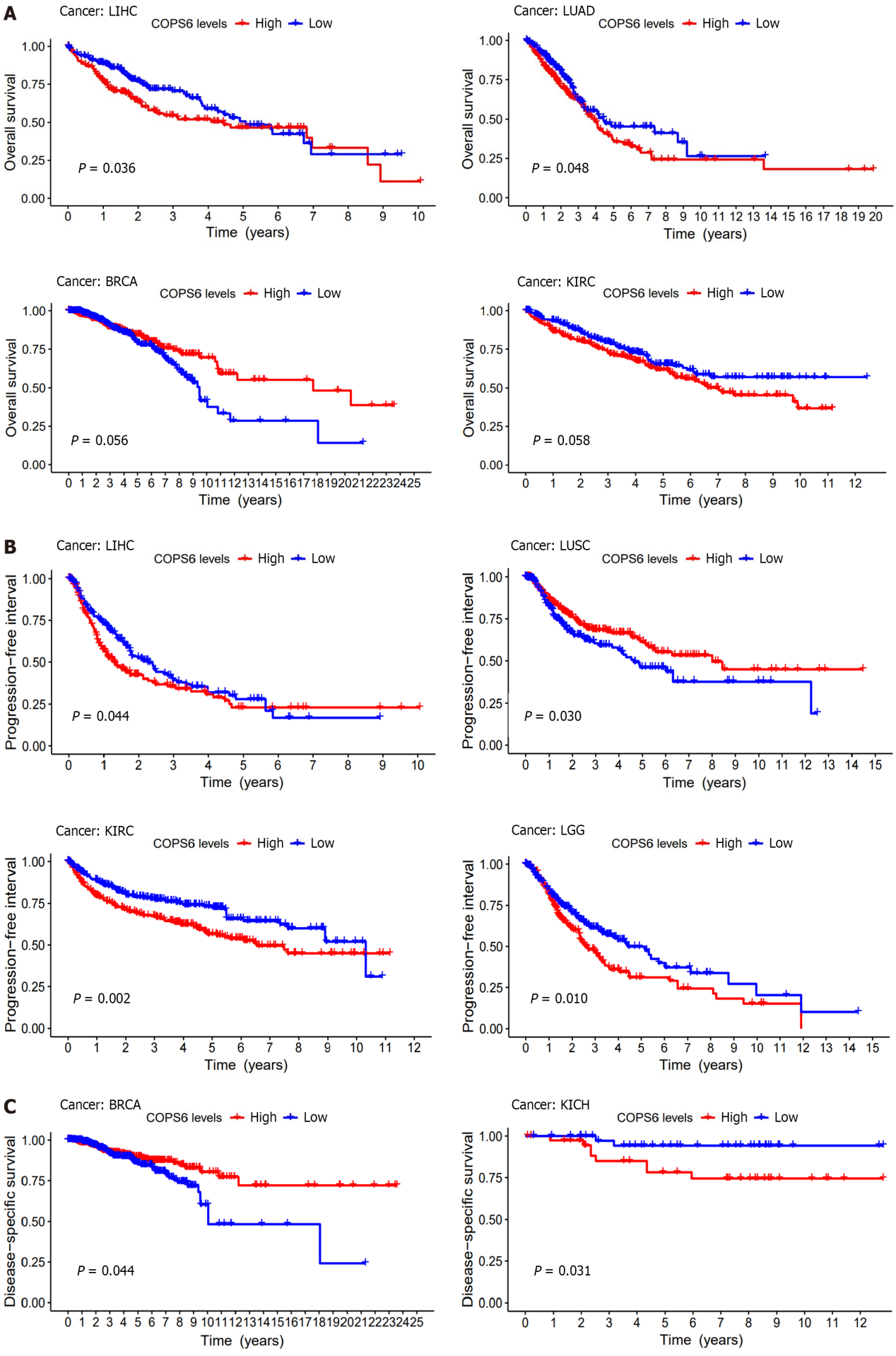


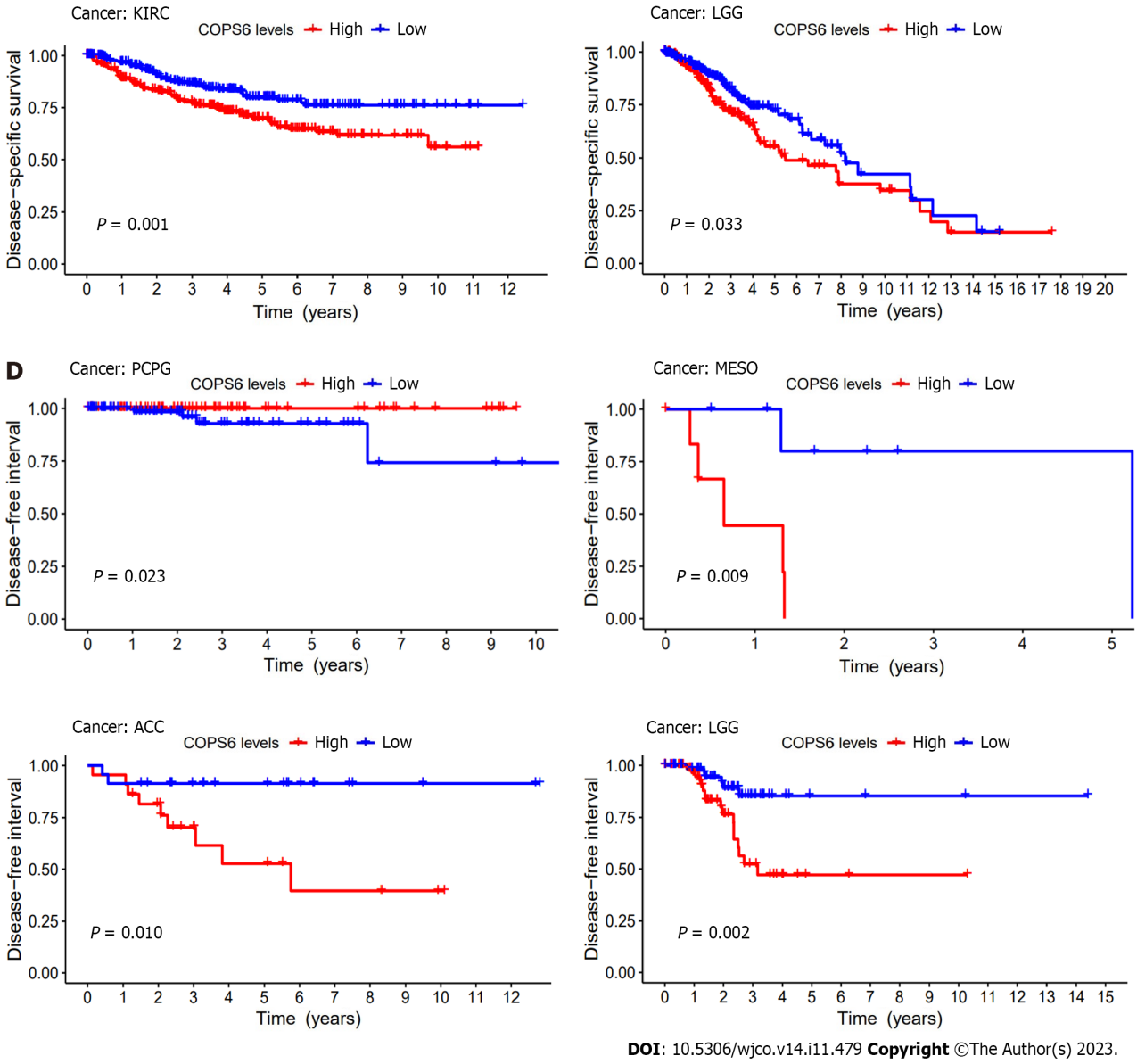
DOI: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

Figure 3 Survival analysis of *COP9 signalosome subunit 6*. A: Overall survival (OS) and disease-free survival analysis conducted with the GEPIA2 website using the log-rank test ($P < 0.05$); B: Forest plots illustrating disease-specific survival (DSS), OS, progression-free interval (PFI), and disease-free interval (DFI) analyzed with Cox analysis in R software. ACC: Adrenocortical carcinoma; GBM: Glioblastoma multiforme; KICH: Kidney chromophobe; KIRP: Kidney renal papillary cell carcinoma; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; MESO: Mesothelioma; STAD: Stomach adenocarcinoma.

level in diverse tumors, and studies have explored its role in cancer[19].

At present, there is a growing interest among researchers in investigating the role of *COPS6* in tumors, as it has been shown to predominantly promote cancer. The CRLs are involved in the ubiquitination of Myc, and Fbxw7, a CRL component, which participates in Myc ubiquitination. In mouse experiments, Chen *et al*[20] demonstrated that *COPS6* enhances Fbxw7 degradation through binding, thereby maintaining Myc stability and promoting tumor progression. Additionally, in a mouse model, Zhao *et al*[21] revealed that *COPS6* attenuates p53-mediated tumor suppression, promotes tumor growth by stabilizing MDM2 protein, and participates in DNA damage-associated apoptosis. In human tumors, *COPS6* also plays a significant role in tumor progression. Fang *et al*[22] demonstrated that *COPS6* overexpression in CRC is associated with a worse prognosis. Mechanistic studies suggested that ERK2 directly binds to CSN6 Leu163/Val165 and phosphorylates *COPS6* at Ser148, thereby regulating β-Trcp and stabilizing β-catenin expression, consequently blocking the ubiquitin-proteasome pathway and promoting CRC development.


A
Cancer: LIHC
COPS6 levels High Low
Overall survival
P = 0.036
Time (years)
Cancer: LUAD
COPS6 levels High Low
Overall survival
P = 0.048
Time (years)
Cancer: BRCA
COPS6 levels High Low
Overall survival
P = 0.056
Time (years)
Cancer: KIRC
COPS6 levels High Low
Overall survival
P = 0.058
Time (years)
B
Cancer: LIHC
COPS6 levels High Low
Progression−free interval
P = 0.044
Time (years)
Cancer: LUSC
COPS6 levels High Low
Progression−free interval
P = 0.030
Time (years)
Cancer: KIRC
COPS6 levels High Low
Progression−free interval
P = 0.002
Time (years)
Cancer: LGG
COPS6 levels High Low
Progression−free interval
P = 0.010
Time (years)
C
Cancer: BRCA
COPS6 levels High Low
Disease−specific survival
P = 0.044
Time (years)
Cancer: KICH
COPS6 levels High Low
Disease−specific survival
P = 0.031
Time (years)

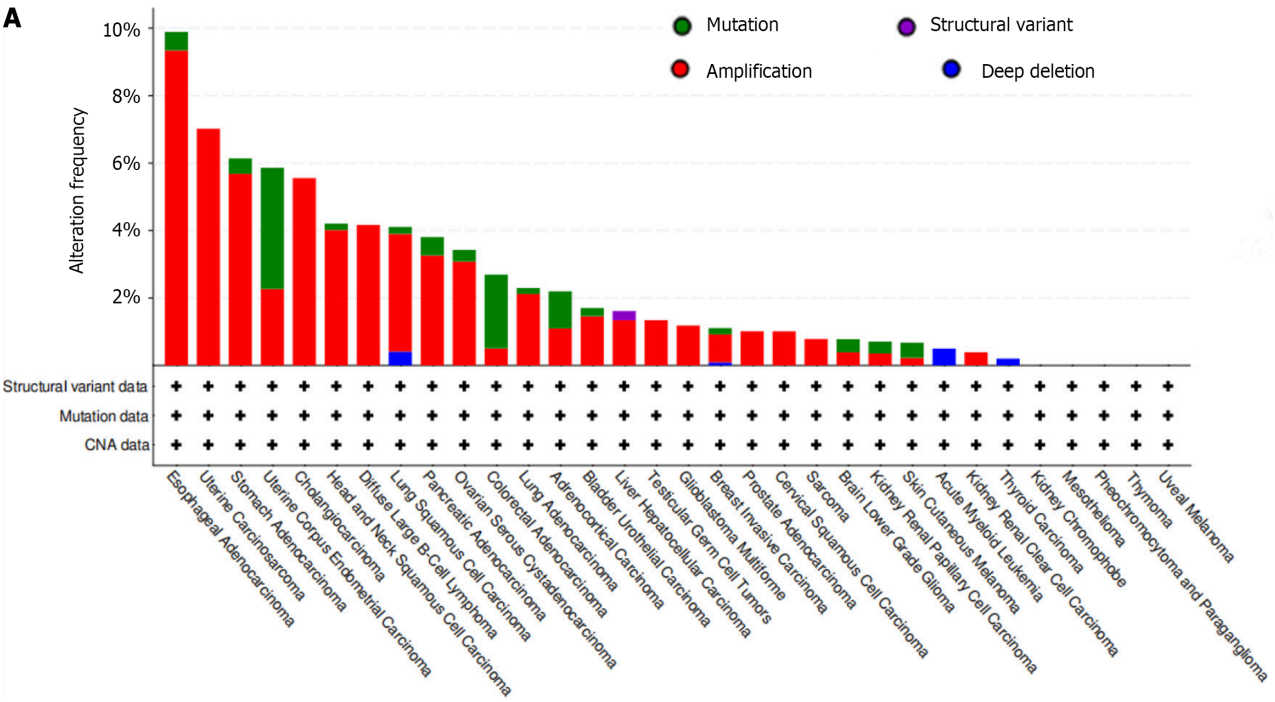


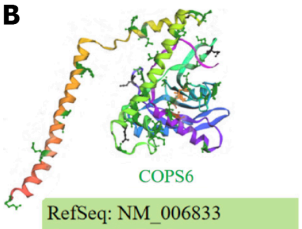
DOI: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

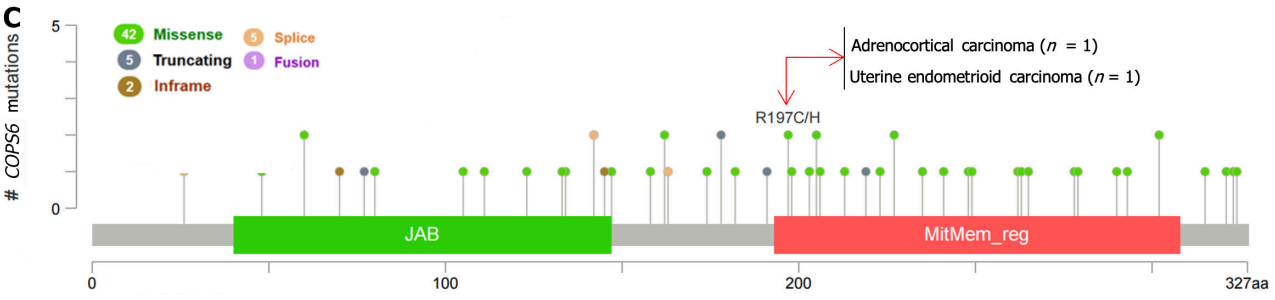
Figure 4 Kaplan-Meier survival analysis of *COP9 signalosome subunit 6* in the The Cancer Genome Atlas database performed in R software. A: Overall survival analysis. B: Progression-free interval analysis; C: Disease-specific survival analysis; D: Disease-free interval analysis. ACC: Adrenocortical carcinoma; BRCA: Breast invasive carcinoma; KIRC: Kidney renal clear cell carcinoma; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma.

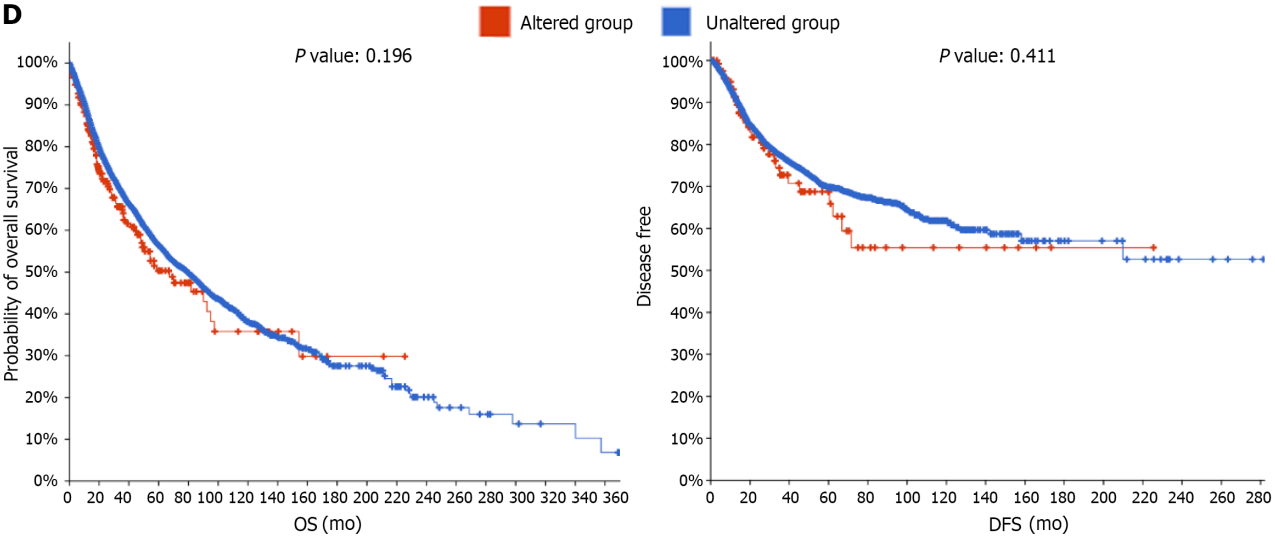
Programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) checkpoint blockade is an emerging immunotherapy modality in various tumors, while its regulatory mechanism remains uncertain. Su *et al*[23] demonstrated that *COPS6* expression level could be regulated by the EGFR-ERK pathway, inhibiting PD-L1 degradation and maintaining PD-L1 stability in GBM. Additionally, several studies have reported the involvement of *COPS6* in the epithelial-mesenchymal transition process in various tumors, promoting tumor invasion and metastasis. For instance, Zhang *et al*[24] revealed that the COPS6-UBR5-CDK9 axis could control melanoma proliferation and metastasis, while Mao *et al*[25] found that *COPS6* could promote migration and invasion of cervical cancer cells by regulating the expression level of cathepsin L through the autophagy-lysosomal system. Furthermore, *COPS6* was found to maintain the key transcription factor Snail1, promoting the invasion of breast cancer cells by inhibiting Snail1 ubiquitination[26].

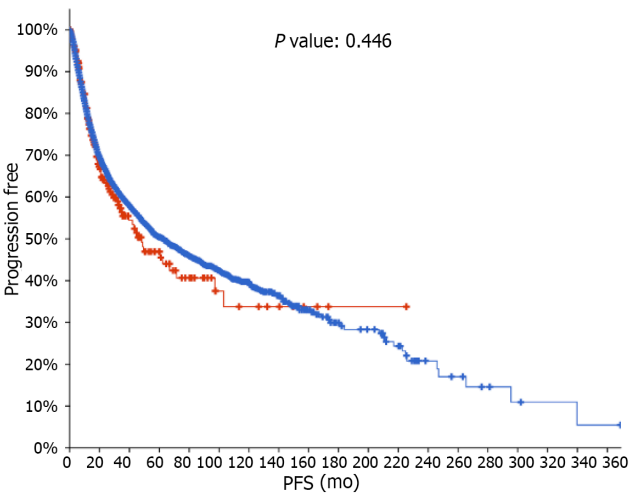
While previous studies have highlighted the significant role of *COPS6* in the progression of specific tumors, the heterogeneity of tumors suggests potential variations in its function across different cancer types. Therefore, a comprehensive analysis and screening are necessary to validate existing findings and provide direction for the future *COPS6*-related studies. In the present study, it was attempted to conduct comprehensive multilevel differential analysis and survival analysis of *COPS6* in pan-cancer data collected from various public databases and online analysis tools, including TCGA, GEO, CPTAC, GEPIA2, TIMER2, and UALCAN. The findings demonstrated that the expression level of the *COPS6* gene was significantly upregulated in the most types of cancer compared with normal tissues, except for KICH and LAML. Prognostic analysis revealed that the high expression level of *COPS6* was typically associated with worse prognosis in the majority of tumors, while showing a favorable prognosis in KIRP, BRCA, LUSC, and PCPG. Mutational analysis

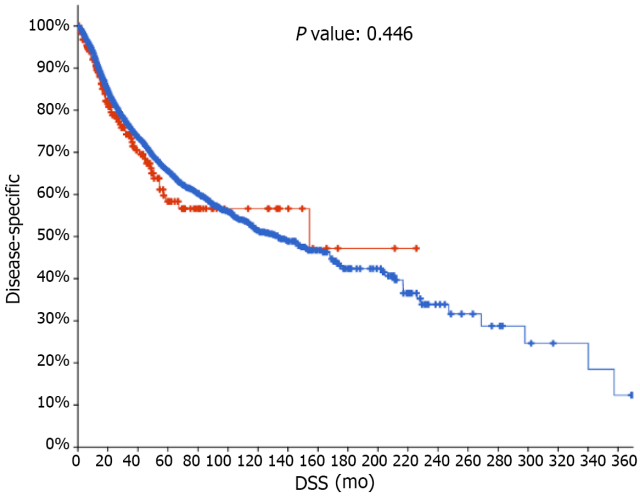

A
Mutation
Structural variant
Amplification
Deep deletion
Alteration frequency
10%
8%
6%
4%
2%
Structural variant data
Mutation data
CNA data
Esophageal Adenocarcinoma
Uterine Carcinosarcoma
Stomach Adenocarcinoma
Uterine Corpus Endometrial Carcinoma
Cholangiocarcinoma
Head and Neck Squamous Cell Carcinoma
Diffuse Large B-Cell Lymphoma
Lung Squamous Cell Carcinoma
Pancreatic Adenocarcinoma
Ovarian Serous Cystadenocarcinoma
Colorectal Adenocarcinoma
Lung Adenocarcinoma
Adrenocortical Carcinoma
Bladder Urothelial Carcinoma
Liver Hepatocellular Carcinoma
Testicular Germ Cell Tumors
Glioblastoma Multiforme
Breast Invasive Carcinoma
Prostate Adenocarcinoma
Cervical Squamous Cell Carcinoma
Sarcoma
Brain Lower Grade Glioma
Kidney Renal Papillary Cell Carcinoma
Skin Cutaneous Melanoma
Acute Myeloid Leukemia
Kidney Renal Clear Cell Carcinoma
Thyroid Carcinoma
Kidney Chromophobe
Mesothelioma
Pheochromocytoma and Paraganglioma
Thymoma
Uveal Melanoma

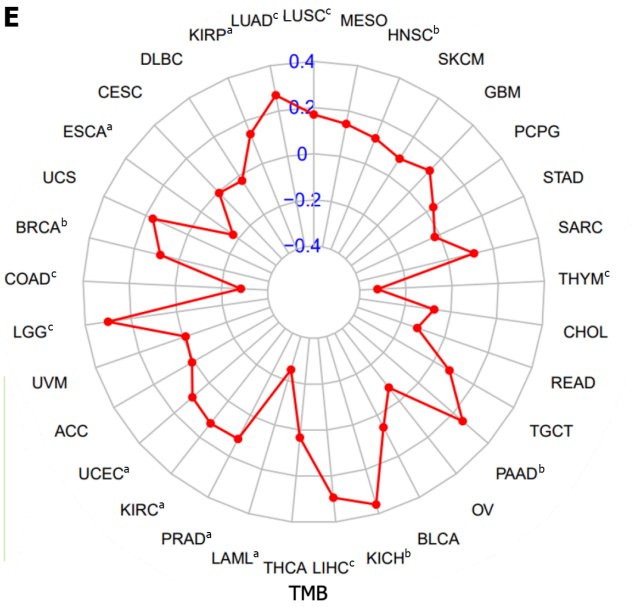

B
COPS6
RefSeq: NM_006833
Ensembl: ENST00000303904
CCDS: CCDS5682
UniProt: CNS6_HUMAN

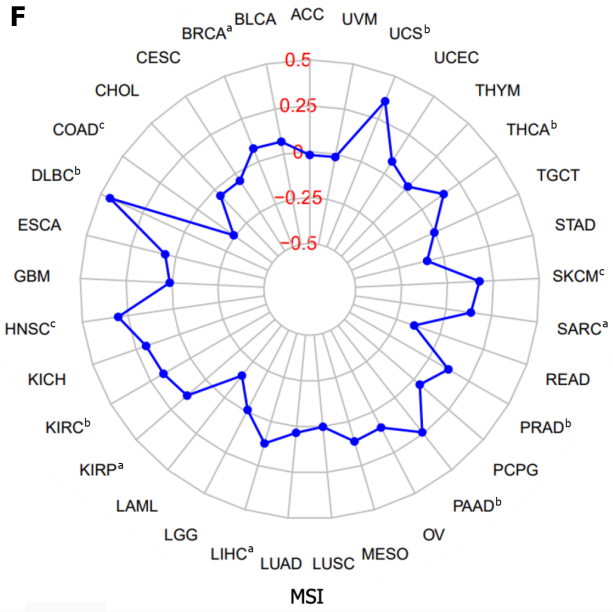

C
COPS6 mutations
42 Missense
5 Truncating
2 Inframe
5 Splice
1 Fusion
Adrenocortical carcinoma (n = 1)
Uterine endometrioid carcinoma (n = 1)
R197C/H
JAB
MitMem_reg
0
100
200
327aa

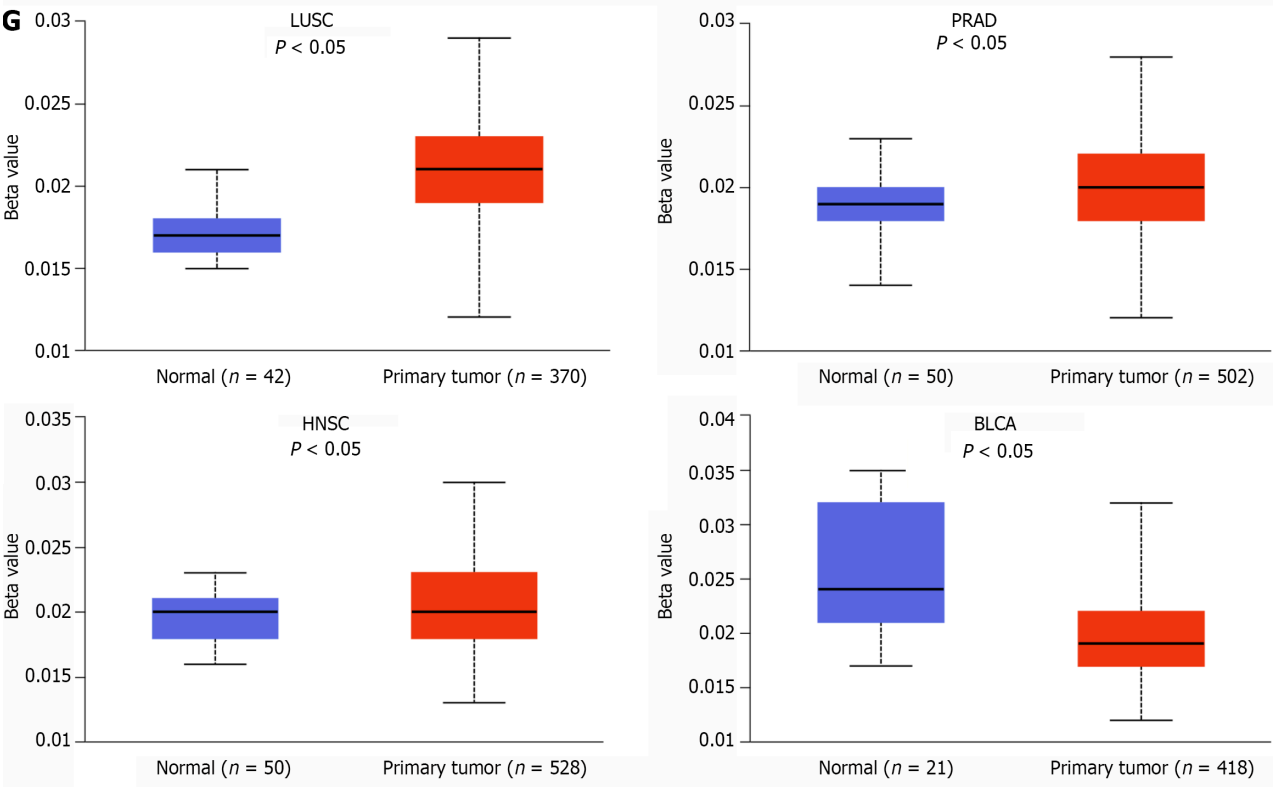

D
Altered group
Unaltered group
P value: 0.196
Probability of overall survival
OS (mo)
P value: 0.411
Disease free
DFS (mo)

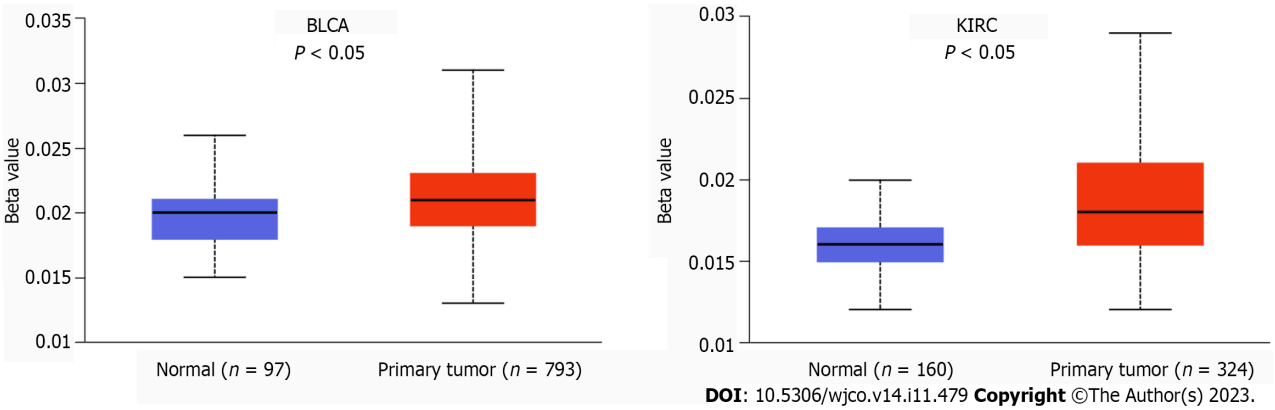

P value: 0.446
Progression free
PFS (mo)


P value: 0.446
Disease-specific
DSS (mo)


E
TMB


F
MSI


G
LUSC
P < 0.05
Normal (n = 42)
Primary tumor (n = 370)
PRAD
P < 0.05
Normal (n = 50)
Primary tumor (n = 502)
HNSC
P < 0.05
Normal (n = 50)
Primary tumor (n = 528)
BLCA
P < 0.05
Normal (n = 21)
Primary tumor (n = 418)
Beta value



DOI: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

Figure 5 Mutation analysis of *COP9 signalosome subunit 6*. A: Mutation frequency and types visualized through the cBioPortal website; B: Three-dimensional structure highlighting the R197C/H mutation site in *COP9 signalosome subunit 6 (COPS6)*; C: Mutation sites depicted in the cBioPortal website; D: Survival analysis of *COPS6* mutations in pan-cancer; E: Correlation of *COPS6* with tumor mutational burden (tumor mutational burden) in pan-cancer using R software; F: Correlation of *COPS6* with microsatellite instability (microsatellite instability) in pan-cancer using R software; G: Promoter methylation levels of *COPS6* in prostate adenocarcinoma (PRAD), lung squamous cell carcinoma (LUSC), head and neck squamous cell carcinoma (HNSC), breast invasive carcinoma (BRCA), bladder cancer (BLCA), and kidney renal clear cell carcinoma (KIRC) accessed through the UALCAN website. OS: Overall survival; DFS: Disease-free survival; PFS: Progression-free survival; DSS: Disease-specific survival.

indicated that missense mutations were the predominant mutation type found in *COPS6*. Additionally, TMB and MSI exhibited a positive correlation with *COPS6* expression level in most of the tumors, and only few tumors showed a negative correlation. Further exploration of the impact of *COPS6* mutations on patient outcomes revealed that these mutations did not significantly contribute to a worse prognosis in any specific tumor types. However, a comprehensive analysis across all tumors indicated a trend towards a shorter OS associated with *COPS6* mutations. Therefore, it can be concluded that *COPS6* mutations have a limited effect on patient prognosis.

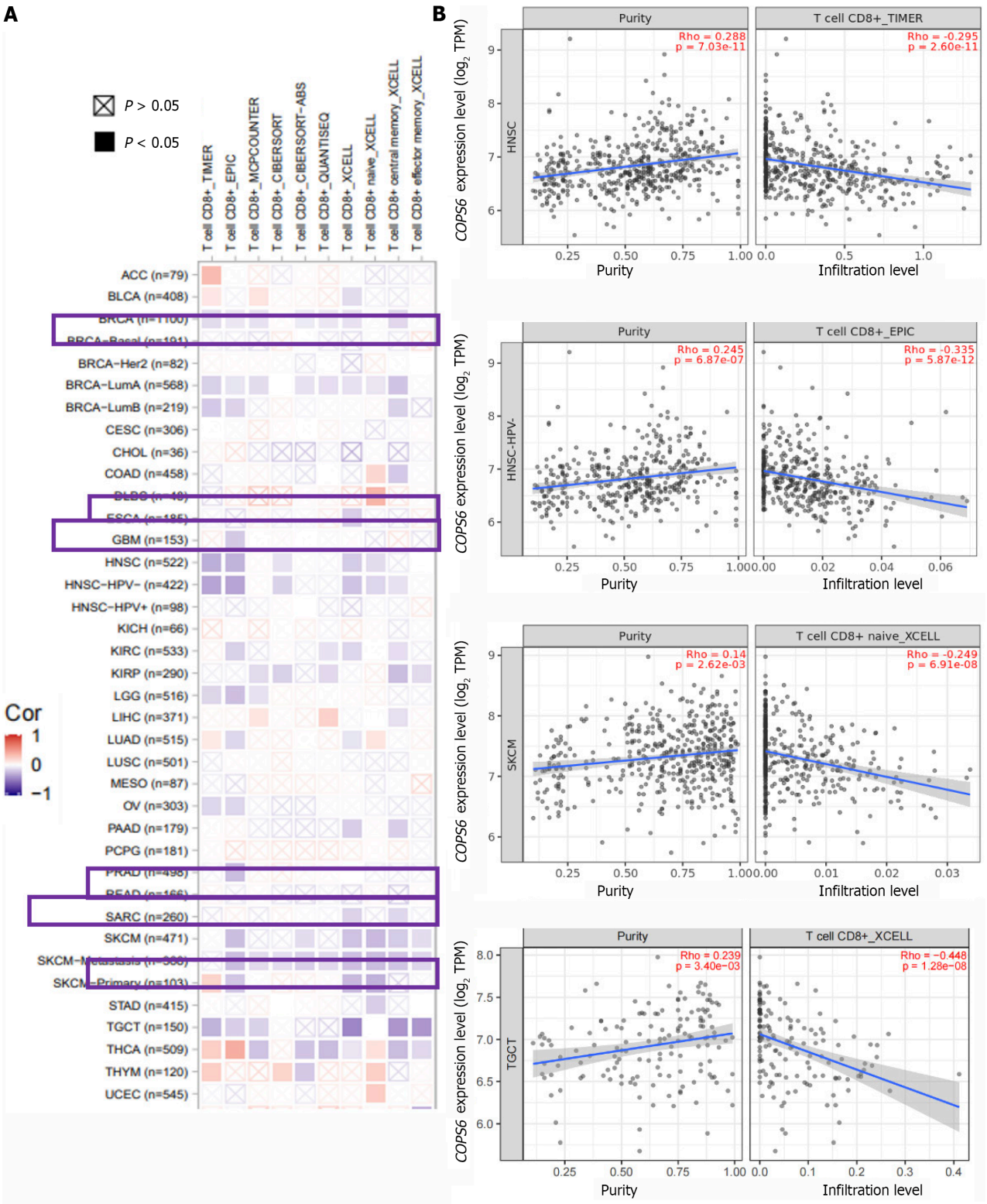
Subsequently, the association between *COPS6* expression level and the tumor immune microenvironment (TMIE) was investigated in various types of cancer. The TMIE plays a pivotal role in tumor progression, immune evasion, and therapeutic resistance, involving key components, such as CD8+ T cells, cancer-associated fibroblasts, macrophages, and NK cells[27]. The findings of the present study demonstrated a negative correlation between *COPS6* expression level and CD8+ T cell infiltration in several tumors, such as BRCA, HNSC, and TGCT. This aligns with Du *et al*[28]'s results, demonstrating that *COPS6* could inhibit CD8+ T cell infiltration within the tumor microenvironment (TME), thereby facilitating tumor immune evasion. Furthermore, a negative correlation was identified between *COPS6* expression level and the M1 phenotype of tumor-associated macrophages (TAMs), while a positive correlation was found with the M2 phenotype. TAMs, which are macrophages that infiltrate tumor tissue and differentiate from monocytes, predominantly adopt the immunosuppressive M2 phenotype in the TMIE[29]. The present study revealed a positive correlation between *COPS6* expression level and the M2 phenotype in the TIDE algorithm for DLBCL, BRCA, KIRC, THCA, THYM, and other tumors, while other algorithms exhibited a negative correlation with the M1 phenotype. However, it is noteworthy that in some tumors, only the TIDE algorithm yielded consistent results, while other algorithms suggested a negative or no correlation between *COPS6* expression level and the M2 phenotype. This discrepancy could be attributed to variations in the statistical methods employed by each algorithm, necessitating further experimental validation of these findings.

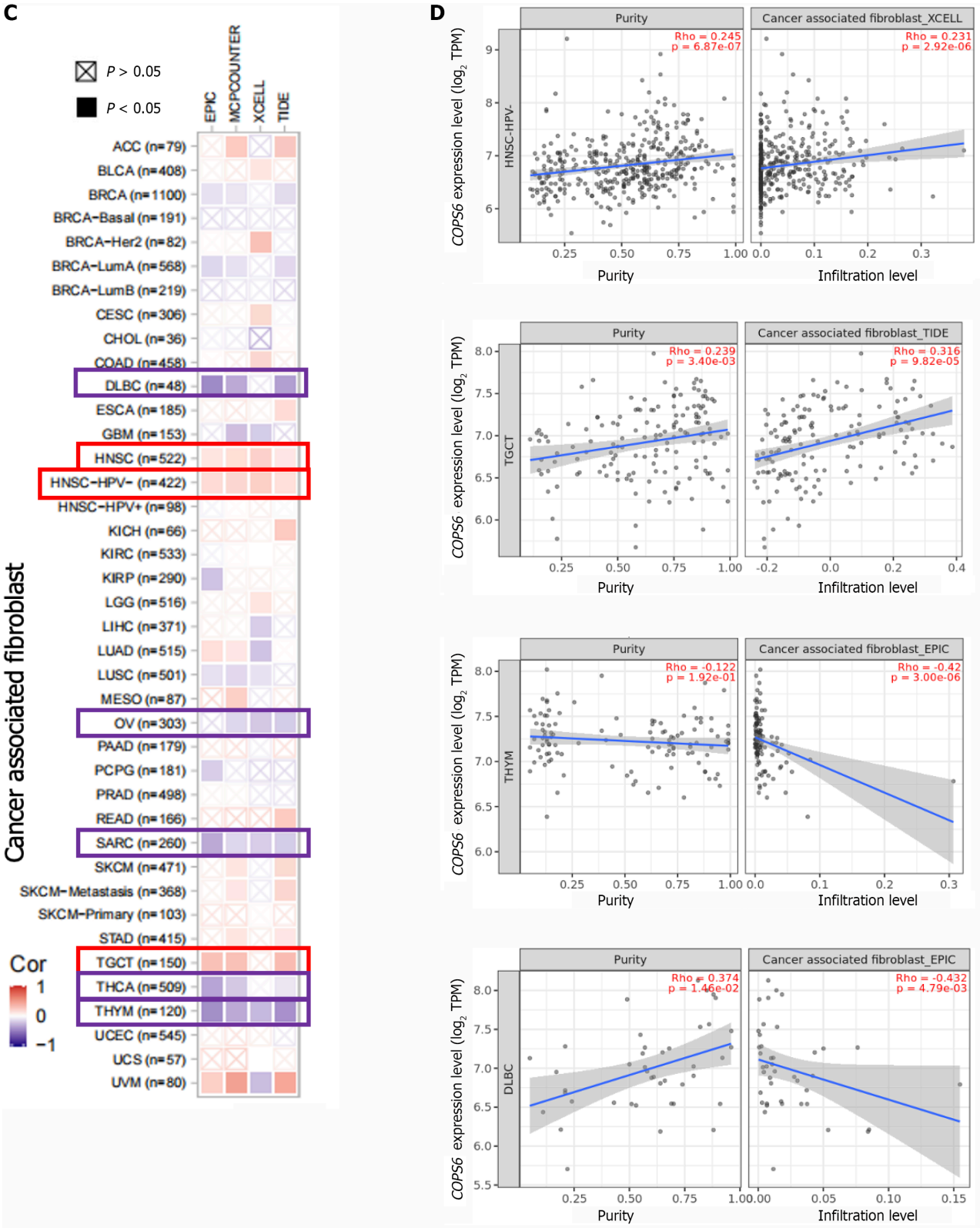
There is a scarcity of research regarding the interaction between *COPS6* Level and TME, highlighting the urgent need to explore the role of *COPS6* in the TME. Furthermore, in the present study, correlation and enrichment analyses of *COPS6* were conducted, and *GPS1* and *TCEB2* were identified as the two genes, exhibiting the strongest correlation. This investigation sheds light on the potential function and significance of *COPS6* as a novel biomarker in cancer, setting the stage for further research on its molecular mechanisms and the development of targeted therapies. Moreover, the findings emphasize the importance of studying the *COPS6*-related TIME. However, it should be noted that the current study of *COPS6* is preliminary, and the specific mechanisms of its action in different types of cancer remain elusive. Therefore, additional resources and efforts are warranted to delve deeper into the role of *COPS6* in cancer.

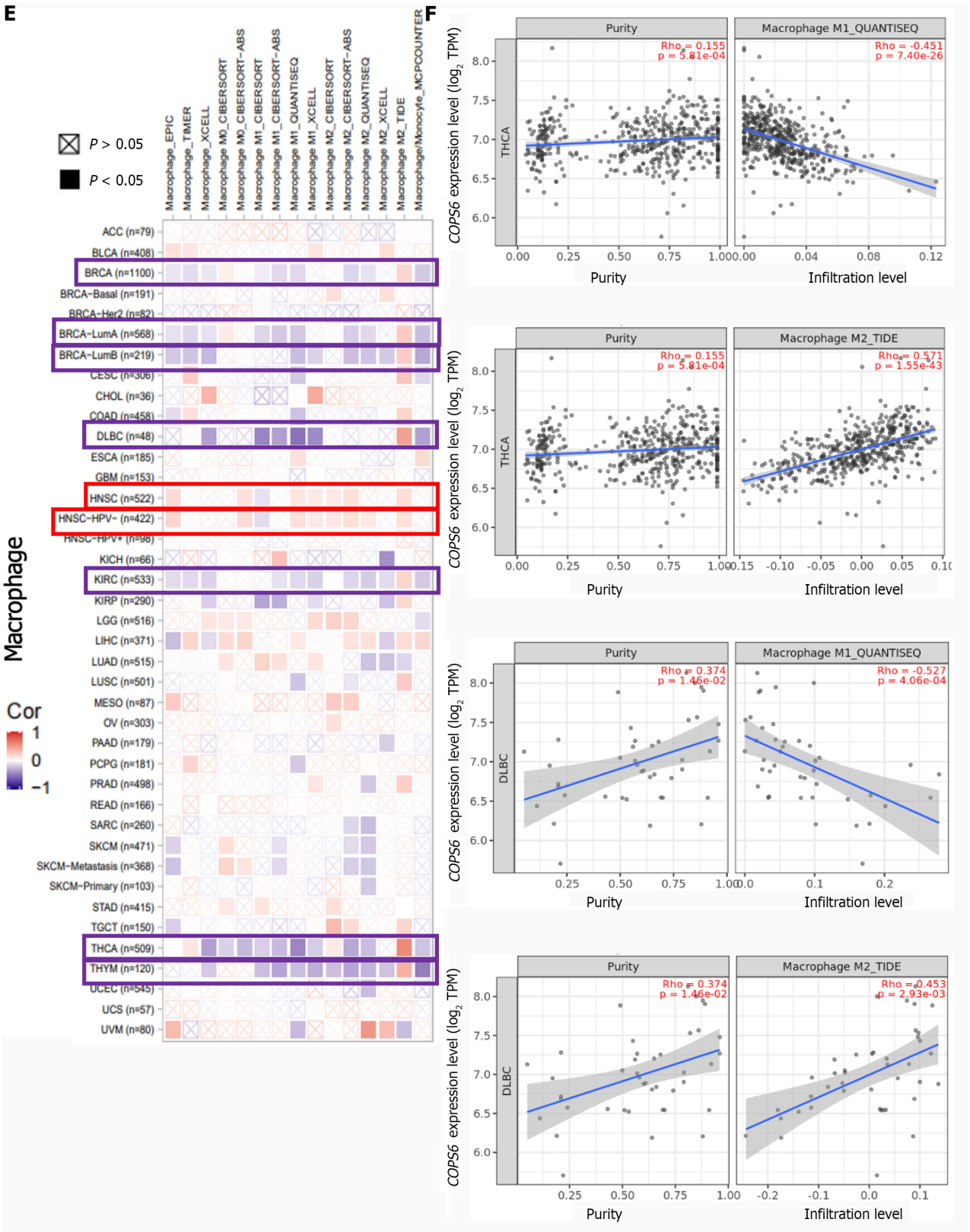
The present study revealed a potential association of *COPS6* with survival outcomes in various tumors. Notably, *GPS1* and *TCEB2* were identified as the two genes exhibiting the strongest correlation with *COPS6* at both the gene and protein levels, making them promising targets for future investigations. Additionally, a significant association was found between *COPS6* expression level and immune infiltration in diverse types of cancer, such as BRCA, HNSC, and TGCT, where research on the TIME remains limited.

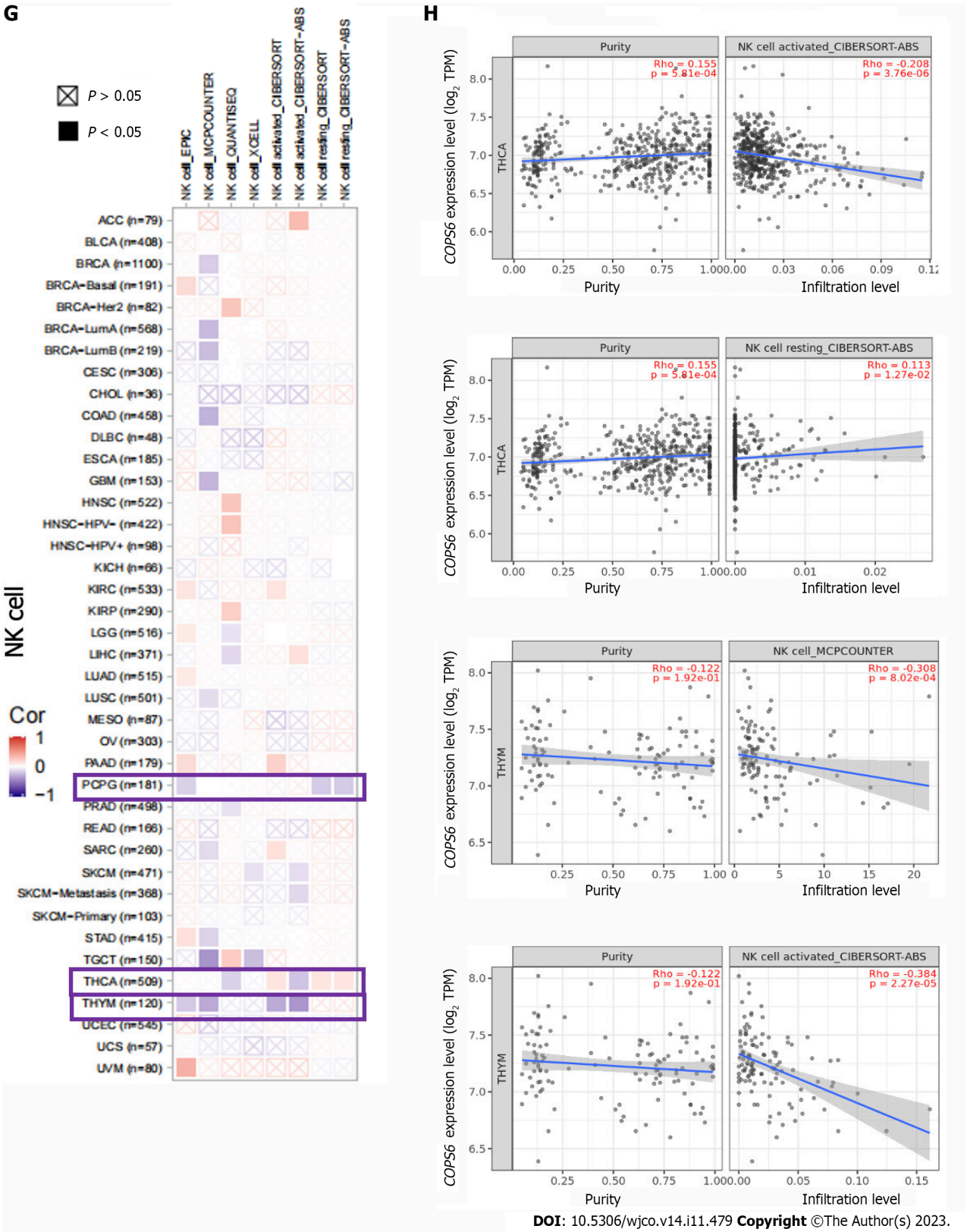
CONCLUSION

This study is the first to explore the role of *COPS6* in pan-cancer, taking full use of the existing public database to investigate *COPS6* from the aspects of gene expression level, mutation, TIME, and prognosis. However, there are also some deficiencies in this study. For instance, only a multifaceted analysis of *COPS6* was conducted through


A
P > 0.05
P < 0.05
T cell CD8+_TIMER
T cell CD8+_EPIC
T cell CD8+_MCPCOUNTER
T cell CD8+_CIBERSORT
T cell CD8+_CIBERSORT-ABS
T cell CD8+_QUANTISEQ
T cell CD8+_XCELL
T cell CD8+ naive_XCELL
T cell CD8+ central memory_XCELL
T cell CD8+ effector memory_XCELL
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA-Basal (n=191)
BRCA-Her2 (n=82)
BRCA-LumA (n=568)
BRCA-LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC-HPV- (n=422)
HNSC-HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM-Metastasis (n=368)
SKCM-Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
Cor
1
0
−1
B
Purity
T cell CD8+_TIMER
Rho = 0.288
p = 7.03e-11
Rho = -0.295
p = 2.60e-11
HNSC
COPS6 expression level (log2 TPM)
Purity
Infiltration level
T cell CD8+_EPIC
Rho = 0.245
p = 6.87e-07
Rho = -0.335
p = 5.87e-12
HNSC-HPV-
T cell CD8+ naive_XCELL
Rho = 0.14
p = 2.62e-03
Rho = -0.249
p = 6.91e-08
SKCM
T cell CD8+_XCELL
Rho = 0.239
p = 3.40e-03
Rho = −0.448
p = 1.28e-08
TGCT

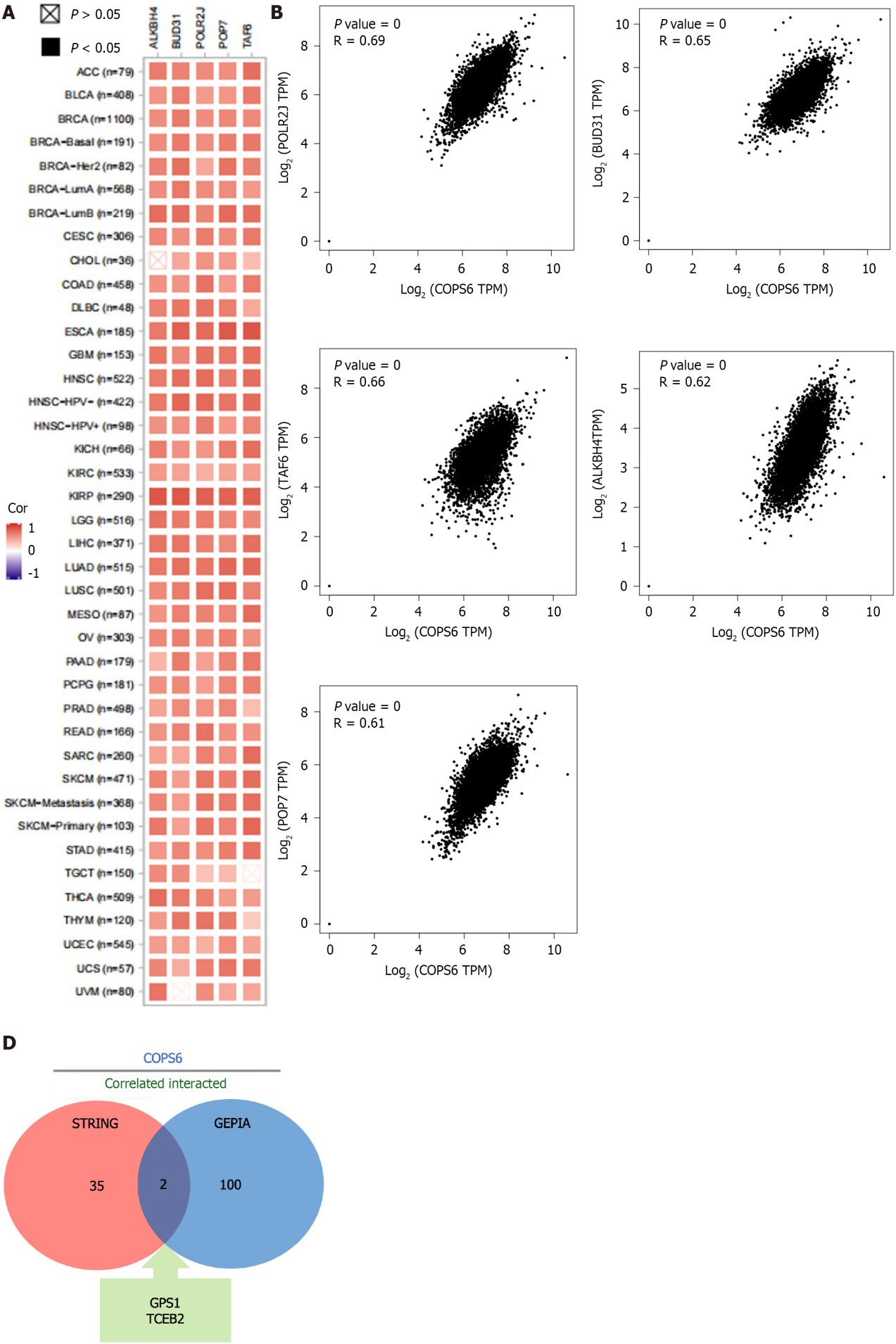

C
P > 0.05
P < 0.05
EPIC
MCPCOUNTER
XCELL
TIDE
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA−Basal (n=191)
BRCA−Her2 (n=82)
BRCA−LumA (n=568)
BRCA−LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC−HPV− (n=422)
HNSC−HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM−Metastasis (n=368)
SKCM−Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
UCS (n=57)
UVM (n=80)
Cancer associated fibroblast
Cor
1
0
−1
D
Purity
Cancer associated fibroblast_XCELL
Rho = 0.245
p = 6.87e-07
Rho = 0.231
p = 2.92e-06
HNSC−HPV−
COPS6 expression level (log2 TPM)
Purity
Infiltration level
Cancer associated fibroblast_TIDE
Rho = 0.239
p = 3.40e-03
Rho = 0.316
p = 9.82e-05
TGCT
Cancer associated fibroblast_EPIC
Rho = -0.122
p = 1.92e-01
Rho = -0.42
p = 3.00e-06
THYM
Rho = 0.374
p = 1.46e-02
Rho = -0.432
p = 4.79e-03
DLBC

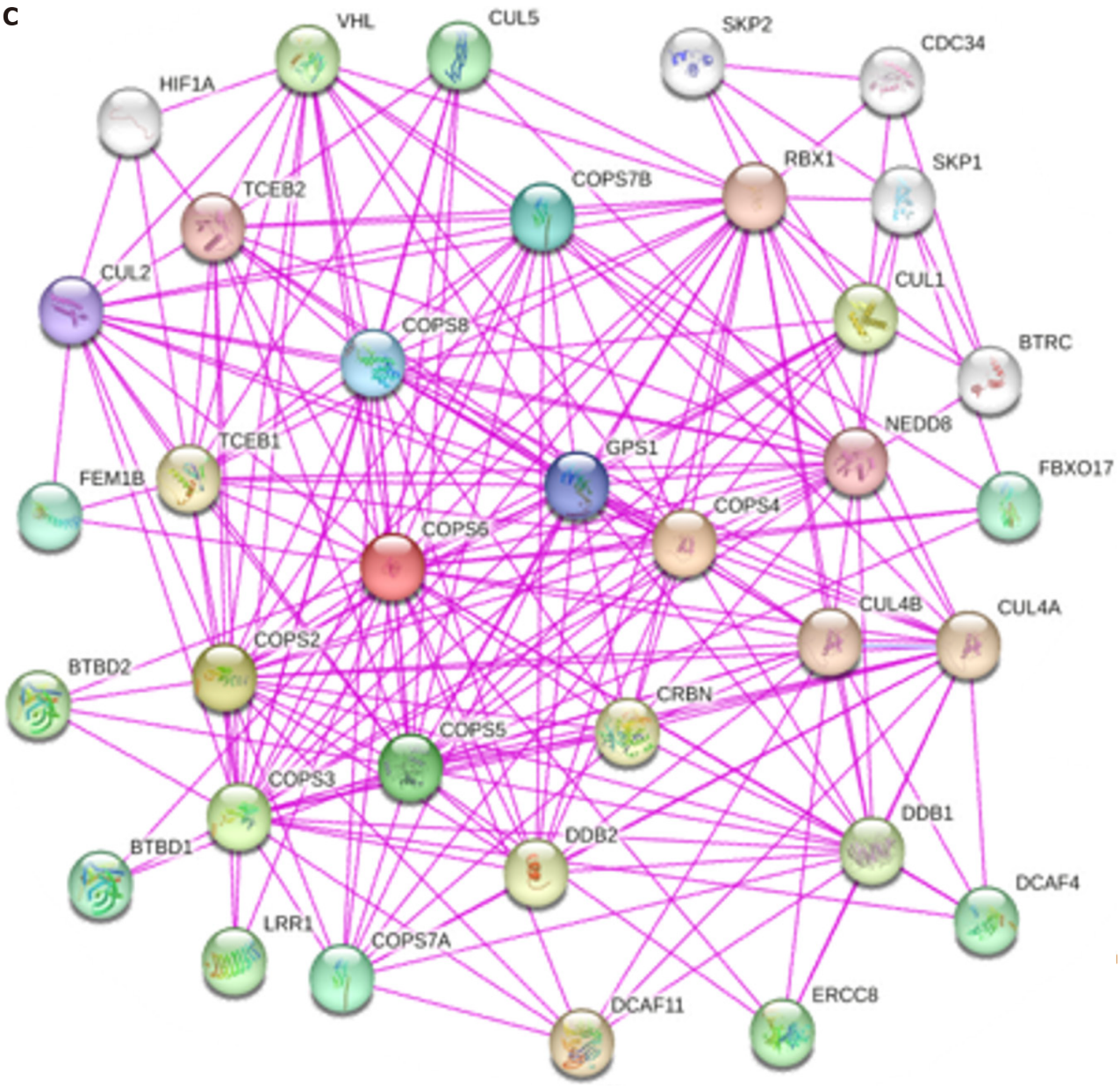

E
F
P > 0.05
P < 0.05
Macrophage_EPIC
Macrophage_TIMER
Macrophage_XCELL
Macrophage M0_CIBERSORT
Macrophage M0_CIBERSORT-ABS
Macrophage M1_CIBERSORT
Macrophage M1_CIBERSORT-ABS
Macrophage M1_QUANTISEQ
Macrophage M1_XCELL
Macrophage M2_CIBERSORT
Macrophage M2_CIBERSORT-ABS
Macrophage M2_QUANTISEQ
Macrophage M2_XCELL
Macrophage M2_TIDE
Macrophage/Monocyte_MCPCOUNTER
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA-Basal (n=191)
BRCA-Her2 (n=82)
BRCA-LumA (n=568)
BRCA-LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC-HPV- (n=422)
HNSC-HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM-Metastasis (n=368)
SKCM-Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
UCS (n=57)
UVM (n=80)
Macrophage
Cor
1
0
−1
Purity
Macrophage M1_QUANTISEQ
Rho = 0.155
p = 5.81e-04
Rho = -0.451
p = 7.40e-26
THCA
COPS6 expression level (log2 TPM)
Purity
Infiltration level
Purity
Macrophage M2_TIDE
Rho = 0.155
p = 5.81e-04
Rho = 0.571
p = 1.55e-43
THCA
COPS6 expression level (log2 TPM)
Purity
Infiltration level
Purity
Macrophage M1_QUANTISEQ
Rho = 0.374
p = 1.46e-02
Rho = -0.527
p = 4.06e-04
DLBC
COPS6 expression level (log2 TPM)
Purity
Infiltration level
Purity
Macrophage M2_TIDE
Rho = 0.374
p = 1.46e-02
Rho = 0.453
p = 2.93e-03
DLBC
COPS6 expression level (log2 TPM)
Purity
Infiltration level

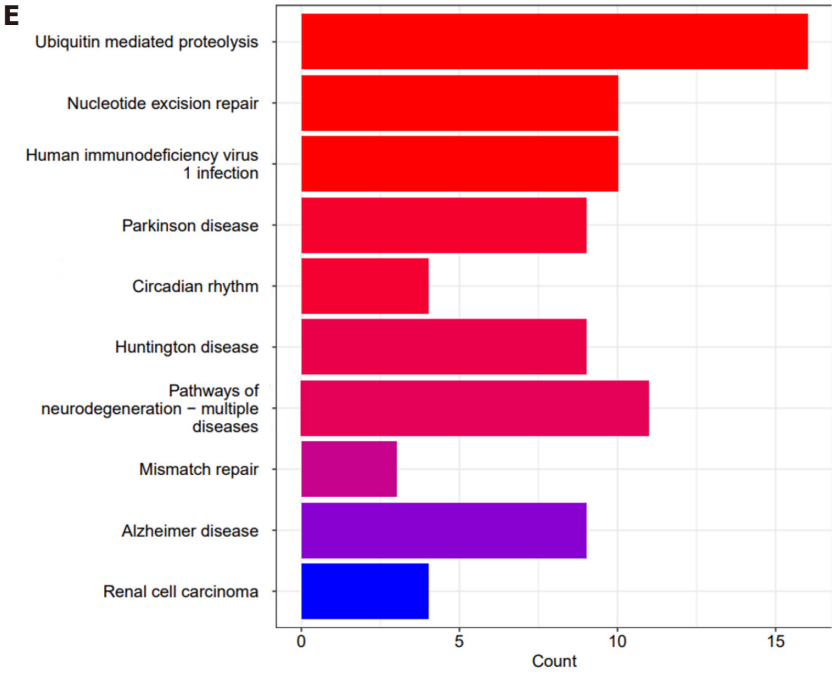


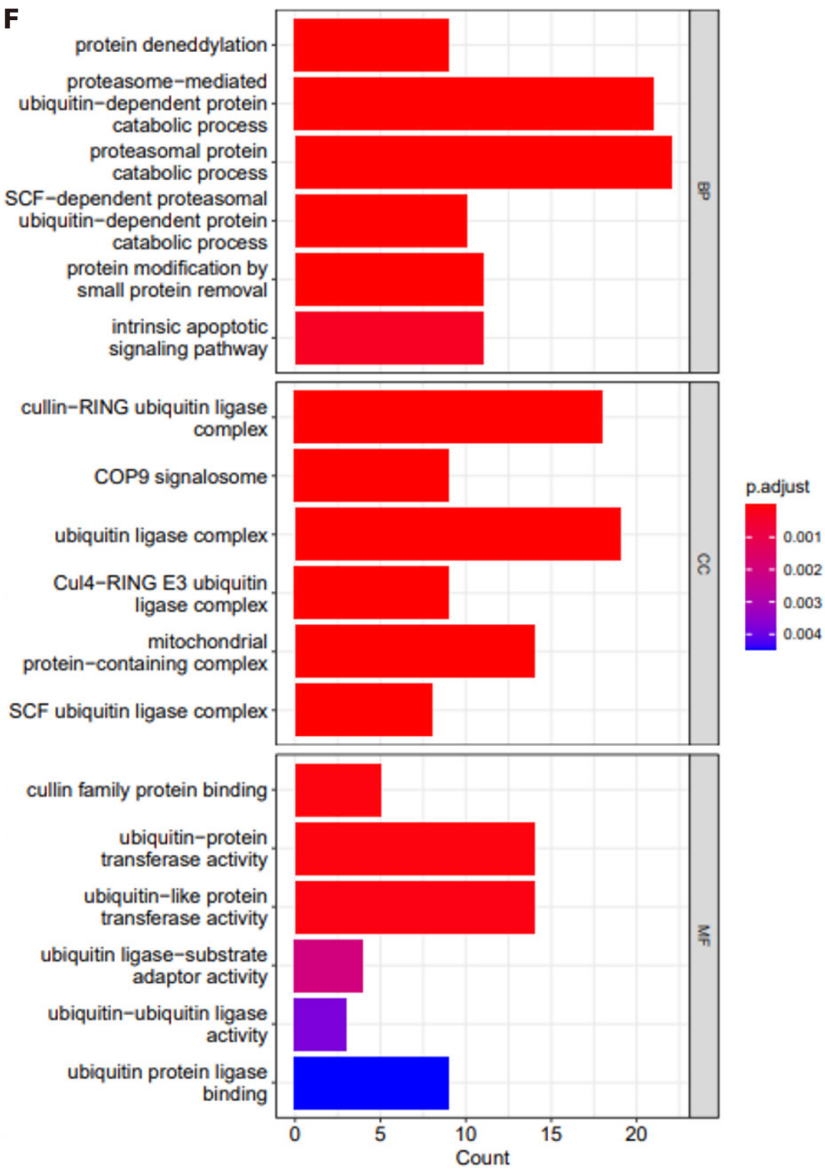
DOI: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

Figure 6 Immune infiltration analysis of *COP9 signalosome subunit 6* in the The Cancer Genome Atlas database using the TIMER2 website. A: Heatmap depicting the correlation between *COP9 signalosome subunit 6 (COPS6)* and CD8+ T cells; B: Scatter plot illustrating the relationship between *COPS6* and CD8+ T cells; C: Heatmap displaying the correlation between *COPS6* and cancer-associated fibroblasts; D: Scatter plot demonstrating the relationship between *COPS6* and cancer-associated fibroblasts; E: Heatmap indicating the correlation between *COPS6* and macrophages; F: Scatter plot showing the relationship between *COPS6* and macrophages; G: Heatmap presenting the correlation between *COPS6* and natural killer (NK) cells; H: Scatter plot depicting the relationship between *COPS6* and NK cells. TPM: Transcripts per million.


A
P > 0.05
P < 0.05
ALKBH4
BUD31
POLR2J
POP7
TAF6
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA−Basal (n=191)
BRCA−Her2 (n=82)
BRCA−LumA (n=568)
BRCA−LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC−HPV− (n=422)
HNSC−HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM−Metastasis (n=368)
SKCM−Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
UCS (n=57)
UVM (n=80)
Cor
1
0
-1
B
P value = 0
R = 0.69
Log2 (POLR2J TPM)
Log2 (COPS6 TPM)
P value = 0
R = 0.65
Log2 (BUD31 TPM)
Log2 (COPS6 TPM)
P value = 0
R = 0.66
Log2 (TAF6 TPM)
Log2 (COPS6 TPM)
P value = 0
R = 0.62
Log2 (ALKBH4TPM)
Log2 (COPS6 TPM)
P value = 0
R = 0.61
Log2 (POP7 TPM)
Log2 (COPS6 TPM)
D
COPS6
Correlated interacted
STRING
GEPIA
35
2
100
GPS1
TCEB2


C
VHL
CUL5
SKP2
CDC34
HIF1A
RBX1
SKP1
TCEB2
COPS7B
CUL2
CUL1
COPS8
BTRC
NEDD8
TCEB1
GPS1
FEM1B
FBXO17
COPS4
COPS6
CUL4B
CUL4A
COPS2
BTBD2
CRBN
COPS5
COPS3
DDB1
BTBD1
DDB2
DCAF4
LRR1
COPS7A
DCAF11
ERCC8


E
Ubiquitin mediated proteolysis
Nucleotide excision repair
Human immunodeficiency virus 1 infection
Parkinson disease
Circadian rhythm
Huntington disease
Pathways of neurodegeneration − multiple diseases
Mismatch repair
Alzheimer disease
Renal cell carcinoma
0
5
10
15
Count



DOI: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

Figure 7 Correlation analysis and enrichment analysis of *COPS6*. A: Heatmap displaying the top 5 genes correlated with *COPS6* in pan-cancer accessed through the TIMER2 website; B: Scatter plot illustrating the correlation between *COPS6* and the top 5 genes in pan-cancer using the GEPIA2 website; C: Protein-protein interaction network of *COPS6* obtained from the STRING database; D: Intersection of *COPS6*-related genes screened in GEPIA2 and STRING, resulting in GPS1 and TCEB2; E: Kyoto Encyclopedia of Genes and Genomes enrichment analysis of the combined *COPS6*-related genes from GEPIA2 and STRING; F: Gene ontology enrichment analysis of the combined *COPS6*-related genes from GEPIA2 and STRING. TPM: Transcripts per million.

bioinformatics, while no experiment was carried out to verify the results, hindering the generalization of the findings.

In conclusion, the present study provided early evidence that *COPS6* could be associated with clinicopathological characteristics in various tumors and could play a role in several cancer hallmarks. Additional research is needed to further elucidate the role of *COPS6* in cancer progression.

ARTICLE HIGHLIGHTS

Research background

The COP9 signaling body subunit 6 (*COPS6*) has been implicated in cancer progression, but its precise role in most types of cancer is unknown.

Research motivation

This study aimed to investigate the functional and clinical relevance of *COPS6* in different tumor types, using publicly available databases.

Research objectives
This study hopes to provide a basis for *COPS6* as a novel biomarker for cancer research by exploring the role of *COPS6* in different cancer types.

Research methods
We used R software and online analysis databases to analyze the differential expression, prognosis, mutation and related functions of *COPS6* in pan-cancer.

Research results
Differential expression analysis and survival analysis demonstrated that *COPS6* was highly expressed and associated with high-risk profiles in the majority of cancer types. Missense mutations are the main type of *COPS6* mutations, and in most types of cancer, the levels of *COPS6* expression are positively correlated with tumor mutation burden and microsatellite instability. Immune infiltration analysis found *COPS6* to play different roles in different cancers. Gene co-expression and enrichment analysis highlighted *COPS6*-related genes were predominantly involved in processes, such as ubiquitin-mediated proteolysis and human immunodeficiency virus 1 infection.

Research conclusions
This study provides early evidence that *COPS6* may be associated with the clinicopathological features of various tumors and may play a role in several cancer features, providing a basis for subsequent studies related to *COPS6*.

Research perspectives
Since this study mainly focused on data analysis, subsequent studies required experimental validation of relevant results.

FOOTNOTES

Co-first authors: Shi-Lin Wang and Guang-Zheng Zhuo.

Co-corresponding authors: Yun-Bao Pan and Yi-Rong Li.

Author contributions: Pan YB designed the research; Wang SL and Zhuo GZ performed the research; Wang SL, Wang LP and Zhuo GZ contributed analytic tools; Wang SL and Zhuo GZ analyzed the data; Wang SL and Pan YB wrote the paper; Pan YB and Li YR were responsible for the supervision. Wang SL and Zhuo GZ contributed equally to this work as co-first authors. The reasons for designating Wang SL and Zhuo GZ as co-first authors are twofold. First, the research was performed as a collaborative effort, and the designation of co-corresponding authorship accurately reflects the distribution of responsibilities and burdens associated with the time and effort required to complete the study and the resultant paper. Second, Wang SL and Zhuo GZ contributed an equally substantial effort throughout the study. They are principal principals of paper writing and data analysis, selecting these researchers as co-first authors, recognizing and respecting this equal contribution. Pan YB and Li YR contributed equally to this work as co-corresponding authors. The reasons for designating Pan YB and Li YR as co-corresponding authors are twofold. First, the research was performed as a collaborative effort. This ensures effective communication and management of post-submission matters, ultimately enhancing the paper's quality and reliability. Second, the overall research team encompassed authors with a variety of expertise and skills from different fields, and the designation of co-corresponding authors best reflects this diversity. Pan YB and Li YR, as heads of both groups, contributed substantially to the experimental design, data analysis and revision, and were therefore listed as co-corresponding authors.

Supported by National Natural Science Foundation of China, No. 31900558; the Hubei Provincial Youth Talents Program for Public Health, No. WSJKRC2022013; Wuhan Young and Middle-Aged Medical Backbone Talents Training Project, No. WHQG201904.

Conflict-of-interest statement: The authors have no relevant financial or non-financial interests to disclose.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at [panyunbao@outlook.com]. Participants gave informed consent for data sharing.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Yun-Bao Pan 0000-0002-6311-2945; Yi-Rong Li 0000-0002-7493-045X.

S-Editor: Lin C
L-Editor: Filipodia
P-Editor: Zhang XD

REFERENCES

1 **Siegel RL**, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin* 2023; **73**: 17-48 [PMID: 36633525 DOI: 10.3322/caac.21763]

2 **Feng R**, Su Q, Huang X, Basnet T, Xu X, Ye W. Cancer situation in China: what does the China cancer map indicate from the first national death survey to the latest cancer registration? *Cancer Commun (Lond)* 2023; **43**: 75-86 [PMID: 36397729 DOI: 10.1002/cac2.12393]

3 **Wang Y**, Yan Q, Fan C, Mo Y, Wang Y, Li X, Liao Q, Guo C, Li G, Zeng Z, Xiong W, Huang H. Overview and countermeasures of cancer burden in China. *Sci China Life Sci* 2023; 1-12 [PMID: 37071289 DOI: 10.1007/s11427-022-2240-6]

4 **Gupta S**, Harper A, Ruan Y, Barr R, Frazier AL, Ferlay J, Steliarova-Foucher E, Fidler-Benaoudia MM. International Trends in the Incidence of Cancer Among Adolescents and Young Adults. *J Natl Cancer Inst* 2020; **112**: 1105-1117 [PMID: 32016323 DOI: 10.1093/jnci/djaa007]

5 **Bhakta N**, Force LM, Allemani C, Atun R, Bray F, Coleman MP, Steliarova-Foucher E, Frazier AL, Robison LL, Rodriguez-Galindo C, Fitzmaurice C. Childhood cancer burden: a review of global estimates. *Lancet Oncol* 2019; **20**: e42-e53 [PMID: 30614477 DOI: 10.1016/S1470-2045(18)30761-7]

6 **Clough E**, Barrett T. The Gene Expression Omnibus Database. *Methods Mol Biol* 2016; **1418**: 93-110 [PMID: 27008011 DOI: 10.1007/978-1-4939-3578-9_5]

7 **Blum A**, Wang P, Zenklusen JC. SnapShot: TCGA-Analyzed Tumors. *Cell* 2018; **173**: 530 [PMID: 29625059 DOI: 10.1016/j.cell.2018.03.059]

8 **Ma XL**, Xu M, Jiang T. Crystal structure of the human CSN6 MPN domain. *Biochem Biophys Res Commun* 2014; **453**: 25-30 [PMID: 25242525 DOI: 10.1016/j.bbrc.2014.09.046]

9 **Lingaraju GM**, Bunker RD, Cavadini S, Hess D, Hassiepen U, Renatus M, Fischer ES, Thomä NH. Crystal structure of the human COP9 signalosome. *Nature* 2014; **512**: 161-165 [PMID: 25043011 DOI: 10.1038/nature13566]

10 **Faull SV**, Lau AMC, Martens C, Ahdash Z, Hansen K, Yebenes H, Schmidt C, Beuron F, Cronin NB, Morris EP, Politis A. Structural basis of Cullin 2 RING E3 ligase regulation by the COP9 signalosome. *Nat Commun* 2019; **10**: 3814 [PMID: 31444342 DOI: 10.1038/s41467-019-11772-y]

11 **Hou J**, Cui H. CSN6: a promising target for cancer prevention and therapy. *Histol Histopathol* 2020; **35**: 645-652 [PMID: 32016946 DOI: 10.14670/HH-18-206]

12 **Tang Z**, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res* 2019; **47**: W556-W560 [PMID: 31114875 DOI: 10.1093/nar/gkz430]

13 **Li T**, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res* 2020; **48**: W509-W514 [PMID: 32442275 DOI: 10.1093/nar/gkaa407]

14 **Chandrashekar DS**, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK, Varambally S. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* 2017; **19**: 649-658 [PMID: 28732212 DOI: 10.1016/j.neo.2017.05.002]

15 **Wu P**, Heins ZJ, Muller JT, Katsnelson L, de Bruijn I, Abeshouse AA, Schultz N, Fenyö D, Gao J. Integration and Analysis of CPTAC Proteomics Data in the Context of Cancer Genomics in the cBioPortal. *Mol Cell Proteomics* 2019; **18**: 1893-1898 [PMID: 31308250 DOI: 10.1074/mcp.TIR119.001673]

16 **Szklarczyk D**, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 2021; **49**: D605-D612 [PMID: 33237311 DOI: 10.1093/nar/gkaa1074]

17 **Birol M**, Enchev RI, Padilla A, Stengel F, Aebersold R, Betzi S, Yang Y, Hoh F, Peter M, Dumas C, Echalier A. Structural and biochemical characterization of the Cop9 signalosome CSN5/CSN6 heterodimer. *PLoS One* 2014; **9**: e105688 [PMID: 25144743 DOI: 10.1371/journal.pone.0105688]

18 **Xu M**, Zhen L, Lin L, Wu K, Wang Y, Cai X. Overexpression of CSN6 promotes the epithelial-mesenchymal transition and predicts poor prognosis in hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol* 2020; **44**: 340-348 [PMID: 31422034 DOI: 10.1016/j.clinre.2019.07.012]

19 **Choi HH**, Lee MH. CSN6-COP1 axis in cancer. *Aging (Albany NY)* 2015; **7**: 461-462 [PMID: 26186957 DOI: 10.18632/aging.100778]

20 **Chen J**, Shin JH, Zhao R, Phan L, Wang H, Xue Y, Post SM, Ho Choi H, Chen JS, Wang E, Zhou Z, Tseng C, Gully C, Velazquez-Torres G, Fuentes-Mattei E, Yeung G, Qiao Y, Chou PC, Su CH, Hsieh YC, Hsu SL, Ohshiro K, Shaikenov T, Yeung SC, Lee MH. CSN6 drives carcinogenesis by positively regulating Myc stability. *Nat Commun* 2014; **5**: 5384 [PMID: 25395170 DOI: 10.1038/ncomms6384]

21 **Zhao R**, Yeung SC, Chen J, Iwakuma T, Su CH, Chen B, Qu C, Zhang F, Chen YT, Lin YL, Lee DF, Jin F, Zhu R, Shaikenov T, Sarbassov D, Sahin A, Wang H, Lai CC, Tsai FJ, Lozano G, Lee MH. Subunit 6 of the COP9 signalosome promotes tumorigenesis in mice through stabilization of MDM2 and is upregulated in human cancers. *J Clin Invest* 2011; **121**: 851-865 [PMID: 21317535 DOI: 10.1172/JCI44111]

22 **Fang L**, Lu W, Choi HH, Yeung SC, Tung JY, Hsiao CD, Fuentes-Mattei E, Menter D, Chen C, Wang L, Wang J, Lee MH. ERK2-Dependent Phosphorylation of CSN6 Is Critical in Colorectal Cancer Development. *Cancer Cell* 2015; **28**: 183-197 [PMID: 26267535 DOI: 10.1016/j.ccell.2015.07.004]

23 **Su L**, Guo W, Lou L, Nie S, Zhang Q, Liu Y, Chang Y, Zhang X, Li Y, Shen H. EGFR-ERK pathway regulates CSN6 to contribute to PD-L1 expression in glioblastoma. *Mol Carcinog* 2020; **59**: 520-532 [PMID: 32134157 DOI: 10.1002/mc.23176]

24 **Zhang Y**, Hou J, Shi S, Du J, Liu Y, Huang P, Li Q, Liu L, Hu H, Ji Y, Guo L, Shi Y, Cui H. CSN6 promotes melanoma proliferation and metastasis by controlling the UBR5-mediated ubiquitination and degradation of CDK9. *Cell Death Dis* 2021; **12**: 118 [PMID: 33483464 DOI: 10.1038/s41419-021-03398-0]

25 **Mao Z**, Sang MM, Chen C, Zhu WT, Gong YS, Pei DS. CSN6 Promotes the Migration and Invasion of Cervical Cancer Cells by Inhibiting Autophagic Degradation of Cathepsin L. *Int J Biol Sci* 2019; **15**: 1310-1324 [PMID: 31223289 DOI: 10.7150/ijbs.32987]

26 **Mou J**, Wei L, Liang J, Du W, Pei D. CSN6 promotes the cell migration of breast cancer cells by positively regulating Snail1 stability. *Int J Med Sci* 2020; **17**: 2809-2818 [PMID: 33162808 DOI: 10.7150/ijms.50206]

27 **Ruffin AT**, Li H, Vujanovic L, Zandberg DP, Ferris RL, Bruno TC. Improving head and neck cancer therapies by immunomodulation of the tumour microenvironment. *Nat Rev Cancer* 2023; **23**: 173-188 [PMID: 36456755 DOI: 10.1038/s41568-022-00531-9]

28 **Du WQ**, Zhu ZM, Jiang X, Kang MJ, Pei DS. COPS6 promotes tumor progression and reduces CD8(+) T cell infiltration by repressing IL-6 production to facilitate tumor immune evasion in breast cancer. *Acta Pharmacol Sin* 2023; **44**: 1890-1905 [PMID: 37095198 DOI: 10.1038/s41401-023-01085-8]

29 **Wang S**, Liu G, Li Y, Pan Y. Metabolic Reprogramming Induces Macrophage Polarization in the Tumor Microenvironment. *Front Immunol* 2022; **13**: 840029 [PMID: 35874739 DOI: 10.3389/fimmu.2022.840029]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 November 24; 14(11): 504-517

DOI: 10.5306/wjco.v14.i11.504

ISSN 2218-4333 (online)

META-ANALYSIS

Circulating tumor cells as potential prognostic biomarkers for early-stage pancreatic cancer: A systematic review and meta-analysis

Zi-Han Zhang, Yi-Wen Bao, Ya-Jun Zhao, Jian-Quan Wang, Jin-Tao Guo, Si-Yu Sun

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Kitamura K, Japan; Mizuno S, Japan

Received: June 28, 2023
Peer-review started: June 28, 2023
First decision: August 25, 2023
Revised: September 14, 2023
Accepted: October 26, 2023
Article in press: October 26, 2023
Published online: November 24, 2023

Zi-Han Zhang, Yi-Wen Bao, Ya-Jun Zhao, Jian-Quan Wang, Jin-Tao Guo, Si-Yu Sun, Department of Gastroenterology, Shengjing Hospital of China Medical University, Shenyang 110004, Liaoning Province, China

Corresponding author: Jin-Tao Guo, MD, PhD, Professor, Department of Gastroenterology, Shengjing Hospital of China Medical University, No. 36 Sanhao Street, Shenyang 110004, Liaoning Province, China. guojt@sj-hospital.org

Abstract

BACKGROUND

Pancreatic cancer is difficult to be diagnosed early clinically, while often leads to poor prognosis. If optimal personalized treatment plan can be provided to pancreatic cancer patient at an earlier stage, this can greatly improve overall survival (OS). Circulating tumor cells (CTCs) are a collective term for various types of tumor cells present in the peripheral blood (PB), which are formed by detachment during the development of solid tumor lesions. Most CTCs undergo apoptosis or are phagocytosed after entering the PB, whereas a few can escape and anchor at distal sites to develop metastasis, increasing the risk of death for patients with malignant tumors.

AIM

To investigate the significance of CTCs in predicting the prognosis of early pancreatic cancer patients.

METHODS

The PubMed, EMBASE, Web of Science, Cochrane Library, China National Knowledge Infrastructure, China Biology Medicine, and ChinaInfo databases were searched for articles published through December 2022. Studies were considered qualified if they included patients with early pancreatic cancer, analyzed the prognostic value of CTCs, and were full papers reported in English or Chinese. Researches were selected and assessed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocol and the Newcastle-Ottawa Scale criteria. We used a funnel plot to assess publication bias.

RESULTS

From 1595 publications, we identified eight eligible studies that collectively enrolled 355 patients with pancreatic cancer. Among these original studies, two were carried out in China; three in the United States; and one each in Italy, Spain, and Norway. All eight studies analyzed the relevance between CTCs and the

prognosis of patients with early-stage pancreatic cancer after surgery. A meta-analysis showed that the patients that were positive pre-treatment or post-treatment for CTCs were associated with decreased OS [hazard ratio (HR) = 1.93, 95% confidence interval (CI): 1.197-3.126, P = 0.007] and decreased relapse-free/disease-free/progression-free survival (HR = 1.27, 95%CI: 1.137-1.419, P < 0.001) in early-stage pancreatic cancer. Additionally, the results suggest no statistically noticeable publication bias for overall, disease-free, progression-free, and recurrence-free survival.

CONCLUSION

This pooled meta-analysis shows that CTCs, as biomarkers, can afford reliable prognostic information for patients with early-stage pancreatic cancer and help develop individualized treatment plans.

Key Words: Pancreatic cancer; Surgery; Prognosis; Systematic review; Meta-analysis; Biomarkers

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: There is no consensus regarding the prognostic value of circulating tumor cells (CTCs) in early-stage pancreatic cancer after surgery. This is the first systematic review and meta-analysis to investigate the potential of CTCs in predicting survival time in early pancreatic cancer. We pooled the analyses of the relationship between CTCs and overall/disease-free/progression-free/relapse-free survival in related studies. Patients testing positive for CTCs pre- or intra-surgery may have worse prognoses, requiring more intense chemotherapy and closer follow-up.

Citation: Zhang ZH, Bao YW, Zhao YJ, Wang JQ, Guo JT, Sun SY. Circulating tumor cells as potential prognostic biomarkers for early-stage pancreatic cancer: A systematic review and meta-analysis. *World J Clin Oncol* 2023; 14(11): 504-517
URL: https://www.wjgnet.com/2218-4333/full/v14/i11/504.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i11.504

INTRODUCTION

Pancreatic cancer is a fatal disease with poor prognosis. In 2018, pancreatic cancer was the seventh leading cause of cancer-related mortality worldwide[1]. The number of annual diagnoses of and deaths related to pancreatic cancer in China have exceeded those in the United States[2]. The incidence of pancreatic cancer continues to increase at a rate of 0.5%-1.0% each year, and it is projected to be the second deadliest cancer by 2030 in Western countries[3]. Despite continuous advances in chemotherapy, radiation, and surgical techniques; the prognosis of patients with pancreatic cancer remains significantly poor. Approximately half of the patients experience reoccurrence within the first year, mainly due to metastatic disease occurrence after surgical resection. Consequently, one of the most important challenges is to identify a factor that can assess the survival outcome of patients with early pancreatic cancer before surgery, optimize treatment, and assist in the development of monitoring strategies.

Currently, technological innovations in imaging and endoscopy are being used to improve the diagnostic accuracy of pancreatic cancer. Computed tomography (CT) and magnetic resonance imaging (MRI) remain first-line diagnostic modalities for clinical suspicion. Endoscopic ultrasound (EUS) and EUS-guided fine-needle aspiration (EUS-FNA), also play important roles in its diagnosis. However, imaging features of early pancreatic cancer are subtle, and the general consensus is that it is difficult to detect early lesions[4]. Liquid biopsy is a new technology that detects biomarkers, such as carcinoembryonic antigen, carbohydrate antigen 19-9 (CA19-9), or CA125, from nonsolid biological tissues, such as blood[5], and has received increased attention because of its convenience and noninvasiveness[6]. However, these biomarkers all lack sufficient sensitivity and specificity for diagnostic purposes.

With the development of medical testing technologies, circulating tumor cells (CTCs) have emerged as a popular research topic over the last few decades. CTCs are a small number of tumor cells that detach from primary tumors, circulate through the bloodstream, and are the main source of its dissemination and metastasis[7]. Unlike traditional histopathological examinations, which are usually complex and risky for difficult-to-biopsy tumors such as pancreatic cancer, CTCs are noninvasive. CTCs can provide real-time and comprehensive information about the tumor because they are enriched in the bloodstream and originate from different regions of the original tumor or metastasis. Moreover, CTCs can present large-scale health information, such as the expression of genes and proteins and alteration of cellular contents and cell membranes, which are essential for improving diagnostic accuracy and developing individualized treatments[8]. Recently, an increasing number of studies have revealed that CTCs show promise for prognostic evaluation in several tumors, including lung[9], renal[10], breast[11], gastric[12], and colorectal cancers[13].

Nonetheless, the prognostic effect of CTCs in early pancreatic cancer remains ambiguous, mainly because of the increasing number of CTC isolation methods and different study designs. Therefore, this study aimed to perform a structural meta-analysis of currently available evidence on the prognostic value of CTCs in early-stage pancreatic cancer.

MATERIALS AND METHODS

Search strategy

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we conducted a comprehensive literature search of all studies published in database repositories before December 2022 that were related to the use of CTCs for the diagnosis of pancreatic cancer. The search was performed only in English and Chinese databases including PubMed, EMBASE, Web of Science, Cochrane Library, China National Knowledge Infrastructure (CNKI), China Biology Medicine (CBM), and ChinaInfo. Only studies published in Chinese and English were included in the analysis. Search terms included these keywords, MeSH (medical subject headings) terms, and their entry terms: "Pancreatic Neoplasms"(MeSH), "Neoplasm, Pancreatic," "Pancreatic Neoplasm," "Pancreas Neoplasms," "Neoplasm, Pancreas," "Neoplasms, Pancreas," "Pancreas Neoplasm," "Neoplasms, Pancreatic," "Cancer of Pancreas," "Pancreas Cancers," "Pancreas Cancer," "Cancer, Pancreas," "Cancers, Pancreas," "Pancreatic Cancer," "Cancer, Pancreatic," "Cancers, Pancreatic," "Pancreatic Cancers," "Cancer of the Pancreas," "Neoplastic Cells, Circulating"(MeSH), "Neoplasm Circulating Cells," "Circulating Neoplastic Cells," "Cell, Circulating Neoplastic," "Cells, Circulating Neoplastic," "Circulating Neoplastic Cell," "Neoplastic Cell, Circulating," "Circulating Tumor Cells," "Cell, Circulating Tumor," "Cells, Circulating Tumor," "Circulating Tumor Cell," "Tumor Cell, Circulating," "Tumor Cells, Circulating," "Cells, Neoplasm Circulating," "Cell, Neoplasm Circulating," "Neoplasm Circulating Cell," "Circulating Cells, Neoplasm," "Tumor Cells, Embolic," "Cell, Embolic Tumor," "Cells, Embolic Tumor," "Embolic Tumor Cell," "Tumor Cell, Embolic," "Embolic Tumor Cells," "Embolism, Tumor," "Embolisms, Tumor," "Tumor Embolism," "Tumor Embolisms," "Prognostic," "Prognosis," "Prognos*". These terms were supplemented by the logical operators "and" and "or." To expand the literature, we reviewed and evaluated the references of the included studies.

Selection criteria

Two independent investigators reviewed the article titles and abstracts according to inclusion and exclusion criteria to exclude irrelevant studies. Subsequently, the full texts of the included studies were analyzed to determine their suitability for meta-analysis. In case of any contradictions, a third reviewer was consulted for adjudication. The inclusion and exclusion criteria were formulated based on the PRISMA of Diagnostic Test Accuracy Studies guidelines. Inclusion criteria: (1) Studies included participants of any age with histologically or cytologically confirmed early pancreatic cancer (early pancreatic cancer was defined as pancreatic adenocarcinoma with a maximum tumor diameter of 4 cm, regional lymph node metastasis of no more than three nodes, and no distant metastasis); (2) studies that investigated pre- or intra-operative CTCs as a prognostic biomarker in blood for early pancreatic cancer patients' survival results after surgery; (3) sufficient published data available for calculating hazard ratio (HR) and 95% confidence interval (CI) of overall survival (OS) and progression-free survival (PFS); (4) studies that were published in English or Chinese; and (5) studies that were reported as full paper publications. Exclusion criteria: (1) Patients were diagnosed with pancreatic cancer at advanced stages or studies that didn't analyze the results of early pancreatic cancer patients independently; (2) studies that did not provide adequate data on the prognostic performance of CTCs for early pancreatic cancer patients; (3) patients didn't receive surgical therapy; and (4) articles were published in languages other than English and Chinese. Overall, five non-human studies, case reports, comments, meta-analyses, reviews, and published clinical and treatment guidelines were excluded.

Data extraction

To decrease systematic errors during data collection, two reviewers independently selected the studies. Any conflicting cases were carefully reviewed, and a third reviewer was consulted to reach a consensus. Data extraction from the eligible papers included the following items: (1) General information about the article: first author, publication date, and country; (2) study information: number of patients, evidence of confirmed pancreatic cancer, CTCs separation solution, CTCs determination criteria, and follow-up time; and (3) data for the meta-analysis: HR with 95%CI for OS, PFS or disease-free survival (DFS), recurrence-free survival (RFS). For articles without HR and 95%CI, we used Engauge Digitizer 11.3 to calculate them based on the survival rate extracted from Kaplan-Meier curves.

Risk of bias

The quality of the included studies was assessed based on the Newcastle-Ottawa Scale (NOS) criteria for non-randomized studies, which included three key domains covering "selection," "comparability," and "outcome." A star rating system was used to semi-quantitatively evaluate study quality, and those who met the standards for each item were awarded one or two stars. Scores range from zero to nine. A score equal to or greater than seven indicates high quality. This tool objectively evaluates the risk of bias and assesses concerns regarding its applicability. The quality assessment was performed by two independent reviewers. Any disagreements were discussed until an agreement was reached. Publication bias was investigated using a funnel plot, with $P < 0.05$ indicating a significant publication bias.

Statistical analysis

Review Manager 5.3 software (The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark) was used to assess the pooled HR effect size. Heterogeneity between studies was assessed using Cochran's Q test and I^2 statistics. According to Higgins and Thompson, $I^2 > 50\%$ or $P < 0.1$ was viewed as consistent significant heterogeneity. A fixed-effect model was used when minor heterogeneity was observed. Otherwise, a random-effects model was used to calculate the pooled HR. Subgroup analyses based on ethnicity, separation solution, treatment, and follow-up time were performed to explore potential sources of heterogeneity. Finally, we conducted a sensitivity analysis to evaluate the effect

of a single study on overall outcomes.

RESULTS

Characteristics and quality of the included studies

A total of 1963 articles were collected, including 91 from CNKI, 273 from ChianInfo, 90 from CBM, 258 from PubMed, 55 from EMBASE, 31 from Cochrane, and 1165 from Web of Science. After deleting 368 duplicate references, we screened the titles and abstracts of 1595 residual articles. In total, 1551 articles were excluded (520 meta-analyses or reviews; 32 non-human studies; 15 articles published in languages other than English and Chinese; 161 meeting abstracts, case reports, guidelines, or letters; and 823 irrelevant studies). According to the inclusion and exclusion criteria, we read the full text of the 44 remaining studies, of which 36 articles were ultimately excluded (5 irrelevant studies and 31 articles with data deficiency). Consequently, eight studies involving 355 patients with early-stage pancreatic cancer were included in our meta-analysis[14-21]. A flow diagram of the literature search and filtering process is shown in Figure 1.

The characteristics of these studies are summarized in Table 1. The overall research quality was rated as moderate by the NOS, with an average score of 6.75 (Table 2). Among these original studies, two were conducted in China; three in the United States; and one each in Italy, Spain, and Norway. The publication years were 2014 (n = 1), 2018 (n = 1), 2021 (n = 5), and 2022 (n = 1). All eight studies analyzed the correlation between CTCs and prognosis in patients with early-stage pancreatic cancer after surgery. While four studies included early pancreatic cancer patients only[14,19-21], the remaining four studies contained early and advanced pancreatic cancer patients with early pancreatic cancer patients accounting for at least 30% of the total patients[15-18]. In total, five studies detected CTCs in the peripheral blood (PB), two studies detected CTCs in the PB and portal venous blood (PVB), and one study detected CTCs in the central venous catheter and portal blood. Blood samples were collected solely before surgery in four studies[16,17,21], before and after surgery in three studies[14,15,18], and during surgery in only one study[19]. Only pre- and intra-operative CTC data were included in this meta-analysis. Although eight studies applied different CTC enrichment and separation methods, including commercially available CTC detection kits[14,17], dielectrophoresis-field flow fractionation (DEP-FFF)[15], and CTC isolation systems[18-21], most methods essentially rely on the density characteristics of CTCs as well as the epithelial and mesenchymal markers expressed by CTCs. Most studies applied DFS, OS, PFS, and RFS as survival outcomes, while only Hugenschmidt *et al*[20] adopted cancer-specific survival as an outcome indicator. Seven of the eight studies reported the adjusted HR and 95%CI for the association between DFS/PFS/RFS and positive CTCs. For the one that did not, we calculated these values according to the Kaplan-Meier curves provided in the articles. In addition, only three of the eight included studies reported the adjusted HR and 95%CI for the association between OS and positive CTCs while another two studies provided Kaplan-Meier curves for OS.

The relationship between CTCs and prognosis of early-stage pancreatic cancer patients after surgery

We conducted a meta-analysis to assess the association between CTCs detected in the blood samples of patients with early-stage pancreatic cancer and their prognosis after surgery. The degree of heterogeneity among the five studies that provided the adjusted HR and 95%CI for OS was low (I^2 = 41%, P = 0.15); therefore, we chose a fixed-effect model for analysis. The pooled analyses of the five studies, containing 169 patients, showed that positive detection of pre-treatment or post-treatment CTCs was associated with decreased OS (HR = 1.93, 95%CI: 1.20-3.13) that was statistically significant (Z = 2.69, P = 0.007) (Figure 2A). There was a moderate degree of heterogeneity among the seven studies available for the adjusted HR and 95%CI of RFS/DFS/PFS (I^2 = 65%, P = 0.01); therefore, we chose a random-effects model for analysis. The results showed that positive pre-treatment or post-treatment CTCs were associated with decreased DFS/PFS/RFS (HR = 1.97, 95%CI: 1.20-3.25) that were statistically significant (Z = 2.67, P = 0.008) (Figure 2B).

Subgroup analysis

Because of the high heterogeneity in the DFS/PFS/RFS results, meta-regression was conducted to explore the sources of heterogeneity. No apparent deviation was found in the ethnicity, treatment, and follow-up time subgroups. Later analysis demonstrated that CTC-positive patients were associated with decreased DFS/PFS/RFS in subgroups that detected CTCs by the CellSearch system (HR = 2.62, 95%CI: 1.65-4.16, Z = 4.09, P < 0.001), and the heterogeneity between subgroups was low (P = 0.018) (Figure 3).

Sensitivity analysis

To explore the potential sources of this difference, we conducted sensitivity analysis by sequentially excluding each study. When the study by Xing *et al*[14] was excluded, the heterogeneity of the remaining studies was significantly reduced (OS: I^2 = 0%, P = 0.44; DFS/PFS/RFS: I^2 = 0%, P = 0.76), and the pooled results for both OS and DFS/PFS/RFS were increased (OS: HR = 3.06 95%CI: 1.59-5.86, Z = 3.36, P < 0.001; DFS/PFS/RFS: HR = 2.60 95%CI: 1.78-3.812, Z = 4.001, P < 0.001) (Figure 4). Nevertheless, the direction of the pooled results for the OS and DFS/PFS/RFS subgroups were not affected, indicating a negative association between CTC positivity and lower OS or DFS/PFS/RFS.

Publication bias

We used a funnel plot to assess publication bias in this meta-analysis. The results showed no publication bias for OS (P = 0.653) and DFS/PFS/RFS (P = 0.117) (Figure 5).

Table 1 Characteristics of the included studies

Ref.	Year	Country	Sample	Separation Solution	Markers and expression level on PC CTC	Cases	No-positive	Treatment	Outcome	Follow-up time
Xing *et al*[14]	2021	China	Peripheral blood	SE-iFISH	CD44+ CTEC: DAPI +/CD45-/CD31 +/CD44 +/Vimentin (+ or -) with aneuploid CEP8	73	Not reported	Surgery	OS/DFS	Median 10.8 mo (1.2-31.8 mo)
Semaan *et al* [15]	2021	United States	Peripheral blood	DEP-FFF	pEMT-CTC: CD45 -, EpCAM + and/or Pan-CK +, Vimentin +, DAPI +	31 early stages (74 total)	28	Surgery/neoadjuvant treatment	OS/PFS	Median 15.4 mo (0-43.1 mo)
Padillo-Ruiz *et al*[21]	2021	Spain	Central venous catheter and portalblood	ICC	DAPI +/CK +/CD45 -	35	35	Surgery/chemotherapy	OS/DFS	24 mo
Court *et al*[16]	2018	United States	Peripheral blood	NanoVelcro chip	DAPI +/CD45 -/CK +; CD45 positivity greater than 2 × background	40 early stages (126 total)	27	Surgery/chemotherapy	OS/RFS	≥ 24 mo
Cheng *et al*[17]	2022	China	Peripheral blood	LT-PCR	FR + CTC	25 early stages (44 total)	13	Surgery/chemotherapy	OS/DFS	Median 20 mo (6-28 mo)
White *et al*[18]	2021	United States	Peripheral blood and portal venous blood	CellSearch	DAPI +/CK +/CD45 -	33 early stages (34 total)	21	Surgery/neoadjuvant treatment	OS/RFS	Median 14.1 mo (0.86-1.97 mo)
Bissolati *et al* [19]	2014	Italy	Peripheral blood and portal venous blood	CellSearch	DAPI +/CK +/CD45 -	20	9	Surgery/chemotherapy	OS/PFS	Median 39.2 mo (36-45 mo)
Hugenschmidt *et al*[20]	2021	Norway	Peripheral blood	CellSearch	EpCAM +/DAPI +/CK +/CD45 -	98	7	Surgery	CSS/DFS	Median 96 mo (63-126 mo)

DEP-FFF: Dielectrophoresis-field flow fractionation; ICC: Immunocytochemistry; SE-iFISH: Subtraction enrichment and immunostaining-fluorescence in situ hybridization; PC: Pancreatic cacner ; CTCs: Circulating tumor cells; LT-PCR: Ligand-targeted polymerase chain reaction; CK: Cytokeratin; DAPI: 4',6-diamidino-2-phenylindole; EpCAM: Epithelial cell adhesion molecule; CSS: Cancer specific survival; DFS: Disease-free survival; OS: Overall survival; PFS: Progression-free survival; pEMT: Partial epithelial-mesenchymal transition.

The relationship between CA19-9 and the prognosis of early-stage pancreatic cancer patients after surgery

In order to compare the potential of CTC and CA19-9 in predicting patient prognosis, we extracted the adjusted HR and 95%CI of DFS related to CA19-9 in the included articles. Only four studies had the required data[14,16-18]. The degree of heterogeneity of the adjusted HR and 95%CI for DFS was high (I^2 =73%, P = 0.01), so we used a random-effects model for the analysis. The pooled analyses of the four studies, containing 171 patients, showed that the CA19-9 level in the PVB of early-stage pancreatic cancer patients was not an independent predictor of a shorter time to recurrence (HR = 1, 95%CI: 1.00-1.00; Z = 0.36, P = 0.72) (Figure 6). Considering the impact of sampling time on the results, we excluded the study of White *et al*[18], which collected venous blood after resection. Though the heterogeneity of the remaining studies was significantly reduced (I^2 = 48%, P = 0.15), the combined HR was still 1 (95%CI: 1.00-1.00; Z =2.20, P = 0.03) (Figure 7).

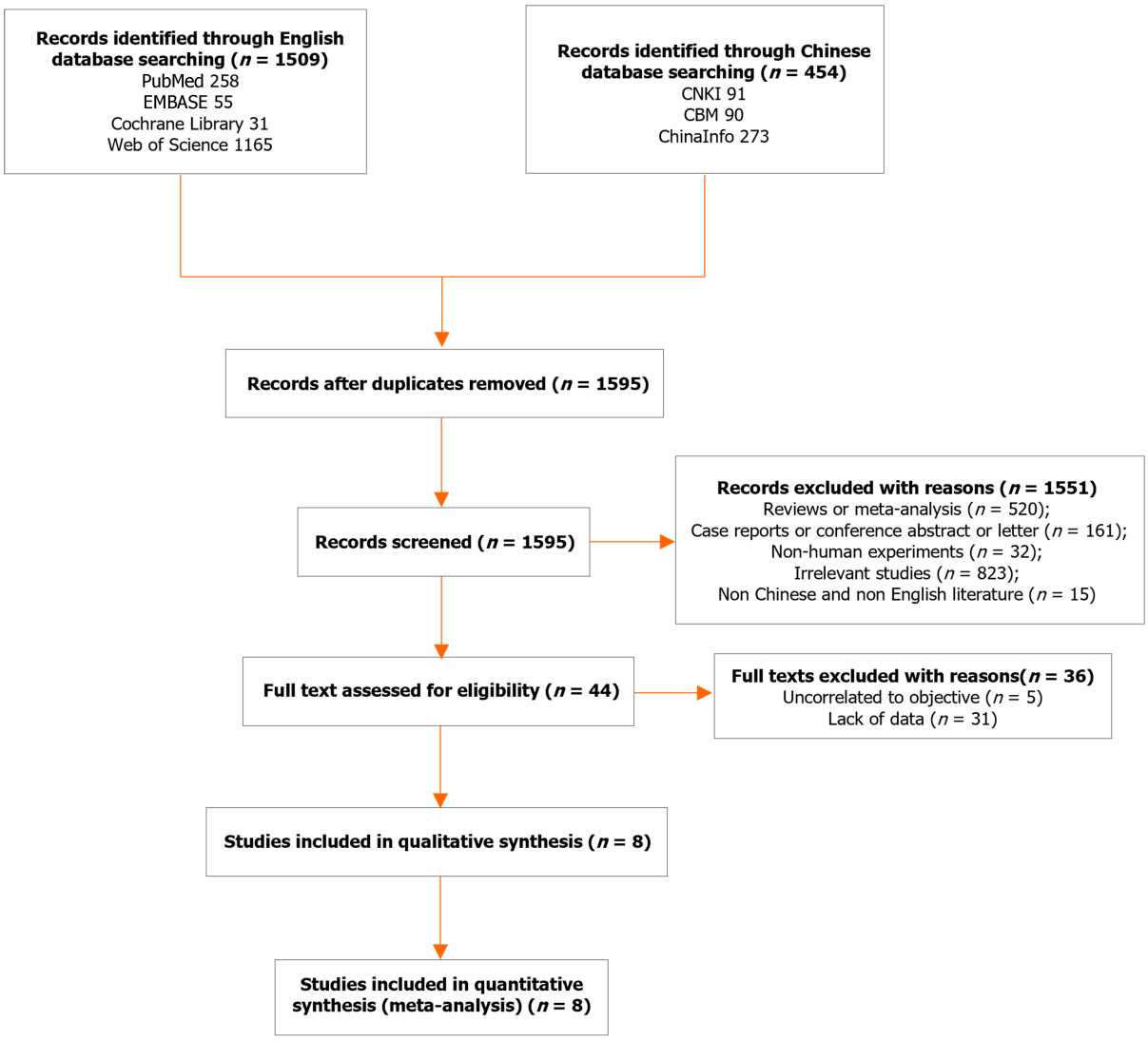
Table 2 Quality assessment of the included studies according to the Newcastle-Ottawa Scale criteria for non-randomized studies

Ref.	Selection				Comparability			Outcome		Total stars
	Representativeness	Selection of non-exposed	Ascertainment of exposure	Outcome not present at start	Comparability on most important factors	Comparability on other risk factors	Assessment of outcome	Long enough follow-up (median 24 mo)	Completeness of follow-up	
Xing *et al*[14]	-	[1]	[1]	[1]	[1]	-	[1]	-	[1]	6
Semaan *et al*[15]	-	[1]	[1]	[1]	[1]	-	[1]	-	[1]	6
Padillo-Ruiz *et al*[21]	-	[1]	[1]	[1]	[1]	-	[1]	[1]	[1]	7
Court *et al*[16]	-	[1]	[1]	[1]	[1]	-	[1]	[1]	[1]	7
Cheng *et al*[17]	[1]	[1]	[1]	[1]	[1]	-	[1]	-	[1]	7
White *et al*[18]	-	[1]	[1]	[1]	[1]	-	[1]	-	[1]	6
Bissolati *et al*[19]	[1]	[1]	[1]	[1]	[1]	-	[1]	[1]	[1]	8
Hugenschmidt *et al*[20]	-	[1]	[1]	[1]	[1]	-	[1]	[1]	[1]	7

[1]Meets the criteria of the scale. A maximum of one 'star' for each item within the 'Selection' and 'Outcome' categories; maximum of two 'stars' for 'Comparability'; -Does not meet the criteria of the scale.

DISCUSSION

In this meta-analysis, we found that CTC detected in patients with early pancreatic cancer is negatively correlated with the prognosis time after surgery. After data collection and filtering, eight studies from 2014 to 2022, including 355 patients with early-stage pancreatic cancer, were included in our analysis. Through data sorting and meta-analysis, we demonstrated that testing positive pre- or intra-treatment for CTCs was associated with decreased OS and RFS/DFS/PFS in early-stage pancreatic cancer. Nevertheless, our meta-analysis showed a high degree of heterogeneity in DFS/PFS/RFS. To determine the potential sources of the heterogeneity, we conducted a subgroup analysis. The pooled results were not affected by ethnicity, treatment, or follow-up time, whereas subgroup analysis by CTCs separation solution decreased the heterogeneity between the groups, indicating that this might have caused the heterogeneity. Studies that enumerated CTCs by the CellSearch system demonstrated a more obvious correlation between CTC-positive patients and decreased DFS/PFS/RFS. However, except for this system, all other separation solutions contained only one study; therefore, we were unable to obtain pooled results. Sensitivity analysis was conducted by using the "leave-one-out method." Notably, when a study by Xing *et al*[14] was removed, the heterogeneity of the remaining studies was significantly reduced, and the pooled results for both OS and DFS/PFS/RFS were relatively elevated. We infer that the differences present in this study may be derived from the separation solutions employed as well as the stemness markers used to identify the stem cell-like phenotype of CTCs. Importantly, the results demonstrating the survival-jeopardizing effects of CTCs were maintained. We suggest that patients testing positive for CTCs pre- or intra-treatment may have a worse prognosis and



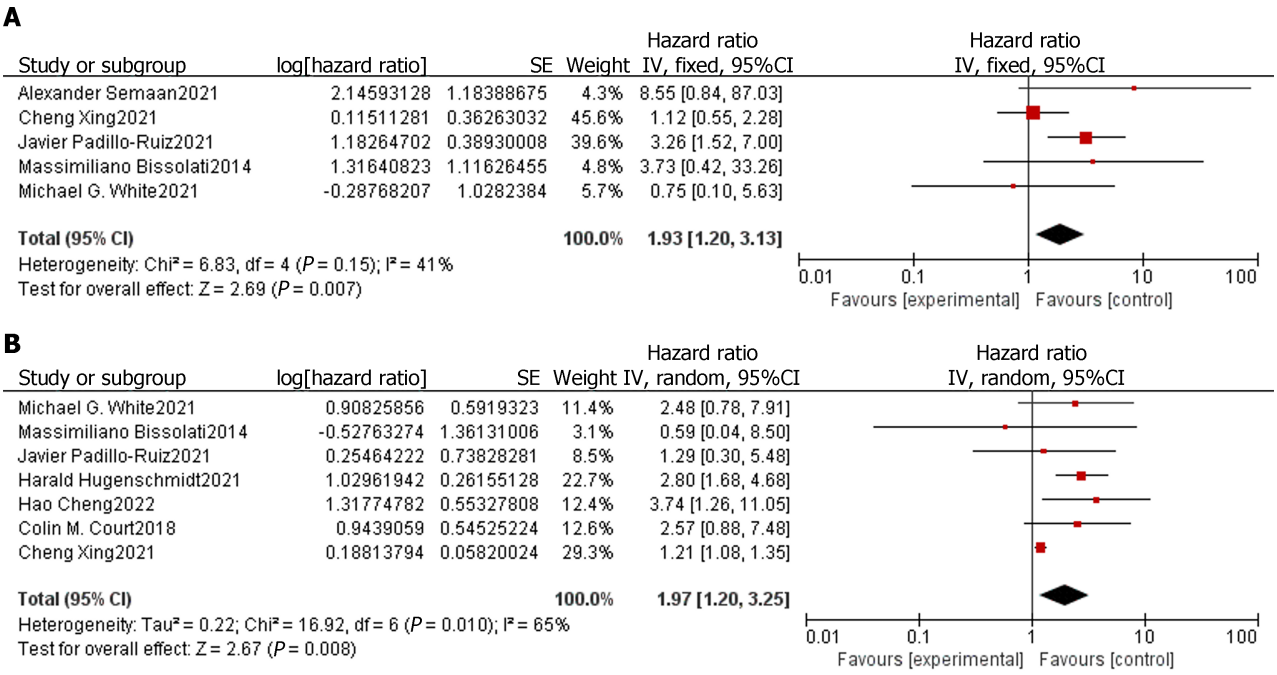
DOI: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

Figure 1 Flow diagram for searching and filtering eligible studies included in the meta-analysis.

require more intense chemotherapy and closer follow-up. Furthermore, we collected data regarding CA19-9 in the included studies and four studies containing 171 patients, that met our requirements. Our meta-analysis showed that the preoperative CA19-9 level in the PVB of early-stage pancreatic cancer patients was not an independent predictor for shorter time of recurrence after resection.

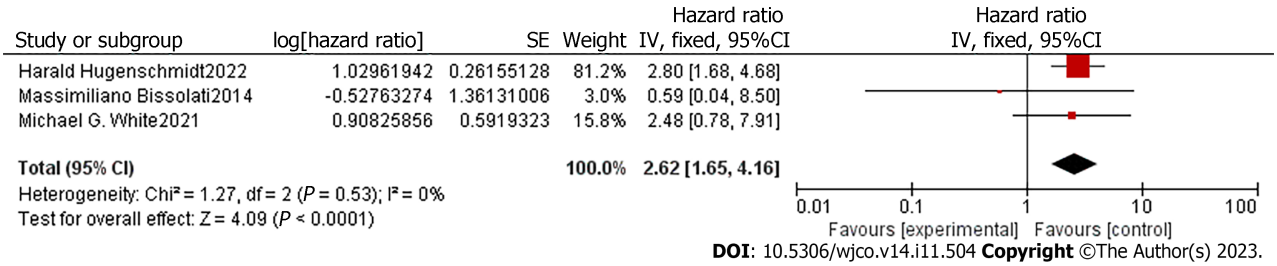
The prevalence of pancreatic cancer has increased dramatically globally, and is expected to become a leading cause of cancer-related mortality[22]. Only 10%-15% of patients have localized pancreatic cancer suitable for surgery[3], which is the only potentially curative therapy known to date. Even for patients with a localized disease, a high proportion experience postoperative recurrence within 5 years, caused by local tumor recurrence or distant metastases. The 1- and 5-year survival rates are 63% and 17%, respectively[23]. Therefore, identifying high-risk populations for screening and prevention, early diagnosis, and establishing personalized treatment plans are currently the primary challenges[24].

Imaging, including EUS, CT and MRI, which can provide a convenient and noninvasive diagnosis, remains the first-line diagnostic modality for pancreatic cancer and is used to evaluate therapeutic efficacy in many organs[25-28]. However, cross-sectional imaging is limited in the visualization of small and metastatic tumors, which can frequently result in underestimation of the pancreatic cancer stage[29]. EUS-FNA or EUS-fine needle biopsy (EUS-FNB) can localize pancreatic lesions measuring < 3 cm, providing a minimally invasive tissue biopsy[30]. EUS-FNB is increasingly becoming a practical tool for diagnosing malignancy in various pancreatic solid lesions[31,32]. EUS-FNA combined with needle-based confocal laser endomicroscopy (nCLE) can achieve real-time imaging for in vivo tissue analysis[33]. However, these examinations requires anesthesia and may be accompanied by complications such as acute pancreatitis, tumor dissemination, and postoperative hemorrhage. Besides, nCLE is limited by the duration of the surgical time and the operability of the 19G FNA needle[33]. More recently, liquid biopsy has received a great deal of attention for its ability to assess a comprehensive cancer profile in a noninvasive and real-time manner[26]. Serum CA19-9 level is the only diagnostic biomarker approved by the United States Food and Drug Administration for pancreatic cancer. Moreover, CA19-9 can be an independent predictor of prognosis for pancreatic cancer patients[34]. An elevated CA19-9 level after



DOI: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

Figure 2 Forest plots of the five studies show a shorter overall survival and disease-free survival/progression-free survival/recurrence-free survival in early pancreatic patients with positive pre-treatment or post-treatment circulating tumor cells detection. A: Overall survival; B: Disease-free survival/progression-free survival/recurrence-free survival. CI: Confidence interval.



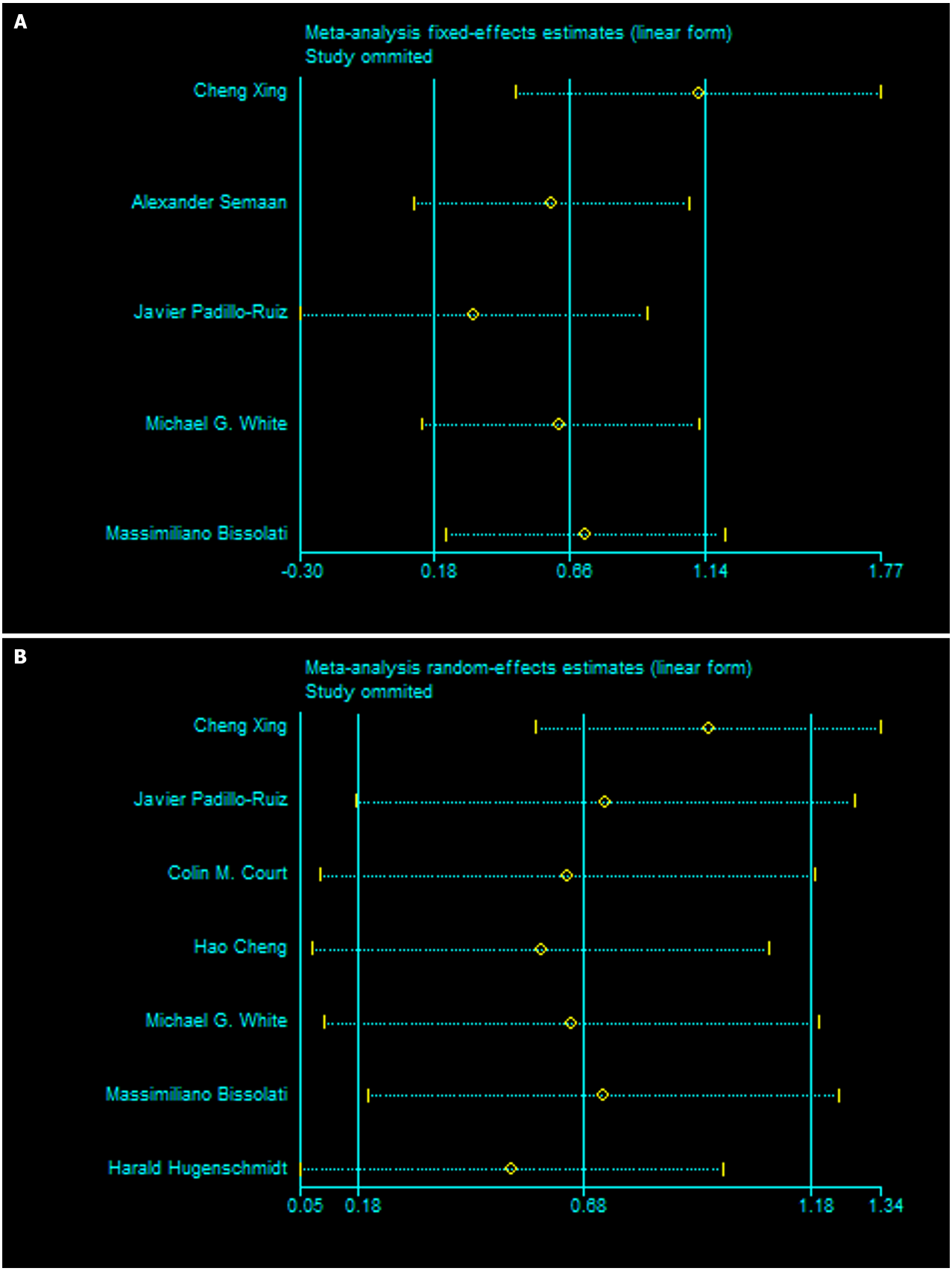
DOI: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

Figure 3 Forest plot of the three studies that used the CellSearch system shows a decreased disease-free survival/progression-free survival/recurrence-free survival in early pancreatic patients that were positive pre-treatment or post-treatment for circulating tumor cells. CI: Confidence interval.

resection or during chemotherapy predicts a high probability of tumor recurrence or progression[35]. However, the prognostic ability of preoperative CA19-9 is still disputed, since it is mostly detected in advanced stages, it is neither sensitive nor specific enough to identify early-stage patients or for the differential diagnosis of patients at different stages; additionally, it is also positive in many other benign and malignant pancreatic diseases such as pancreatitis, cholestasis, and gastric cancer[7]. In this meta-analysis, the pooled results of the four studies, that we examined, demonstrated that the prognostic effect of CA19-9 in peripheral venous blood on early postoperative recurrence of early-stage pancreatic cancer is not as obvious as that of CTCs.

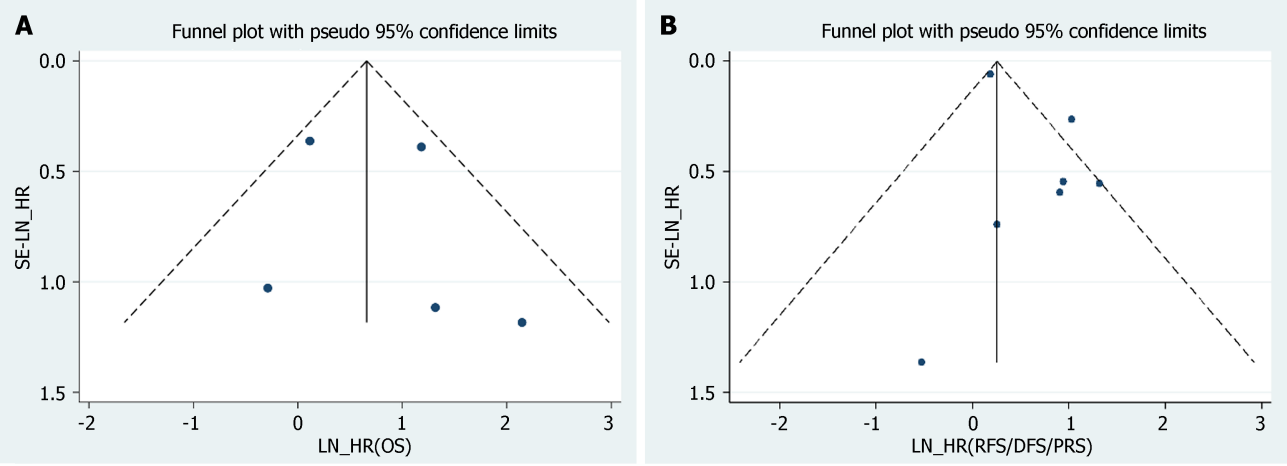
In the past decade, an increasing number of studies have examined the prognostic value of CTCs in cancers of various organs, as the formation of tumor metastases relies heavily on the survival of CTCs and their ability to mediate angiogenesis in target organs[36]. CTCs are tumor cells shed from both primary and secondary foci and are found circulating in the bloodstream; therefore, they can provide valuable information about primary tumors and secondary deposits. In addition, isolation and in vivo cultures of animal xenografts provide deeper information on individual tumor characteristics[37]. Prospective observational studies have also revealed that CTC numbers rarely drop to zero even after complete resection in both chemo-naïve and post-neoadjuvant patients and can be observed longitudinally before disease recurrence[38]. However, the deficiency of relevant studies and different research designs make the clinical significance of CTCs in early pancreatic cancer prognosis a controversial topic. A thorough analysis of the prognostic performance of CTCs is critical for monitoring and developing treatment strategies for patients with pancreatic cancer.

CTCs in the bloodstream are difficult to capture and identify because their concentration in the circulatory system is extremely low (1-10 cells/10 mL) in most cases[39]. Furthermore, CTCs are scattered among an enormous number of erythrocytes and leukocytes, posing tremendous challenges for their complete collection and accurate detection. To



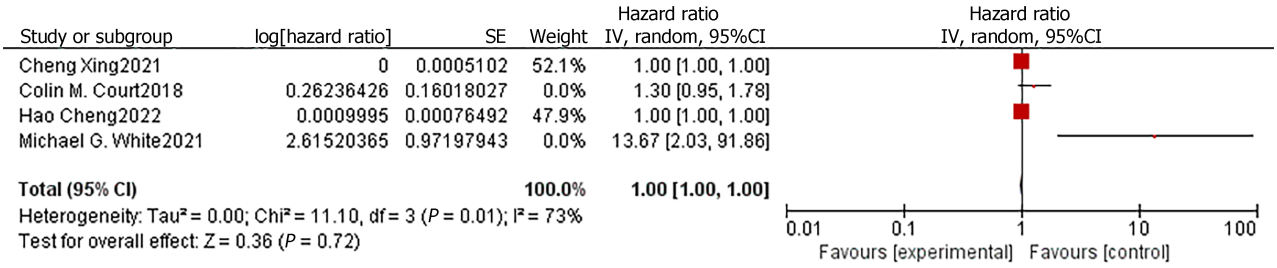
DOI: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

Figure 4 Sensitivity analysis of the influence of each study on the pooled results for the overall survival and disease-free survival/progression-free survival/recurrence-free survival subgroups. A: Overall survival; B: Disease-free survival/progression-free survival/recurrence-free survival.



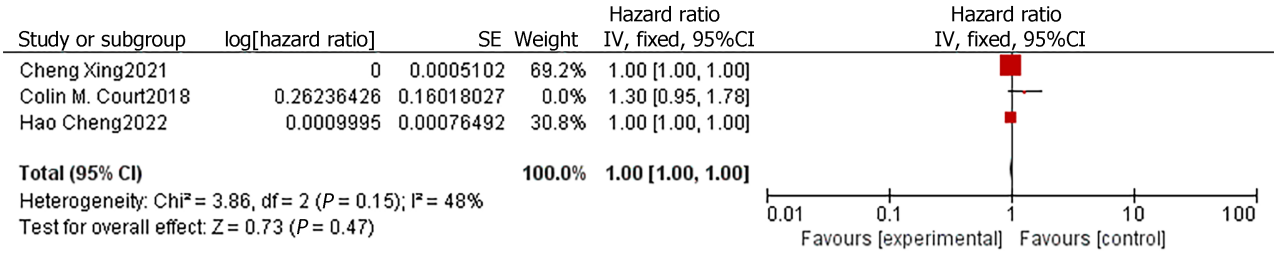
DOI: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

Figure 5 Funnel plots were generated for the included studies to determine whether or not publication bias was found for both the overall survival and disease-free survival/progression-free survival/recurrence-free survival subgroups. A: Overall survival; B: Disease-free survival/progression-free survival/recurrence-free survival.



DOI: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

Figure 6 Forest plots of the four studies show that the carbohydrate antigen 19-9 level in the venous blood of early-stage pancreatic cancer patients was not an independent predictor of a shorter time to recurrence. CI: Confidence interval.



DOI: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

Figure 7 Forest plots of the three studies, that detected the carbohydrate antigen 19-9 level in the venous blood before surgery, show that it was not an independent predictor of a shorter time to recurrence for early-stage pancreatic cancer patients. CI: Confidence interval.

improve this situation, several methods have been used for CTC enrichment, such as density centrifugation, immunomagnetic enrichment with anti-CD45 monoclonal antibodies, epithelial cell adhesion molecule (EpCAM), and cell filtration technology. Additionally, epithelial cell-specific markers, such as the cytokeratin (CK) family, and mesenchymal markers, such as N-cadherin and vimentin, have been used to identify epithelial and mesenchymal CTCs, respectively[40, 41]. Numerous platforms based on these technologies have been developed. The CellSearch system is the only platform approved by the Food and Drug Administration for confirming the presence of CTCs in patients with pancreatic cancer. This system first enriches CTCs by taking advantage of their characteristic expression of the EpCAM on their membrane surface and then distinguishing different cells by immunostaining markers such as CD45, 4',6-diamidino-2-phenylindole (DAPI), and CK 8, 18, 19[42]. However, the detection rates are only 20% and 5%-42% for resectable and advanced pancreatic cancers, respectively [19,43]. This is because most CTCs undergo epithelial-mesenchymal transition (EMT) and thus lack or express low levels of epithelial markers that are generally used as the basis for the detection of these cells [44]. In addition, separation solutions based on these epithelial markers may exclude cells that play important roles in

metastasis and chemoresistance[45]. In our included studies, Court *et al*[16] used the microfluidic NanoVelcro CTC chip to evaluate the presence and number of CTCs. This platform greatly improves CTC capture and identification by utilizing anti-EpCAM-coated 3D-nanosubstrates in conjunction with microfluidic chaotic mixers and by adding tumor identification markers, which enhance the synergistic effects of cell–substrate contact frequency as well as its affinity[46]. This platform also allows seamless integration with laser capture microdissection for single CTC isolation[47]; moreover, the separated cells can be subjected to downstream molecular analyses[48]. However, an important limitation of this assay is that it is similar to the CellSearch system. In addition, using membrane filtration may reduce specificity, as some CTCs are found to be equal or smaller in size than nucleated blood cells[49]. The combination of specific mesenchymal markers may be a future direction for these assays. Considering their failure to detect EpCAM- or CK-negative CTCs, Lin *et al*[50] developed an integrated tumor cell surface molecule-independent SE-iFISH platform in 2015. Using fluorescence in situ hybridization with a specific chromosome centromere probe, this system can detect aneuploidy in the PB, which is a common manifestation of chromosome instability and malignant solid tumors. Moreover, Semaan *et al*[15] used an antigen-independent approach called DEP-FFF, which utilizes the physical properties of cells and allows the isolation of phenotypically distinct CTCs. In doing so, they obtained not only epithelial and mesenchymal CTCs, but also intermediate-state CTCs, which may show greater invasiveness and therapeutic resistance. Another valid method to detect the molecular characterization of CTCs is PCR. This is most likely due to the detection of multiple tumor markers, which could downgrade the effect of the heterogeneity that exists in CTCs. Among the included studies, Cheng *et al*[17] used immunomagnetic depletion and ligand-targeted polymerase chain reaction to detect the expression rate of folate receptor + CTCs in patients with different stages of pancreatic cancer. Traditional genetic studies of CTCs are limited by the low specificity of the enrichment methods, which contributes to the presence of nuclear blood cells, necrotic cells, tumor-derived exosomes, and cellular fragments. Therefore, the obtained nucleic acids may not accurately reflect the hereditary properties of CTCs[42]. In recent years, single-cell separation and whole-genome amplification of CTCs have been developed to overcome this challenge. However, studies on pancreatic cancer are still lacking.

Although the pooled results of our meta-analysis indicated that CTCs were associated with shorter OS and DFS/PFS/RFS in patients with early pancreatic cancer after surgery, three of the eight included studies showed no significant correlation between the existence of CTCs and both OS and DFS/PFS/RFS. Colin *et al*[48] found that CTCs were independent predictors of RFS following surgery and could correctly identify patients with occult metastatic disease preoperatively. However, when the analysis was limited to the early-stage subset, CTC count was no longer associated with shorter RFS. One possibility is that they used only 4 mL of PB, which may be too small to detect CTCs in patients with early-stage pancreatic cancer. For example, White *et al*[18], the CTC number in the PB did not contribute to predicting the OS or RFS of patients after resection, and the CTC number in the PVB was not associated with RFS. However, their results showed complete collinearity between the number of CTCs detected in the PVB and the OS. They hypothesized that this was caused by pancreatic venous drainage, as well as the capture and dilution effects of the liver for CTCs in the portal circulation. Similarly, the research of Bissolati *et al*[19] showed no significant correlation between the number of CTCs and survival time. However, they found that patients with a positive intraoperative detection of CTCs in the PVB had a higher liver metastasis rate, indicating that CTCs are of great significance for guiding adjuvant chemotherapy and postoperative follow-up monitoring. Overall, these studies revealed that CTCs in the blood of patients with pancreatic cancer are difficult to detect, particularly in early-stage patients.

In contrast, despite the different separation technologies and biomarkers used, the prognostic value of CTCs as a survival indicator for patients with early-stage pancreatic cancer was demonstrated in the remaining five studies. Moreover, they indicated that CTCs could provide tumor characteristics. Xing *et al*[14] used CD44+ as a marker to detect CTCs, which represent a more stem-like phenotype and can cause tumor metastasis, promoting tumor growth, angiogenesis, and drug resistance. Semaan *et al*[15] detected the longitudinal characterization of CTC subtypes and described EMT-CTCs as a more aggressive phenotype that is more prone to therapeutic resistance. In addition, the preoperative CTCs level in the PVB of early-stage pancreatic cancer patients may be a better prognostic marker then the preoperative CA19-9 level. This may broaden the selectivity of biomarkers for pancreatic cancer.

Our study has several limitations. First, the small number of included studies as well as the numerous types of separation strategies and research designs limited the power of our analysis. Second, several studies did not provide HR and 95%CI directly; therefore, we estimated them based on Kaplan-Meier curves, which may cause deviations. Finally, four of the eight studies recruited patients with pancreatic cancer at all stages, and we only extracted data about patients with early-stage pancreatic cancer; hence, the information was not detailed.

CONCLUSION

This meta-analysis reveals the potential of CTCs as a prognostic biomarker for early-stage pancreatic cancer. Although we rigorously gathered and analyzed the data, the essential limitations of the included studies caused a high degree of heterogeneity and hindered deeper exploration, which reduced the confidence level of our study. To overcome these difficulties, large-scale multicenter cohort studies are urgently needed to explore the full potential of CTCs.

ARTICLE HIGHLIGHTS

Research background

Pancreatic cancer is a terribly invasive and poorly prognosis disease with a five-year survival less than 10%. Recently, more and more studies demonstrated that circulating tumor cells (CTCs) can be a significant prognostic marker of pancreatic cancer.

Research motivation

In this study, we conducted a meta-analysis to analyse the prognostic role of CTCs in patients with pancreatic cancer and investigated whether CTCs can provide prognostic information and assist develop personalized treatment plans.

Research objectives

Our research aims at exploring the predictive effect of CTCs on survival indicators of pancreatic cancer patients in different studies.

Research methods

A standardized literature search of databases was conducted for articles about CTCs published through December 2022. After screening based on inclusion and exclusion criteria, data relevant to prognosis were extracted for analysis. We used a fixed- or random-effect model to calculate the pooled hazard ratio (HR) and 95% confidence interval (CI) of overall survival (OS) and progression-free survival (PFS) according to the degree of heterogeneity.

Research results

Eight eligible studies with a total number of 355 patients with early-stage pancreatic cancer were included. This meta-analysis showed that positive pre-treatment or post-treatment CTCs was associated with shorter OS (HR = 1.93, 95%CI: 1.197-3.126, P = 0.007) and decreased relapse-free/disease-free/PFS (HR = 1.27, 95%CI: 1.137-1.419, P < 0.001) in patients with early-stage pancreatic cancer. While the CA19-9 level in the portal venous blood of early-stage pancreatic cancer patients showed no significant correlation with postoperative recurrence time of patients (HR = 1, 95%CI: 1.00-1.00, P = 0.03).

Research conclusions

Our meta-analysis indicates that CTCs are closely related to the prognosis of early pancreatic cancer patients and can serve as a guiding indicator for developing patient important treatment plans.

Research perspectives

Researchers should extend follow-up time to observe the relationship between CTC and OS. Besides, large-scale multicenter cohort studies are urgently needed to explore the full potential of CTCs.

ACKNOWLEDGEMENTS

We thank Professor Si-Yu Sun, for his support and guidance, as well as all other doctors who participated in this study.

FOOTNOTES

Author contributions: Zhang ZH contributed to the data acquisition and analysis, and article drafting; Bao YW, Zhao YJ, and Wang JQ and Sun SY oversaw acquiring and analyzing data; Guo JT was the study supervisor and was responsible for the revision of the manuscript; all authors read and gave their final approval for this version of the manuscript to be submitted.

Supported by Liaoning Province Applied Basic Research Program Joint Program Project, No. 2022JH2/101500076; Shenyang Young and Middle-aged Science and Technology Innovation Talent Support Program, No. RC200438; Tree planting program of Shengjing Hospital, No. M1595.

Conflict-of-interest statement: The authors deny any conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Zi-Han Zhang 0000-0001-7636-9400; Yi-Wen Bao 0000-0002-2224-1118; Ya-Jun Zhao 0009-0008-6326-6590; Jian-Quan Wang 0009-0008-8907-5311; Jin-Tao Guo 0000-0001-5722-6359; Si-Yu Sun 0000-0002-7308-0473.

S-Editor: Qu XL
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **Park W**, Chawla A, O'Reilly EM. Pancreatic Cancer: A Review. *JAMA* 2021; **326**: 851-862 [PMID: 34547082 DOI: 10.1001/jama.2021.13027]
2 **Lin QJ**, Yang F, Jin C, Fu DL. Current status and progress of pancreatic cancer in China. *World J Gastroenterol* 2015; **21**: 7988-8003 [PMID: 26185370 DOI: 10.3748/wjg.v21.i26.7988]
3 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 7-30 [PMID: 31912902 DOI: 10.3322/caac.21590]
4 **Kamyabi N**, Bernard V, Maitra A. Liquid biopsies in pancreatic cancer. *Expert Rev Anticancer Ther* 2019; **19**: 869-878 [PMID: 31533487 DOI: 10.1080/14737140.2019.1670063]
5 **Crowley E**, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; **10**: 472-484 [PMID: 23836314 DOI: 10.1038/nrclinonc.2013.110]
6 **Marrugo-Ramírez J**, Mir M, Samitier J. Blood-Based Cancer Biomarkers in Liquid Biopsy: A Promising Non-Invasive Alternative to Tissue Biopsy. *Int J Mol Sci* 2018; **19** [PMID: 30248975 DOI: 10.3390/ijms19102877]
7 **Hou J**, Li X, Xie KP. Coupled liquid biopsy and bioinformatics for pancreatic cancer early detection and precision prognostication. *Mol Cancer* 2021; **20**: 34 [PMID: 33593396 DOI: 10.1186/s12943-021-01309-7]
8 **Gall TMH**, Belete S, Khanderia E, Frampton AE, Jiao LR. Circulating Tumor Cells and Cell-Free DNA in Pancreatic Ductal Adenocarcinoma. *Am J Pathol* 2019; **189**: 71-81 [PMID: 30558725 DOI: 10.1016/j.ajpath.2018.03.020]
9 **Kanayama M**, Kuwata T, Mori M, Nemoto Y, Nishizawa N, Oyama R, Matsumiya H, Taira A, Shinohara S, Takenaka M, Yoneda K, Kuroda K, Ohnaga T, Tanaka F. Prognostic impact of circulating tumor cells detected with the microfluidic "universal CTC-chip" for primary lung cancer. *Cancer Sci* 2022; **113**: 1028-1037 [PMID: 34964211 DOI: 10.1111/cas.15255]
10 **Guan Y**, Xu F, Tian J, Gao K, Wan Z, Wang Y, Gao M, Wang Z, Chong T. The prognostic value of circulating tumour cells (CTCs) and CTC white blood cell clusters in patients with renal cell carcinoma. *BMC Cancer* 2021; **21**: 826 [PMID: 34271857 DOI: 10.1186/s12885-021-08463-7]
11 **Strati A**, Nikolaou M, Georgoulias V, Lianidou ES. RNA-Based CTC Analysis Provides Prognostic Information in Metastatic Breast Cancer. *Diagnostics (Basel)* 2021; **11** [PMID: 33799422 DOI: 10.3390/diagnostics11030513]
12 **Zong W**, Feng W, Jiang Y, Ju S, Cui M, Jing R. Evaluating the diagnostic and prognostic value of serum long non-coding RNA CTC-497E21.4 in gastric cancer. *Clin Chem Lab Med* 2019; **57**: 1063-1072 [PMID: 30763257 DOI: 10.1515/cclm-2018-0929]
13 **Cai D**, Li N, Jin L, Qi X, Hua D, Wang T. High CTC-TRPC5 Expression Significantly Associated With Poor Prognosis in Radical Resected Colorectal Cancer Patients. *Front Mol Biosci* 2021; **8**: 727864 [PMID: 34422911 DOI: 10.3389/fmolb.2021.727864]
14 **Xing C**, Li Y, Ding C, Wang S, Zhang H, Chen L, Li P, Dai M. CD44+ Circulating Tumor Endothelial Cells Indicate Poor Prognosis in Pancreatic Ductal Adenocarcinoma After Radical Surgery: A Pilot Study. *Cancer Manag Res* 2021; **13**: 4417-4431 [PMID: 34103996 DOI: 10.2147/CMAR.S309115]
15 **Semaan A**, Bernard V, Kim DU, Lee JJ, Huang J, Kamyabi N, Stephens BM, Qiao W, Varadhachary GR, Katz MH, Shen Y, San Lucas FA, Gascoyne P, Alvarez HA, Maitra A, Guerrero PA. Characterisation of circulating tumour cell phenotypes identifies a partial-EMT sub-population for clinical stratification of pancreatic cancer. *Br J Cancer* 2021; **124**: 1970-1977 [PMID: 33785875 DOI: 10.1038/s41416-021-01350-9]
16 **Court CM**, Ankeny JS, Sho S, Winograd P, Hou S, Song M, Wainberg ZA, Girgis MD, Graeber TG, Agopian VG, Tseng HR, Tomlinson JS. Circulating Tumor Cells Predict Occult Metastatic Disease and Prognosis in Pancreatic Cancer. *Ann Surg Oncol* 2018; **25**: 1000-1008 [PMID: 29442211 DOI: 10.1245/s10434-017-6290-8]
17 **Cheng H**, Yang J, Fu X, Mao L, Chu X, Lu C, Li G, Qiu Y, He W. Folate receptor-positive circulating tumor cells predict survival and recurrence patterns in patients undergoing resection for pancreatic cancer. *Front Oncol* 2022; **12**: 1012609 [PMID: 36313690 DOI: 10.3389/fonc.2022.1012609]
18 **White MG**, Lee A, Vicente D, Hall C, Kim MP, Katz MHG, Lee JE, Ikoma N, Lucci A, Tzeng CD. Measurement of Portal Vein Blood Circulating Tumor Cells is Safe and May Correlate With Outcomes in Resected Pancreatic Ductal Adenocarcinoma. *Ann Surg Oncol* 2021; **28**: 4615-4622 [PMID: 33415562 DOI: 10.1245/s10434-020-09518-y]
19 **Bissolati M**, Sandri MT, Burtulo G, Zorzino L, Balzano G, Braga M. Portal vein-circulating tumor cells predict liver metastases in patients with resectable pancreatic cancer. *Tumour Biol* 2015; **36**: 991-996 [PMID: 25318603 DOI: 10.1007/s13277-014-2716-0]
20 **Hugenschmidt H**, Labori KJ, Borgen E, Brunborg C, Schirmer CB, Seeberg LT, Naume B, Wiedswang G. Preoperative CTC-Detection by CellSearch((R)) Is Associated with Early Distant Metastasis and Impaired Survival in Resected Pancreatic Cancer. *Cancers (Basel)* 2021; **13** [DOI: 10.20944/preprints202012.0556.v1]
21 **Padillo-Ruiz J**, Suarez G, Pereira S, Calero-Castro FJ, Tinoco J, Marin L, Bernal C, Cepeda-Franco C, Alamo JM, Almoguera F, Macher HC, Villanueva P, García-Fernandez FJ, Gallego I, Romero M, Gomez-Bravo MA, Denninghoff V, Serrano MJ. Circulating Tumor Cells Enumeration from the Portal Vein for Risk Stratification in Early Pancreatic Cancer Patients. *Cancers (Basel)* 2021; **13** [PMID: 34944773 DOI: 10.3390/cancers13246153]
22 **Klein AP**. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 493-502 [PMID: 34002083 DOI: 10.1038/s41575-021-00457-x]
23 **Forgensen J**. Resected adenocarcinoma of the pancreas--616 patients: results, outcomes, and prognostic indicators. *J Gastrointest Surg* 2001; **5**: 681; author reply 681 [PMID: 12086909 DOI: 10.1016/s1091-255x(01)80113-x]
24 **Neoptolemos JP**, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer DH. Therapeutic developments in pancreatic cancer: current and future

perspectives. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 333-348 [PMID: 29717230 DOI: 10.1038/s41575-018-0005-x]

25 **Liu P**, Zhu H, Zhang X, Feng A, Zhu X, Sun Y. Predicting Survival for Hepatic Arterial Infusion Chemotherapy of Unresectable Colorectal Liver Metastases: Radiomics Analysis of Pretreatment Computed Tomography. *J Transl Int Med* 2022; **10**: 56-64 [PMID: 35702189 DOI: 10.2478/jtim-2022-0004]

26 **Yang J**, Xu R, Wang C, Qiu J, Ren B, You L. Early screening and diagnosis strategies of pancreatic cancer: a comprehensive review. *Cancer Commun (Lond)* 2021; **41**: 1257-1274 [PMID: 34331845 DOI: 10.1002/cac2.12204]

27 **Yin H**, Yang X, Sun L, Pan P, Peng L, Li K, Zhang D, Cui F, Xia C, Huang H, Li Z. The value of artificial intelligence techniques in predicting pancreatic ductal adenocarcinoma with EUS images: A meta-analysis and systematic review. *Endosc Ultrasound* 2023; **12**: 50-58 [PMID: 35313419 DOI: 10.4103/EUS-D-21-00131]

28 **Sagami R**, Hayasaka K, Ujihara T, Iwaki T, Katsuyama Y, Harada H, Ome Y, Honda G, Horiguchi SI, Murakami K, Amano Y. Role of EUS combined with a newly modified scoring system to detect pancreatic high-grade precancerous lesions. *Endosc Ultrasound* 2023; **12**: 111-119 [PMID: 36861510 DOI: 10.4103/EUS-D-21-00187]

29 **Bian Y**, Jiang H, Zheng J, Shao C, Lu J. Basic Pancreatic Lesions: Radiologic-pathologic Correlation. *J Transl Int Med* 2022; **10**: 18-27 [PMID: 35702187 DOI: 10.2478/jtim-2022-0003]

30 **Hewitt MJ**, McPhail MJ, Possamai L, Dhar A, Vlavianos P, Monahan KJ. EUS-guided FNA for diagnosis of solid pancreatic neoplasms: a meta-analysis. *Gastrointest Endosc* 2012; **75**: 319-331 [PMID: 22248600 DOI: 10.1016/j.gie.2011.08.049]

31 **Facciorusso A**, Gkolfakis P, Tziatzios G, Ramai D, Papanikolaou IS, Triantafyllou K, Lisotti A, Fusaroli P, Mangiavillano B, Chandan S, Mohan BP, Crinò SF. Comparison between EUS-guided fine-needle biopsy with or without rapid on-site evaluation for tissue sampling of solid pancreatic lesions: A systematic review and meta-analysis. *Endosc Ultrasound* 2022; **11**: 458-465 [PMID: 36537383 DOI: 10.4103/EUS-D-22-00026]

32 **Thomsen MM**, Larsen MH, Di Caterino T, Hedegaard Jensen G, Mortensen MB, Detlefsen S. Accuracy and clinical outcomes of pancreatic EUS-guided fine-needle biopsy in a consecutive series of 852 specimens. *Endosc Ultrasound* 2022; **11**: 306-318 [PMID: 35708361 DOI: 10.4103/EUS-D-21-00180]

33 **Saghir SM**, Dhindsa BS, Daid SGS, Mashiana HS, Dhaliwal A, Cross C, Singh S, Bhat I, Ohning GV, Adler DG. Efficacy of EUS-guided needle-based confocal laser endomicroscopy in the diagnosis of pancreatic lesions: A systematic review and meta-analysis. *Endosc Ultrasound* 2022; **11**: 275-282 [PMID: 33666181 DOI: 10.4103/EUS-D-20-00122]

34 **Hartwig W**, Strobel O, Hinz U, Fritz S, Hackert T, Roth C, Büchler MW, Werner J. CA19-9 in potentially resectable pancreatic cancer: perspective to adjust surgical and perioperative therapy. *Ann Surg Oncol* 2013; **20**: 2188-2196 [PMID: 23247983 DOI: 10.1245/s10434-012-2809-1]

35 **Ferrone CR**, Finkelstein DM, Thayer SP, Muzikansky A, Fernandez-delCastillo C, Warshaw AL. Perioperative CA19-9 levels can predict stage and survival in patients with resectable pancreatic adenocarcinoma. *J Clin Oncol* 2006; **24**: 2897-2902 [PMID: 16782929 DOI: 10.1200/JCO.2005.05.3934]

36 **Wang Y**, Yu X, Hartmann D, Zhou J. Circulating tumor cells in peripheral blood of pancreatic cancer patients and their prognostic role: a systematic review and meta-analysis. *HPB (Oxford)* 2020; **22**: 660-669 [PMID: 31786054 DOI: 10.1016/j.hpb.2019.11.003]

37 **Khoo BL**, Grenci G, Lim YB, Lee SC, Han J, Lim CT. Expansion of patient-derived circulating tumor cells from liquid biopsies using a CTC microfluidic culture device. *Nat Protoc* 2018; **13**: 34-58 [PMID: 29215634 DOI: 10.1038/nprot.2017.125]

38 **Gemenetzis G**, Groot VP, Yu J, Ding D, Teinor JA, Javed AA, Wood LD, Burkhart RA, Cameron JL, Makary MA, Weiss MJ, He J, Wolfgang CL. Circulating Tumor Cells Dynamics in Pancreatic Adenocarcinoma Correlate With Disease Status: Results of the Prospective CLUSTER Study. *Ann Surg* 2018; **268**: 408-420 [PMID: 30080739 DOI: 10.1097/SLA.0000000000002925]

39 **Li Y**, Wu S, Bai F. Molecular characterization of circulating tumor cells-from bench to bedside. *Semin Cell Dev Biol* 2018; **75**: 88-97 [PMID: 28899718 DOI: 10.1016/j.semcdb.2017.09.013]

40 **Pantel K**, Alix-Panabières C. The clinical significance of circulating tumor cells. *Nat Clin Pract Oncol* 2007; **4**: 62-63 [PMID: 17259923 DOI: 10.1038/ncponc0737]

41 **Alix-Panabières C**, Pantel K. Challenges in circulating tumour cell research. *Nat Rev Cancer* 2014; **14**: 623-631 [PMID: 25154812 DOI: 10.1038/nrc3820]

42 **Cen P**, Ni X, Yang J, Graham DY, Li M. Circulating tumor cells in the diagnosis and management of pancreatic cancer. *Biochim Biophys Acta* 2012; **1826**: 350-356 [PMID: 22683404 DOI: 10.1016/j.bbcan.2012.05.007]

43 **Bidard FC**, Huguet F, Louvet C, Mineur L, Bouché O, Chibaudel B, Artru P, Desseigne F, Bachet JB, Mathiot C, Pierga JY, Hammel P. Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. *Ann Oncol* 2013; **24**: 2057-2061 [PMID: 23676420 DOI: 10.1093/annonc/mdt176]

44 **Pecot CV**, Bischoff FZ, Mayer JA, Wong KL, Pham T, Bottsford-Miller J, Stone RL, Lin YG, Jaladurgam P, Roh JW, Goodman BW, Merritt WM, Pircher TJ, Mikolajczyk SD, Nick AM, Celestino J, Eng C, Ellis LM, Deavers MT, Sood AK. A novel platform for detection of CK+ and CK- CTCs. *Cancer Discov* 2011; **1**: 580-586 [PMID: 22180853 DOI: 10.1158/2159-8290.CD-11-0215]

45 **Liu H**, Sun B, Wang S, Liu C, Lu Y, Li D, Liu X. Circulating Tumor Cells as a Biomarker in Pancreatic Ductal Adenocarcinoma. *Cell Physiol Biochem* 2017; **42**: 373-382 [PMID: 28558380 DOI: 10.1159/000477481]

46 **Wang S**, Liu K, Liu J, Yu ZT, Xu X, Zhao L, Lee T, Lee EK, Reiss J, Lee YK, Chung LW, Huang J, Rettig M, Seligson D, Duraiswamy KN, Shen CK, Tseng HR. Highly efficient capture of circulating tumor cells by using nanostructured silicon substrates with integrated chaotic micromixers. *Angew Chem Int Ed Engl* 2011; **50**: 3084-3088 [PMID: 21374764 DOI: 10.1002/anie.201005853]

47 **Hou S**, Zhao L, Shen Q, Yu J, Ng C, Kong X, Wu D, Song M, Shi X, Xu X, OuYang WH, He R, Zhao XZ, Lee T, Brunicardi FC, Garcia MA, Ribas A, Lo RS, Tseng HR. Polymer nanofiber-embedded microchips for detection, isolation, and molecular analysis of single circulating melanoma cells. *Angew Chem Int Ed Engl* 2013; **52**: 3379-3383 [PMID: 23436302 DOI: 10.1002/anie.201208452]

48 **Court CM**, Ankeny JS, Sho S, Hou S, Li Q, Hsieh C, Song M, Liao X, Rochefort MM, Wainberg ZA, Graeber TG, Tseng HR, Tomlinson JS. Reality of Single Circulating Tumor Cell Sequencing for Molecular Diagnostics in Pancreatic Cancer. *J Mol Diagn* 2016; **18**: 688-696 [PMID: 27375074 DOI: 10.1016/j.jmoldx.2016.03.006]

49 **Zou D**, Cui D. Advances in isolation and detection of circulating tumor cells based on microfluidics. *Cancer Biol Med* 2018; **15**: 335-353 [PMID: 30766747 DOI: 10.20892/j.issn.2095-3941.2018.0256]

50 **Lin PP**. Integrated EpCAM-independent subtraction enrichment and iFISH strategies to detect and classify disseminated and circulating tumors cells. *Clin Transl Med* 2015; **4**: 38 [PMID: 26718583 DOI: 10.1186/s40169-015-0081-2]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 November 24; 14(11): 518-534

DOI: 10.5306/wjco.v14.i11.518

ISSN 2218-4333 (online)

SCIENTOMETRICS

Bibliometric analysis of the global research status and trends of mechanotransduction in cancer

Yi-Zhan Zhang, Meng-Zhu Li, Guang-Xin Wang, Da-Wei Wang

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): C
Grade D (Fair): D, D
Grade E (Poor): 0

P-Reviewer: Fu L, China; Jeong KY, South Korea; Safarzadeh Kozani P, Iran; Gupta MK, Germany

Received: August 2, 2023
Peer-review started: August 2, 2023
First decision: August 16, 2023
Revised: September 14, 2023
Accepted: October 16, 2023
Article in press: October 16, 2023
Published online: November 24, 2023

Yi-Zhan Zhang, Meng-Zhu Li, Da-Wei Wang, Department of Endocrinology, Shandong Provincial Hospital, Shandong University, Jinan 250021, Shandong Province, China

Yi-Zhan Zhang, Meng-Zhu Li, Da-Wei Wang, Key Laboratory of Endocrine Glucose & Lipids Metabolism and Brain Aging, Ministry of Education, Jinan 250021, Shandong Province, China

Guang-Xin Wang, Shandong Innovation Center of Intelligent Diagnosis, Central Hospital Affiliated to Shandong First Medical University, Jinan 250013, Shandong Province, China

Corresponding author: Da-Wei Wang, DPhil, Associate Professor, Postdoc, Postdoctoral Fellow, Department of Endocrinology, Shandong Provincial Hospital, Shandong University, No. 544 Jingsi Road, Huaiyin District, Jinan 250021, Shandong Province, China.
wangdawei@sdfmu.edu.cn

Abstract

BACKGROUND

The development of cancer is thought to involve the dynamic crosstalk between the tumor cells and the microenvironment they inhabit. Such crosstalk is thought to involve mechanotransduction, a process whereby the cells sense mechanical cues such as stiffness, and translate these into biochemical signals, which have an impact on the subsequent cellular activities. Bibliometric analysis is a statistical method that involves investigating different aspects (including authors' names and affiliations, article keywords, journals and citations) of large volumes of literature. Despite an increase in mechanotransduction-related research in recent years, there are currently no bibliometric studies that describe the global status and trends of mechanotransduction-related research in the cancer field.

AIM

To investigate the global research status and trends of mechanotransduction in cancer from a bibliometric viewpoint.

METHODS

Literature on mechanotransduction in cancer published from January 1, 1900 to December 31, 2022 was retrieved from the Web of Science Core Collection. Excel and GraphPad software carried out the statistical analysis of the relevant author, journal, organization, and country information. The co-authorship, keyword co-occurrence, and keyword burst analysis were visualized with VOSviewer and CiteSpace.

RESULTS

Of 597 publications from 745 institutions in 45 countries were published in 268 journals with 35510 citation times. With 270 articles, the United States is a well-established global leader in this field, and the University of California system, the most productive (n = 36) and influential institution (n = 4705 citations), is the most highly active in collaborating with other organizations. *Cancers* was the most frequent publisher with the highest H-index. The most productive researcher was Valerie M. Weaver, with 10 publications. The combined analysis of concurrent and burst keywords revealed that the future research hotspots of mechanotransduction in cancer were related to the plasma membrane, autophagy, piezo1/2, heterogeneity, cancer diagnosis, and post-transcriptional modifications.

CONCLUSION

Mechanotransduction-related cancer research remains a hot topic. The United States is in the leading position of global research on mechano-oncology after almost 30 years of investigations. Research group cooperations exist but remain largely domestic, lacking cross-national communications. The next big topic in this field is to explore how the plasma membrane and its localized mechanosensor can transduce mechanical force through post-transcriptional modifications and thereby participate in cellular activity regulations and cancer development.

Key Words: Bibliometric analysis; Mechanotransduction; Cancer; Visualization; Signal transduction

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Through bibliometric analysis, we found that mechanotransduction-related cancer research remains a hot topic, with approximately 100 papers and 5000 citations generated per year in the past three years. Additionally, the United States is a well-established global leader of this field, and the University of California system is the most influential organization in this field. We predict that investigating how the plasma membrane and its localized mechanosensors transduce mechanical forces *via* post-transcriptional modifications and thereby participate in the regulation of cellular activity will be the next big research topic in the cancer field.

Citation: Zhang YZ, Li MZ, Wang GX, Wang DW. Bibliometric analysis of the global research status and trends of mechanotransduction in cancer. *World J Clin Oncol* 2023; 14(11): 518-534
URL: https://www.wjgnet.com/2218-4333/full/v14/i11/518.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i11.518

INTRODUCTION

Cancer is a devastating disease characterized by the transduction of abnormal cell signals, which leads to oncogenic cellular behaviors such as uncontrolled proliferation and resistance to death[1]. According to the latest statistics, cancer is the second most common cause of death worldwide after cardiovascular disease[2]. Therefore, it is crucial to determine the pathogenesis of this disease so that effective therapeutic approaches can be identified. Although cancer is primarily regarded as being a genetic disease caused by the stepwise accumulation of gene mutations, an increasing number of studies suggest that environmental factors might also have a significant influence on the development of this disease[3]. The tumor microenvironment (TME) consists of cancer-associated fibroblasts (CAFs), endothelial cells, immune cells, and the extracellular matrix (ECM), and together these provide the biochemical and mechanical signals required to stimulate the occurrence, survival, and development of cancer[4]. While the importance of biochemical stimuli (such as small molecules, growth factors, and cytokines) in cancer progression is well-established, the role of mechanical force is still relatively underexplored although recent investigations suggest that it is on par with the chemical factors[5-7].

In cancerous tissues, mechanical forces such as shear stress, tension, and compression, are suggested to be generated during cell-TME and cell-cell contact events[8]. These forces are then translated into biochemical signaling cascades with the help of various mechanoreceptors, including G protein-coupled receptors, ion channels, and cell junction proteins[9, 10]. This process of transducing specific mechanosignals into distinct intracellular biochemical signals is called mechanotransduction[5]. Abnormal mechanical signals (generated by the environment or cells), can alter the expression of genes and transduction of signaling pathways. This leads to the dysregulated cell behaviors that are associated with many diseases, including cancer[11]. For example, when compared with normal tissues, cancerous tissues have a more rigid ECM, which contributes to the aberrant mechanotransduction observed[12]. This leads to an increase in the expression of many oncogenic transcription factors (such as YAP/TAZ, Twist1, and β-catenin), which enhances cell proliferation, the epithelial-mesenchymal transition (EMT), and/or cell migration in cancer cells[13]. Interestingly, YAP/TAZ has also been reported to contribute to tissue stiffness by increasing the expression of the crucial ECM modifiers, CTGF and CYR61[14-17]. Therefore, a positive mechanotransductive feedback loop between cancer cells and the ECM is regarded as one of the major culprits for cancer malignancy[17]. In addition, when cancer cells are surrounded by a rigid, crosslinked ECM, then this impedes immune cell invasion and drug distribution, which leads to immune surveillance escape and drug resistance

[6,18].

The concept of mechanotransduction also helps to explain the mechanisms of cancer initiation, invasion, and metastasis from a new perspective. Considering the fundamental role of mechanotransduction in cancer development, researchers are now developing new cancer therapeutics based on the specific mechanical properties of and mechano-signal transducers generated by tumors. For example, Simtuzumab and Simvastatin, which reduce ECM stiffness and inhibit YAP/TAZ hyperactivation, respectively, have been evaluated for their anti-cancer efficacy in preclinical studies [18-20]. Mechanotransduction is therefore already showing great promise for clinical applications, and so further comprehensive investigations in this field will likely reveal more therapeutic strategies for cancer treatment. Thus, gaining a better understanding of the current research status of mechanotransduction in cancer is likely to shed light on important research questions and directions for future study.

Bibliometric analysis is a useful quantitative method to comprehensively analyze publications from many different aspects (including the authors and their affiliations, journal title and article keywords), to reveal collaborative networks and emerging trends in specific research areas[21-23]. Although increasing numbers of research papers now focus on the role of mechanotransduction in cancer, to date, no bibliometric analysis has been conducted to quantify the situation. To this end, here we present the updated bibliometric analysis of research conducted on mechanotransduction in cancer and reveal the current research being conducted in this field. In this way, we aim to provide a better understanding of the present status of this study area and predict key topics of future investigation in this promising research field.

MATERIALS AND METHODS

Searching strategies

We retrieved literature on 'mechanotransduction in cancer' research from the Web of Science Core Collection (WoSCC). The publication period was set from January 1, 1900 to December 31, 2022, and only English publications were included. The search strategy is illustrated in Figure 1. All recorded data, including the authors' names, institutions, and countries, as well as keywords, were downloaded from the WoSCC and normalized to a standard format. To avoid ambiguity, we cross-checked duplicate authors among the documents. Meeting abstracts, editorial materials, corrections, and retractions were excluded from our research.

Keyword analysis

The keywords were extracted from the keyword section of articles. To avoid potential deviations, similar or same keywords with different expressions were manually standardized to correct and/or group similarities as previously suggested[23-25], before VOSviewer or CiteSpace analysis. Burst keywords were assessed using CiteSpace (V6.2R4 SE) with the following parameters: time slicing (from January 1994 to December 2022), years per slice (1), node type (keyword), the minimum burst duration (1 year), γ (0.39) and others (default). A keyword co-occurrence analysis was conducted with VOSviewer (version 1.6.18) with the following parameters: Type of analysis (co-occurrence), unit of analysis (keywords), counting method (full counting), minimum number of occurrences of a keyword (3).

Data visualization

The number of publications and citations in the indicated time was presented using GraphPad Prism 8. Visualization of bibliometric information, including co-authorship analysis, keyword co-occurrence, and burst analysis, was conducted with VOSviewer (version 1.6.18) and CiteSpace (V6.2R4 SE).

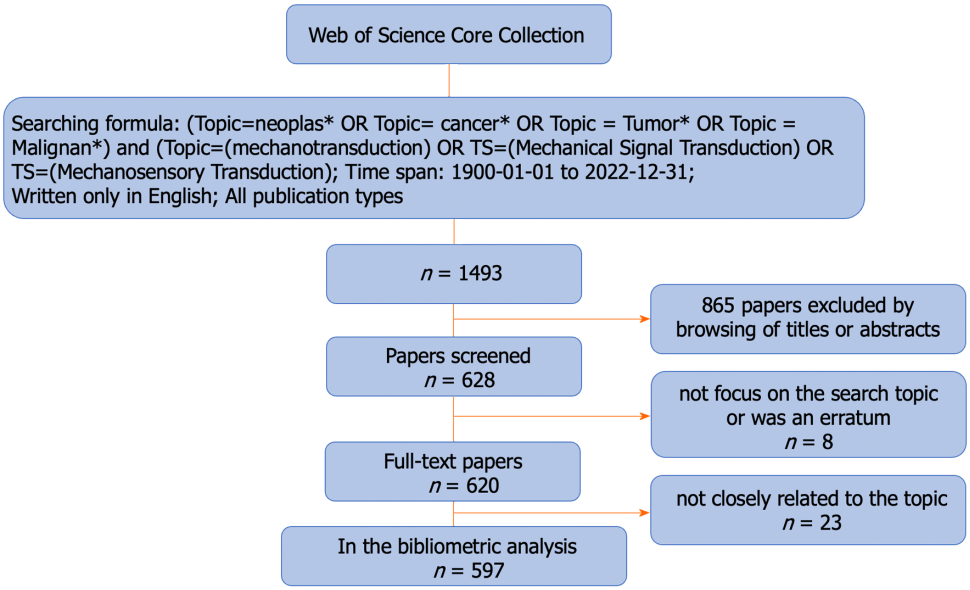
RESULTS

General information

A total of 597 publications comprising 388 research and 209 review articles, were extracted for deep analysis. Although the concept of mechanotransduction was established progressively from the 1950s to the 1980s[26-28], the first paper describing mechanotransduction in cancer was only published in 1994[29]. Since then, this topic has gradually gained more attention from researchers in the cancer research field. Based on the number of publications and citations analysed in our bibliometric study on mechanotransduction-related cancer research, we prepared a growth curve comprising three clear stages. The first stage described the period from 1994 to 2010, during which time < 10 papers were published each year. In the second stage, which ran from 2012 to 2017, a slow growth rate was observed, and in the third stage (2018-2022), the growth rate started to accelerate, with almost 100 articles being published in the final year (Figure 2A).

In the 597 articles that were published, there was an average of 59.48 citations per paper. The top 10 most cited papers included six reviews and four research articles; these are listed in Supplementary Table 1 and they are ranked by the number of times they were cited. The most highly cited review article (n = 2882 citations) was published by Chambers *et al*[30], and the most highly cited research article (n = 1021 citations) was contributed by Aragona *et al*[31].

During our exploration of mechanotransduction in cancer, we sub-divided the publications into 56 research categories to indicate the multidisciplinary crossovers that occurred. After the top 10 categories were generated (Figure 2B), we found that cell biology (n = 191), oncology (n = 130), and biochemistry molecular biology (n = 117), accounted for 73.37% of all the publications. This indicates that molecular cytology is a key contributor to research on the role of mechanical force in cancer development.



DOI: 10.5306/wjco.v14.i11.518 **Copyright** ©The Author(s) 2023.

Figure 1 The strategy we used to search for publications about mechanotransduction in cancer. 597 publications closely correlated with mechanotransduction in cancer were extracted based on this strategy.

Analysis of countries and institutions

Although a total of 45 countries was found to contribute publications on the topic of mechanotransduction in cancer, the top 10 most productive countries generated 87.6% of all the papers. Of these, the United States was the most productive as it was responsible for 270 (*i.e.*, 45.23%) of the publications. China (n = 119, 19.93%) and Italy (n = 41, 6.87%) held the second and third positions, respectively, but both lagged behind the United States (Table 1). However, when we ranked the countries in terms of the average citation times, Canada occupied the first place with an average of 197.33 citations per paper. This was followed by Italy and Spain with values of 109.17 and 93.09, respectively. In addition, according to this ranking approach, the United States (73.74 times) held a middle position, whereas China (20.34 times) was at the bottom of the ranking list. To evaluate the cooperation between different countries, a co-authorship network was established with a criterion of at least three publications in each country. As shown in Figure 3, the United States (with the most extended research history in this field), collaborated most with other countries, followed by the United Kingdom. In addition, it was interesting to find that although 37.78% (*i.e.*, 17/45) of the countries only published one or two papers, most of this work was conducted in the recent five years. This indicates that more countries are stepping into this research field.

Our data analysis also showed that 745 organizations were involved in research related to mechanotransduction in cancer. After ranking these according to the number of publications, we found that the top 10 organizations accounted for 35.85% (214/597) of all papers. The University of California system ranked first with 36 articles, and this was followed sequentially by UDICE-French Research Universities (n = 27), Centre National de la Recherche Scientifique (n = 25), the University of Illinois system (n = 21), and Institut National de la Sante et de la Recherche Medicale (Inserm). It was striking to find that three institutions from France occupied three of the top five positions with a total of 31 publications (Table 2). Another analysis of the global inter-institutional network showed that the University of California system collaborated most with other organizations, followed sequentially by the University of Padua, Harvard University, and University College London (Figure 4).

Analysis of journals and authors

Articles related to the field of mechanotransductive cancer research were distributed among 268 different academic journals. The top 10 journals to publish papers in this field accounted for 22.61% (135/597) of all the articles (Supplementary Table 2), but 162 journals (60.45%) only published one paper in this research area. *Cancers* ranked first with 29 (4.86%) publications, followed by the *International Journal of Molecular Sciences* (n = 20, 3.35%) and the *Journal of Cell Science* (n = 17, 2.85%).

We also found that a total of 3001 authors contributed to these 597 publications. After analyzing the top 10 authors in terms of the number of papers (Supplementary Table 3), we found that Valerie M. Weaver from the University of California system was the most productive researcher with n = 10 publications. She was followed by Stefano Piccolo from the University of Padua (n = 9), Antonios Gargalionis from the National & Kapodistrian University of Athens (n = 9), and Marc D. Basson from John D. Dingell Veterans Affairs Medical Center (n = 8). Notably, according to the total (T) and average (A) citation times of the papers generated from each author, Stefano Piccolo (T = 3167; A = 351.89), Tito Panciera (T = 2076, A = 296.57), Valerie M. Weaver (T = 2495, A = 249.5), Michelangelo Cordenonsi (T = 1983, A = 330.5), and Patricia J. Keely (T = 1478, A = 246.33) ranked the top 5, indicating that they were in positions of authority. To investigate if there were any collaborations between the various authors in this field, an authorship network analysis was performed by identifying those with at least three publications. As shown in Figure 5, the authors were distributed into 43 clusters,

Table 1 The top 10 productive countries that published papers about mechanotransduction in cancer

Rank	Country	Publications	%	Total citations	Average citations	Connections	H-index
1	United States	270	45.23	19911	73.74	22	69
2	China	119	19.93	2421	20.34	10	25
3	Italy	41	6.87	4476	109.17	12	22
4	Germany	37	6.20	2072	56.00	10	18
5	United Kingdom	34	7.54	2098	61.71	15	20
6	France	31	5.19	2466	79.55	12	19
7	Spain	22	3.69	2048	93.09	11	14
8	Canada	21	3.52	4144	197.33	8	13
9	Japan	20	3.35	761	38.05	4	14
10	South Korea	20	3.35	355	17.75	2	9

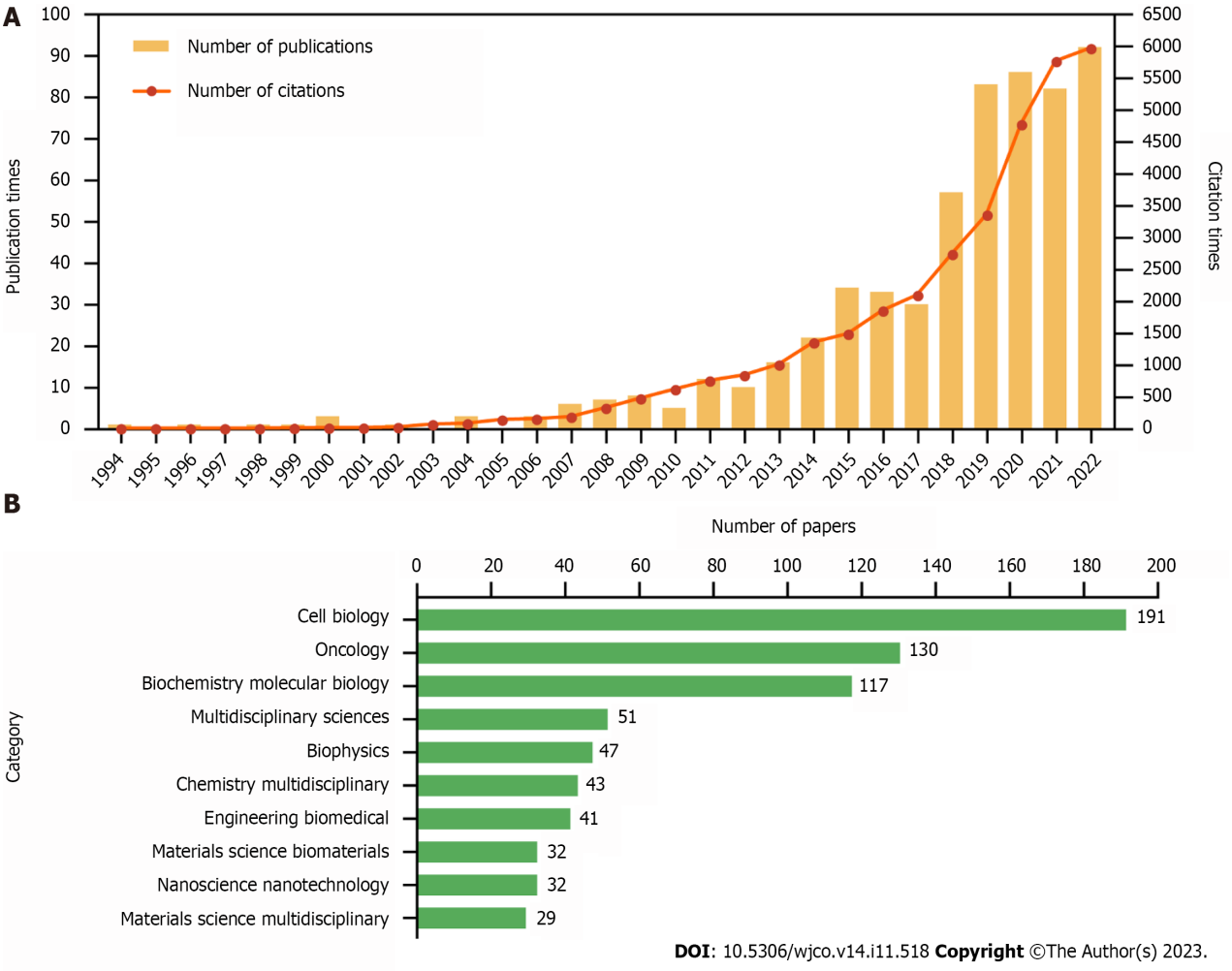
Table 2 The top 10 productive organizations publishing papers about mechanotransduction in cancer

Rank	Organization	Publications	Citations	Average citation	Country	%	H-index
1	University of California System	36	4705	130.69	United States	6.03	21
2	UDICE-French Research Universities	27	1875	69.44	France	4.52	17
3	Centre National de la Recherche Scientifique	25	1843	73.72	France	4.19	16
4	University of Illinois System	21	1013	48.24	United States	3.52	14
5	Institut National de la Sante et de la Recherche Medicale	20	1845	92.25	France	3.35	13
6	University of Texas System	20	1619	80.95	United States	3.35	15
7	University of London	18	1388	77.11	United Kingdom	3.02	13
8	University of Padua	16	3399	212.44	Italy	2.68	12
9	Harvard University	16	2463	153.94	United States	2.68	13
10	Vanderbilt University	15	691	46.07	United States	2.51	10

with 4 clusters containing at least ten authors. This indicates that there were partial connections between the different groups in this research area.

Keyword analysis

To explore the current research themes and discover hot topics in mechanotransduction-related cancer research, we acquired 666 keywords by collating those with the same meaning or category from all 597 papers. Among them, the top 4 co-occurrent keywords that were consistent with our research topic, were: Mechanotransduction, cancer, mechanical force, and mechanical property. However, when these keywords were restricted to at least 3 co-occurrences, only 93 items could satisfy this criterion. We next established a network based on these 93 keywords and found that they could be further subdivided into eight clusters (Figure 6A and Supplementary Table 4). We found that 19 keywords were included in cluster 1 (red) as follows: Angiogenesis, cadherin, caveolin, cell death, endothelial cell, epithelial cell, growth factor/receptor, hypoxia, integrin, invadopodia, mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK), mechanical force, matrix metalloproteinases (MMPs), mechanistic target of rapamycin (mTOR), NF-κB, phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), plasma membrane, signal transduction, and traction force microscopy. Cluster 2 (green) also contained 19 items. These were: AMP-activated protein kinase, cell cycle, cell morphology, cytoskeleton or cytoskeleton remodeling, epigenetic regulation, gene regulation, heterogeneity, lamin, linc complex, mechanotransduction, metabolism, microfluidics, migration, nuclear envelope, nuclear mechanics, nucleoskeleton, nucleus, pancreatic stellate cell, and SRC. Cluster 3 (blue) contained the following 16 items: Calcium, cancer, DNA damage, dormancy, immunology, invasion, ion channel, mechanobiology, metastasis, motility, oxidative stress, piezo1/2, post-translational modification, SRF, stem cell, and TRP. Cluster 4 (yellow) contained 11 items: 3D culture, autophagy, cancer diagnosis, cancer stem cell (CSC), drug resistance, hydrogel, immunotherapy, organoid, programmed cell death protein 1/programmed cell death ligand 1, pharmacology, and polycystin. Cluster 5 (purple) contained 8 items: Atomic force microscopy, CAF, ECM or ECM remodeling, mechanical property, oncophysics, therapy, TME, and tumor model. Cluster 6 (cyan) contained 8 items: Contact inhibition, G protein-coupled receptor (GPCR), growth, Hippo, polarity, TAZ, transcription factor, and YAP. Cluster 7 (orange) contained 7 items: Catenin, EMT, fibrosis, noncoding



DOI: 10.5306/wjco.v14.i11.518 **Copyright** ©The Author(s) 2023.

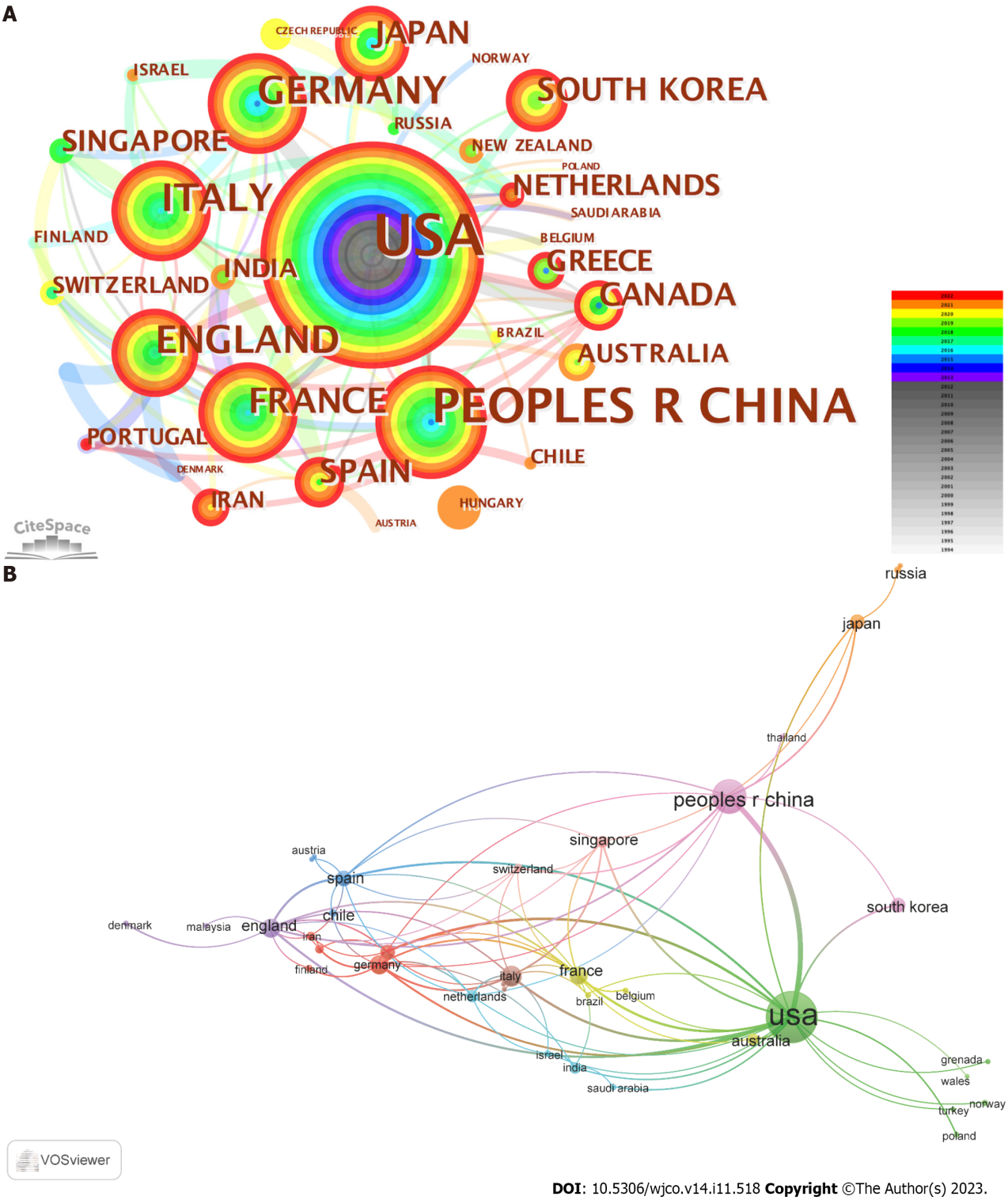
Figure 2 Trends and categories of publications about mechanotransduction in cancer. A: The annual number of publications and citations from 1994 to 2022; B: The research categories of publications related to mechanotransduction in cancer.

RNA, transforming growth factor-beta (TGF-β), transcription, and Wnt, and cluster 8 (brown) had 5 items: Adhesion, focal adhesion kinase (FAK), mechanical microenvironment, morphogenesis, and RhoA/Rho/ROCK. These grouped keywords were further marked according to the average publication year to reflect their yearly development. Figure 6B shows that the keywords in clusters 1 and 8 were mainly labeled in purple, suggesting that they appeared relatively early on in this field. In contrast, there were more keywords labeled in yellow in clusters 4 and 5, indicating that the topics in these two clusters gained more attention in recent years. Of note, some keywords exhibited a relatively late mean publication year and low mean frequency of occurrence, and so we suggest that these might be the next big topics to be investigated in this area. These include "plasma membrane [average appearing year (AAY) = 2021.00]", "autophagy (AAY = 2021.00)", "piezo1/2 (AAY = 2021.17)", and "heterogeneity (AAY = 2021.25)".

In addition to our analysis of co-occurring keywords, a burst keyword analysis (which identifies keywords that were frequently used over a certain period), was carried out with the CiteSpace software. We found that "mechanical force" was the most potent keyword; this appeared in 2006 with a burst strength of 3.99. In addition, the longest burst duration occurred for "signal transduction" (with a burst strength of 2.19); this started in 2004 and lasted for 9 years. Apart from these two keywords, "cancer diagnosis", "post-transcriptional modification" and "plasma membrane" were shown to have the highest more recent burst time (Figure 6C), indicating possible new research topics in mechanotransduction-associated cancer studies.

DISCUSSION

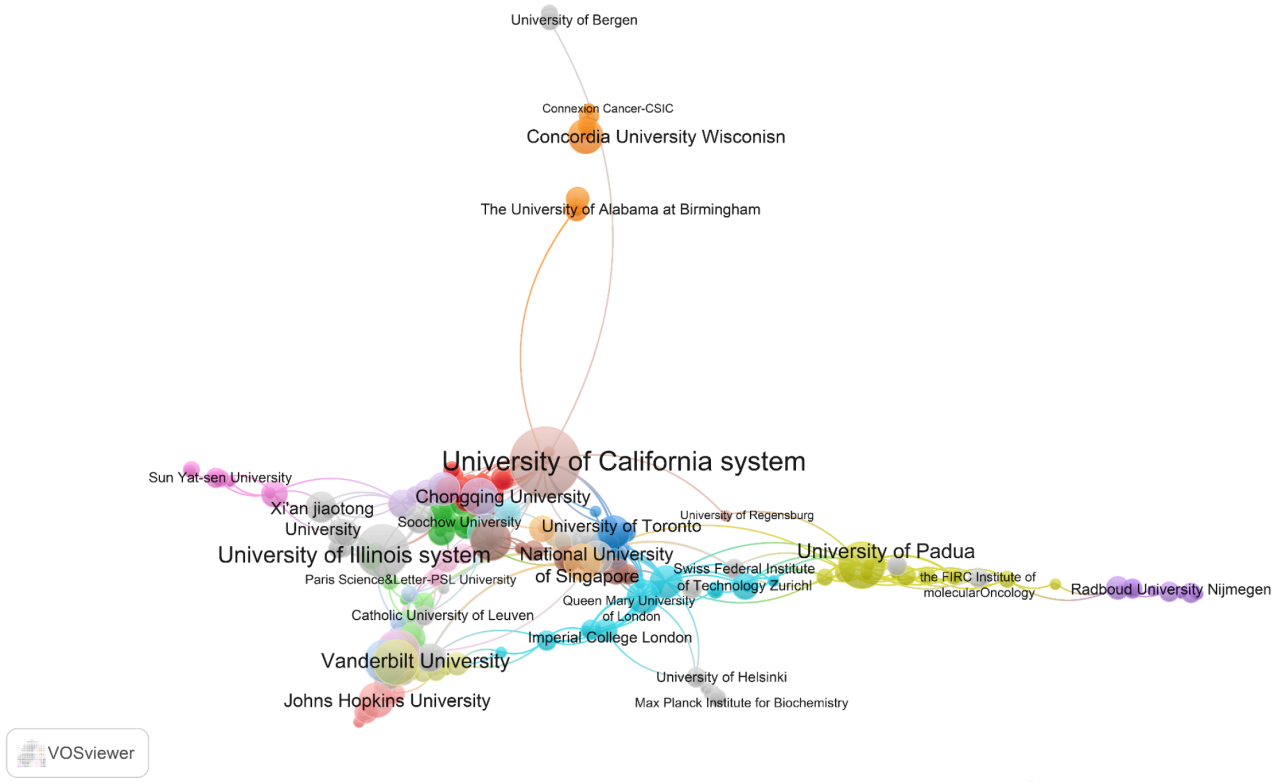
The concept of mechanotransduction developed gradually between the 1950s and 1980s as researchers studied how stretching forces influenced membrane depolarization in excitable cells such as nerves[26-28]. The fact that mechanotransduction might play a role in cancer was first suggested in 1994, and since then it has helped to explain many puzzles in the cancer research field[29]. However, compared to biochemical signal transduction, the role of mechanotransduction in cancer development was largely overlooked from 1994 to 2006. The field then gradually received more attention, and more than 40 papers were published between 2006 and 2016, probably due to the boom in research on the Hippo



DOI: 10.5306/wjco.v14.i11.518 **Copyright** ©The Author(s) 2023.

Figure 3 Analysis of countries working on mechanotransduction in cancer. A: CiteSpace was used to conduct the cooperation network between countries. The number of publications is represented by the node size and the different publication years are indicated by the different colors; B: VOSviewer was used to visualize the cooperation between country network. The number of publications is represented by the size of the node and the connection strength is indicated by the line thickness.

pathway[32]. Notably, there was then a slight reduction in the number of publications in 2017, followed by a substantial increase in 2018. Since then, this field has become far more popular among cancer researchers, such that in the last three years, > 80 papers were published per year. As the number of publications increased, so did the number of times that the publications were cited. Indeed, the average citation time reached 59.48 citations per paper, suggesting the high quality of research conducted in this field. Furthermore, Drs David Julius and Ardem Patapoutian were awarded the 2021 Nobel Prize in Physiology or medicine for their work on mechanosensitive ion channels[33]. Therefore, this might encourage more groups to conduct mechanotransduction-related cancer research.

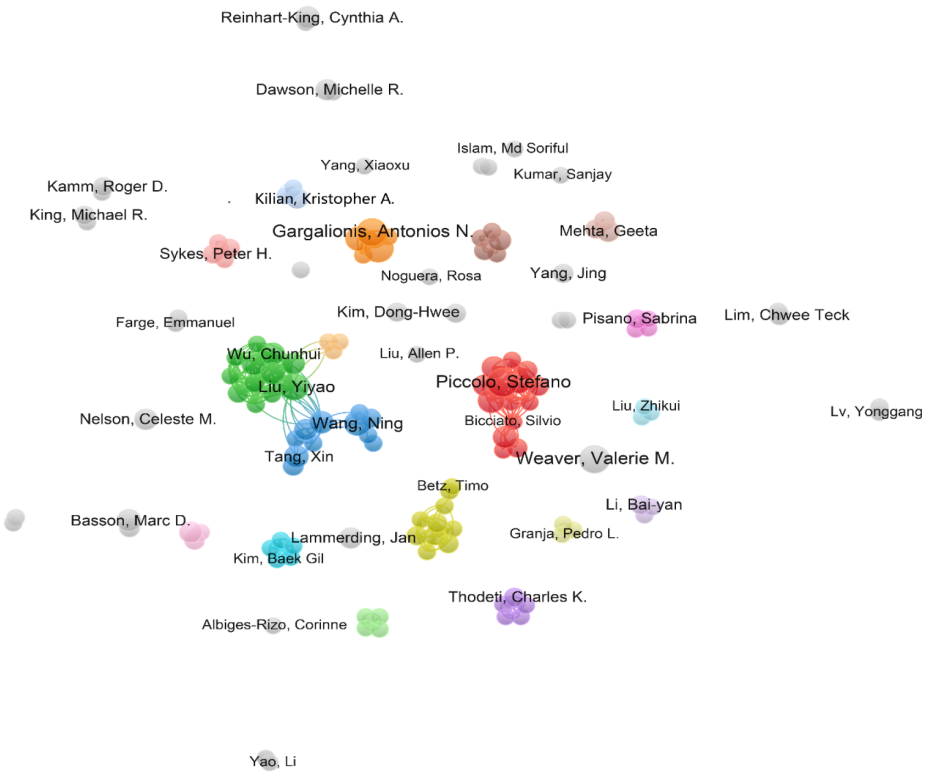


DOI: 10.5306/wjco.v14.i11.518 **Copyright** ©The Author(s) 2023.

Figure 4 Network analysis of organizations. The collaboration between organizations was analysed with VOSviewer.

By ranking the top 10 productive countries in this area, we found that all except China are advanced countries. The United States has established the lead position of global research on mechano-oncology after nearly 30 years of investigations. However, the number of publications from China indicates that they are in a catch-up position, although the average citation time for their papers is still relatively low. This might be due to the relatively low quality of the publications so far, even though the quantity is high. In addition, the low number of publications in the past from China and the more recent fast growth rate might inherently overly inflate the contribution of highly cited papers to the average citation time. To address this phenomenon, the Chinese government should provide more financial and political support for this research field and encourage original research. For Chinese scholars, discovering new research frontiers as early as possible and carrying out in-depth research is indispensable for improving their international influence and academic standing. It is also important to note that although Canada published 21 articles and is ranked only eighth according to the number of publications, it has the highest average citation rate. One reason for this might be that a review paper published by a Canadian oncology group (Chambers *et al*[30]), systematically describes the role of mechanical factors on the various physiological stages of cancer metastasis, and this attracts the highest citation frequency in this field.

We also found that cooperations existed between each country, with China and the United States having the most exchanges and collaborations. At the institutional level, we found that cooperations existed in developed countries such as the United States, France, and the United Kingdom. However, in other countries mutual partnerships tended to be insufficient, especially in undeveloped countries, including China. In addition, although several connections between different research groups were found on our authorship network map, these group cooperations largely remained domestic, lacking cross-national communications. Furthermore, three of the top productive and influential scientists in this field (*i.e.*, Valerie M. Weaver, Stefano Piccolo, and Antonios Gargalionis), have relatively few collaborations with other researchers. The study of mechanotransduction in cancer not only requires an in-depth exploration of molecular mechanisms but also necessitates a large number of clinical samples or populations for clinical validation or translation. At this point, inter-country or inter-institutional collaboration should be advocated, either by sharing clinical databases or by dividing the project into concrete tasks based on the respective expertise. With this approach, significant breakthroughs in this field might be achieved at the earliest time. In addition to collaboration between research groups, interdisciplinary collaborations are also essential for a research field to flourish. Mechanotransduction-related cancer research involves various different disciplines, including biology, physics, and medicine, and so interdisciplinary exchanges are beneficial for the diversity of research and to create new perspectives and questions. For example, *in vivo* mechanosensing is based on force-dependent protein deformation and reorganisation[34]. However, due to a lack of molecular resolution in cellular imaging techniques, the intracellular mechanisms are unknown. Recently, with the development of super-resolution microscopy and molecular force sensors, it is now possible to gain molecular insights into mechanosensing in living cells[35]. Moreover, the development of novel imaging techniques has helped to advance our knowledge of the molecular mechanisms involved in mechanotransduction[36].



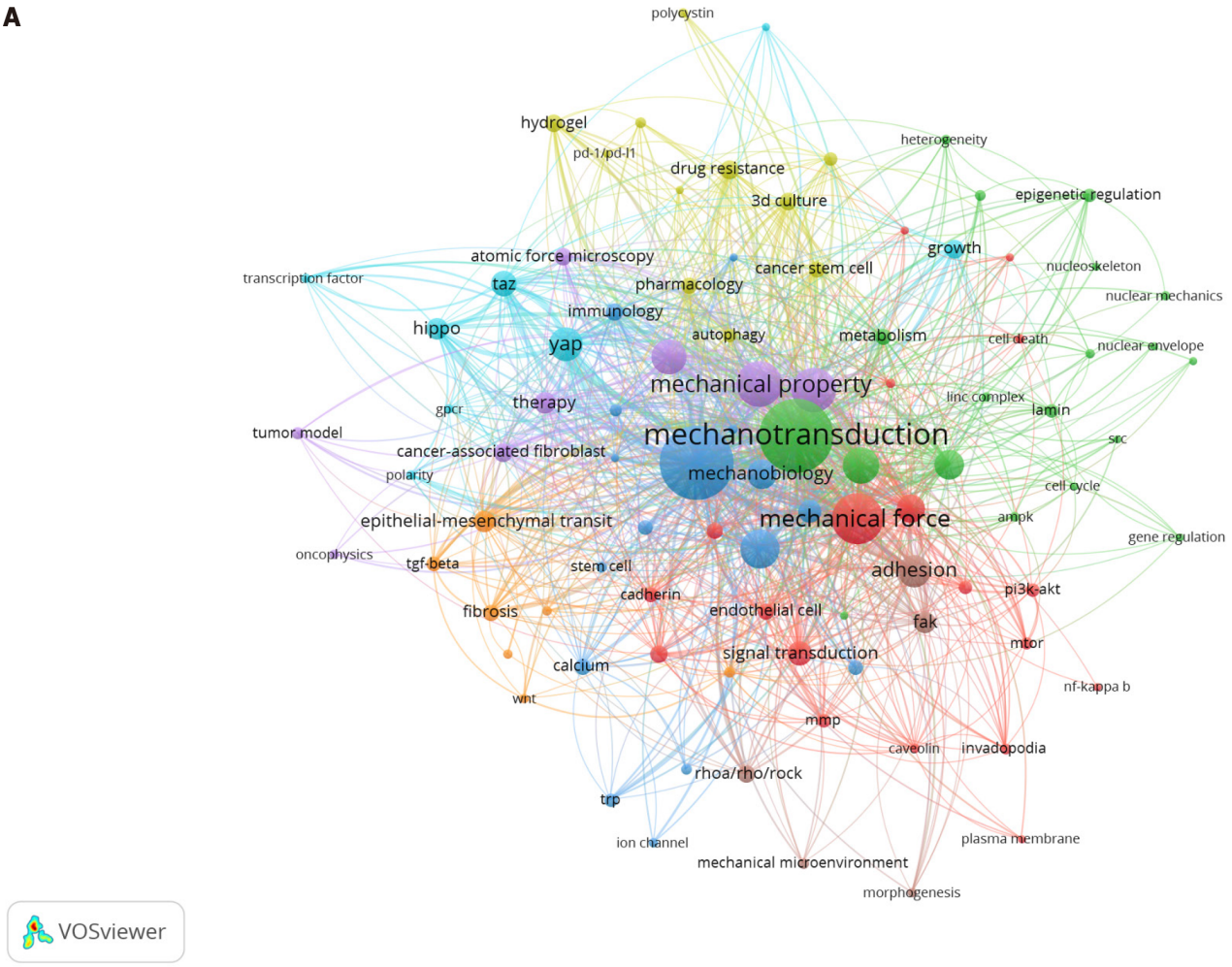
DOI: 10.5306/wjco.v14.i11.518 **Copyright** ©The Author(s) 2023.

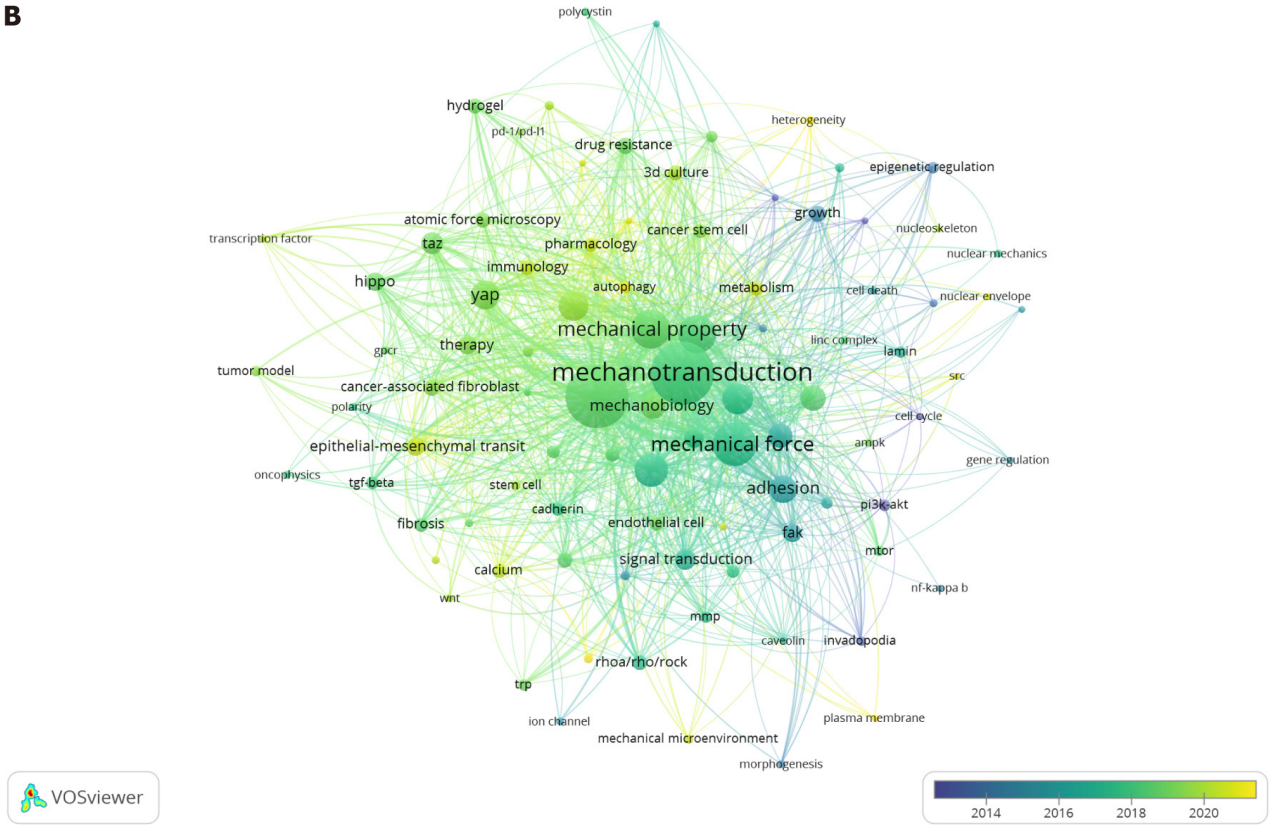
Figure 5 Visualization of author cooperation. VOSviewer was used to visualize the co-authorship network. The clusters are indicated by the different colors and the lines represent the various collaborations between authors.

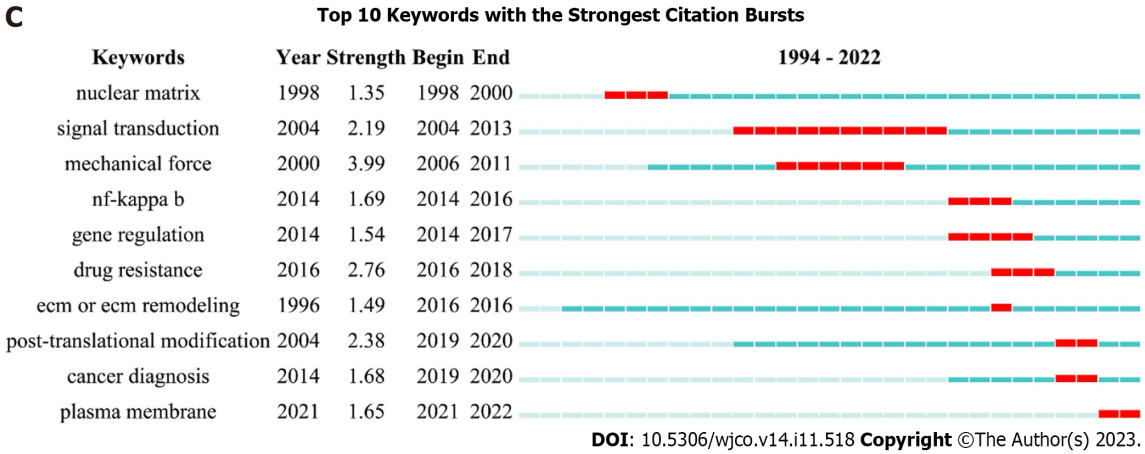
The 597 papers we found were published in almost 300 journals. This suggests that this field is widely recognized by publishers. Notably, some highly prestigious and influential journals, such as *Nature*, *Cell*, and *Cancer Cell*, have accepted relevant articles in recent years, which indicates that this research direction is significant and holds great potential for future investigations. We also found that there was a positive correlation between the average number of times that papers published in the top 10 most productive journals were cited, and the impact factor of the journal. This reflects the vibrancy and attractiveness of this research field. However, except for the *Proceedings of the National Academy of Science of the United States of America* and *Cancer Research*, we found that most of these journals such as *Cancers* and *International Journal of Molecular Sciences* are less highly qualified; therefore, more in-depth explorations are required in the future.

Eight clusters were enriched through a keywords co-occurrence analysis. Cluster 1, with 19 keywords, appeared to elucidate the mechanisms of tumor angiogenesis from the viewpoint of mechanotransduction. Indeed, by providing oxygen and nutrition, tumor angiogenesis, which is initiated by vascular endothelial cell activation, is one of the most fundamental factors for tumor growth and metastasis[37,38]. Hypoxia and growth factors plus their receptors (including vascular endothelial-derived growth factor/vascular endothelial-derived growth factor receptor and fibroblast growth factor/fibroblast growth factor receptor) are known to induce endothelial cell proliferation *via* various signal transduction pathways such as *via* hypoxia-inducible factor-1, PI3K/Akt/mTOR, MAPK/ERK, integrin, and NF-κB signaling[39,40]. In addition, MMPs facilitate endothelial cell invasion by degrading the surrounding ECM components[41,42]. As well as these biochemical pathways, the shear force of the blood flow and stiffness of the tumor tissues are also considered to be essential parameters that influence angiogenesis by regulating endothelial (tip and stalk) cell migration and vessel stabilization[43,44]. For example, it has been demonstrated that caveolin-1 and caveolae act as mechanosensors to respond to altered shear forces for endothelial cell stimulation[45,46]. The mechanical forces exerted on cells can be measured using traction force microscopy, and so this technique might help researchers discover more plasma membrane-localized mechanosensors during angiogenesis[47,48].

Clusters 2 and 3 emphasize the molecular mechanisms of mechanotransduction in cancer metastasis from the perspective of cytoskeletal and nucleoskeletal remodeling, and ion channels, respectively. During metastasis, cancer cells detach from the site of the primary tumor. They then migrate and invade the surrounding microenvironments, and after intravasation into vessels and escaping immune surveillance, they seed and colonize distant sites[49]. As essential initial steps during metastasis, the migration and invasion processes subject cancer cells to different mechanical forces. These are generated due to contact with the TME, and are characterized by increased solid stress, fluid pressure, and tissue stiffness when compared with their counterparts in normal tissue[17]. Although the rigid TME imposes compressive stress on migrating cancer cells, which can cause DNA damage, these mechanical forces can in turn activate the DNA repair system, which limits genotoxicity and maintains regular cellular activity[50]. Various ion channels, such as TRP, polycystin, and piezo1/2, are involved in this process as mechanosensors. For example, in the presence of membrane


A
polycystin
hydrogel
pd-1/pd-l1
heterogeneity
drug resistance
3d culture
epigenetic regulation
growth
atomic force microscopy
cancer stem cell
nucleoskeleton
transcription factor
taz
pharmacology
nuclear mechanics
immunology
hippo
autophagy
metabolism
cell death
nuclear envelope
yap
mechanical property
therapy
linc complex
gpcr
lamin
tumor model
mechanotransduction
src
cancer-associated fibroblast
polarity
mechanobiology
cell cycle
epithelial-mesenchymal transit
mechanical force
ampk
gene regulation
oncophysics
tgf-beta
stem cell
adhesion
pi3k-akt
cadherin
fibrosis
endothelial cell
fak
mtor
calcium
signal transduction
nf-kappa b
wnt
mmp
caveolin
invadopodia
rhoa/rho/rock
trp
ion channel
plasma membrane
mechanical microenvironment
morphogenesis
VOSviewer


B
polycystin
hydrogel
pd-1/pd-l1
heterogeneity
drug resistance
3d culture
epigenetic regulation
growth
atomic force microscopy
cancer stem cell
nucleoskeleton
transcription factor
taz
pharmacology
nuclear mechanics
immunology
hippo
autophagy
metabolism
cell death
nuclear envelope
yap
mechanical property
therapy
linc complex
gpcr
lamin
tumor model
mechanotransduction
src
cancer-associated fibroblast
polarity
mechanobiology
cell cycle
epithelial-mesenchymal transit
mechanical force
ampk
gene regulation
oncophysics
tgf-beta
stem cell
adhesion
pi3k-akt
cadherin
fibrosis
endothelial cell
fak
mtor
calcium
signal transduction
nf-kappa b
wnt
mmp
caveolin
invadopodia
rhoa/rho/rock
trp
ion channel
plasma membrane
mechanical microenvironment
morphogenesis
VOSviewer
2014
2016
2018
2020



DOI: 10.5306/wjco.v14.i11.518 **Copyright** ©The Author(s) 2023.

Figure 6 Keyword co-occurrence and burst analysis. A: VOSviewer was applied in our keyword co-occurrence analysis. The colors indicate different clusters and the node size indicates the number of publications; B: A keyword overlay was visualized with VOSviewer. The circle size reflects the number of publications and the average published year is shown by different colored circles; C: CiteSpace was used to conduct the keyword burst analysis. The red rectangles represent the duration of a keyword.

tension, TRP or piezo1/2 undergo conformational changes, which leads to their activation and an influx of extracellular Ca^{2+}[51-53]. Such alterations in the Ca^{2+} influx often result in cytoskeleton rearrangements, which directly affect downstream signaling by changing the affinity of cytoskeletal protein binding and regulatory proteins[54]. Then, with the assistance of the linker of the nucleoskeleton and cytoskeleton complex, the mechanical forces are transferred to the nucleoskeleton, which distorts the nuclear envelope, impacts the epigenetic state and regulates gene expression[55].

Clusters 4 and 5 highlight the contributing factors that determine the mechanical properties during cancer progression and so these might be useful for the development of relevant pharmaceutical interventions and therapeutical applications. CSCs are the main culprits for the heterogeneity of cancer as they are responsible for cancer initiation, invasive front formation, and drug resistance. It is suggested that the mechanical forces induced by the dense ECM might activate the autophagy and Hippo pathways to promote CSC proliferation and stemness[56,57]. Thus, an alternative explanation for the generation of heterogenous CSC populations (except for their intrinsic differences) might be that the heterogenous TME exerts differential mechanical forces on these cells[58]. CAFs are a key component of the TME in most solid tumor tissues, and the secretomes that originate from these cells contribute to the formation of the ECM[59]. During cancer development, CAFs and cancer cells secrete proteolytic enzymes, cytokines, growth factors, and/or other ECM components, which result in high ECM deposition and increased stiffness[60]. The upregulation of programmed cell death ligand 1 under these stiffer ECM conditions enables cancer cells to evade the immune system; a characteristic that is positively correlated with increased malignancy[61]. As expected, these unique cancer ECM characteristics have already been applied to cancer diagnosis[62]. The three-way biochemical and mechanical crosstalk between CAFs, cancer cells, and the ECM facilitate the remodeling of the latter. The resulting altered mechanical properties of the ECM are favorable for cancer survival, proliferation, drug resistance, and metastasis[63]. Therefore, mechanistic investigations of mechanotransduction during oncogenesis are crucial for the development of relevant pharmaceutical interventions and therapeutical applications[64]. Due to the development of 3D cell culture systems and atomic force microscopy, the mechanical properties can now be mimicked and evaluated to reflect the TME and physical cell-matrix interactions *in vivo*. These methods could therefore be used to screen anti-cancer drugs and in mechanotransduction-related cancer research[65,66].

Cluster 6 shows how cancer cells can override contact inhibition from the viewpoint of mechanotransduction. In cell culture conditions, when the density of normal cells reaches a high enough level, the close physical contact between the cells leads to cell cycle arrest and the suppression of proliferation[67]. Several GPCRs can sense and transduce the higher pressure resulting from the increased cell contacts, to downstream signals. This leads to the cytoplasmic translocation of YAP and TAZ, which nullifies their transcriptional activation[68]. In addition to an inhibition of proliferation in cell dense regions, cell migration is also affected when two normal cells collide. Indeed, cell repolarisation is necessary to separate cells after a collision, and RACK1-dependent cytoskeletal reorganization at the migratory front is also crucial for this process[69,70]. However, these events do not occur during carcinogenesis, and so cancer outgrowth and metastasis is the result[71]. The hyperactivation of YAP and TAZ (caused by a loss of E-cadherin or spectrin), helps to explain the reduced contact inhibition that occurs in cancer from the proliferation perspective[72,73]. However, the reasons for the loss of contact inhibition from the cell migration viewpoint, remain elusive and require further investigation.

Cluster 7 describes the role of Wnt signaling in the mechanical force-driven EMT. The EMT is a process by which epithelial cells are transformed into mesenchymal cells, which then migrate and secrete more ECM components, and so it is crucial for cancer metastasis and drug resistance[74,75]. The activation of the WNT/β-catenin signaling pathway contributes to the transcription of EMT regulators such as *Snail* and *Slug*, both of which inhibit the expression of E-cadherin, and therefore reduce intercellular adhesion and increase cell motility[76]. In addition, TGF-β and some long noncoding RNAs respond to the increase in ECM stiffness, and in this way, they regulate WNT/β-catenin activity and participate in the EMT[18,77].

Finally, cluster 8 demonstrates the impact of the mechanical microenvironment on cell adhesion. The three main characteristics of tumor mechanical microenvironments are an increase in matrix stiffness, solid stress, and interstitial fluid pressure, and these are proposed to activate FAK, which drives focal adhesion formation and primes the RhoA/ROCK signaling cascade[78,79]. This signaling pathway is involved in regulating the organization of the actin cytoskeleton and, therefore, it enables cells to alter their shape and migrate from their primary sites[80].

After performing co-occurring and burst keyword analyses, we found that the keywords "plasma membrane", "autophagy", "piezo1/2", "heterogeneity", "cancer diagnosis", and "post-transcriptional modification" are likely to be the next topics of interest in this field. Interestingly, both keyword analysis methods indicated that the plasma membrane, a mechanosensing structure that transduces the mechanical stimulus to downstream biochemical signal transduction pathways, is a popular topic for future research. Contrary to the increase in substrate stiffness that occurs in cancerous tissues, the plasma membrane of cancer cells is softer than in normal cells, which means that they can migrate more easily [81]. Moreover, the softer plasma membrane along with the underlying cytoskeleton, are more likely to undergo alterations in configuration in response to external mechanical stimuli, and this affects the subsequent transmission of biochemical signals[82]. Since membrane tension is closely associated with cancer cell behavior, this characteristic has recently been used for the diagnosis and prognosis of a low-grade glioma *via* the establishment of a membrane tension-related gene signature[83]. In addition, many cell membrane-localized proteins such as ion channels and other mechano-sensitive proteins are reported to be highly expressed in cancers and act as mechanosensors, which respond to the rigid TME by changing their conformation[84,85]. Of note, the 2021 Nobel Prize was awarded for the discovery of piezo1/2 as mechanosensitive ion channel proteins, and this has initiated a burst of related studies, especially in the cancer research field[33]. Indeed, piezo proteins are closely associated with several cancers and so their potential as diagnostic and prognostic cancer biomarkers is indisputable. In addition, due to the contribution of mechanical force from the TME in the regulation of tumor heterogeneity, identifying the mechanical properties of areas surrounding a tumor and developing therapeutics to counter the mechanical forces with carcinogenic impact would be favorable for precise cancer diagnosis and treatment[86].

The main biochemical signals generated from the mechanical forces, are transduced in the form of phosphorylations. Therefore, identifying more post-transcriptional modification types under different mechanical stimuli might provide some novel perspectives for determining how extracellular cues influence intracellular activities[87,88]. For example, it is now accepted that autophagy is activated by mechanical stress and plays a role in tumorigenesis[89-91]. Therefore, further investigations to explore how the plasma membrane and its localized mechanosensors transduce mechanical forces through post-transcriptional modifications (and thereby participate in the regulation of cellular activity), will not only help to reveal the reasons behind tumor heterogeneity but will also benefit the diagnosis, treatment, and prognosis of cancer. For example, the increase in stiffness is a well-recognized feature of cancer mechanics that has been used previously for cancer diagnosis and prognosis[12]. The continued development and validation of mechanobiological biomarkers that reflect the mechanical properties of tissue microenvironments are likely to facilitate the clinical application of mechano-oncology. Moreover, the mechanosensitivity of cancer cells is suggested to promote malignant cell behaviors[92,93], and mechanical abnormalities are the main culprit that drives cancer chemoresistance *via* the activation of cellular drug efflux or DNA repair systems[94]. Therefore, deciphering the detailed signaling pathways such as autophagy and post-transcriptional modifications involved in mechanotransduction might allow the development of new drugs that can be used in combination with current cancer therapies. This would increase the likelihood of therapeutic success and minimize the chance of developing drug resistance, which is advantageous for the prognosis of cancer patients.

Limitations

Here, we used a bibliometric approach to analyze the trends and important issues regarding mechanotransduction studies in cancer research. While this analysis provides a relatively complete and understandable picture of the state of research today, there are several inevitable limitations. First, while the WoSCC used in this study, is regarded as being a reliable database for bibliometric analysis, the use of additional database sources would provide a more comprehensive view of the situation. Second, some papers that are already included in the associated databases might be delayed being included in the WoSCC, leading to statistical bias and a loss of precision. Finally, analyzing and summarizing research trends based on keywords alone, might be subjective and therefore lack a depth of exploration when compared with traditional literature reviews.

CONCLUSION

Our results show that mechanotransduction-related cancer research is an increasingly popular topic in the world today. The United States is in the leading position of global research on mechano-oncology after almost 30 years of investigations, and the University of California system (with the largest number of collaborators), is the most influential organization based on its publication and citation times. Research group cooperations exist but remain largely domestic, lacking cross-national communications. Our findings suggest that investigations exploring how the plasma membrane and its localized mechanosensors might transduce mechanical force through post-transcriptional modifications and thereby participate in the cellular activity regulations and cancer development, will be the next big topic in this field.

ARTICLE HIGHLIGHTS

Research background

Mechanical stimuli, generated by the contact between cells (both tumor and non-tumur) or with the non-cellular microenvironment, have been demonstrated to play a significant role in the development of cancer. Unlike biochemical transduction, which depends on small molecules, growth factors, and cytokines, mechanotransduction is a process whereby cells sense mechanical cues in their external environment and translate them into biochemical signals to impact their intracellular activities. Indeed, in recent investigations, the importance of mechanical stimulation in cancer development is described as being on par with biochemical factors. Drs David Julius and Ardem Patapoutian were awarded the 2021 Nobel Prize in Physiology or Medicine for their work on mechanosensitive ion channels, which has already acted as a new catalyst for the increasing numbers of researchers to conduct mechanotransduction-related cancer research. Bibliometrics is a useful quantitative method to comprehensively analyze publications in multiple aspects, including the authors, organizations, countries, journals, and keywords, to uncover collaboration conditions and emerging trends in specific research areas. Although increasing numbers of research papers are now starting to focus on the role of mechanotransduction in cancer, to date no bibliometric analysis has been conducted to quantify the situation.

Research motivation

The deep understanding of mechanotransduction in cancer will not only help determine the reasons behind the tumor heterogeneity, but also facilitate the development of more versatile approaches to cancer diagnosis and therapy.

Research objectives

To provides an objective evaluation of the dynamics and emerging trends of mechanotransduction-related cancer research.

Research methods

We present the first bibliometric analysis of research conducted on mechanotransduction in cancer and reveal the current trends and hot topics in this field.

Research results

This study showed that mechanotransduction-related cancer research remains a hot topic, with approximately 100 papers and 5000 citations generated per year in the past three years. Additionally, the United States is a well-established global leader of this field, and the University of California system is the most influential organization in this field. The keywords "plasma membrane", "autophagy", "piezo1/2", "heterogeneity", "cancer diagnosis", and "post-transcriptional modification" are likely to be the next topics of interest in this field.

Research conclusions

Our results found that mechanotransduction-related cancer research is an increasingly popular topic in the world today. The United States is in the leading position of global research on mechano-oncology after almost 30 years of investigations, and the University of California system (with the largest number of collaborators), is the most influential organization based on its publication and citation times. Research group cooperations exist but remain largely domestic, lacking cross-national communications.

Research perspectives

We predict that the next 'hot' topic in cancer research will be investigating how localized mechanosensors in the plasma membrane transduce mechanical forces *via* post-transcriptional modifications to participate in the regulation of cellular activity.

FOOTNOTES

Author contributions: Wang DW conceptualized and designed this study; Wang DW, Zhang YZ, Li MZ, and Wang GX collected and analyzed the database; Wang DW, Zhang YZ, and Li MZ wrote the manuscript; and all authors have read and approve the final manuscript.

Supported by the National Natural Science Foundation of China, No. 32200557; Natural Science Foundation of Shandong Province, No. ZR2022QH271; and the Postdoctoral Innovative Projects of Shandong Province, No. SDCX-ZG-202203047.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to

distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Da-Wei Wang 0000-0001-7902-001X.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Vaghari-Tabari M**, Ferns GA, Qujeq D, Andevari AN, Sabahi Z, Moein S. Signaling, metabolism, and cancer: An important relationship for therapeutic intervention. *J Cell Physiol* 2021; **236**: 5512-5532 [PMID: 33580511 DOI: 10.1002/jcp.30276]

2 **Global Burden of Disease 2019 Cancer Collaboration**, Kocarnik JM, Compton K, Dean FE, Fu W, Gaw BL, Harvey JD, Henrikson HJ, Lu D, Pennini A, Xu R, Ababneh E, Abbasi-Kangevari M, Abbastabar H, Abd-Elsalam SM, Abdoli A, Abedi A, Abidi H, Abolhassani H, Adedeji IA, Adnani QES, Advani SM, Afzal MS, Aghaali M, Ahinkorah BO, Ahmad S, Ahmad T, Ahmadi A, Ahmadi S, Ahmed Rashid T, Ahmed Salih Y, Akalu GT, Aklilu A, Akram T, Akunna CJ, Al Hamad H, Alahdab F, Al-Aly Z, Ali S, Alimohamadi Y, Alipour V, Aljunid SM, Alkhayyat M, Almasi-Hashiani A, Almasri NA, Al-Maweri SAA, Almustanyir S, Alonso N, Alvis-Guzman N, Amu H, Anbesu EW, Ancuceanu R, Ansari F, Ansari-Moghaddam A, Antwi MH, Anvari D, Anyasodor AE, Aqeel M, Arabloo J, Arab-Zozani M, Aremu O, Ariffin H, Aripov T, Arshad M, Artaman A, Arulappan J, Asemi Z, Asghari Jafarabadi M, Ashraf T, Atorkey P, Aujayeb A, Ausloos M, Awedew AF, Ayala Quintanilla BP, Ayenew T, Azab MA, Azadnajafabad S, Azari Jafari A, Azarian G, Azzam AY, Badiye AD, Bahadory S, Baig AA, Baker JL, Balakrishnan S, Banach M, Bärnighausen TW, Barone-Adesi F, Barra F, Barrow A, Behzadifar M, Belgaumi UI, Bezabhe WMM, Bezabih YM, Bhagat DS, Bhagavathula AS, Bhardwaj N, Bhardwaj P, Bhaskar S, Bhattacharyya K, Bhojaraja VS, Bibi S, Bijani A, Biondi A, Bisignano C, Bjørge T, Bleyer A, Blyuss O, Bolarinwa OA, Bolla SR, Braithwaite D, Brar A, Brenner H, Bustamante-Teixeira MT, Butt NS, Butt ZA, Caetano Dos Santos FL, Cao Y, Carreras G, Catalá-López F, Cembranel F, Cerin E, Cernigliaro A, Chakinala RC, Chattu SK, Chattu VK, Chaturvedi P, Chimed-Ochir O, Cho DY, Christopher DJ, Chu DT, Chung MT, Conde J, Cortés S, Cortesi PA, Costa VM, Cunha AR, Dadras O, Dagnew AB, Dahlawi SMA, Dai X, Dandona L, Dandona R, Darwesh AM, das Neves J, De la Hoz FP, Demis AB, Denova-Gutiérrez E, Dhamnetiya D, Dhimal ML, Dhimal M, Dianatinasab M, Diaz D, Djalalinia S, Do HP, Doaei S, Dorostkar F, Dos Santos Figueiredo FW, Driscoll TR, Ebrahimi H, Eftekharzadeh S, El Tantawi M, El-Abid H, Elbarazi I, Elhabashy HR, Elhadi M, El-Jaafary SI, Eshrati B, Eskandarieh S, Esmaeilzadeh F, Etemadi A, Ezzikouri S, Faisaluddin M, Faraon EJA, Fares J, Farzadfar F, Feroze AH, Ferrero S, Ferro Desideri L, Filip I, Fischer F, Fisher JL, Foroutan M, Fukumoto T, Gaal PA, Gad MM, Gadanya MA, Gallus S, Gaspar Fonseca M, Getachew Obsa A, Ghafourifard M, Ghashghaee A, Ghith N, Gholamalizadeh M, Gilani SA, Ginindza TG, Gizaw ATT, Glasbey JC, Golechha M, Goleij P, Gomez RS, Gopalani SV, Gorini G, Goudarzi H, Grosso G, Gubari MIM, Guerra MR, Guha A, Gunasekera DS, Gupta B, Gupta VB, Gupta VK, Gutiérrez RA, Hafezi-Nejad N, Haider MR, Haj-Mirzaian A, Halwani R, Hamadeh RR, Hameed S, Hamidi S, Hanif A, Haque S, Harlianto NI, Haro JM, Hasaballah AI, Hassanipour S, Hay RJ, Hay SI, Hayat K, Heidari G, Heidari M, Herrera-Serna BY, Herteliu C, Hezam K, Holla R, Hossain MM, Hossain MBH, Hosseini MS, Hosseini M, Hosseinzadeh M, Hostiuc M, Hostiuc S, Househ M, Hsairi M, Huang J, Hugo FN, Hussain R, Hussein NR, Hwang BF, Iavicoli I, Ibitoye SE, Ida F, Ikuta KS, Ilesanmi OS, Ilic IM, Ilic MD, Irham LM, Islam JY, Islam RM, Islam SMS, Ismail NE, Isola G, Iwagami M, Jacob L, Jain V, Jakovljevic MB, Javaheri T, Jayaram S, Jazayeri SB, Jha RP, Jonas JB, Joo T, Joseph N, Joukar F, Jürisson M, Kabir A, Kahrizi D, Kalankesh LR, Kalhor R, Kaliyadan F, Kalkonde Y, Kamath A, Kameran Al-Salihi N, Kandel H, Kapoor N, Karch A, Kasa AS, Katikireddi SV, Kauppila JH, Kavetskyy T, Kebede SA, Keshavarz P, Keykhaei M, Khader YS, Khalilov R, Khan G, Khan M, Khan MN, Khan MAB, Khang YH, Khater AM, Khayamzadeh M, Kim GR, Kim YJ, Kisa A, Kisa S, Kissimova-Skarbek K, Kopec JA, Koteeswaran R, Koul PA, Koulmane Laxminarayana SL, Koyanagi A, Kucuk Bicer B, Kugbey N, Kumar GA, Kumar N, Kurmi OP, Kutluk T, La Vecchia C, Lami FH, Landires I, Lauriola P, Lee SW, Lee SWH, Lee WC, Lee YH, Leigh J, Leong E, Li J, Li MC, Liu X, Loureiro JA, Lunevicius R, Magdy Abd El Razek M, Majeed A, Makki A, Male S, Malik AA, Mansournia MA, Martini S, Masoumi SZ, Mathur P, McKee M, Mehrotra R, Mendoza W, Menezes RG, Mengesha EW, Mesregah MK, Mestrovic T, Miao Jonasson J, Miazgowski B, Miazgowski T, Michalek IM, Miller TR, Mirzaei H, Mirzaei HR, Misra S, Mithra P, Moghadaszadeh M, Mohammad KA, Mohammad Y, Mohammadi M, Mohammadi SM, Mohammadian-Hafshejani A, Mohammed S, Moka N, Mokdad AH, Molokhia M, Monasta L, Moni MA, Moosavi MA, Moradi Y, Moraga P, Morgado-da-Costa J, Morrison SD, Mosapour A, Mubarik S, Mwanri L, Nagarajan AJ, Nagaraju SP, Nagata C, Naimzada MD, Nangia V, Naqvi AA, Narasimha Swamy S, Ndejjo R, Nduaguba SO, Negoi I, Negru SM, Neupane Kandel S, Nguyen CT, Nguyen HLT, Niazi RK, Nnaji CA, Noor NM, Nuñez-Samudio V, Nzoputam CI, Oancea B, Ochir C, Odukoya OO, Ogbo FA, Olagunju AT, Olakunde BO, Omar E, Omar Bali A, Omonisi AEE, Ong S, Onwujekwe OE, Orru H, Ortega-Altamirano DV, Otstavnov N, Otstavnov SS, Owolabi MO, P A M, Padubidri JR, Pakshir K, Pana A, Panagiotakos D, Panda-Jonas S, Pardhan S, Park EC, Park EK, Pashazadeh Kan F, Patel HK, Patel JR, Pati S, Pattanshetty SM, Paudel U, Pereira DM, Pereira RB, Perianayagam A, Pillay JD, Pirouzpanah S, Pishgar F, Podder I, Postma MJ, Pourjafar H, Prashant A, Preotescu L, Rabiee M, Rabiee N, Radfar A, Radhakrishnan RA, Radhakrishnan V, Rafiee A, Rahim F, Rahimzadeh S, Rahman M, Rahman MA, Rahmani AM, Rajai N, Rajesh A, Rakovac I, Ram P, Ramezanzadeh K, Ranabhat K, Ranasinghe P, Rao CR, Rao SJ, Rawassizadeh R, Razeghinia MS, Renzaho AMN, Rezaei N, Rezapour A, Roberts TJ, Rodriguez JAB, Rohloff P, Romoli M, Ronfani L, Roshandel G, Rwegerera GM, S M, Sabour S, Saddik B, Saeed U, Sahebkar A, Sahoo H, Salehi S, Salem MR, Salimzadeh H, Samaei M, Samy AM, Sanabria J, Sankararaman S, Santric-Milicevic MM, Sardiwalla Y, Sarveazad A, Sathian B, Sawhney M, Saylan M, Schneider IJC, Sekerija M, Seylani A, Shafaat O, Shaghaghi Z, Shaikh MA, Shamsoddin E, Shannawaz M, Sharma R, Sheikh A, Sheikhbahaei S, Shetty A, Shetty JK, Shetty PH, Shibuya K, Shirkoohi R, Shivakumar KM, Shivarov V, Siabani S, Siddappa Malleshappa SK, Silva DAS, Singh JA, Sintayehu Y, Skryabin VY, Skryabina AA, Soeberg MJ, Sofi-Mahmudi A, Sotoudeh H, Steiropoulos P, Straif K, Subedi R, Sufiyan MB, Sultan I, Sultana S, Sur D, Szerencsés V, Szócska M, Tabarés-Seisdedos R, Tabuchi T, Tadbiri H, Taherkhani A, Takahashi K, Talaat IM, Tan KK, Tat VY, Tedla BAA, Tefera YG, Tehrani-Banihashemi A, Temsah MH, Tesfay FH, Tessema GA, Thapar R, Thavamani A, Thoguluva Chandrasekar V, Thomas N, Tohidinik HR, Touvier M,

Tovani-Palone MR, Traini E, Tran BX, Tran KB, Tran MTN, Tripathy JP, Tusa BS, Ullah I, Ullah S, Umapathi KK, Unnikrishnan B, Upadhyay E, Vacante M, Vaezi M, Valadan Tahbaz S, Velazquez DZ, Veroux M, Violante FS, Vlassov V, Vo B, Volovici V, Vu GT, Waheed Y, Wamai RG, Ward P, Wen YF, Westerman R, Winkler AS, Yadav L, Yahyazadeh Jabbari SH, Yang L, Yaya S, Yazie TSY, Yeshaw Y, Yonemoto N, Younis MZ, Yousefi Z, Yu C, Yuce D, Yunusa I, Zadnik V, Zare F, Zastrozhin MS, Zastrozhina A, Zhang J, Zhong C, Zhou L, Zhu C, Ziapour A, Zimmermann IR, Fitzmaurice C, Murray CJL, Force LM. Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life Years for 29 Cancer Groups From 2010 to 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *JAMA Oncol* 2022; **8**: 420-444 [PMID: 34967848 DOI: 10.1001/jamaoncol.2021.6987]

3 **Mbemi A**, Khanna S, Njiki S, Yedjou CG, Tchounwou PB. Impact of Gene-Environment Interactions on Cancer Development. *Int J Environ Res Public Health* 2020; **17** [PMID: 33153024 DOI: 10.3390/ijerph17218089]

4 **de Visser KE**, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. *Cancer Cell* 2023; **41**: 374-403 [PMID: 36917948 DOI: 10.1016/j.ccell.2023.02.016]

5 **Broders-Bondon F**, Nguyen Ho-Bouldoires TH, Fernandez-Sanchez ME, Farge E. Mechanotransduction in tumor progression: The dark side of the force. *J Cell Biol* 2018; **217**: 1571-1587 [PMID: 29467174 DOI: 10.1083/jcb.201701039]

6 **Mierke CT**. The fundamental role of mechanical properties in the progression of cancer disease and inflammation. *Rep Prog Phys* 2014; **77**: 076602 [PMID: 25006689 DOI: 10.1088/0034-4885/77/7/076602]

7 **Butcher DT**, Alliston T, Weaver VM. A tense situation: forcing tumour progression. *Nat Rev Cancer* 2009; **9**: 108-122 [PMID: 19165226 DOI: 10.1038/nrc2544]

8 **Liu Q**, Luo Q, Ju Y, Song G. Role of the mechanical microenvironment in cancer development and progression. *Cancer Biol Med* 2020; **17**: 282-292 [PMID: 32587769 DOI: 10.20892/j.issn.2095-3941.2019.0437]

9 **Fang Y**, Wu D, Birukov KG. Mechanosensing and Mechanoregulation of Endothelial Cell Functions. *Compr Physiol* 2019; **9**: 873-904 [PMID: 30873580 DOI: 10.1002/cphy.c180020]

10 **Lin HH**, Ng KF, Chen TC, Tseng WY. Ligands and Beyond: Mechanosensitive Adhesion GPCRs. *Pharmaceuticals (Basel)* 2022; **15** [PMID: 35215331 DOI: 10.3390/ph15020219]

11 **Panciera T**, Azzolin L, Cordenonsi M, Piccolo S. Mechanobiology of YAP and TAZ in physiology and disease. *Nat Rev Mol Cell Biol* 2017; **18**: 758-770 [PMID: 28951564 DOI: 10.1038/nrm.2017.87]

12 **Winkler J**, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat Commun* 2020; **11**: 5120 [PMID: 33037194 DOI: 10.1038/s41467-020-18794-x]

13 **Abdollahiyan P**, Oroojalian F, Baradaran B, de la Guardia M, Mokhtarzadeh A. Advanced mechanotherapy: Biotensegrity for governing metastatic tumor cell fate *via* modulating the extracellular matrix. *J Control Release* 2021; **335**: 596-618 [PMID: 34097925 DOI: 10.1016/j.jconrel.2021.06.002]

14 **Chaqour B**, Goppelt-Struebe M. Mechanical regulation of the Cyr61/CCN1 and CTGF/CCN2 proteins. *FEBS J* 2006; **273**: 3639-3649 [PMID: 16856934 DOI: 10.1111/j.1742-4658.2006.05360.x]

15 **Hong W**, Guan KL. The YAP and TAZ transcription co-activators: key downstream effectors of the mammalian Hippo pathway. *Semin Cell Dev Biol* 2012; **23**: 785-793 [PMID: 22659496 DOI: 10.1016/j.semcdb.2012.05.004]

16 **Nasrollahi S**, Walter C, Loza AJ, Schimizzi GV, Longmore GD, Pathak A. Past matrix stiffness primes epithelial cells and regulates their future collective migration through a mechanical memory. *Biomaterials* 2017; **146**: 146-155 [PMID: 28918264 DOI: 10.1016/j.biomaterials.2017.09.012]

17 **Mohammadi H**, Sahai E. Mechanisms and impact of altered tumour mechanics. *Nat Cell Biol* 2018; **20**: 766-774 [PMID: 29950570 DOI: 10.1038/s41556-018-0131-2]

18 **Jiang Y**, Zhang H, Wang J, Liu Y, Luo T, Hua H. Targeting extracellular matrix stiffness and mechanotransducers to improve cancer therapy. *J Hematol Oncol* 2022; **15**: 34 [PMID: 35331296 DOI: 10.1186/s13045-022-01252-0]

19 **Er EE**, Tello-Lafoz M, Huse M. Mechanoregulation of Metastasis beyond the Matrix. *Cancer Res* 2022; **82**: 3409-3419 [PMID: 35877197 DOI: 10.1158/0008-5472.CAN-22-0419]

20 **Ni W**, Mo H, Liu Y, Xu Y, Qin C, Zhou Y, Li Y, Zhou A, Yao S, Zhou R, Huo J, Che L, Li J. Targeting cholesterol biosynthesis promotes anti-tumor immunity by inhibiting long noncoding RNA SNHG29-mediated YAP activation. *Mol Ther* 2021; **29**: 2995-3010 [PMID: 33992804 DOI: 10.1016/j.ymthe.2021.05.012]

21 **Donthu N**, Kumar S, Mukherjee D, Pandey N, Lim WM. How to conduct a bibliometric analysis: An overview and guidelines. *J Bus Res* 2021; **133**: 285-296 [DOI: 10.1016/j.jbusres.2021.04.070]

22 **Ugolini D**, Puntoni R, Perera FP, Schulte PA, Bonassi S. A bibliometric analysis of scientific production in cancer molecular epidemiology. *Carcinogenesis* 2007; **28**: 1774-1779 [PMID: 17548902 DOI: 10.1093/carcin/bgm129]

23 **Song M**, Lu Q, Xu M, Li Y, Zhao Y, Gong C, Ou X. The global research and emerging trends in autophagy of pancreatic cancer: A bibliometric and visualized study. *Front Oncol* 2022; **12**: 987026 [PMID: 36263211 DOI: 10.3389/fonc.2022.987026]

24 **Li M**, Zhang Y, Zhao J, Wang D. The global landscape and research trend of phase separation in cancer: a bibliometric analysis and visualization. *Front Oncol* 2023; **13**: 1170157 [PMID: 37333812 DOI: 10.3389/fonc.2023.1170157]

25 **Kong H**, Li M, Deng CM, Wu YJ, He ST, Mu DL. A comprehensive overview of clinical research on dexmedetomidine in the past 2 decades: A bibliometric analysis. *Front Pharmacol* 2023; **14**: 1043956 [PMID: 36865921 DOI: 10.3389/fphar.2023.1043956]

26 **KATZ B**. Depolarization of sensory terminals and the initiation of impulses in the muscle spindle. *J Physiol* 1950; **111**: 261-282 [PMID: 14795439 DOI: 10.1113/jphysiol.1950.sp004479]

27 **Guharay F**, Sachs F. Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J Physiol* 1984; **352**: 685-701 [PMID: 6086918 DOI: 10.1113/jphysiol.1984.sp015317]

28 **Erxleben C**. Stretch-activated current through single ion channels in the abdominal stretch receptor organ of the crayfish. *J Gen Physiol* 1989; **94**: 1071-1083 [PMID: 2482327 DOI: 10.1085/jgp.94.6.1071]

29 **Weiss L**. Cell adhesion molecules: a critical examination of their role in metastasis. *Invasion Metastasis***14**: 192-197 [PMID: 7657512]

30 **Chambers AF**, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* 2002; **2**: 563-572 [PMID: 12154349 DOI: 10.1038/nrc865]

31 **Aragona M**, Panciera T, Manfrin A, Giulitti S, Michielin F, Elvassore N, Dupont S, Piccolo S. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell* 2013; **154**: 1047-1059 [PMID: 23954413 DOI: 10.1016/j.cell.2013.07.042]

32 **Ma S**, Meng Z, Chen R, Guan KL. The Hippo Pathway: Biology and Pathophysiology. *Annu Rev Biochem* 2019; **88**: 577-604 [PMID: 30566373 DOI: 10.1146/annurev-biochem-013118-111829]

33 **Martinac B**. 2021 Nobel Prize for mechanosensory transduction. *Biophys Rev* 2022; **14**: 15-20 [PMID: 35340591 DOI: 10.1007/s12551-022-00935-9]

34 **Massou S**, Nunes Vicente F, Wetzel F, Mehidi A, Strehle D, Leduc C, Voituriez R, Rossier O, Nassoy P, Giannone G. Cell stretching is amplified by active actin remodelling to deform and recruit proteins in mechanosensitive structures. *Nat Cell Biol* 2020; **22**: 1011-1023 [PMID: 32719553 DOI: 10.1038/s41556-020-0548-2]

35 **Nunes Vicente F**, Chen T, Rossier O, Giannone G. Novel imaging methods and force probes for molecular mechanobiology of cytoskeleton and adhesion. *Trends Cell Biol* 2023; **33**: 204-220 [PMID: 36055943 DOI: 10.1016/j.tcb.2022.07.008]

36 **Lavrenyuk K**, Conway D, Dahl KN. Imaging methods in mechanosensing: a historical perspective and visions for the future. *Mol Biol Cell* 2021; **32**: 842-854 [PMID: 33788578 DOI: 10.1091/mbc.E20-10-0671]

37 **Folkman J**. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**: 1182-1186 [PMID: 4938153 DOI: 10.1056/nejm197111182852108]

38 **Folkman J**. Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 2002; **29**: 15-18 [PMID: 12516034 DOI: 10.1053/sonc.2002.37263]

39 **Liu G**, Chen T, Ding Z, Wang Y, Wei Y, Wei X. Inhibition of FGF-FGFR and VEGF-VEGFR signalling in cancer treatment. *Cell Prolif* 2021; **54**: e13009 [PMID: 33655556 DOI: 10.1111/cpr.13009]

40 **Pugh CW**, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med* 2003; **9**: 677-684 [PMID: 12778166 DOI: 10.1038/nm0603-677]

41 **Alaseem A**, Alhazzani K, Dondapati P, Alobid S, Bishayee A, Rathinavelu A. Matrix Metalloproteinases: A challenging paradigm of cancer management. *Semin Cancer Biol* 2019; **56**: 100-115 [PMID: 29155240 DOI: 10.1016/j.semcancer.2017.11.008]

42 **Kessenbrock K**, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010; **141**: 52-67 [PMID: 20371345 DOI: 10.1016/j.cell.2010.03.015]

43 **Kai F**, Laklai H, Weaver VM. Force Matters: Biomechanical Regulation of Cell Invasion and Migration in Disease. *Trends Cell Biol* 2016; **26**: 486-497 [PMID: 27056543 DOI: 10.1016/j.tcb.2016.03.007]

44 **Kretschmer M**, Rüdiger D, Zahler S. Mechanical Aspects of Angiogenesis. *Cancers (Basel)* 2021; **13** [PMID: 34638470 DOI: 10.3390/cancers13194987]

45 **Shihata WA**, Michell DL, Andrews KL, Chin-Dusting JP. Caveolae: A Role in Endothelial Inflammation and Mechanotransduction? *Front Physiol* 2016; **7**: 628 [PMID: 28066261 DOI: 10.3389/fphys.2016.00628]

46 **Frank PG**, Lisanti MP. Role of caveolin-1 in the regulation of the vascular shear stress response. *J Clin Invest* 2006; **116**: 1222-1225 [PMID: 16670766 DOI: 10.1172/jci28509]

47 **Boldock L**, Wittkowske C, Perrault CM. Microfluidic traction force microscopy to study mechanotransduction in angiogenesis. *Microcirculation* 2017; **24** [PMID: 28164414 DOI: 10.1111/micc.12361]

48 **Hur SS**, Jeong JH, Ban MJ, Park JH, Yoon JK, Hwang Y. Traction force microscopy for understanding cellular mechanotransduction. *BMB Rep* 2020; **53**: 74-81 [PMID: 31964473 DOI: 10.5483/BMBRep.2020.53.2.308]

49 **Fares J**, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: a hallmark of cancer revisited. *Signal Transduct Target Ther* 2020; **5**: 28 [PMID: 32296047 DOI: 10.1038/s41392-020-0134-x]

50 **Montagner M**, Dupont S. Mechanical Forces as Determinants of Disseminated Metastatic Cell Fate. *Cells* 2020; **9** [PMID: 31963820 DOI: 10.3390/cells9010250]

51 **Pethő Z**, Najder K, Bulk E, Schwab A. Mechanosensitive ion channels push cancer progression. *Cell Calcium* 2019; **80**: 79-90 [PMID: 30991298 DOI: 10.1016/j.ceca.2019.03.007]

52 **Pedersen SF**, Nilius B. Transient receptor potential channels in mechanosensing and cell volume regulation. *Methods Enzymol* 2007; **428**: 183-207 [PMID: 17875418 DOI: 10.1016/s0076-6879(07)28010-3]

53 **Jiang Y**, Yang X, Jiang J, Xiao B. Structural Designs and Mechanogating Mechanisms of the Mechanosensitive Piezo Channels. *Trends Biochem Sci* 2021; **46**: 472-488 [PMID: 33610426 DOI: 10.1016/j.tibs.2021.01.008]

54 **Li X**, Wang J. Mechanical tumor microenvironment and transduction: cytoskeleton mediates cancer cell invasion and metastasis. *Int J Biol Sci* 2020; **16**: 2014-2028 [PMID: 32549750 DOI: 10.7150/ijbs.44943]

55 **Janota CS**, Calero-Cuenca FJ, Gomes ER. The role of the cell nucleus in mechanotransduction. *Curr Opin Cell Biol* 2020; **63**: 204-211 [PMID: 32361559 DOI: 10.1016/j.ceb.2020.03.001]

56 **Li Y**, Randriantsilefisoa R, Chen J, Cuellar-Camacho JL, Liang W, Li W. Matrix Stiffness Regulates Chemosensitivity, Stemness Characteristics, and Autophagy in Breast Cancer Cells. *ACS Appl Bio Mater* 2020; **3**: 4474-4485 [PMID: 35025446 DOI: 10.1021/acsabm.0c00448]

57 **Li YR**, Fang Y, Lyu Z, Zhu Y, Yang L. Exploring the dynamic interplay between cancer stem cells and the tumor microenvironment: implications for novel therapeutic strategies. *J Transl Med* 2023; **21**: 686 [PMID: 37784157 DOI: 10.1186/s12967-023-04575-9]

58 **Prasetyanti PR**, Medema JP. Intra-tumor heterogeneity from a cancer stem cell perspective. *Mol Cancer* 2017; **16**: 41 [PMID: 28209166 DOI: 10.1186/s12943-017-0600-4]

59 **Wu F**, Yang J, Liu J, Wang Y, Mu J, Zeng Q, Deng S, Zhou H. Signaling pathways in cancer-associated fibroblasts and targeted therapy for cancer. *Signal Transduct Target Ther* 2021; **6**: 218 [PMID: 34108441 DOI: 10.1038/s41392-021-00641-0]

60 **Najafi M**, Farhood B, Mortezaee K. Extracellular matrix (ECM) stiffness and degradation as cancer drivers. *J Cell Biochem* 2019; **120**: 2782-2790 [PMID: 30321449 DOI: 10.1002/jcb.27681]

61 **Miyazawa A**, Ito S, Asano S, Tanaka I, Sato M, Kondo M, Hasegawa Y. Regulation of PD-L1 expression by matrix stiffness in lung cancer cells. *Biochem Biophys Res Commun* 2018; **495**: 2344-2349 [PMID: 29274784 DOI: 10.1016/j.bbrc.2017.12.115]

62 **Petersen EV**, Chudakova DA, Skorova EY, Anikin V, Reshetov IV, Mynbaev OA. The Extracellular Matrix-Derived Biomarkers for Diagnosis, Prognosis, and Personalized Therapy of Malignant Tumors. *Front Oncol* 2020; **10**: 575569 [PMID: 33425730 DOI: 10.3389/fonc.2020.575569]

63 **Jang I**, Beningo KA. Integrins, CAFs and Mechanical Forces in the Progression of Cancer. *Cancers (Basel)* 2019; **11** [PMID: 31137693 DOI: 10.3390/cancers11050721]

64 **Gargalionis AN**, Basdra EK, Papavassiliou AG. Tumor mechanosensing and its therapeutic potential. *J Cell Biochem* 2018; **119**: 4304-4308 [PMID: 29479734 DOI: 10.1002/jcb.26786]

65 **Trache A**, Xie L, Huang H, Glinsky VV, Meininger GA. Applications of Atomic Force Microscopy for Adhesion Force Measurements in Mechanotransduction. *Methods Mol Biol* 2018; **1814**: 515-528 [PMID: 29956252 DOI: 10.1007/978-1-4939-8591-3_30]

66 **Zonderland J**, Moroni L. Steering cell behavior through mechanobiology in 3D: A regenerative medicine perspective. *Biomaterials* 2021; **268**: 120572 [PMID: 33285439 DOI: 10.1016/j.biomaterials.2020.120572]

67 **McClatchey AI**, Yap AS. Contact inhibition (of proliferation) redux. *Curr Opin Cell Biol* 2012; **24**: 685-694 [PMID: 22835462 DOI: 10.1016/j.ceb.2012.06.009]

68 **Luo J**, Yu FX. GPCR-Hippo Signaling in Cancer. *Cells* 2019; **8** [PMID: 31072060 DOI: 10.3390/cells8050426]

69 **Duff D**, Long A. Roles for RACK1 in cancer cell migration and invasion. *Cell Signal* 2017; **35**: 250-255 [PMID: 28336233 DOI: 10.1016/j.cellsig.2017.03.005]

70 **Roycroft A**, Mayor R. Molecular basis of contact inhibition of locomotion. *Cell Mol Life Sci* 2016; **73**: 1119-1130 [PMID: 26585026 DOI: 10.1007/s00018-015-2090-0]

71 **Mayor R**, Carmona-Fontaine C. Keeping in touch with contact inhibition of locomotion. *Trends Cell Biol* 2010; **20**: 319-328 [PMID: 20399659 DOI: 10.1016/j.tcb.2010.03.005]

72 **Kim NG**, Koh E, Chen X, Gumbiner BM. E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components. *Proc Natl Acad Sci U S A* 2011; **108**: 11930-11935 [PMID: 21730131 DOI: 10.1073/pnas.1103345108]

73 **Fletcher GC**, Elbediwy A, Khanal I, Ribeiro PS, Tapon N, Thompson BJ. The Spectrin cytoskeleton regulates the Hippo signalling pathway. *EMBO J* 2015; **34**: 940-954 [PMID: 25712476 DOI: 10.15252/embj.201489642]

74 **Pastushenko I**, Blanpain C. EMT Transition States during Tumor Progression and Metastasis. *Trends Cell Biol* 2019; **29**: 212-226 [PMID: 30594349 DOI: 10.1016/j.tcb.2018.12.001]

75 **Bakir B**, Chiarella AM, Pitarresi JR, Rustgi AK. EMT, MET, Plasticity, and Tumor Metastasis. *Trends Cell Biol* 2020; **30**: 764-776 [PMID: 32800658 DOI: 10.1016/j.tcb.2020.07.003]

76 **Kalluri R**, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009; **119**: 1420-1428 [PMID: 19487818 DOI: 10.1172/JCI39104]

77 **Xu X**, Zhang Y, Wang X, Li S, Tang L. Substrate Stiffness Drives Epithelial to Mesenchymal Transition and Proliferation through the NEAT1-Wnt/β-Catenin Pathway in Liver Cancer. *Int J Mol Sci* 2021; **22** [PMID: 34769497 DOI: 10.3390/ijms222112066]

78 **Zebda N**, Dubrovskyi O, Birukov KG. Focal adhesion kinase regulation of mechanotransduction and its impact on endothelial cell functions. *Microvasc Res* 2012; **83**: 71-81 [PMID: 21741394 DOI: 10.1016/j.mvr.2011.06.007]

79 **Chuang HH**, Zhen YY, Tsai YC, Chuang CH, Hsiao M, Huang MS, Yang CJ. FAK in Cancer: From Mechanisms to Therapeutic Strategies. *Int J Mol Sci* 2022; **23** [PMID: 35163650 DOI: 10.3390/ijms23031726]

80 **Crosas-Molist E**, Samain R, Kohlhammer L, Orgaz JL, George SL, Maiques O, Barcelo J, Sanz-Moreno V. Rho GTPase signaling in cancer progression and dissemination. *Physiol Rev* 2022; **102**: 455-510 [PMID: 34541899 DOI: 10.1152/physrev.00045.2020]

81 **Nguyen HL**, Man VH, Li MS, Derreumaux P, Wang J, Nguyen PH. Elastic moduli of normal and cancer cell membranes revealed by molecular dynamics simulations. *Phys Chem Chem Phys* 2022; **24**: 6225-6237 [PMID: 35229839 DOI: 10.1039/d1cp04836h]

82 **Uray IP**, Uray K. Mechanotransduction at the Plasma Membrane-Cytoskeleton Interface. *Int J Mol Sci* 2021; **22** [PMID: 34768998 DOI: 10.3390/ijms222111566]

83 **Li J**, Lv F, Jin T. Structuring and validating a prognostic model for low-grade gliomas based on the genes for plasma membrane tension. *Front Neurol* 2022; **13**: 1024869 [PMID: 36408514 DOI: 10.3389/fneur.2022.1024869]

84 **Haining AW**, Lieberthal TJ, Del Río Hernández A. Talin: a mechanosensitive molecule in health and disease. *FASEB J* 2016; **30**: 2073-2085 [PMID: 27252130 DOI: 10.1096/fj.201500080R]

85 **Yu JL**, Liao HY. Piezo-type mechanosensitive ion channel component 1 (Piezo1) in human cancer. *Biomed Pharmacother* 2021; **140**: 111692 [PMID: 34004511 DOI: 10.1016/j.biopha.2021.111692]

86 **Bejarano L**, Jordão MJC, Joyce JA. Therapeutic Targeting of the Tumor Microenvironment. *Cancer Discov* 2021; **11**: 933-959 [PMID: 33811125 DOI: 10.1158/2159-8290.CD-20-1808]

87 **Buwa N**, Mazumdar D, Balasubramanian N. Caveolin1 Tyrosine-14 Phosphorylation: Role in Cellular Responsiveness to Mechanical Cues. *J Membr Biol* 2020; **253**: 509-534 [PMID: 33089394 DOI: 10.1007/s00232-020-00143-0]

88 **Iqbal J**, Zaidi M. Molecular regulation of mechanotransduction. *Biochem Biophys Res Commun* 2005; **328**: 751-755 [PMID: 15694410 DOI: 10.1016/j.bbrc.2004.12.087]

89 **Hernández-Cáceres MP**, Munoz L, Pradenas JM, Pena F, Lagos P, Aceiton P, Owen GI, Morselli E, Criollo A, Ravasio A, Bertocchi C. Mechanobiology of Autophagy: The Unexplored Side of Cancer. *Front Oncol* 2021; **11**: 632956 [PMID: 33718218 DOI: 10.3389/fonc.2021.632956]

90 **Ravasio A**, Morselli E, Bertocchi C. Mechanoautophagy: Synergies Between Autophagy and Cell Mechanotransduction at Adhesive Complexes. *Front Cell Dev Biol* 2022; **10**: 917662 [PMID: 35721483 DOI: 10.3389/fcell.2022.917662]

91 **Levy JMM**, Towers CG, Thorburn A. Targeting autophagy in cancer. *Nat Rev Cancer* 2017; **17**: 528-542 [PMID: 28751651 DOI: 10.1038/nrc.2017.53]

92 **Abidine Y**, Constantinescu A, Laurent VM, Sundar Rajan V, Michel R, Laplaud V, Duperray A, Verdier C. Mechanosensitivity of Cancer Cells in Contact with Soft Substrates Using AFM. *Biophys J* 2018; **114**: 1165-1175 [PMID: 29539402 DOI: 10.1016/j.bpj.2018.01.005]

93 **Chen X**, Momin A, Wanggou S, Wang X, Min HK, Dou W, Gong Z, Chan J, Dong W, Fan JJ, Xiong Y, Talipova K, Zhao H, Chen YX, Veerasammy K, Fekete A, Kumar SA, Liu H, Yang Q, Son JE, Dou Z, Hu M, Pardis P, Juraschka K, Donovan LK, Zhang J, Ramaswamy V, Selvadurai HJ, Dirks PB, Taylor MD, Wang LY, Hui CC, Abzalimov R, He Y, Sun Y, Li X, Huang X. Mechanosensitive brain tumor cells construct blood-tumor barrier to mask chemosensitivity. *Neuron* 2023; **111**: 30-48.e14 [PMID: 36323321 DOI: 10.1016/j.neuron.2022.10.007]

94 **Kalli M**, Poskus MD, Stylianopoulos T, Zervantonakis IK. Beyond matrix stiffness: targeting force-induced cancer drug resistance. *Trends Cancer* 2023; **9**: 937-954 [PMID: 37558577 DOI: 10.1016/j.trecan.2023.07.006]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 November 24; 14(11): 535-543

DOI: 10.5306/wjco.v14.i11.535

ISSN 2218-4333 (online)

CASE REPORT

Autoimmune diabetes from pembrolizumab: A case report and review of literature

Hardikkumar Bhanderi, Farhan Khalid, Zubair Hassan Bodla, Tayyeb Muhammad, Doantrang Du, Trishala Meghal

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Wu QN, China; Zhang H, China

Received: July 13, 2023
Peer-review started: July 13, 2023
First decision: September 26, 2023
Revised: October 13, 2023
Accepted: October 30, 2023
Article in press: October 30, 2023
Published online: November 24, 2023

Hardikkumar Bhanderi, Farhan Khalid, Tayyeb Muhammad, Doantrang Du, Department of Internal Medicine, Monmouth Medical Center, Long branch, NJ 07740, United States

Zubair Hassan Bodla, Department of Internal Medicine, University of Central Florida College of Medicine, Gainesville, FL 32303, United States

Trishala Meghal, Department of Hematology-Oncology, Monmouth Medical Center, Long Branch, NJ 07740, United States

Corresponding author: Hardikkumar Bhanderi, MD, Staff Physician, Department of Internal Medicine, Monmouth Medical Center, 300 2nd Avenue, Long Branch, NJ 07740, United States. dr.hardikkumar.bhanderi@gmail.com

Abstract

BACKGROUND

Immunotherapy, specifically the use of checkpoint inhibitors such as pembrolizumab, has become an important tool in personalized cancer therapy. These inhibitors target proteins on T-cells that regulate the immune response against tumor cells. Pembrolizumab, which targets the programmed cell death 1 receptor on T-cells, has been approved for the treatment of metastatic melanoma and non-small cell lung cancer. However, it can also lead to immune-related side effects, including pneumonitis, colitis, thyroid abnormalities, and rare cases of type 1 diabetes.

CASE SUMMARY

The case presented involves an adult patient in 30s with breast cancer who developed hyperglycemia after receiving pembrolizumab treatment. The patient was diagnosed with diabetic ketoacidosis and further investigations were performed to evaluate for new-onset type 1 diabetes. The patient had a history of hypothyroidism and a family history of breast cancer. Treatment for diabetic ketoacidosis was initiated, and the patient was discharged for close follow-up with an endocrinologist.

CONCLUSION

This literature review highlights the occurrence of diabetic ketoacidosis and new-onset type 1 diabetes in patients receiving pembrolizumab treatment for different types of cancer. Overall, the article emphasizes the therapeutic benefits of immunotherapy in cancer treatment, particularly pembrolizumab, while also

highlighting the potential side effect of immune-related diabetes that can occur in a small percentage of patients. Here we present a case where pembrolizumab lead to development of diabetes after a few cycles highlighting one of the rare yet a serious toxicity of the drug.

Key Words: Pembrolizumab; Breast cancer; Autoimmune diabetes; Keytruda; Immunotherapy; Case report

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Our review highlights an important and rare adverse effect of Pembrolizumab. We have also reviewed the number of cycles patients were treated with Keytruda before the onset of diabetes. Clinicians should be watchful for the signs and symptoms. Early discontinuation of immunotherapy is needed to prevent significant morbidity and mortality.

Citation: Bhanderi H, Khalid F, Bodla ZH, Muhammad T, Du D, Meghal T. Autoimmune diabetes from pembrolizumab: A case report and review of literature. *World J Clin Oncol* 2023; 14(11): 535-543
URL: https://www.wjgnet.com/2218-4333/full/v14/i11/535.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i11.535

INTRODUCTION

Immunotherapy has become an essential tool in the treatment of cancers and represents therapeutic advancement in the individualized cancer therapy[1]. The role of immunotherapy is based on the ability to recognize abnormal tissue and enhance body's immune system against tumor cells. Immune system has both stimulators and inhibitors for the immune response generation in order to maintain balance and avoid auto-immune response to self antigens by means of positive selection of T cells. But sometimes this positive selection leads to lack of required immune response against tumor cells, which leads to tumor growth[2]. There are multiple check-points in cell production have been identified like T cell immunoglobulin and mucin-domain containing-3, T cell immunoglobulin and ITIM domain, lymphocyte activation gene-3, indoleamine 2, 3-dioxygenase 1, and V-domain immunoglobulin suppressor of T cell activation, but to date only United States Food and Drug Administration (FDA) approved check-point inhibitors are those which targets cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), or programmed cell death protein-ligand 1 (PD-L1)[3]. The mechanism behind is the inhibition of check point inhibitors namely CTLA-4, PD-1, or PD-L1 which results in the increased anti-tumor immune response. These check point inhibitors are expressed on T-cells and their activation leads to the decreased T-cell proliferation from inhibition of T-cell receptor mediated signaling, reduced cytokines secretion limiting inflammatory response and autoimmunity[4]. The immune check point inhibitors are the monoclonal antibodies directed against the above mentioned ligands which results in the immune activation against the tumor cells[5]. Pembrolizumab is a monoclonal antibody designed against check point inhibitor PD-1 receptor on surface of T-cells resulting in the proliferation of T-cells and enhanced intrinsic immune mediated anticancer activity[6]. PD-1 receptor is a cell surface protein expressed on activated T cells which on binding with the ligands PD-L1 and PD-L2 leads to the inhibition of kinase signaling pathways causing suppression of T-cell[7]. Pembrolizumab was originally approved by FDA for metastatic melanoma in 2014 and for non-small cell lung cancer in 2014[1]. Since then it has been widely used in the treatment of different cancers especially those with resistance to first line therapies. Excessive immune activation has been a frequent and serious side effect of the immune therapies. Most common adverse effects reported from the clinical trials are pneumonitis, colitis, thyroid abnormalities, liver and kidney issues[8]. Type 1 diabetes was only reported in 0.1% of the patients in the clinical trials making this rare but significant side effect of the treatment[1]. Here we present a case of a young female who presented with hyperglycemia after getting treatment with the pembrolizumab for the breast cancer.

CASE PRESENTATION

Chief complaints

Nausea, Vomiting and Hyperglycemia at outpatient chemotherapy infusion center.

History of present illness

The patient presented to the emergency department for the evaluation of hyperglycemia, which was found at the infusion center during 4th cycle. The patient complained of nausea, vomiting which was non-bilious and non-bloody associated with dizziness. The patient denied any fever, shortness of breath, chest pain, abdominal pain or loss of consciousness, recent weight loss, travel history, constipation or diarrhea.

History of past illness

Hypothyroidism and triple negative left invasive mammary breast carcinoma with Ki 67%-90% diagnosed an year ago which was at anatomical stage 2A/Clinical prognostic stage 2B status post chemotherapy with carboplatin and Paclitaxel along with 3 cycles of Pembrolizumab.

Personal and family history

The patient social history was significant for 2-3 cigarettes a day before getting diagnosed with breast cancer and occasional alcohol consumption and marijuana consumption. The family history was significant for breast cancer in mother and sister.

Physical examination

Physical examination was unremarkable.

Laboratory examinations

The initial blood work up revealed Hemoglobin level of 12.8 g/dL, white cell count of 4.7 K/CMM, Hematocrits of 37.2% and platelet count of 332 K/CMM. Complete metabolic panel was significant for sodium level of 133 mEq/L, Bicarbonate level of < 10 mEq/L with anion gap of > 19 mEq/L, blood glucose level of 343 mg/dL. Liver and Kidney functions were benign. Beta hydroxy butyrate level was found to be elevated > 46.8 mg/dL. Urinalysis was positive for glucose and ketones. Amylase and lipase level were within normal limits. Arterial blood gas analysis showed pH of 7.13 with pCO2 of 23 mmHg, pO2 of 65 mmHg, and bicarbonate level of 8 mmol/L. HbA1c level was found to be 6.8. During hospitalization, work up for the new onset type 1 diabetes mellitus (T1DM) was done. IA-2 antibody, Insulin antibody, glutamic acid decarboxylase antibody was negative. C-peptide level was found to be low at 0.24. Cortisol and Thyroid stimulating hormone level was within normal limits. So, involvements of other endocrine abnormalities were ruled out.

Imaging examinations

No imaging studies were done.

MULTIDISCIPLINARY EXPERT CONSULTATION

Endocrinologist was consulted because of labile glucose level and to optimize insulin regimen on discharge.

FINAL DIAGNOSIS

Diabetic ketoacidosis.

TREATMENT

Intensive care unit was consulted and the patient was managed as per protocol for diabetic ketoacidosis.

OUTCOME AND FOLLOW-UP

Patient was discharged for the close follow up with endocrinologist. Pembrolizumab was stopped and the chemotherapy was continued.

Our literature review mentions the prior studies highlighting the effects of pembrolizumab leading to autoimmune diabetes. The mean number of cycles was 4 and the mean number of weeks leading to presentation after the start of treatment was 15.4. The mean HbA1c of the patients was 7.97. Below mentioned are the baseline characteristics of the patients along with the disease presentation (Tables 1 and 2)[9-56].

DISCUSSION

This study presents a comprehensive literature review of similar cases that were reported on various databases. These patients were started on various chemotherapy regimens for different cancer, but after no or little improvement from those modalities, were eventually started on immunotherapy particularly pembrolizumab. These patients presented to the emergency department with various chief complaints including from asymptomatic hyperglycemia to diabetic ketoacidosis (DKA) and were eventually diagnosed with insulin dependent diabetes mellitus. The time of presentation for all these patients varied a lot in terms of range from after just one cycle to as long as 19 cycles with average beingcycles. This average number of cycles is skewed most probably, because the most number of patients developed this

Table 1 Baseline characteristics

Ref.	No. of patients	Age/Sex	Type of cancer	Time from first administration (wk)	No. of cycle
de Filette *et al*[9], 2019		61/M	NSCLC	8	2
de Filette *et al*[9], 2019	91	65	Melanoma/NSCLC		4.5
Clotman *et al*[10], 2018		73/F	Melanoma	8	2
Clotman *et al*[10], 2018	14	63		6	3
Farina *et al*[11], 2019	10	62	Melanoma/Lung cancer		5
Kyriacou *et al*[12], 2020		68/F	Lung cancer	7	2
Banatwalla *et al*[13], 2021		83/F	Melanoma	23	7
Hernandez *et al*[14], 2021		67/M	SCC tongue	3	1
Bansal *et al*[15], 2022		85/F	Lung adeno	9	3
Kedzior *et al*[16], 2021		51/F	Lung adeno	8	2
Cunha *et al*[17], 2022		59/F	Lung adeno	3	1
Kähler *et al*[18], 2020	5	74-85, 3F and 2M	Melanoma		4
Tohi *et al*[19], 2019		75/M	Urothelial CA	10	3
Edahiro *et al*[20], 2019		61/F	Lung adeno	25	8
Magis *et al*[21], 2018		41/F	Melanoma	57	19
Samoa *et al*[22], 2020		12/M	Hodgkin's lymphoma	15	5
Li *et al*[23], 2018		67/M	NSCLC	10	3
Boyle *et al*[24], 2019		56/M	Melanoma	22 months	
Boyle *et al*[24], 2019		74/F	Merkel cell cancer	23	7
Sankar *et al*[25], 2021		85/F	Bladder CA	9 months	
Hakami *et al*[26], 2019		52/M	Melanoma	21	7
Chaudry *et al*[27], 2020		75/M	NSCLC	12	4
Kotwal *et al*[28], 2019	11	61			4
Zand *et al*[29], 2022		81/F	Melanoma	26	8
Maamari *et al*[30], 2019		47/F	Cardiac angiosarcoma	6	1
Alrifai *et al*[31], 2019		69/M	NSCLC	15	4
Hong *et al*[32], 2020		76/M	Lung	11	3
Hong *et al*[32], 2020		78/F	Melanoma	4	1
Hong *et al*[32], 2020		65/F	Biliary CA	21	7
Skorpen *et al*[33], 2019		60s/M	Lung adeno	8	2
Martin-Liberal *et al*[34], 2015		54/F	Melanoma	9	3
Gaudy *et al*[35], 2015		44/F	Melanoma	8	2
Aleksova *et al*[36], 2016		61/M	Melanoma	6	1
M A *et al*[37], 2016		55/M	Melanoma	27	9
Hansen *et al*[38], 2016		58/M	Melanoma		17
Alhusseini *et al*[39], 2017		65/M	Lung adenocarcinoma	3	1
F A *et al*[40], 2017		48/F	Melanoma	2	1
Tay *et al*[41], 2017		74/F	Melanoma	3	1
Chae *et al*[42], 2017		76/M	Lung adenocarcinoma	1	1
Smith-Cohn *et al*[43], 2017		61/F	Cholangiocarcinoma	18	6
C M *et al*[44], 2017		58/M	Melanoma		4

Abayev *et al*[45], 2018	71/M	Melanoma	26	
Ioana *et al*[46], 2018	52/M	Melanoma	13	
Kalkan *et al*[47], 2018	73/F	NSCLC	9	3
Reslan *et al*[48], 2018	79/M	Melanoma	24	5
Fernandez *et al*[49], 2019	15/M	Soft tissue sarcoma	2	1
Sfeir *et al*[50], 2019	90/M	Melanoma		
Talib *et al*[51], 2019	67/F	Esophageal squamous cell CA	8	2
Gunjur *et al*[52], 2019	77/F	Melanoma	3 Days	1
Singh *et al*[53], 2019	70/M	Melanoma	10	3
Akopyan *et al*[54], 2020	66/F	Urothelial CA	6 months	
Zagouras *et al*[55], 2020	52/M	Lung adenocarcinoma	9	3
Kethireddy *et al*[56], 2021	85/M		9	3

NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma.

diabetic complication earlier rather than later in the course of starting immunotherapy. This observation is also supported by a relatively lower value of glycated hemoglobin value as compared to classic type 1 diabetic patients who develop diabetic ketoacidosis[57]. At the same time, a diagnosis of T1DM was established by presence of one or the other classic antibodies in most of the patients. Among patients who were tested for these antibodies, many of them were positive for anti-glutamic acid decarboxylase antibodiesand some of them were positive for others like islet cell antibodies or insulin antigen 2 antibodies. This conclusion is based on the data from the patients who were tested for these antibodies. To some extent this data suggest that presence of these antibodies is lower in these patients as compared to patients with classic T1DM, where a presence of at least one antibody in 97.8%[58]. On reviewing the literature it was found that incidence of newly diagnosed diabetic ketoacidosis is more in patients receiving pembrolizumab dose of 400 mg every 6 wk as compared to conventional 200 mg every 3 wk[18]. These patients are more prone to develop other endocrinopathies as well particularly thyroid related issues along with diabetes[59]. The pathophysiology of these immune checkpoint inhibitors induced diabetes mellitus is still not clear. Human leukocyte antigen is the key structure involved in the presentation of different peptides, one of which might be containing "diabetogenic peptide" in genetically susceptible individuals[60]. Recognition of this complex by T cell receptor stimulates cytotoxic T-cells that lead to destruction of B-cells in pancreas. Alternatively, these auto-antigenic peptides gets presented to the regulatory T cells, stimulation of which leads to secretion of different kind of cytokines like Interleukin 1, Interleukin 2, Interferon gamma, Tumor necrosis factor alpha and beta. These cytokines in turn stimulate cytotoxic T cells and eventual destruction of B cells ensues. To avoid this phenomenon, interaction between PD-1 and its PD-L1 is really important to maintain self tolerance against pancreatic islets[9]. Different Immune checkpoint inhibitors affect different pathways. Pembrolizumab in particular inhibits the PD-1/PD-L1 pathway, which leads to destruction of pancreatic islet cells and development of T1DM.

The predisposing factors in an individual for development of immune checkpoint inhibitors induced diabetes is not well defined as opposed to individuals with classic T1DM. Individuals with certain genotypes like DR3-DQ2 and DR4-DQ8 have shown to have higher risk of developing classic T1DM as compared to the general population[61]. In our study, we have not included genotypes of patients as there was not much data available regarding that in most cases, but studies particularly focusing on these aspects have shown that individuals with high risk genotypes have developed diabetes more while being on immune checkpoint inhibitors as compared individuals with other genotypes[9]. So, these individuals were at a high risk, but rapid onset of diabetes with presentation of ketoacidosis and relatively a low glycated hemoglobin value as compared to classic T1DM makes it different. In our study, some of the patients also had history of autoimmune disease, which makes them more susceptible to develop other autoimmune disease. Patients with already diagnosed and well controlled type 2 diabetes are also shown to be at high risk of worsening diabetes and presenting with diabetic ketoacidosis along with blood work showing presence of autoantibodies.

Although this is one of the rare side effects of the immunotherapies but with development of new immunotherapy agents, these cases should be kept in mind particularly while giving therapies to patients with high risk factors. Initial check for glycated haemoglobin before starting therapy for both diagnosed and undiagnosed diabetic patients can be useful for the risk stratification. A regular and timely checkup for glucose along with education for signs and symptoms of hyperglycemia should be introduced in patients receiving these agents. This could lead to detection of early development or worsening diabetes. Another significant finding in most of the patients was continuation of immuno-therapy after initial management of diabetic ketoacidosis was possible with introduction of as needed long and short acting insulin regimen. This is not the best option, but stopping immunotherapy in advanced malignancies, where very few treatment options are available is not desirable. The prognosis particularly because of the development of these endocrinopathies did not seem to change in most of the patients.

Table 2 Diabetes characteristics

Ref.	Presentation	HbA1C	C-peptide	Auto Ab	Outcome
de Filette *et al*[9]	DKA		0.02 nmol/L	GADA	Not known
de Filette *et al*[9]	71% DKA	7.6	Low in 84%	51% GADA 18% IA213% ICA26% Anti-Insulin	Not known
Clotman *et al*[10]	DKA	7.1	Low	GADA, ICA	Stayed on insulin
Clotman *et al*[10]	70% DKA	7.5	Low in 93%	56% GADA	
Farina *et al*[11]	69% DKA	7.76	0.1	50% GADA+	97% remained on Insulin therapy
Kyriacou *et al*[12]	DKA	7	Low	GADA+	Stayed on insulin
Banatwalla *et al*[13]	DKA	8.2	0.09	All neg	Stayed on insulin
Hernandez *et al*[14]	DKA	6.9			Stayed on insulin
Bansal *et al*[15]	HHS	8.3	Normal	GADA +	Stayed on insulin
Kedzior *et al*[16]	DKA	8.3	Undetected	GADA+	
Cunha *et al*[17]	DKA	5.6	Undetected	GADA+	Stayed on insulin
Kähler *et al*[18]	DKA	9.7, 6.5, 7.5, No data for other 2	Low in 1	GADA+ in 2	
Tohi *et al*[19]	DKA	6.7	Undetected	GADA negative	Stayed on insulin
Edahiro *et al*[20]	DKA	8.4	Low	GADA negative	Stayed on insuline
Magis *et al*[21]	DKA	6.8	< 0.003	GADA negative,IA-2 Positive	Stayed on insulin
Samoa *et al*[22]	DKA	8.9, Intial was 6.0	Low	GADA negative,IA-2 PositiveIA Positive	Stayed on insulin
Li *et al*[23]	DKA	8	Low	All ab negative	Stayed on insuline
Boyle *et al*[24]	DKA	7.4	Low	All ab negative	Stayed on insulin
Boyle *et al*[24]	DKA		Low	All ab negative	
Sankar *et al*[25]	DKA	6.8		All ab negative	Stayed on insulin
Hakami *et al*[26]	DKA	8.3	< 0.001	All ab negative	Stayed on insulin
Chaudry *et al*[27]	DKA			GADA +	Stayed on insulin
Kotwal *et al*[28]	8 DKA,1 HHS,1 Ketosis,1 Hyperglycemia	9.7	5/6 Low	4/7 GADA+, 1/7 IAA+, 1/7 IA2A+	Stayed on insulin
Zand *et al*[29]	DKA	8.9		All ab negative	Stayed on insulin
Maamari *et al*[30]	DKA	6.4	Low	GADA+	Stayed on insulin
Alrifai *et al*[31]	DKA	9.2	Low	GADA+	Stayed on insulin
Hong *et al*[32]	DKA	10.4	Low	All ab negative	Stayed on insulin
Hong *et al*[32]	DKA	11.4	Low	All ab negative	Stayed on insulin
Hong *et al*[32]	DKA	5.8	Low	All ab negative	Stayed on insulin
Skorpen *et al*[33]	DKA	8.4	Undetected	All ab negative	Stayed on insulin
Martin-Liberal *et al* [34]	DKA			GADA+	Stayed on insulin
Gaudy *et al*[35]	DKA	6.85	Undetected	All ab negative	Stayed on insulin
Aleksova *et al*[36]	DKA		Low	All ab negative	Stayed on insulin
M A *et al*[37]	DKA	10.7		All ab negative	Stayed on insulin
Hansen *et al*[38]	Simple T1DM	7.1	Low	GADA+	Dced insulin
Alhusseini *et al*[39]	DKA	8.5	Undectable	GADA+, ICA+	Stayed on insulin
F A *et al*[40]	DKA	8	Undectable	GADA+, IA+	Stayed on insulin

Tay *et al*[41]	DKA	9.3	Undectable	All ab Negative	Stayed on insulin
Chae *et al*[42]	DKA	5.8	Low	GADA+, ICA+	Stayed on insulin
Smith-Cohn *et al*[43]	DKA	8.7		GADA+	Stayed on insulin
C M *et al*[44]	DKA	7.4	Undetectable	All ab Negative	
Abayev *et al*[45]	DKA	11.8	Normal	All ab Negative	Stayed on insulin
Ioana *et al*[46]	DKA	8.3	Undetectable	All ab Negative	
Kalkan *et al*[47]	DKA		Low	All ab Negative	
Reslan *et al*[48]	DKA	7.5			Stayed on insulin
Fernandez *et al*[49]	DKA	5.5	Low	GADA+	
Sfeir *et al*[50]	DKA	10.2	Low	All ab negative	Stayed on insulin
Talib *et al*[51]	DKA	7.9	Low	GADA+	
Gunjur *et al*[52]	DKA	6.9	Low	GADA+, ICA+	Stayed on insulin
Singh *et al*[53]	DKA			GADA+	Stayed on insulin
Akopyan *et al*[54]	DKA			All ab negative	Stayed on insulin
Zagouras *et al*[55]	Hyperglycemia	5.7	Low	GADA+	Stayed on insulin
Kethireddy *et al*[56]	T1DM	9		GADA+	Stayed on insulin

NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma; DKA: Diabetic ketoacidosis; GADA: Glutamic acid decarboxylase antibody; IA-2: Islet antibody; ICA: Islet cell antibodies; IAA: Insulin autoantibodies; ab: Antibodies.

CONCLUSION

In the end, there is a need for a lot of research in this particular aspect regarding recognition of high risk individuals for developing these rare side effects, which might eventually help patients to avoid these side effects. Identifying different biomarkers apart from classic autoantibodies can also help in early detection of diabetes. More studies are needed to find out exact pathophysiology behind this side effect which is also the need of the hour.

FOOTNOTES

Author contributions: Bhanderi H did conceptualization and did literature review; Khalid F wrote case presentation; Bodla ZH and Muhammad T helped in discussion; Du D and Meghal T helped in final editing.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: Authors does not have any conflict of interest.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: United States

ORCID number: Hardikkumar Bhanderi 0000-0002-7939-0958.

S-Editor: Liu JH
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **Abdel-Wahab N**, Shah M, Suarez-Almazor ME. Adverse Events Associated with Immune Checkpoint Blockade in Patients with Cancer: A Systematic Review of Case Reports. *PLoS One* 2016; **11**: e0160221 [PMID: 27472273 DOI: 10.1371/journal.pone.0160221]

2 **Wang Y**, Zhang H, Liu C, Wang Z, Wu W, Zhang N, Zhang L, Hu J, Luo P, Zhang J, Liu Z, Peng Y, Tang L, Cheng Q. Immune checkpoint modulators in cancer immunotherapy: recent advances and emerging concepts. *J Hematol Oncol* 2022; **15**: 111 [PMID: 35978433 DOI: 10.1186/s13045-022-01325-0]

3 **Zhang H**, Dai Z, Wu W, Wang Z, Zhang N, Zhang L, Zeng WJ, Liu Z, Cheng Q. Regulatory mechanisms of immune checkpoints PD-L1 and CTLA-4 in cancer. *J Exp Clin Cancer Res* 2021; **40**: 184 [PMID: 34088360 DOI: 10.1186/s13046-021-01987-7]

4 **Pardoll DM**. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12**: 252-264 [PMID: 22437870 DOI: 10.1038/nrc3239]

5 **Guleria I**, Gubbels Bupp M, Dada S, Fife B, Tang Q, Ansari MJ, Trikudanathan S, Vadivel N, Fiorina P, Yagita H, Azuma M, Atkinson M, Bluestone JA, Sayegh MH. Mechanisms of PDL1-mediated regulation of autoimmune diabetes. *Clin Immunol* 2007; **125**: 16-25 [PMID: 17627890 DOI: 10.1016/j.clim.2007.05.013]

6 **Faghfuri E**, Faramarzi MA, Nikfar S, Abdollahi M. Nivolumab and pembrolizumab as immune-modulating monoclonal antibodies targeting the PD-1 receptor to treat melanoma. *Expert Rev Anticancer Ther* 2015; **15**: 981-993 [PMID: 26313415 DOI: 10.1586/14737140.2015.1074862]

7 **Godwin JL**, Jaggi S, Sirisena I, Sharda P, Rao AD, Mehra R, Veloski C. Nivolumab-induced autoimmune diabetes mellitus presenting as diabetic ketoacidosis in a patient with metastatic lung cancer. *J Immunother Cancer* 2017; **5**: 40 [PMID: 28515940 DOI: 10.1186/s40425-017-0245-2]

8 **Chan MM**, Kefford RF, Carlino M, Clements A, Manolios N. Arthritis and tenosynovitis associated with the anti-PD1 antibody pembrolizumab in metastatic melanoma. *J Immunother* 2015; **38**: 37-39 [PMID: 25415286 DOI: 10.1097/CJI.0000000000000060]

9 **de Filette JMK**, Pen JJ, Decoster L, Vissers T, Bravenboer B, Van der Auwera BJ, Gorus FK, Roep BO, Aspeslagh S, Neyns B, Velkeniers B, Kharagjitsingh AV. Immune checkpoint inhibitors and type 1 diabetes mellitus: a case report and systematic review. *Eur J Endocrinol* 2019; **181**: 363-374 [PMID: 31330498 DOI: 10.1530/EJE-19-0291]

10 **Clotman K**, Janssens K, Specenier P, Weets I, De Block CEM. Programmed Cell Death-1 Inhibitor-Induced Type 1 Diabetes Mellitus. *J Clin Endocrinol Metab* 2018; **103**: 3144-3154 [PMID: 29955867 DOI: 10.1210/jc.2018-00728]

11 **Farina KA**, Kane MP. Programmed Cell Death-1 Monoclonal Antibody Therapy and Type 1 Diabetes Mellitus: A Review of the Literature. *J Pharm Pract* 2021; **34**: 133-140 [PMID: 31269868 DOI: 10.1177/0897190019850929]

12 **Kyriacou A**, Melson E, Chen W, Kempegowda P. Is immune checkpoint inhibitor-associated diabetes the same as fulminant type 1 diabetes mellitus? *Clin Med (Lond)* 2020; **20**: 417-423 [PMID: 32675150 DOI: 10.7861/clinmed.2020-0054]

13 **Banatwalla R**, Kirresh OZ, Ahmed FW. Pembrolizumab-induced diabetes. *Endokrynol Pol* 2021; **72**: 414-415 [PMID: 34057191 DOI: 10.5603/EP.a2021.0053]

14 **Hernandez A**, Zeidan B, Desai P, Frunzi J. Diabetic Ketoacidosis Secondary to New Onset Type 1 Diabetes Mellitus Related to Pembrolizumab Therapy. *Cureus* 2021; **13**: e13302 [DOI: 10.7759/cureus.13302]

15 **Bansal S**, Saca D, Getz D, Rab A, Singhania N. New-Onset Type 1 Diabetes in an Elderly Female on Pembrolizumab. *Am J Med Sci* 2022; **363**: 371-372 [PMID: 34995577 DOI: 10.1016/j.amjms.2021.10.024]

16 **Kedzior SK**, Jacknin G, Hudler A, Mueller SW, Kiser TH. A Severe Case of Diabetic Ketoacidosis and New-Onset Type 1 Diabetes Mellitus Associated with Anti-Glutamic Acid Decarboxylase Antibodies Following Immunotherapy with Pembrolizumab. *Am J Case Rep* 2021; **22**: e931702 [PMID: 34185763 DOI: 10.12659/AJCR.931702]

17 **Cunha C**, Silva E, Vieira AC, Saraiva C, Duarte S. New onset autoimmune diabetes mellitus and hypothyroidism secondary to pembrolizumab in a patient with metastatic lung cancer. *Endocrinol Diabetes Metab Case Rep* 2022; **2022** [PMID: 35140188 DOI: 10.1530/EDM-21-0123]

18 **Kähler KC**, Kosova K, Bohne AS, Schreiber S, Hauschild A. Increased risk of immune checkpoint inhibitor-induced type 1 diabetes mellitus with the new approved 6-week scheme of pembrolizumab in patients with melanoma? *Eur J Cancer* 2020; **138**: 169-171 [PMID: 32890812 DOI: 10.1016/j.ejca.2020.07.028]

19 **Tohi Y**, Fujimoto K, Suzuki R, Suzuki I, Kubota M, Kawakita M. Fulminant type 1 diabetes mellitus induced by pembrolizumab in a patient with urothelial carcinoma: A case report. *Urol Case Rep* 2019; **24**: 100849 [PMID: 31211060 DOI: 10.1016/j.eucr.2019.100849]

20 **Edahiro R**, Ishijima M, Kurebe H, Nishida K, Uenami T, Kanazu M, Akazawa Y, Yano Y, Mori M. Continued administration of pembrolizumab for adenocarcinoma of the lung after the onset of fulminant type 1 diabetes mellitus as an immune-related adverse effect: A case report. *Thorac Cancer* 2019; **10**: 1276-1279 [PMID: 30964601 DOI: 10.1111/1759-7714.13065]

21 **Magis Q**, Gaudy-Marqueste C, Basire A, Loundou A, Malissen N, Troin L, Monestier S, Mallet S, Hesse S, Richard MA, Valéro R, Beliard S, Grob JJ. Diabetes and Blood Glucose Disorders Under Anti-PD1. *J Immunother* 2018; **41**: 232-240 [PMID: 29481423 DOI: 10.1097/CJI.0000000000000218]

22 **Samoa RA**, Lee HS, Kil SH, Roep BO. Anti-PD-1 Therapy-Associated Type 1 Diabetes in a Pediatric Patient With Relapsed Classical Hodgkin Lymphoma. *Diabetes Care* 2020; **43**: 2293-2295 [PMID: 32616607 DOI: 10.2337/dc20-0740]

23 **Li S**, Zhang Y, Sun Z, Hu J, Fang C. Anti-PD-1 pembrolizumab induced autoimmune diabetes in Chinese patient: A case report. *Medicine (Baltimore)* 2018; **97**: e12907 [PMID: 30407284 DOI: 10.1097/MD.0000000000012907]

24 **Boyle V**, Cundy T, Cutfield R. Rapid onset type 1 diabetes associated with the programmed cell death-1 inhibitor pembrolizumab. *Intern Med J* 2019; **49**: 930-931 [PMID: 31295781 DOI: 10.1111/imj.14340]

25 **Sankar K**, Macfarlane M, Cooper O, Falk J. Pembrolizumab-Induced Diabetic Ketoacidosis: A Review of Critical Care Case. *Cureus* 2021; **13**: e18983 [DOI: 10.7759/cureus.18983]

26 **Hakami OA**, Ioana J, Ahmad S, Tun TK, Sreenan S, McDermott JH. A case of pembrolizumab-induced severe DKA and hypothyroidism in a patient with metastatic melanoma. *Endocrinol Diabetes Metab Case Rep* 2019; **2019** [PMID: 30836329 DOI: 10.1530/EDM-18-0153]

27 **Chaudry A**, Chaudry M, Aslam J. Pembrolizumab: An Immunotherapeutic Agent Causing Endocrinopathies. *Cureus* 2020; **12**: e8836 [DOI: 10.7759/cureus.8836]

28 **Kotwal A**, Haddox C, Block M, Kudva YC. Immune checkpoint inhibitors: an emerging cause of insulin-dependent diabetes. *BMJ Open Diabetes Res Care* 2019; **7**: e000591 [PMID: 30899528 DOI: 10.1136/bmjdrc-2018-000591]

29 **Zand Irani A**, Almuwais A, Gibbons H. Immune checkpoint inhibitor-induced diabetes mellitus with pembrolizumab. *BMJ Case Rep* 2022; **15** [PMID: 35039353 DOI: 10.1136/bcr-2021-245846]

30 **Maamari J**, Yeung SJ, Chaftari PS. Diabetic ketoacidosis induced by a single dose of pembrolizumab. *Am J Emerg Med* 2019; **37**: 376.e1-376.e2 [PMID: 30361152 DOI: 10.1016/j.ajem.2018.10.040]

31 **Alrifai T**, Ali FS, Saleem S, Ruiz DCM, Rifai D, Younas S, Qureshi F. Immune Checkpoint Inhibitor Induced Diabetes Mellitus Treated with Insulin and Metformin: Evolution of Diabetes Management in the Era of Immunotherapy. *Case Rep Oncol Med* 2019; **2019**: 8781347 [PMID: 31781446 DOI: 10.1155/2019/8781347]

32 **Hong AR**, Yoon JH, Kim HK, Kang HC. Immune Checkpoint Inhibitor-Induced Diabetic Ketoacidosis: A Report of Four Cases and Literature Review. *Front Endocrinol (Lausanne)* 2020; **11**: 14 [PMID: 32047478 DOI: 10.3389/fendo.2020.00014]

33 **Skorpen PK**, Margull J. Diabetic ketoacidosis following immunotherapy for lung cancer. *Tidsskr Nor Laegeforen* 2019; **139** [PMID: 30808100 DOI: 10.4045/tidsskr.18.0597]

34 **Martin-Liberal J**, Furness AJ, Joshi K, Peggs KS, Quezada SA, Larkin J. Anti-programmed cell death-1 therapy and insulin-dependent diabetes: a case report. *Cancer Immunol Immunother* 2015; **64**: 765-767 [PMID: 25828465 DOI: 10.1007/s00262-015-1689-1]

35 **Gaudy C**, Clévy C, Monestier S, Dubois N, Préau Y, Mallet S, Richard MA, Grob JJ, Valéro R, Béliard S. Anti-PD1 Pembrolizumab Can Induce Exceptional Fulminant Type 1 Diabetes. *Diabetes Care* 2015; **38**: e182-e183 [PMID: 26310693 DOI: 10.2337/dc15-1331]

36 Abstracts of the Endocrine Society of Australia Annual Scientific Meeting 2015, 23-26 August 2015. *Clin Endocrinol (Oxf)* 2016; **84** Suppl 1: 1-49 [PMID: 26935124 DOI: 10.1111/cen.13010]

37 **Humayun MA**, Poole R. A case of multiple immune toxicities from Ipilimumab and pembrolizumab treatment. *Hormones (Athens)* 2016; **15**: 303-306 [PMID: 27376434 DOI: 10.14310/horm.2002.1656]

38 **Hansen E**, Sahasrabudhe D, Sievert L. A case report of insulin-dependent diabetes as immune-related toxicity of pembrolizumab: presentation, management and outcome. *Cancer Immunol Immunother* 2016; **65**: 765-767 [PMID: 27067877 DOI: 10.1007/s00262-016-1835-4]

39 **Alhusseini M**, Samantray J. Autoimmune diabetes superimposed on type 2 diabetes in a patient initiated on immunotherapy for lung cancer. *Diabetes Metab* 2017; **43**: 86-88 [PMID: 27291328 DOI: 10.1016/j.diabet.2016.05.007]

40 **Abdul Aziz MHariz F**, Fernando IP, Lenkanpally A, Fernando DJS. Diabetic ketoacidosis after treatment with pembrolizumab. *J Clin Transl Endocrinol Case Rep* 2017; **5**: 4-5 [DOI: 10.1016/j.jecr.2017.05.002]

41 **Tay WL**, Loh WJ, Kek PC. A case of pembrolizumab-induced type 1 diabetes mellitus presenting with severe diabetic ketoacidosis. *J Clin Transl Endocrinol Case Rep* 2017; **6**: 9-10 [DOI: 10.1016/j.jecr.2017.07.002]

42 **Chae YK**, Chiec L, Mohindra N, Gentzler R, Patel J, Giles F. A case of pembrolizumab-induced type-1 diabetes mellitus and discussion of immune checkpoint inhibitor-induced type 1 diabetes. *Cancer Immunol Immunother* 2017; **66**: 25-32 [PMID: 27761609 DOI: 10.1007/s00262-016-1913-7]

43 **Smith-Cohn MA**, Gill D, Voorhies BN, Agarwal N, Garrido-Laguna I. Case report: pembrolizumab-induced Type 1 diabetes in a patient with metastatic cholangiocarcinoma. *Immunotherapy* 2017; **9**: 797-804 [PMID: 28877632 DOI: 10.2217/imt-2017-0042]

44 **Mizab Mellah C**, Sánchez Pérez M, Santos Rey MD, Hernández García M. Fulminant type 1 diabetes mellitus associated with pembrolizumab. *Endocrinol Diabetes Nutr* 2017; **64**: 272-273 [PMID: 29056250 DOI: 10.1016/j.endinu.2017.01.005]

45 **Abayev J**, Dhawan N, Jetty V, Khan A, Goldenberg N. Abstract #100 Atypical Presentation of Congenitial Adrenal Hyperplasia. *Endocr Pract* 2018; **24**: 1 [DOI: 10.4158/1530-891X-24.s1.1]

46 Irish Endocrine Society 42nd Annual Meeting, 19th and 20th October 2018. *Ir J Med Sci* 2018; **187**: 173-226 [PMID: 30120710 DOI: 10.1007/s11845-018-1877-z]

47 **Ryder M**, Randolph G. 88(th) Annual Meeting of the American Thyroid Association October 3-7, 2018 Marriott Marquis, Washington, DC. *Thyroid* 2018; **28**: 1227-1228 [PMID: 30141375 DOI: 10.1089/thy.2018.0524]

48 **Reslan Z**, Bennett M, Wong E, Jacques S, Lee A. Pembrolizumab-induced auto-immune type-1 diabetes in a patient with metastatic melanoma. *J Pharm Pract Res* 2018; **48**: 262-264 [DOI: 10.1002/jppr.1329]

49 9 2019 ASPHO ABSTRACTS. Pediatr Blood Cancer. 2019; 66: e27713 [DOI: 10.1002/pbc.27713]

50 Paper Abstract. *J Am GeriatrSoc* 2019; **67**: S1-384 [DOI: 10.1111/jgs.15898]

51 **Talib U**, ALpert A, Mena J, Matos A, Nagus I, Sycheva T, Blaski C. Pembrolizumab-induced type 1 diabetes presenting as diabetic ketoacidosis. *Chest* 2019; **156**: A2146-A2147

52 **Gunjur A**, Klein O, Kee D, Cebon J. Anti-programmed cell death protein 1 (anti-PD1) immunotherapy induced autoimmune polyendocrine syndrome type II (APS-2): a case report and review of the literature. *J Immunother Cancer* 2019; **7**: 241 [PMID: 31488221 DOI: 10.1186/s40425-019-0713-y]

53 **Singh NR**. Pembrolizumab: a case of drug-induced autoimmune diabetes mellitus and colitis. *J Pharm Pract Res* 2019; **49**: 50-54 [DOI: 10.1002/jppr.1436]

54 **Akopyan K**, Siddiqui A, Otekeiwebia A, Kroker-Bode C. Finding the culprit: a case of new onset type i diabetes mellitus (DM) and severe diabetic ketoacidosis (DKA) after the initiation of pembrolizumab. *Chest* 2020; **158**: A869 [DOI: 10.1016/j.chest.2020.08.807]

55 **Zagouras A**, Patil PD, Yogi-Morren D, Pennell NA. Cases from the Immune-Related Adverse Event Tumor Board: Diagnosis and Management of Immune Checkpoint Blockade-Induced Diabetes. *Oncologist* 2020; **25**: 921-924 [PMID: 32564463 DOI: 10.1634/theoncologist.2019-0806]

56 **Kethireddy N**, Thomas S, Bindal P, Shukla P, Hegde U. Multiple autoimmune side effects of immune checkpoint inhibitors in a patient with metastatic melanoma receiving pembrolizumab. *J Oncol Pharm Pract* 2021; **27**: 207-211 [PMID: 32390537 DOI: 10.1177/1078155220921543]

57 **Lamsal R**, Klyachman L, Adeyinka A, Kondamudi N, Pierre L. Is HbA1c an Indicator of Diabetic Ketoacidosis Severity in the Pediatric Population? *Pediatr Emerg Care* 2023; **39**: 216-218 [PMID: 36727771 DOI: 10.1097/PEC.0000000000002859]

58 **Basu M**, Pandit K, Banerjee M, Mondal SA, Mukhopadhyay P, Ghosh S. Profile of Auto-antibodies (Disease Related and Other) in Children with Type 1 Diabetes. *Indian J Endocrinol Metab* 2020; **24**: 256-259 [PMID: 33083265 DOI: 10.4103/ijem.IJEM_63_20]

59 **Paepegaey AC**, Lheure C, Ratour C, Lethielleux G, Clerc J, Bertherat J, Kramkimel N, Groussin L. Polyendocrinopathy Resulting From Pembrolizumab in a Patient With a Malignant Melanoma. *J Endocr Soc* 2017; **1**: 646-649 [PMID: 29264517 DOI: 10.1210/js.2017-00170]

60 **Abel M**, Krokowski M. Pathophysiology of immune-mediated (type 1) diabetes mellitus: potential for immunotherapy. *BioDrugs* 2001; **15**: 291-301 [PMID: 11437693 DOI: 10.2165/00063030-200115050-00002]

61 **Steck AK**, Rewers MJ. Genetics of type 1 diabetes. *Clin Chem* 2011; **57**: 176-185 [PMID: 21205883 DOI: 10.1373/clinchem.2010.148221]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 November 24; 14(11): 544-548

DOI: 10.5306/wjco.v14.i11.544

ISSN 2218-4333 (online)

CASE REPORT

Calcitriol induced hypercalcemia - a rare phenomenon in lung cancer: A case report

Amulya Prakash, Farhan Khalid, Ahmad Alalwan, Husam Bader, Doantrang Du, Trishala Meghal

Specialty type: Medicine, research and experimental

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Haddadi S, Algeria; Musetti C, Italy

Received: July 26, 2023
Peer-review started: July 26, 2023
First decision: September 4, 2023
Revised: September 20, 2023
Accepted: October 23, 2023
Article in press: October 23, 2023
Published online: November 24, 2023

Amulya Prakash, Department of Internal Medicine, Haywood Regional Medical Center, Clyde, NC 28721, United States

Amulya Prakash, Farhan Khalid, Ahmad Alalwan, Husam Bader, Doantrang Du, Department of Internal Medicine, Monmouth Medical Center, Long Branch, NJ 07740, United States

Trishala Meghal, Department of Hematology-Oncology, Monmouth Medical Center, Long Branch, NJ 07740, United States

Corresponding author: Farhan Khalid, MD, Chief Physician, Department of Internal Medicine, Monmouth Medical Center, 200 Second Avenue, Long Branch, NJ 07740, United States. farhan.khalid.md@gmail.com

Abstract

BACKGROUND

Calcitriol-induced hypercalcemia has been rarely reported in cases of lung cancer; however, it is frequently reported in cases of lymphoid malignancy and granulomatous disease. We present a rare case of hypercalcemia associated with squamous cell cancer of the lung with elevated calcitriol level.

CASE SUMMARY

A 61-year-old Caucasian female with severe hypercalcemia of 15 mg/dL, which led to a new diagnosis of metastatic lung cancer. Since the parathyroid hormone-related peptide (PTHrP) level was minimally elevated at 2.1 pmol/L, we believe excessive calcitriol production by tumor cells was the underlying mechanism for hypercalcemia. Calcitriol was significantly elevated at 130 pg/mL with a low 25-hydroxyvitamin D level of 25.9 ng/mL and suppressed PTH level of 8 pg/mL. Corticosteroids are generally used to treat calcitriol-induced hypercalcemia, but we successfully treated our patient with bisphosphonate, highlighting the further utility of bisphosphonates in hypercalcemia treatment.

CONCLUSION

We believe that the underlying cause of hypercalcemia, in this case of metastatic squamous cell lung carcinoma, was elevated calcitriol, which was likely produced by the tumor cells. In addition to PTHrP, calcitriol levels should be included in the workup for hypercalcemia in cases of lung cancer. However, the pathophysiology and prognostic significance of dysregulated calcitriol production in solid tumors remain unclear and warrant further research. Bisphosphonate may be used as a steroid-sparing therapy even in cases of calcitriol-induced hypercalcemia and

warrants further investigation.

Key Words: Hypercalcemia associated malignancy; Lung cancer; Denosumab; Calcitriol; Vitamin D; Case report

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Our case report illuminates a rare mechanism of hypercalcemia in lung malignancies, characterized by elevated calcitriol. Despite its rarity, it sheds light on the pathophysiology of hypercalcemia in solid malignancies, notably lung cancers. To the best of our knowledge, this is the first documented case in medical literature to present this mechanism. Moreover, the successful management of this condition with bisphosphonates highlights the potential efficacy of this treatment approach for future cases involving similar symptoms. By emphasizing this novel observation, our report contributes to the expanding body of knowledge regarding hypercalcemia in lung cancers and paves the way for the development of novel therapeutic strategies for the treatment of such cases.

Citation: Prakash A, Khalid F, Alalwan A, Bader H, Du D, Meghal T. Calcitriol induced hypercalcemia - a rare phenomenon in lung cancer: A case report. *World J Clin Oncol* 2023; 14(11): 544-548
URL: https://www.wjgnet.com/2218-4333/full/v14/i11/544.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i11.544

INTRODUCTION

Hypercalcemia associated with malignancy (HAM) is a common clinical finding and may even present as an oncologic emergency. It has been found in up to 30% of cases of malignancy[1]. The estimated yearly prevalence of hypercalcemia for all cancers is 1.46% to 2.74%[2]. Calcitriol overproduction is a rare etiology of HAM and accounts for merely 1% of cases of HAM[3]. It has been frequently reported in cases of Hodgkin and non-Hodgkin lymphoma and also in some cases of ovarian dysgerminoma, pancreatic neuroendocrine tumors, seminomas, and renal cell carcinoma[4-6]. In our extensive literature search, we came across just one case report of squamous cell lung cancer by Akai *et al*[7], where calcitriol overproduction was exclusively responsible for hypercalcemia and treated with tumor resection. We present a rare case of squamous cell lung carcinoma with hypercalcemia and elevated calcitriol levels, which was treated successfully with bisphosphonate. To the best of our knowledge, bisphosphonates have never been reported for the treatment of calcitriol-induced hypercalcemia in the case of squamous cell lung carcinoma.

CASE PRESENTATION

Chief complaints

Hypercalcemia on routine blood work investigation.

History of present illness

The patient didn't complain of any significant symptoms at the time of presentation. However, during detailed history taking, she reported vague complaints of nausea, fatigue, and generalized weakness but denied any other symptoms like constipation, palpitation, confusion, *etc.*

History of past illness

She has a past medical history of diabetes, hyperlipidemia, and depression.

Personal and family history

The patient has a remote history of tobacco smoking more than 15 years ago. She denies any supplementation with vitamin A, vitamin D, or calcium, frequent use of antacids, or excessive consumption of dairy products. Her current medications include atorvastatin and sertraline. Patient denies any significant family history.

Physical examination

Her vital signs and physical exam were within normal limits.

Laboratory examinations

Three months prior to the presentation, her calcium level was noted to be within the normal range of 10.1 (range: 8.6-10.3 mg/dL). In a routine lab work performed one week before the presentation, her corrected serum calcium was noted to be elevated first time at 13 mg/dL. She was asymptomatic at that point so decision was made to monitor with serial labs.

Our patient presented for repeat lab draw a week later and now her corrected serum calcium was 14.3 mg/dL, so she was instructed to visit the emergency department for further evaluation. She was admitted 12 h later on the same day and her corrected serum calcium further worsened to 15 mg/dL with an ionized calcium value of 7.5 mg/dL (range: 4.5-5.6). Her albumin was 3.4 mg/dL, creatinine 0.5 mg/dL, estimated glomerular filtration rate > 60 mL/min/1.73 m^2, magnesium 1.9 mg/dL (range: 1.8-2.4), phosphorus 2.4 mg/dL (range: 2.5-4.9). 25-hydroxyvitamin D was noted to be low: 25.9 ng/mL (30-100 ng/mL), with elevated calcitriol level: 130 pg/mL (24.8-81.5 pg/mL) and suppressed intact parathyroid hormone (PTH) level: 8 pg/mL (15-65 pg/mL) and minimally elevated PTH-related peptide (PTHrP) 2.1 pmol/L (normal < 2.0 pmol/L). Complete blood count and urinalysis were within normal limits.

Imaging examinations

Chest X-ray reveals a left hilar opacity, which was concerning for lung neoplasm. Upon further investigation with contrasted pan-computed tomography, the patient was noted to have a left lower lobe mass with an epicenter in the left lower lobe bronchus with invasion into the mediastinum and multiple hepatic metastases. No metastasis to bones, brain or spleen was noted. A core biopsy was performed on one of the liver metastases. Histopathology findings were consistent with metastatic squamous cell carcinoma, moderately differentiated. Immunohistochemistry of biopsied tissue sample was positive for markers p63, cytokeratin 5/6 heterogeneously positive; negative for thyroid transcription factor, cytokeratins 7, caudal-related homeobox transcription factor 2 and GATA binding protein 3. Thus, immunohistochemistry findings were also consistent with squamous cell carcinoma of lung. The tissue sample shows high programmed cell death ligand 1 expression (Figure 1).

FINAL DIAGNOSIS

Calcitriol induced hypercalcemia.

TREATMENT

Our patient was paucisymptomatic but had rapid rise in serum calcium level so we decided to treat her hypercalcemia. The patient was managed with intravenous normal saline, subcutaneous calcitonin administered twice, and a single infusion of zoledronic acid 4 mg during the course of the hospital stay. Her calcium level improved rapidly to 9.8 mg/dL within 48 h and she was discharged from the hospital. Her calcium level 2-wk later was noted to be elevated again at 13 mg/dL and was given another dose of zolendronic acid. Four weeks after discharge, her calcium level was noted to be within the normal range at 9.9 mg/dL.

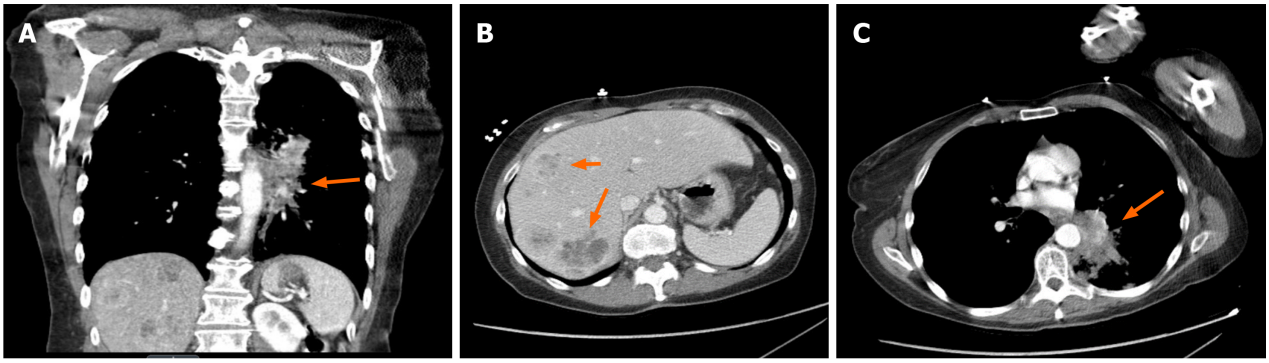
OUTCOME AND FOLLOW-UP

The patient is scheduled to receive infusion of zolendronic acid once every month to manage hypercalcemia. She is planned to undergo chemotherapy with carboplatin, paclitaxel and pembrolizumab.

DISCUSSION

Hypercalcemia results from dysregulation between normal bone formation and the degradation cycle. The pathophysiology of HAM can be broadly classified into: (1) Local osteolytic hypercalcemia; (2) Humoral hypercalcemia mediated through PTHrP; and (3) Excess production of 1,25-dihydroxy vitamin D (calcitriol).

In the current case, calcitriol level was noted to be significantly elevated with suppressed PTH level and minimal elevation of PTHrP. The question is about what's causing the calcitriol elevation in this case. Squamous cell cancer of the lung is usually associated with hypercalcemia driven by PTHrP elevation. In granulomatous disease such as sacrcoidosis, there is extrarenal production of calcitriol *via* autonomous 1-α-hydroxylase activity in tissue macrophages. PTHrP can also upregulate 1-alpha hydroxylase activity and calcitriol production in mice models, but it does not increase calcitriol production in humans[8,9]. In this patient, we tend to believe that hypercalcemia was due to a PTHrP-independent mechanism since PTHrP was minimally elevated. Additionally, extrarenal synthesis of calcitriol is dependent on its substrate 25-hydroxyvitamin D, which was low in this case, thus excluding extrarenal calcitriol production as an underlying mechanism. It's very possible that it was being ectopically produced by tumor cells in an autonomous fashion [10]. Although staining of the biopsy sample for 1,25-dihydroxy vitamin D and 1-alpha-hydroxylase was not done to confirm calcitriol's ectopic production, the treatment response further solidifies our hypothesis. Increased calcitriol level in cases of granulomatous disease is believed to be due to the upregulation of 1-alpha hydroxylase activity, and its autocrine regulation is sensitive to corticosteroid therapy. Hence, corticosteroid is indicated in the treatment of hypercalcemia in such cases[11,12]. However, in our case, hypercalcemia responded remarkably to treatment with bispho -sphonate and didn't require any steroid treatment, making us doubtful of any increased 1-alpha-hydroxylase activity.



DOI: 10.5306/wjco.v14.i11.544 **Copyright** ©The Author(s) 2023.

Figure 1 Computed tomography images. A: Computed tomography (CT) showing left lower lobe mass involving left bronchus; B: CT abdomen with contrast showing liver metastases; C: CT chest showing left lower lobe mass invasion into mediastinum.

The standard treatment approach for HAM is aimed at: (1) Promoting renal calcium excretion through intravenous normal saline administration and even loop diuretics, sometimes; and (2) Reducing bone absorption through the use of bisphosphonates and denosumab in refractory cases. Calcitonin can also be used as an adjunctive therapy with bisphosphonates, but tachyphylaxis develops within 48 h. Corticosteroids are the first-line agents for the treatment of calcitriol-mediated hypercalcemia by inhibiting the transcription of 25-hydroxyvitamin D-1-hydroxylase; however, it was not required in our case. That being said, bisphosphonates may have more of a role in the treatment of HAM. Bisphosphonate may inhibit the adhesion of osteoclast precursors to stromal osteoblasts through the increased expression of intercellular adhesion molecule-1, which is promoted by calcitriol[13,14]. In a case series reported by Rizzoli *et al*[15], bisphosphonate was more effective than steroids in the treatment of hypercalcemia, probably due to its bone anti-resorptive effect. Bisphosphonates may have additional effects, including induction of apoptosis, inhibition of invasion, and antiangiogenic properties, as seen in some preclinical studies[16]. It would be worthwhile to conduct further research to investigate the cellular effects of calcitriol and bisphosphonate in patients with lung cancer.

CONCLUSION

We believe that the underlying cause of hypercalcemia, in this case of metastatic squamous cell lung carcinoma, was elevated calcitriol, which was likely produced by the tumor cells. In addition to PTHrP, calcitriol levels should be included in the workup for hypercalcemia in cases of lung cancer. However, the pathophysiology and prognostic significance of dysregulated calcitriol production in solid tumors remain unclear and warrant further research. Bisphosphonate may be used as a steroid-sparing therapy even in cases of calcitriol-induced hypercalcemia and warrants further investigation.

FOOTNOTES

Author contributions: Alalwan A conceived the idea, did the literature search, managed the case on medical floor, contributed to case presenrtation and the introduction; Khalid F and Bader H contributed the discussion; Du D and Meghal T edited and revised the manuscript.

Informed consent statement: Informed consent was obtained by the patient.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: United States

ORCID number: Farhan Khalid 0000-0002-8456-5118.

S-Editor: Wang JJ

L-Editor: A
P-Editor: Wang JJ

REFERENCES

1 **Stewart AF**. Clinical practice. Hypercalcemia associated with cancer. *N Engl J Med* 2005; **352**: 373-379 [PMID: 15673803 DOI: 10.1056/NEJMcp042806]
2 **Jick S**, Li L, Gastanaga VM, Liede A. Prevalence of hypercalcemia of malignancy among cancer patients in the UK: analysis of the Clinical Practice Research Datalink database. *Cancer Epidemiol* 2015; **39**: 901-907 [PMID: 26520619 DOI: 10.1016/j.canep.2015.10.012]
3 **Goldner W**. Cancer-Related Hypercalcemia. *J Oncol Pract* 2016; **12**: 426-432 [PMID: 27170690 DOI: 10.1200/JOP.2016.011155]
4 **Rodríguez-Gutiérrez R**, Zapata-Rivera MA, Quintanilla-Flores DL, Camara-Lemarroy CR, Lavalle-Gonzalez FJ, González-González JG, Villarreal-Pérez JZ. 1,25-dihydroxyvitamin D and PTHrP mediated malignant hypercalcemia in a seminoma. *BMC Endocr Disord* 2014; **14**: 32 [PMID: 24721620 DOI: 10.1186/1472-6823-14-32]
5 **Shivnani SB**, Shelton JM, Richardson JA, Maalouf NM. Hypercalcemia of malignancy with simultaneous elevation in serum parathyroid hormone--related peptide and 1,25-dihydroxyvitamin D in a patient with metastatic renal cell carcinoma. *Endocr Pract* 2009; **15**: 234-239 [PMID: 19364692 DOI: 10.4158/EP.15.3.234]
6 **Hibi M**, Hara F, Tomishige H, Nishida Y, Kato T, Okumura N, Hashimoto T, Kato R. 1,25-dihydroxyvitamin D-mediated hypercalcemia in ovarian dysgerminoma. *Pediatr Hematol Oncol* 2008; **25**: 73-78 [PMID: 18231957 DOI: 10.1080/08880010701774033]
7 **Akai PS**, Wong T, Chang-Poon V, Green F, Whitelaw WA, Hanley DA. Resectable bronchogenic carcinoma presenting with hypercalcemia: tumor-associated granulomatous reaction and probable production of 1,25-dihydroxyvitamin D. *Clin Invest Med* 1989; **12**: 212-216 [PMID: 2743640]
8 **Horwitz MJ**, Tedesco MB, Sereika SM, Syed MA, Garcia-Ocaña A, Bisello A, Hollis BW, Rosen CJ, Wysolmerski JJ, Dann P, Gundberg C, Stewart AF. Continuous PTH and PTHrP infusion causes suppression of bone formation and discordant effects on 1,25(OH)2 vitamin D. *J Bone Miner Res* 2005; **20**: 1792-1803 [PMID: 16160737 DOI: 10.1359/JBMR.050602]
9 **Schilling T**, Pecherstorfer M, Blind E, Leidig G, Ziegler R, Raue F. Parathyroid hormone-related protein (PTHrP) does not regulate 1,25-dihydroxyvitamin D serum levels in hypercalcemia of malignancy. *J Clin Endocrinol Metab* 1993; **76**: 801-803 [PMID: 8445039 DOI: 10.1210/jcem.76.3.8445039]
10 **Chukir T**, Liu Y, Hoffman K, Bilezikian JP, Farooki A. Calcitriol Elevation Is Associated with a Higher Risk of Refractory Hypercalcemia of Malignancy in Solid Tumors. *J Clin Endocrinol Metab* 2020; **105**: e1115-e1123 [PMID: 31841590 DOI: 10.1210/clinem/dgz278]
11 **Fuss M**, Pepersack T, Gillet C, Karmali R, Corvilain J. Calcium and vitamin D metabolism in granulomatous diseases. *Clin Rheumatol* 1992; **11**: 28-36 [PMID: 1582115 DOI: 10.1007/BF02207080]
12 **Kallas M**, Green F, Hewison M, White C, Kline G. Rare causes of calcitriol-mediated hypercalcemia: a case report and literature review. *J Clin Endocrinol Metab* 2010; **95**: 3111-3117 [PMID: 20427501 DOI: 10.1210/jc.2009-2673]
13 **Mateo RCI**, Ortiz R, Rosen HN. BISPHOSPHONATES FOR THE TREATMENT OF CALCITRIOL-INDUCED HYPERCALCEMIA. *AACE Clin Case Rep* 2019; **5**: e316-e320 [PMID: 31967061 DOI: 10.4158/ACCR-2019-0101]
14 **Okada Y**, Morimoto I, Ura K, Watanabe K, Eto S, Kumegawa M, Raisz L, Pilbeam C, Tanaka Y. Cell-to-Cell adhesion *via* intercellular adhesion molecule-1 and leukocyte function-associated antigen-1 pathway is involved in 1alpha,25(OH)2D3, PTH and IL-1alpha-induced osteoclast differentiation and bone resorption. *Endocr J* 2002; **49**: 483-495 [PMID: 12402981 DOI: 10.1507/endocrj.49.483]
15 **Rizzoli R**, Stoermann C, Ammann P, Bonjour JP. Hypercalcemia and hyperosteolysis in vitamin D intoxication: effects of clodronate therapy. *Bone* 1994; **15**: 193-198 [PMID: 8086237 DOI: 10.1016/8756-3282(94)90707-2]
16 **Clezardin P**. Potential anticancer properties of bisphosphonates: insights from preclinical studies. *Anticancer Agents Med Chem* 2012; **12**: 102-113 [PMID: 21864232 DOI: 10.2174/1871520127990149777]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.

ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2023 December 24; 14(12): 549-627

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 12 December 24, 2023

REVIEW

549 Adenosine triphosphate induced cell death: Mechanisms and implications in cancer biology and therapy

Zhang HL, Sandai D, Zhang ZW, Song ZJ, Babu D, Tabana Y, Dahham SS, Adam Ahmed Adam M, Wang Y, Wang W, Zhang HL, Zhao R, Barakat K, Harun MSR, Shapudin SNM, Lok B

570 Update on current diagnosis and management of anaplastic thyroid carcinoma

Pavlidis ET, Galanis IN, Pavlidis TE

MINIREVIEWS

584 Current perspectives on the management of lateral pelvic lymph nodes in rectal cancer

Chua JYJ, Ngu JCY, Teo NZ

593 Anti-tumor effect of coix seed based on the theory of medicinal and food homology

Meng FD, Yuan L, Lu DD, Yang YT, Xu DJ, Che MY, Nan Y

ORIGINAL ARTICLE

Retrospective Study

606 Clinical outcomes of newly diagnosed primary central nervous system lymphoma treated with zanubrutinib-based combination therapy

Wang N, Chen FL, Pan L, Teng Y, Wei XJ, Guo HG, Jiang XM, Huang L, Liu SC, Liang ZL, Li WY

CASE REPORT

620 Rapid transformation of branched pancreatic duct-derived intraductal tubulopapillary neoplasm into an invasive carcinoma: A case report

Yamamoto K, Takada Y, Kobayashi T, Ito R, Ikeda Y, Ota S, Adachi K, Shimada Y, Hayashi M, Itani T, Asai S, Nakamura K

World Journal of Clinical Oncology

Monthly Volume 14 Number 12 December 24, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Oncology*, Qiang Huo, MD, MSc(Med), Doctor-In-Charge, Center for Translational Medicine, Zibo Central Hospital, No. 54 West Gongqingtuan Road, Zibo 255036, Shandong Province, China. qianghuo@mail.sdu.edu.cn

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJCO mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJCO* as 2.8; IF without journal self cites: 2.8; 5-year IF: 3.0; Journal Citation Indicator: 0.36.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Xu Guo*.

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
December 24, 2023

COPYRIGHT
© 2023 Baishideng Publishing Group Inc

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

© 2023 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 December 24; 14(12): 549-569

DOI: 10.5306/wjco.v14.i12.549

ISSN 2218-4333 (online)

REVIEW

Adenosine triphosphate induced cell death: Mechanisms and implications in cancer biology and therapy

Hao-Ling Zhang, Doblin Sandai, Zhong-Wen Zhang, Zhi-Jing Song, Dinesh Babu, Yasser Tabana, Sabbar Saad Dahham, Mowaffaq Adam Ahmed Adam, Yong Wang, Wei Wang, Hao-Long Zhang, Rui Zhao, Khaled Barakat, Mohammad Syamsul Reza Harun, Siti Nurfatimah Mohd Shapudin, Bronwyn Lok

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Peng XC, China

Received: September 6, 2023
Peer-review started: September 6, 2023
First decision: October 24, 2023
Revised: November 8, 2023
Accepted: November 21, 2023
Article in press: November 21, 2023
Published online: December 24, 2023

Hao-Ling Zhang, Doblin Sandai, Hao-Long Zhang, Mohammad Syamsul Reza Harun, Siti Nurfatimah Mohd Shapudin, Bronwyn Lok, Department of Biomedical Science, Advanced Medical and Dental Institute, University Sains Malaysia, Penang 13200, Malaysia

Zhong-Wen Zhang, School of Public Health, Gansu University of Chinese Medicine, Lanzhou 730000, Gansu Province, China

Zhi-Jing Song, Rui Zhao, Clinical College of Chinese Medicine, Gansu University of Chinese Medicine, Lanzhou 730000, Gansu Province, China

Dinesh Babu, Yasser Tabana, Khaled Barakat, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton AB T6G 2E1, Canada

Sabbar Saad Dahham, Department of Science, University of Technology and Applied Sciences Rustaq, Rustaq 10 P.C. 329, Oman

Mowaffaq Adam Ahmed Adam, Department of Chemistry and Biochemistry, San Diego State University, San Diego, CA 92182, United States

Yong Wang, Pathology Center, Gansu University of Chinese Medicine, Lanzhou 730000, Gansu Province, China

Wei Wang, College of Acupuncture-Moxibustion and Tuina, Gansu University of Chinese Medicine, Lanzhou 730000, Gansu Province, China

Corresponding author: Doblin Sandai, Doctor, PhD, Academic Editor, Researcher, Department of Biomedical Sciences, Advanced Medical and Dental Institute, University Sains Malaysia, Kepala Batas, Penang 13200, Malaysia. doblin@usm.my

Abstract

Adenosine triphosphate (ATP) induced cell death (AICD) is a critical cellular process that has garnered substantial scientific interest for its profound relevance to cancer biology and to therapeutic interventions. This comprehensive review unveils the intricate web of AICD mechanisms and their intricate connections with cancer biology. This review offers a comprehensive framework for comprehending the multifaceted role of AICD in the context of cancer. This is achieved by elucidating the dynamic interplay between systemic and cellular ATP

homeostasis, deciphering the intricate mechanisms governing AICD, elucidating its intricate involvement in cancer signaling pathways, and scrutinizing validated key genes. Moreover, the exploration of AICD as a potential avenue for cancer treatment underscores its essential role in shaping the future landscape of cancer therapeutics.

Key Words: Adenosine triphosphate induced cell death; Adenosine triphosphate homeostasis; Mechanism; Cancer signaling pathways; Prognosis and clinical values; Cancer treatment

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The research delves deeply into the pivotal realm of adenosine triphosphate (ATP)-induced cell death (AICD), a fundamental cellular phenomenon that has captured significant scholarly interest owing to its pertinence in cancer biology and therapeutic strategies. Our review is dedicated to delivering an all-encompassing grasp of the intricate mechanisms underpinning AICD and its far-reaching ramifications within the cancer context. By meticulously dissecting the dynamic interplay between systemic and cellular ATP homeostasis, unraveling the governing mechanisms steering AICD, and probing its intricate entanglement with cancer signaling pathways, we present an exhaustive framework that illuminates the multifaceted role of AICD in the realm of cancer.

Citation: Zhang HL, Sandai D, Zhang ZW, Song ZJ, Babu D, Tabana Y, Dahham SS, Adam Ahmed Adam M, Wang Y, Wang W, Zhang HL, Zhao R, Barakat K, Harun MSR, Shapudin SNM, Lok B. Adenosine triphosphate induced cell death: Mechanisms and implications in cancer biology and therapy. *World J Clin Oncol* 2023; 14(12): 549-569
URL: https://www.wjgnet.com/2218-4333/full/v14/i12/549.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i12.549

INTRODUCTION

In recent years, adenosine triphosphate (ATP) induced cell death (AICD) has emerged as a discernible mode of cell death triggered by elevated extracellular ATP (eATP) levels, exhibiting intimate association with the progression of various cancer types[1-3]. ATP, or adenosine triphosphate, a nucleotide crucial for cellular energy metabolism, assumes a pivotal role in multiple tumor-related signaling pathways and biological processes[4,5]. Nonetheless, the precise mechanisms and modalities underlying AICD have long remained elusive. Subsequent investigations have unveiled the distinctive features and regulatory mechanisms of AICD, setting it apart from other forms of cell demise such as apoptosis and necrosis. This review provides a concise summary of key discoveries in the field of AICD that have propelled advancements (Figure 1)[5-12].

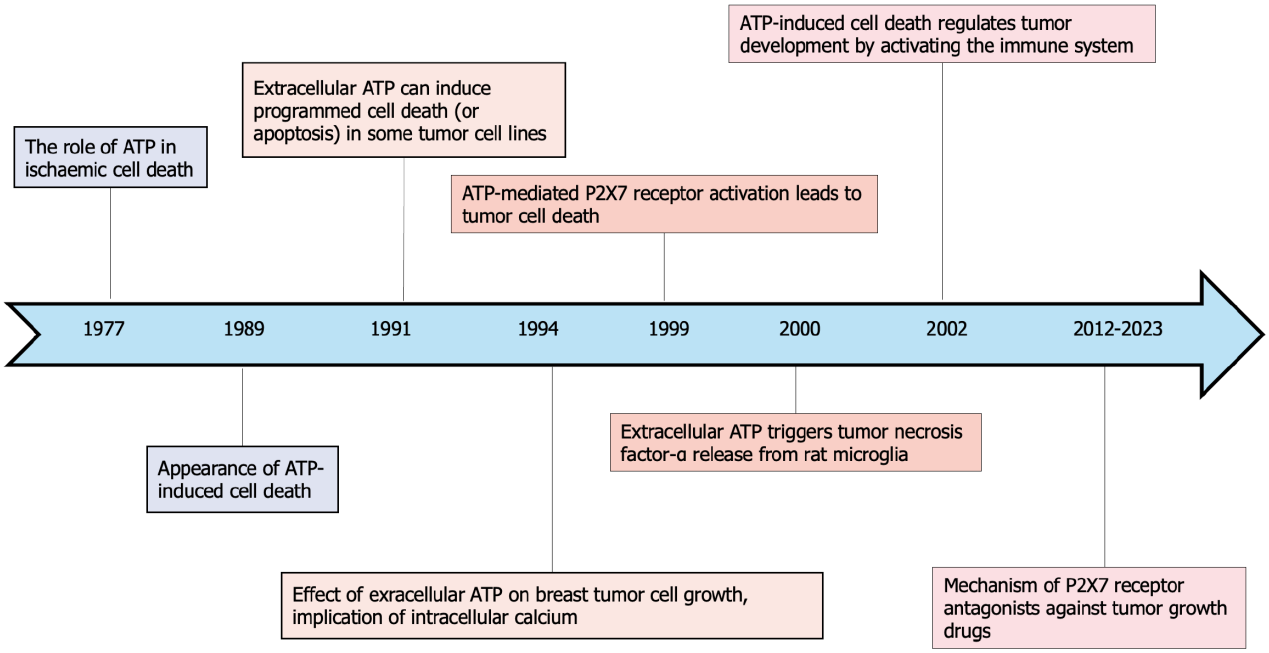
The identification of AICD represents a significant milestone in the realm of cell biology. Initially, researchers noted that the addition of exogenous ATP to cells resulted in cell death, thereby generating considerable interest and instigating extensive investigations[1]. AICD, being an inevitable facet of the cell's life cycle, assumes a pivotal role in maintaining tissue homeostasis and functionality, holding profound significance for tissue development, as well as the etiology and progression of various diseases. The mechanisms and specific manifestations of AICD remain unknown.

In the realm of oncology, aberrant regulation of AICD is a crucial determinant in tumor initiation and progression. It exerts direct influence on the fate of tumor cells, impeding their proliferation, invasion, and metastasis, while also indirectly suppressing tumor development through immune system activation[13-15]. Furthermore, AICD elicits transformative changes in the tumor microenvironment, having an impact on the proliferative, invasive, and migratory capabilities of tumor cells. Consequently, an extensive exploration of the interconnections and correlations between AICD and cancer provides novel targets and strategies for cancer therapy, facilitating a profound comprehension of the mechanisms underlying cancer onset and progression.

This paper presents a comprehensive review of the mechanisms underlying AICD and its association with cancer. The primary objective is to outline potential avenues for future research, investigating various aspects related to AICD and its relevance to cancer. Through an in-depth exploration of these mechanisms and their functions , this paper aspires to unveil novel breakthroughs in cancer treatment development and to enhance our comprehension of the occurrence and progression of cancer.

SYSTEMIC AND CELLULAR ATP HOMEOSTASIS

ATP homeostasis in biological systems and cells is a dynamic state of balance that involves the precise regulation of ATP concentration within a specific range. This is achieved through intricate processes including ATP synthesis, degradation, transport, and exchange both within and outside the cell, as well as regulation by the intracellular environment. Maintaining ATP homeostasis is crucial for sustaining cellular energy metabolism and overall physiological function. Various external factors can impact ATP production and stability, thereby perturbing ATP homeostasis.



DOI: 10.5306/wjco.v14.i12.549 **Copyright** ©The Author(s) 2023.

Figure 1 Chronological depiction of key milestones in the exploration of adenosine triphosphate induced cell death. ATP: Adenosine triphosphate.

These factors encompass fluctuations in oxygen levels, alterations in nutrient availability, exposure to toxins and pharmacological agents, variations in temperature and thermal stress, changes in potential of hydrogen (pH), activation of inflammatory and immune responses, oxidative stress resulting from the accumulation of reactive oxygen species, infections and pathogen invasions, exposure to environmental toxins, as well as prolonged or intense physical and psychological stressors. Internally, several factors participate in the regulation of ATP homeostasis. This includes the coordinated regulation of ATP synthesis pathways, ATP consumption pathways, ATP transport pathways, and ATP hydrolase activity. Additionally, ATP homeostasis can be affected by disruptions in intracellular ATP leakage, alterations in eATP transport pathways, and dysregulation of eATP metabolic pathways (Figure 2).

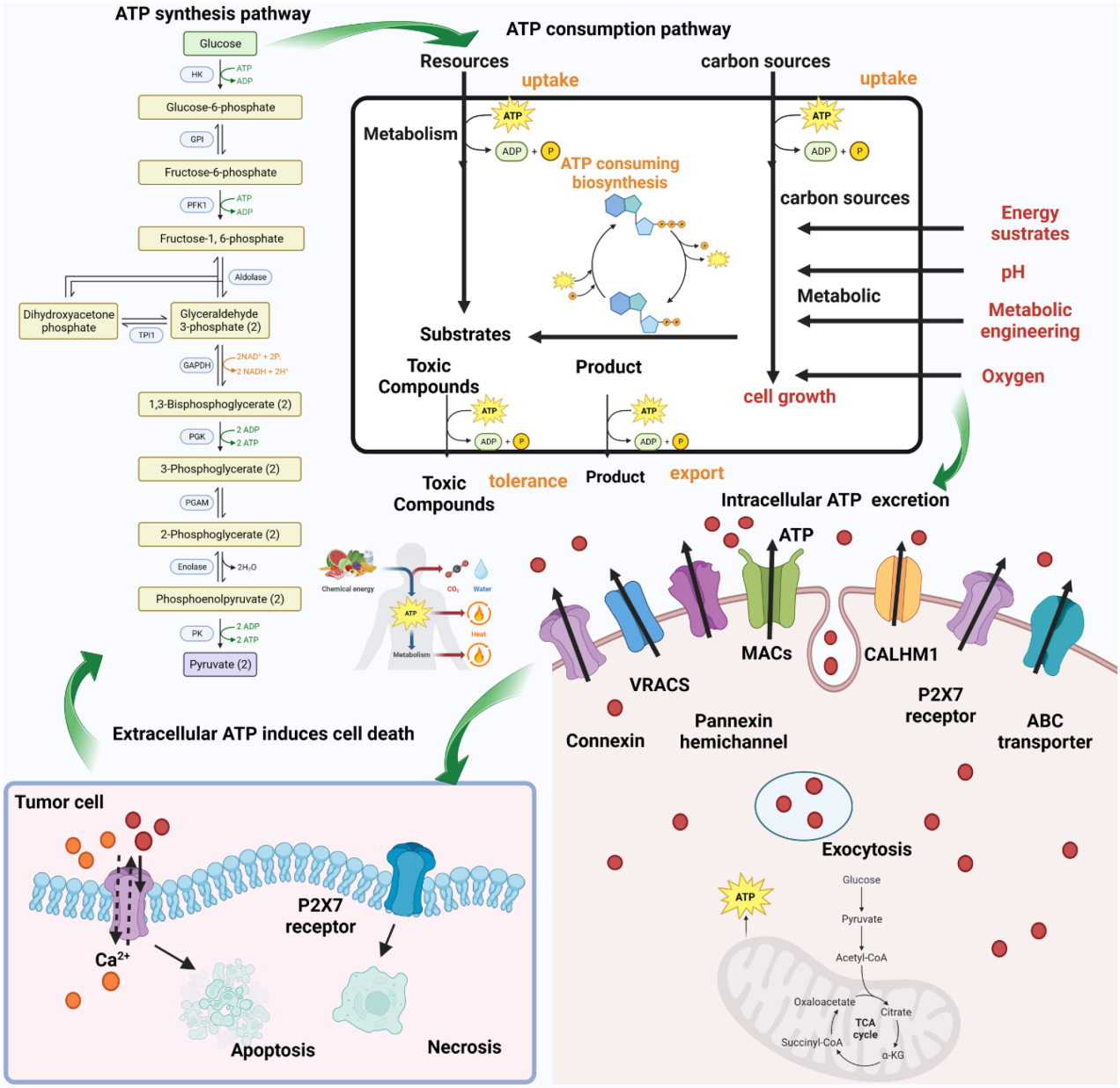
EXTERNAL FACTORS THAT AFFECT ATP HOMEOSTASIS IN SYSTEMS AND CELLS

Hypoxia induces an elevation in eATP levels, which can be attenuated by the administration of L-type Ca^{2+} channel blockers and reduced by the activity of a nucleoside hydrolase such as apyrase. Furthermore, the application of iberiotoxin (100 nM), a specific blocker of O^{2-}sensitive Ca^{2+}-dependent K^+ channels, has been shown to enhance the release of ATP[16]. Nutrient deficiency also affects ATP synthesis and metabolism[17].

Chemotherapeutic agents trigger the release of ATP through two main mechanisms: Caspase-gated pannexin-1 (Panx1) channels and caspase/Panx1-independent pathways. Various pro-apoptotic drugs, such as topoisomerase II inhibitors, kinase inhibitors, and proteomic inhibitors, induce the functional activation of Panx1 channels by inhibiting the C-terminal cleavage of Panx1 mediated by caspase-3. The activation of caspase-activated Panx1 channels facilitates the efflux of ATP, as well as adenosine diphosphate (ADP) and adenosine monophosphate (AMP), which collectively constitute over 90% of the adenine nucleotide pool released during the transition from early to late apoptosis[18].

Blood flow undergoes a substantial increase in response to elevated temperatures, most likely attributed to physiological mechanisms governed by temperature-sensitive regulatory processes. ATP exhibits sensitivity to physiological temperature elevations observed both *in vitro* and in vivo, potentially as a result of the activation of cystic fibrosis transmembrane conductance regulator (CFTR)-like channels that disrupt ATP synthesis and stability[19]. Brainstem astrocytes possess the capacity to directly perceive alterations in blood and brain carbon dioxide and pH levels, and potentially govern the function of respiratory neuronal networks to modulate respiration. The reduction in extracellular pH triggers the release of ATP, which results in the depolarization of neighboring astrocytes and neurons. Perturbations in acid-base equilibrium can impede the regular progression of intracellular energy metabolism and impact ATP synthesis and stability[20]. Clodronate, as a highly effective and specific inhibitor of vesicular ATP release, represents a distinctive therapeutic approach to the management of chronic pain. Its inhibitory action on vesicular ATP release implicates its potential efficacy in the treatment of various purinergic-mediated disorders, such as inflammatory conditions, diabetes, and neurological ailments.

These discoveries underscore the contribution of chronic inflammation and immune responses to the dysregulation of cellular ATP homeostasis[21]. These findings imply that hydrogen peroxide triggers the release of ATP from intracellular compartments into the extracellular milieu *via* lysosomal exocytosis. The generation of reactive oxygen species during



DOI: 10.5306/wjco.v14.i12.549 **Copyright** ©The Author(s) 2023.

Figure 2 The process of adenosine triphosphate production necessitates the sequential progression through a series of reactions encompassing glycolysis, pyruvate decarboxylation, the krebs cycle, and the respiratory chain. Cellular entities harness carbon sources to generate adenosine triphosphate (ATP) *via* glycolysis and the respiratory chain. Engineered cellular systems, when designed along specific pathways to facilitate targeted product synthesis, incur heightened ATP consumption for processes such as sugar uptake, cellular proliferation, biosynthesis, product efflux, and the acquisition of tolerance to cytotoxic agents. Furthermore, the equilibrium of ATP is influenced by a range of factors, including pH levels and oxygen availability. Perturbations in these dynamics can result in the overproduction of intracellular ATP, leading to its efflux through membrane-associated signaling channels or extracellular vesicles. Subsequent activation of cell membrane-associated P2 receptors by extracellular ATP triggers the influx of intracellular calcium ions, culminating in apoptotic cell demise. ATP: Adenosine triphosphate.

oxidative stress disrupts the delicate balance of ATP homeostasis[22]. Accumulating evidence suggests that the ATP/P2X7 signaling pathway confers extensive protection against viral infections in the host. The eATP exerts inhibitory effects on the replication of various viruses, including vesicular stomatitis virus, Newcastle disease virus, mouse leukemia virus, and herpes simplex virus, both *in vivo* and *in vitro*, by activating P2X7 receptors [P2X7R/purinergic receptor P2X7 (P2X7Rs)]. Concurrently, ATP administration leads to a significant upregulation of interferon-beta (IFN-β) expression in a concentration- and time-dependent manner. Mechanistically, ATP stimulates the secretion of IFN-β through the activation of the (p38 mitogen-activated protein kinase/c-jun n-terminal kinase/activating transcription factor 2) P38/JNK signaling pathway, which plays a crucial role in facilitating antiviral immune responses[23]. Furthermore, cellular energy homeostasis, particularly ATP production and stability, can be disrupted by environmental toxins (*e.g.*, heavy metals, organic pollutants) and prolonged or heightened stress. These external factors can disrupt the delicate balance of energy metabolism within cells, leading to alterations in ATP synthesis and stability[24,25].

INTERNAL FACTORS AFFECTING ATP HOMEOSTASIS IN SYSTEMS AND CELLS

The ATP synthesis pathway exerts a considerable influence on the cellular release of ATP. Oxidative phosphorylation and photophosphorylation, catalyzed by F1F0-ATP synthetase, represent the fundamental mechanisms by which cells generate energy through ATP synthesis[26]. Enhanced enzymatic activity of F1F0-ATP synthetase results in increased ATP production. Mitochondrial exposure to shear stress induces mitochondrial ATP production *via* the involvement of a specific protein called fossa or fossa protein-1, thereby converting the mechanical shear stress into a novel modulator of ATP production. This process leads to the release of ATP from vesicles and initiates purinergic Ca^{2+} signaling[25]. These findings indicate that under conditions of metabolic activity or stress, the ATP synthesis pathway can be activated in response to mitochondrial dysfunction, resulting in an upregulation of ATP production. Additionally, aberrant ion channels[27], transporters, and membrane vesicles can also contribute to augmented ATP synthesis in cells, thereby increasing the pool of available ATP for subsequent release.

Furthermore, the ATP-consuming pathway plays a crucial role in the release of ATP by cells. Cell proliferation, for instance, is associated with heightened ATP consumption[28]. In muscle protein synthesis, citrulline has been shown to induce ATP redistribution, resulting in increased ATP consumption during the process[29]. As a consequence, cells release more ATP to fulfill their heightened energy demands. Similarly, during the shortening of rabbit psoas muscle skin fibers, ATP consumption is elevated[30]. Studies have also demonstrated that certain abused drugs, such as degeneration of optic atrophy, exhibit increased ATP consumption during their transport across filter-grown CACO-2-monolayers[31]. ATPase and ATP-dependent enzyme reactions are implicated in this increased ATP consumption, which subsequently affects the quantity of ATP released by cells. These findings underscore the significance of the ATP-consuming pathway in modulating ATP release dynamics in cellular processes.

ATP transport channels play a vital role in cellular ATP release. Notably, the opening of the Panx1 half-channel is modulated by the activity of P2X7Rs. Evidence suggests that P2X7Rs are activated under pathological conditions like ischemia, leading to the opening of the PANX1 half-channel. This allows substantial Ca^{2+} influx from the extracellular space and the release of ATP from the cytoplasm, ultimately triggering cell death[32]. These findings indicate that activated Pannexin channels facilitate ATP release from the intracellular space through the cell membrane to the extracellular environment.

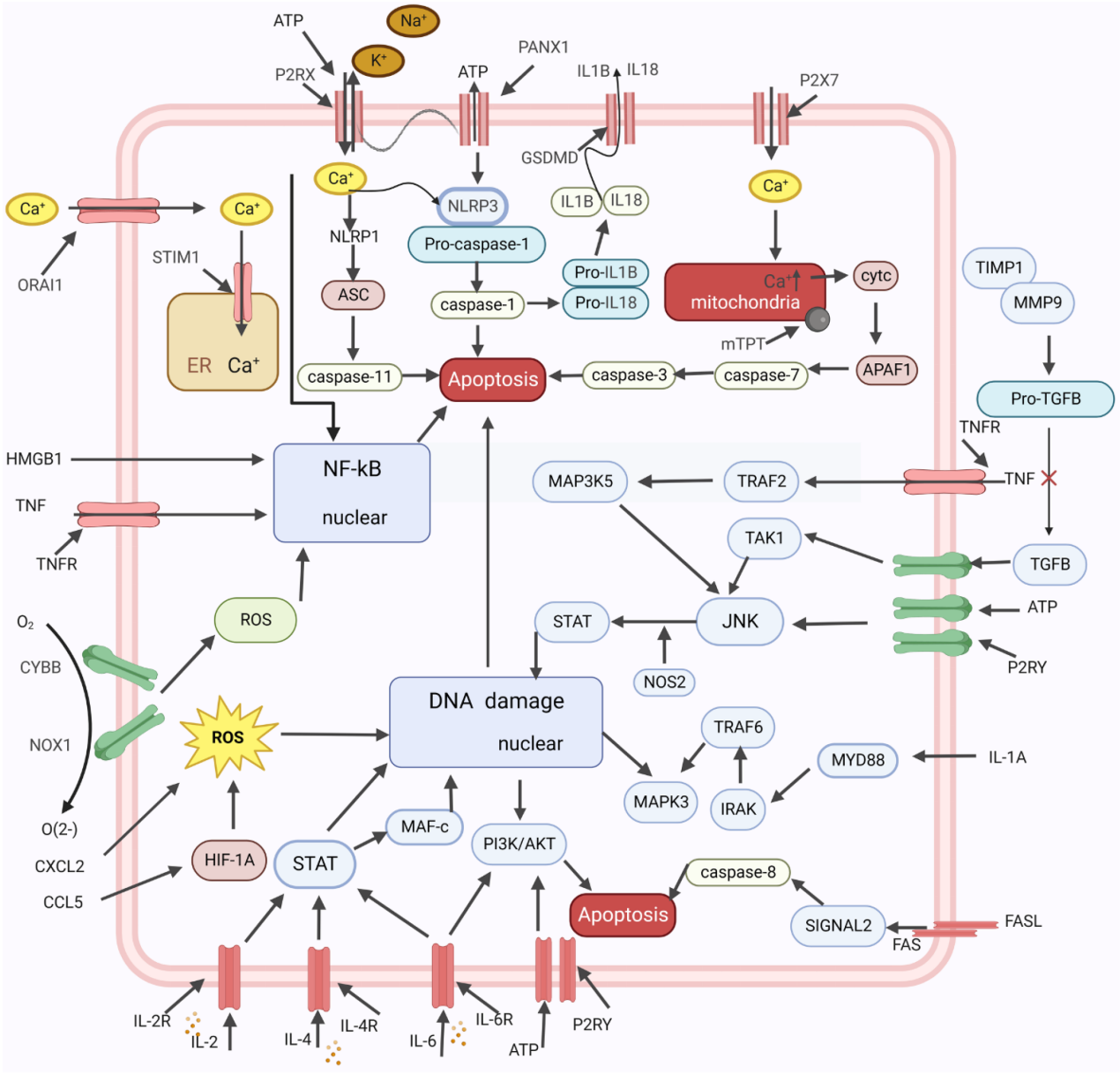
CFTR also promote ATP release by stimulating independent ATP release channels, thus governing cellular autocrine signaling[27]. Studies have demonstrated that CFTR forms pores in the cell membrane, enhancing the efflux of ATP from the cytoplasm to the extracellular milieu. Furthermore, eATP plays a regulatory role in various signaling systems, including the propagation of intercellular Ca^{2+} signaling (ICS). Nexin semi-channels, P2X7Rs, pannexin channels, anion channels, vesicles, and transporters are recognized as potential ATP-released channels; however, their precise contributions to ICS remain subject to debate. In the inner ear, these connexins play a dual and crucial role in Ca^{2+} signaling: serving as semi-channels, they promote ATP release and sustain long-range ICS propagation; acting as gap junction channels, as well as facilitating the diffusion of Ca^{2+}-mobilized second messengers among coupled cells[33]. Additionally, the binding of ATP facilitates the release of substrates by multidrug resistant protein [34]. Simultaneously, multidrug-resistant protein participates in intracellular substance transport and excretion, contributing to the transport of ATP from the cytoplasm to the extracellular space, thus promoting ATP release.

Cells can regulate the balance of ATP concentration inside and outside the cell by modulating the activity of ATP hydrolase. Among the ATP hydrolases, exonucleoside triphosphate diphosphate hydrolases form a significant enzyme family, with members including ectonucleoside triphosphate diphosphohydrolase 1 (CD39) and ENTPD3. These enzymes are capable of catalyzing the hydrolysis of ATP to ADP, leading to the degradation and subsequent release of ATP[35]. Moreover, the ectonucleotide pyrophosphatase/phosphodiesterase family includes members such as ectonucleotide pyrophosphatase/phosphodiesterase 1 and ectonucleotide pyrophosphatase/phosphodiesterase 2, which are also involved in ATP hydrolysis. These enzymes catalyze the hydrolysis of ATP to AMP and two inorganic phosphate ions. The impact of eATP on the release of ATP from cells is a multifaceted and intricately regulated process that entails the interplay of various cell surface receptors, channels, and enzymes.

AICD MECHANISMS

The complexity of AICD can vary depending on the specific cell type and the surrounding microenvironment. Nevertheless, several general mechanisms have been elucidated. One of these mechanisms involves the activation of purinergic receptors, particularly the P2X7R, which can initiate a cascade of events leading to cell death. Another mechanism is associated with the elevation of intracellular calcium ion concentration. Moreover, ATP-triggered cell death may also contribute to the activation of inflammatory responses. Lastly, AICD is linked to the perturbation of mitochondrial function, with the release of cytochrome c being strongly associated with the activation of apoptosis signaling pathways (Figure 3).

EATP stimulates the activation of the P2X7R, leading to inflammasome activation and the release of pro-inflammatory cytokines in monocytes. Native-like T cells effectively respond to innate stimuli by secreting a multitude of pro-inflammatory cytokines, and human T cell compartments exhibit the highest expression of the P2X7R. Within the innate lymphoid population, Tγδ cells demonstrate heightened sensitivity to P2X7R activation compared to conventional T cells, influencing fundamental cellular mechanisms such as calcium signaling and AICD[36]. Neuroinflammation is positively linked to P2X7R activation through risk-associated molecular patterns, with eATP being the most prominent among them. The P2X7R is expressed in various retinal cells, including retinal endothelial cells, and ATP serves as the sole



DOI: 10.5306/wjco.v14.i12.549 **Copyright** ©The Author(s) 2023.

Figure 3 Illustration of the mechanism of adenosine triphosphate induced cell death, which involves several interconnected pathways. Upon binding to the purinergic receptor P2X7 (P2X7R), extracellular adenosine triphosphate (ATP) induces a surge in intracellular calcium levels, leading to caspase activation and subsequent cell death. Additionally, ATP activates the NOD-like receptor family pyrin domain containing 3 inflammasome by releasing High Mobility Group Box 1/Toll-Like Receptor 4, triggering caspase-1 activation and promoting cell apoptosis. The interaction between ATP and P2X7Rs also activates the Nuclear Factor-kappa B and Phosphatidylinositol 3-kinase-protein kinase B/hypoxia-inducible factor pathways, resulting in DNA damage and cell death. Simultaneously, the continuous accumulation of intracellular Ca^{2+} stimulates the opening of the mitochondrial permeability transition pore, leading to DNA damage and ultimately cell necrosis. Ca^{2+} induces mitochondria to release cytochrome c, further contributing to the apoptotic process. Moreover, ATP-triggered cellular demise instigates a transformative shift within the extracellular microenvironment, concurrently unleashing a plethora of cytokines. Lastly, apart from elucidating the fundamental underpinnings of ATP induced cell death, this Figure also encapsulates a synthesized appraisal of the plausible mechanisms governing microenvironmental equilibrium, as extrapolated from relevant literature. ATP: Adenosine triphosphate; NF-κB: Nuclear Factor-kappa B; NLRP3: NOD-like receptor family pyrin domain containing 3; PI3K-AKT: Phosphatidylinositol 3-kinase-protein kinase B; ROS: Reactive oxygen species; TNF-α: Tumor necrosis factor-alpha; IL: Interleukin; ASC: Apoptosis-related speckle-like protein; STAT: Signal transducer and activator of transcription.

physiological agonist for P2X7. High glucose induces periretinal cell death by activating P2X7R, and the ATP released by the deceased cells functions as a "danger signal," further amplifying the inflammatory response caused by glucose-induced injury[37]. Research has demonstrated that brief (1-4 min) stimulation of mouse macrophages with high eATP leads to delayed (hourly) cell death, as evidenced by DEVDase (caspase-3 and caspase-7) activity. "Transient" P2X7R activation and Ca^{2+} overload have been identified as triggers for death in native mouse macrophages, independent of Panx1 and pro-inflammatory caspase-1 and toll-like receptor (TLR) signaling[38]. Furthermore, knockdown of chloride intracellular channel protein 4 enhances ATP-induced apoptosis of HN4 cells through mitochondrial and endoplasmic reticulum pathways[39].

AICD AND CANCER SIGNALING PATHWAYS

AICD is directly associated with multiple signaling pathways in tumor cells, achieved through the binding and activation of key molecules in these pathways. Among them, a correlation exists between the mitochondrial pathway and AICD. Upon eATP activation of the P2X7R, intracellular mitochondrial Ca^{2+} levels increase, leading to the formation of Bcl-2-associated X /Bcl-2 homologous antagonist/killer oligomer complexes that insert into the outer membrane pores of mitochondria. This causes changes in mitochondrial osmotic pressure and transmembrane potential loss, subsequently facilitating the release of cytochrome c from mitochondria into the cytoplasm and activating the caspase-9 precursor.

Consequently, caspase-3 and caspase-7 are activated, triggering a Caspase cascade reaction, and ultimately inducing cell apoptosis[40-47]. ATP promotes apoptosis by activating extracellular P2X7Rs. The apoptosis of tumor cells can induce apoptosis in surrounding cells, resulting in proliferative necrosis, providing an environment favorable for cancer spread. P2X7R activation leads to tumor necrosis factor (TNF) activation, stimulating Caspase activation, and initiating the execution phase of apoptosis[48,49]. Simultaneously, P2X7R activation alters membrane permeability, leading to an outflow of intracellular ions, cell swelling, and rupture, ultimately causing cell necrosis[50,51]. Necrosis is an internal tumor death that creates an ideal environment for cancer dissemination. ATP activates immune cell membrane P2X7Rs, triggering the release of necrosis factors, and activating serine-threonine kinases such as receptor-interacting protein kinase 1 and receptor-interacting protein kinase 3 after TNF receptor 1 or TLR stimulation, ultimately inducing necrosis [50-54].

The autophagy pathway plays a crucial role in recycling metabolic waste in tumor cells, ensuring their energy requirements are met, or facilitating evasion of apoptosis, ultimately leading to tumor cell proliferation. ATP can promote autophagy initiation by activating the AMP-activated protein kinase (AMPK) signaling pathway[55,56]. When intracellular ATP levels decrease, AMPK becomes phosphorylated and activated, subsequently activating the unc-51-like autophagy activating kinase 1 complex and initiating the autophagy process.

Nuclear factor kappaB (NF-κB) assumes a critical role in numerous biological processes of tumor cells, encompassing inflammation, proliferation, survival, apoptosis, angiogenesis, epithelial-mesenchymal transition (EMT), metastasis, stem-cell characteristics, metabolism, and therapeutic resistance. Prior investigations have established that NF-κB activation leads to DNA damage and initiates the signaling pathway of NF-κB[57]. The Wnt signaling pathway holds paramount significance in embryonic development by preserving stem cell properties and dictating cell fate. When ATP binds to the P2 purinergic receptor, it activates protein kinase C and phosphoinositide 3-kinase (PI3K) signaling pathways, thereby inhibiting the activity of glycogen synthesis kinase-3β (GSK-3β)[57-60].

Consequently, β-catenin is no longer phosphorylated and degraded by GSK-3β, which regulates cell growth and differentiation. Several studies have indicated that ATP can promote the activation of the PI3K/protein kinase B (Akt) pathway through P2 purinergic receptor activation. This process results in PI3K catalyzing the transformation of phosphatidylinositol diphosphate into phosphatidylinositol triphosphate (PIP3). Subsequently, PIP3 attracts Akt kinase to the cell membrane, resulting in its phosphorylation and activation. Activated Akt kinase modulates cancer development by phosphorylating a diverse array of downstream effector proteins.

MAPK comprises a cluster of evolutionarily conserved serine-threonine kinases, encompassing extracellular signal-regulated kinase (ERK), p38, JNK, and big mitogen-activated protein kinase 1, with each representing distinct classical MAPK pathways. ATP phosphorylates and activates MAPK protein kinases (such as ERK, JNK, and p38) by engaging P2 purinergic receptors[61].

Research has revealed that AICD may incite DNA damage, consequently activating tumor protein 53 (p53) expression and function. Activated p53 effectively regulates multiple target genes, including cyclin-dependent kinase inhibitor 1 (p21), Bax, p53 upregulated modulator of apoptosis, *etc.*, which are closely associated with cancer development[62,63]. The induction of AICD exerts a direct or indirect impact on cancer signaling pathways and cancer characteristics, thus further underscoring its vital role in cancer.

VALIDATED KEY GENES IN AICD KEY GENES: FUNCTIONS, PROGNOSIS, AND CLINICAL VALUES

The underlying mechanism of AICD remains incompletely understood. However, several overarching mechanisms have been revealed. Among them, a pivotal pathway involves the activation of the P2 receptor family, specifically the P2X7R, by eATP. Perturbation or activation of these genes may modify susceptibility to AICD. Furthermore, investigations into ATP homeostasis have highlighted the regulatory role of PANX1 protein in intracellular ATP concentration, thus influencing AICD. Also, activation of P2X7R triggers an elevation in intracellular calcium levels, which is balanced by the calcium release-activated calcium channel protein 1 (ORAI1) and stromal interaction molecule (STIM) 1 proteins to maintain intracellular calcium homeostasis. Besides these mechanisms, apoptotic and mitochondrial pathways also participate in AICD. Consequently, 37 genes have been identified as crucial players in the AICD mechanism. As the concept of AICD gains prominence, researchers are increasingly focusing on its role in diverse tumor types, implying that the expression levels and clinical significance of AICD may hold significant relevance across different tumors.

Therefore, this paper will discuss prevalent cancer types globally. Table 1 below enumerates the functions and subcellular localizations of these genes during AICD. Due to the limited availability of cancer prognosis-related information regarding AICD genes, an extensive analysis was conducted using clinical data from the database provided by the American Cancer Letters and Biology Institute (https://www.aclbi.com/static/index.html/). Table 1, establishes a comprehensive gene prognosis model centered on AICD, aiming to assess the prognostic significance of individual genes across several types of cancer.

Table 1 List of adenosine triphosphate induced cell death core genes and their relationship with tumors

Gene	Full name	Risk factor	Protective factor	Clinical prognostic value	Role in ATP induced cell death	Ref.
P2RX7	Purinergic receptor P2X7	NA	NA	HNSC, KIRC, LAML, SARC	Activates inflammatory mediators and increases calcium ions	Tamajusuku *et al* [89]
CASP3	Caspase-3	DPG, HNSC, MESO	OV, THYM	ACC, COAD, LGG, LIHC, LUSC, PAAD	Caspase-3 cleavage by caspase-1/4/5/11 forms pores, releasing pro-inflammatory cytokines	Souza *et al*[90]
PANX1	Pannexin-1	NSCLC, BRCA, RCA, SARC, MESO		LUAD, MESO, PAAD, STAD	P2X7 activation opens PANX1 channels, releasing ATP and triggering cell death pathways	Shoji *et al*[91]
NLRP3	NOD-like receptor family pyrin domain-containing protein 3	SARC, TGCT	PAAD	LAML, SKCM	NLRP3 activated by stimuli forms inflammasome, triggers caspase-1 activation, releases cytokines, induces apoptosis	Sadatomi *et et al* [92]
CASP1	Caspase-1	DPG, HNSC, PAAD, LAML, THYM	BRCA, MESO	BRCA, LAML, LGG, MESO, SARC, THYM	Caspase-1 induces cytokine processing, pyrosis, and inflammation	Sadatomi *et al*[92]
P2RY1	P2Y purinoceptor 1	DPG, PAAD	NA	BLCA, KIRC	P2RY1 can increase calcium ions in the Golgi apparatus	Ohishi *et al*[93]
P2RY11	P2Y purinoceptor 11	NA	HNSC,PAAD,UCEC, Rb, TGCT	ACC, BLCA, LGG, UCEC, UVM	Involved in immune inflammatory mechanisms	Yoon *et al*[94]
ORAI1	Calcium release-activated calcium channel protein 1	RCA, SARC, MESO	HNSC	ACC, BLCA, KIRP, LGG, MESO,	Increased intracellular calcium ions	Peng *et al*[95]
STIM1	Stromal interaction molecule 1	HNSC, PCPG	SARC	KIRP, PAAD, UVM	STIM1 responds to ATP-induced calcium influx, activating ORAI1 and promoting cell death	Peng *et al*[95]
CASP8	Caspase-8	CESC, RCA	DPG, BRCA, OV, SKCM, SARC	LGG, PAAD, SKCM	CASP8 causes apoptosis	Zeng *et al*[96]
CASP9	Caspase-9	DPG, NSCLC, ACC, THYM	PAAD,BRCA, Rb, MESO	ACC, BLCA, BRCA, LAML, LGG, MESO	CASP9 causes apoptosis	Zeng *et al*[96]
CASP7	Caspase-7	HCC, THYM	BRCA, MESO	ACC, KIRC, LGG, LIHC, STAD	CASP7 causes apoptosis	Zeng *et al*[96]
P2RX3	Purinergic receptor P2X3	DPG	PAAD,NSCLC, CESC, Rb	KIRC, KIRP, LUAD	NA	Ohishi *et al*[93]
NLRP1	NLR family pyrin domain-containing protein 1	RCA, MESO, THYM	HNSC, NSCLC, SARC	LGG, LUAD, SKCM	NLRP1 activates caspase-1, induces pyrodeath, and releases IL-1β and IL-18	Zhao *et al*[97]
P2RX4	P2X purinoceptor 4	HNSC, HCC, RCA, Rb, MESO	DPG, UCEC	LGG, LIHC, MESO, UCEC, UVM	P2RX4 contributes to AICD (pyroptosis) by activating the NLRP3 inflammasome, leading to IL-1β and IL-18 production	Ohishi *et al*[93]
P2RX5	P2X purinoceptor 5	RCA, ACC	HNSC	ACC, KIRC, LGG, SKCM	NA	Ohishi *et al*[93]
SAPK	Stress-Activated Protein Kinase	NA	NA	NA	ATP induces cell death *via* SAPK pathways, regulating apoptosis, necrosis, and stress	Humphreys *et al* [98]

					signaling	
p38 MAPK	p38 mitogen-activated protein kinases (p38 MAPK)	NA	NA	NA	ATP activates p38 MAPK, which leads to cell death through apoptosis and necrosis	Noguchi *et al*[99]
ASK1	Apoptosis Signal-Regulating Kinase 1	OV, THYM	DPG, HNSC, RCA	KIRC, LAML, LGG, MESO, PAAD, READ, SKCM	Excessive ATP induces cellular stress, activating ASK1 and downstream pathways for cell death	Noguchi *et al*[99]
NOX2	NADPH oxidase 2	NA	NA	CESC, KIRC, LIHC, LUAD, SKCM	ATP activates NOX2, generating ROS causing oxidative stress and potential cell death	Noguchi *et al*[99]
bax	BCL2 Associated X	NA	PAAD, BRCA, CESC, RCA	LGG, LIHC, MESO, SKCM, UVM	Excessive ATP triggers BAX activation, mitochondrial dysfunction, and apoptotic cell deat	Wen *et al*[100]
MLC	Myosin Light Chain	UCEC, MESO	HNSC, PAAD, BRCA, CESC, RCA, PCPG	CESC, KIRC	ATP depletion hampers muscle contraction, affecting myosin function and cellular viability	Hwang *et al*[101]
ROCK I	Rho-associated, coiled-coil containing protein kinase 1	THYM	BRCA, RCA	KIRC, LGG, PAAD	ATP activates P2X7 receptors, inducing apoptosis *via* the Rho/ROCK pathway, potentially involving ROCK I	Hwang *et al*[101]
ERK1/2	Extracellular Signal-Regulated Kinase 1 and 2	NA	NA	NA	ERK1/2 promotes cell survival or antagonizes apoptosis, but prolonged activation may lead to cell death. Activates the ERK1/2 pathway, affecting cell fate	Tsukimoto *et al* [102]
P2X6	P2X purinoceptor 6	DPG, HNSC, BRCA, OV, UCEC, RCA, MESO	SARC, ACC	ACC, HNSC, KIRC, LGG, OV, UVM	Activation may raise calcium levels, potentially triggering cell death	Banfi *et al*[103]
CYTC	Cytochrome c	HNSC, NSCLC, Rb, MESO, THYM	DPG, RCA	ACC, BRCA, COAD, HNSC, KIRP, LAML, LGG, LUAD, MESO, UCEC	Cytochrome c released by mitochondria during cell stress triggers cell apoptosis	Sadatomi *et al*[92]
TNF-α	Tumor necrosis factor alpha	CESC, Rb, MESO	HNSC, PAAD, RCA, SARC	SKCM, THYM	ATP induces cell death, activating TNF-α and triggering apoptosis or necroptosis pathways. Immune cells produce TNF-α in response to ATP, amplifying the cellular response	Hide *et al*[5]
P2RY5	P2R purinoceptor 5	NA	NA	NA	NA	Yoon *et al*[94]
P2RY14	P2R purinoceptor14	RCA	HNSC, HCC, OV, UCEC MESO	HNSC, KIRP, LUAD, SKCM, UCEC	NA	Ohishi *et al*[93]
P2RY13	P2R purinoceptor 13	NA	PAAD, NSCLC, CESC, SKCM, RCA, SARC	ACC, CESC, KIRC, LUAD, SARC, SKCM, UCEC	P2Y13 may play a role in ADP receptors, mainly involved in ATP homeostasis	Ohishi *et al*[93]
P2RY12	P2R purinoceptor 12	DPG,PAAD,OV, SARC, MESO, THYM,	NSCLC	LAML, LUAD, SKCM	P2Y12 may play a role in ADP receptors, mainly involved in ATP homeostasis	Ohishi *et al*[93]
P2RY6	P2R purinoceptor 6	DPG, HNSC, PAAD, HCC, BRCA, RCA	SARC,	KIRC, LGG, SKCM, UVM	P2Y6 may be involved in calcium signaling	Ohishi *et al*[93]

					leading to cell death	
P2RY4	P2R purinoceptor 4	HCC, SARC	HNSC, PAAD, RCA	PRAD	P2Y6 may be involved in calcium signaling leading to cell death	Ohishi *et al*[93]
P2RY2	P2R purinoceptor 2	DPG, UCEC, BRCA, OV	RCA, SARC	BLCA, GBM, LAML, LGG, MESO, OV, PAAD, UCEC, UVM	ATP binding triggers intracellular signaling pathways that may lead to cell death	Ohishi *et al*[93]
ANO6	Anoctamin-6	HNSC, PAAD, OV, NSCLC, BRCA, CESC		BRCA, CESC, KIRC, LGG, MESO, OV, PAAD	As a calcium-activating channel and superburning enzyme, it may influence cell death pathways	Ousingsawat *et al*[104]
cyclinE2	cyclinE2	DPG, HCC, UCEC, RCA, SARC, Rb, ACC, MESO	HNSC	ACC, BRCA, KICH, KIRP, LGG, LIHC, LUAD, MESO, THYM	NA	Wang *et al*[105]
cyclinD2	Cyclin D2	HNSC	PAAD, NSCLC, BRCA, LAML, MESO, PCPG	LAML, LGG, LUSC, MESO, PAAD, SKCM, THCA, UCEC	NA	Wang *et al*[105]

ACC: Adrenocortical carcinoma; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; COADREAD: Colon adenocarcinoma/rectum adenocarcinoma esophageal carcinoma; DLBC: Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; GBMLGG: Glioma; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIPAN: Pan-kidney cohort (KICH + KIRC + KIRP); KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute myeloid leukemia; LGG: Brain lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcomav; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; STES: Stomach and esophageal carcinoma; GCT: Testicular germ cell tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine corpus endometrial carcinoma; UCS: Uterine carcinosarcoma; UVM: Uveal melanoma.

AICD IN GLOBALLY-PREVALENT CANCER TYPES

Breast cancer

Breast cancer is the predominant malignancy among women globally, holding the foremost position in cancer-related mortalities. Emerging investigations have revealed a significant elevation of P2X7Rs in breast cancer, implicating their involvement in mediating crucial cellular processes. Specifically, P2X7Rs have been associated with the activation of the Akt signaling pathway, the calcium-activated small conductance calcium-activated potassium channel 3 potassium channel, and the induction of EMT. Additionally, they play a regulatory role in the secretion of extracellular vesicles, thereby fostering breast cancer invasion and migration. These mechanisms are influenced by factors such as hypoxia and ATP exposure[64]. In T47D cells, the silencing of the P2X7R remarkably hindered the invasion and migration induced by ATP stimulation. Moreover, the activation of P2X7Rs by ATP led to a down-regulation of E-cadherin protein levels and an up-regulation of matrix metalloproteinase-13 (MMP-13) production[65]. This suggests that ATP-induced activation of P2X7Rs may facilitate breast cancer cell invasion and migration through the activation of the Akt pathway and the regulation of E-cadherin and MMP-13 expression. Furthermore, the glycoprotein PANX1 has emerged as a key player in breast cancer metastases, bearing similarities in structure and function to connexins and contributing to cell-environment communication. Elevated PANX1 expression has been associated with a shift towards an EMT phenotype and has been implicated in the tumor-promoting role of breast cancer, correlating with unfavorable clinical outcomes in breast cancer patients[66].

The expression levels of ORAI1 were also found to be upregulated in breast cancer cell lines. Employing ORAI1 small interfering RNA (siRNA) interference in breast cancer cells resulted in reduced calcium ion entry related to storage operations and altered calcium inflow linked to invasive stimulation. Microarray data analysis of 295 breast cancer cases indicated that the transcriptional breast cancer subtype with the worst prognosis (basal type) exhibited alterations in the relationship between ORAI1 regulatory factors, namely STIM1 and STIM2. Notably, breast cancer patients with tumors expressing high levels of STIM1 and low levels of STIM2 had significantly worse prognoses[67]. *In vitro* investigations have further validated the pivotal role of STIM1 in the proliferation and metastasis of breast cancer. STIM1 was found to be expressed in 66.1% of breast cancer cases, a significantly higher proportion than in adjacent non-tumor tissues. Moreover, STIM1 overexpression demonstrated positive associations with larger tumors, lymph node metastasis, and negative estrogen receptor status. Additionally, in breast cancer patients, increased STIM1 expression was significantly linked to poorer disease-free survival but did not exhibit a significant correlation with overall survival[68].

The P2Y2 receptor plays a pivotal role in the progression of various tumor types. It exhibits high expression levels in MCF7 and Hs578T breast cancer cells. Targeting the P2Y2 receptor with siRNA leads to a significant attenuation of ATP- or uridine 5′-triphosphate-driven migration and invasion of breast cancer cells, along with down-regulation of the EMT-related genes snail family transcriptional repressor 1 and E-cadherin. Consistent with *in vitro* findings, the expression of the P2Y2 receptor was markedly higher at the tumor infiltrating margin, invasive tumor cells within breast adipose tissue, and/or cancer embolus of lymphatic sinus compared to the tumor core[69]. Abnormal expression and mutations of the P2Y6 receptor have been observed in most tumor types and strongly correlated with poor prognosis in breast cancer patients. Additionally, uridine diphosphate significantly enhances the migration and invasion of breast cancer cells, and this effect can be blocked by P2Y6 receptor-specific inhibitors MRS2578 and P2Y6 short hairpin RNA (shRNA)[70]. Furthermore, the expression of P2Y12 is significantly up-regulated in cisplatin-treated 4T1 breast cancer cell lines. The combined use of P2Y12 inhibitors and cisplatin significantly enhances the cytotoxic response of 4T1 cancer cells[71]. Notably, a certain relationship exists between AICD and breast cancer. Being an intracellular energy molecule, ATP plays critical biological functions within the cell. Therefore, further investigations are warranted to elucidate the mechanism of action and potential therapeutic value of ATP in breast cancer.

Lung cancer

Lung cancer, one of the most prevalent cancer types globally, is directly associated with smoking, but it can also affect non-smokers. It involves the uncontrolled proliferation of lung cells, leading to the formation of malignant tumors. Recent research has demonstrated a significant relationship between the dysregulated expression of the P2X7R and the occurrence and progression of lung cancer. Particularly, the P2X7R is prominently expressed in tumor-associated macrophages (TAMs), and its deficiency impairs the "M2-like" polarization of TAM by reducing the phosphorylation of signal transducer and activator of transcription 6 and interferon regulatory factor 4. Consequently, P2X7 deficiency curtails lung cancer and Lewis lung cancer progression by inhibiting tumor cell proliferation and angiogenesis, promoting T cell mobilization, and reverting M2-like TAM polarization[72]. Furthermore, relevant data has verified the functional presence of P2X1, P2X4, and P2X7Rs in laboratory of allergic disease 2 cells and HLMC[73].

Overexpression of ORAI1/calcium release activated calcium modulator 1 (CRACM1) has a suppressive effect on extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt phosphorylation. This overexpression induces the expression of the cell cycle regulator p21 while reducing the expression of cyclin D3. As a result, cell cycle arrest occurs in the G0/G1 phase. Of particular significance is that the heightened expression of ORAI1/CRACM1 significantly diminishes epidermal growth factor-triggered calcium influx[74]. In non-small cell lung cancer (NSCLC), the expression of STIM1 is substantially elevated compared to benign lesions and is positively correlated with advanced T stages of NSCLC. STIM1 knockdown in NSCLC cell lines A549 and lung cancer (SK-MES-1) Leads to significant inhibition of cell proliferation and arrests A549 and SK-MES-1 cells in the G2/M and S phases of the cell cycle. Moreover, STIM1 knockdown markedly reduces the growth of xenografted tumors in nude mice[74,75].

While some studies have indicated the potential involvement of ATP in the regulation of lung cancer occurrence and development, further research is needed to confirm and clarify whether ATP acts as an independent risk factor for lung cancer. Additionally, exploring how ATP-related mechanisms can be applied for clinical intervention remains an essential area of investigation.

Colorectal cancer (CRC)

CRC stands as a prominent contributor to cancer-related mortality on a global scale. In CRC patients, distinct phenotypes characterized by high and low P2X7R expressions have been identified. Those exhibiting high P2X7R expression displayed shorter survival, elevated serum carcinoembryonic antigen levels, and more advanced tumor stages. Moreover, P2X7R expression showed significant upregulation in metastatic CRC and metastatic CRC cell lines, indicating a positive correlation between P2X7R expression and metastasis[75,76]. P2X7R, through inducing glucose transporter protein 1 (GLUT-1) expression, aids in tumor cells' resistance to unfavorable conditions. GLUT1, a principal glucose transporter in CRC cells, serves as a prognostic marker for adverse outcomes in CRC patients. Recent investigations have identified P2X7R and GLUT-1 as potential prognostic biomarkers for the development of novel treatment strategies. Higher P2X7R expression was found in patients with poorly differentiated tumors, and those with GLUT-1 overexpression experienced reduced overall survival and disease-free survival. Therefore, P2X7R and GLUT-1 may independently serve as prognostic markers, offering a novel avenue for targeted therapy in CRC patients[77].

Purinergic receptors, particularly P2Y2 receptors, have been identified to exert an anti-apoptotic effect in ursolic acid-induced CRC HT-29 and prostate cancer DU145 cells. P2Y2 receptor activation leads to Src activation, subsequently phosphorylating p38, resulting in cyclooxygenase-2 (COX-2) overexpression and thereby inducing resistance to apoptosis in HT-29 and DU145 cells[78]. Current investigations indicate that sustained activation of P2Y6R may contribute to the development of intestinal tumors by inhibiting the apoptotic process and promoting chemotherapy resistance, which poses a critical challenge in the management of CRC patients[79].

STIM1 overexpression is prevalent in CRC patients. Notably, elevated STIM1 expression is significantly associated with tumor size, depth of invasion, lymph node metastasis, and serum carcinoembryonic antigen levels in CRC. Furthermore, ectopic STIM1 expression enhances the motility of CRC cells, while STIM1 depletion through shRNA inhibits CRC cell migration[80]. Additionally, ORAI1 is upregulated in human CRC tissues, and its high expression is closely linked to tumor invasion depth, lymph node metastasis, and peri-nerve invasion. Patients with high ORAI1 expression experience shortened overall survival. CRC cell lines also exhibit upregulated ORAI1 expression. Although ORAI1 downregulation suppresses cell proliferation, this growth inhibition is not attributed to augmented apoptosis, and STIM1 does not participate in the regulation of CRC cell proliferation[81].

Prostate cancer

Prostate cancer, one of the most prevalent malignancies in men, is characterized by the aberrant proliferation and propagation of malignant cells within prostate tissue. In the context of prostate cancer, the expression profile of P2X7R exhibits a distinctive stage-specific pattern, initially appearing in the nucleus, progressing to the cytoplasm, and ultimately localizing to the apical membrane of epithelial cells. Early biopsy findings revealed that all 114 prostate tissues examined exhibited positive P2X7 staining, indicating the presence of P2X7 at the early stage of prostate cancer[82]. Subsequent investigations demonstrated that the downregulation of P2X7 by siRNA substantially attenuated the *in vitro* migration and invasion of prostate cancer cells driven by ATP or 2′,3′-O-(Benzoyl-4-benzoyl)-adenosine 5′-triphosphate, while also suppressing tumor invasion and metastasis in nude mice. Additionally, the silencing of P2X7 significantly reduced the expression of EMT/invasion-related genes, namely Snail, e-cadherin, claudin-1, interleukin (IL)-8, and matrix metalloproteinase-3, along with dampening the phosphorylation of PI3K/AKT and ERK1/2[83].

Moreover, P2X4 protein exhibits expression in prostate epithelial cells, a specific subset of CD66+ neutrophils, and the majority of CD68+ macrophages. Elevated P2X4 expression in prostate cancer has been associated with post-radical prostatectomy metastasis. Depletion of the P2X4 gene leads to a reduction in the growth, migration, and invasion of prostate cancer cells. Furthermore, knockout of P2X4 in Myc-CaP cells results in a significant decrease in the subcutaneous growth of allografts in FVB/NJ mice[84]. Additionally, other investigations have demonstrated that indoline derivatives can activate the P2Y1R receptor and induce mitochondrial apoptosis signaling[85]. In prostate cancer cells, the P2Y2 receptor shows a notable expression. Suppression of the P2Y2 receptor inhibits cell invasion and metastasis. Moreover, ATP presence promotes the expression of IL-8 and Snail genes while inhibiting the expression of E-cadherin and Claudin-1. Consequently, knockdown of the P2Y2 receptor affects the expression of these EMT/invasion-related genes both *in vitro* and *in vivo*[86].

The functional interplay between STIM1 and ORAI1, as well as the calcium channel selectivity of ORAI1, are crucial for its pro-apoptotic effect. Furthermore, it was observed that resistance to apoptosis in androgen-independent prostate cancer cells was associated with the down-regulation of ORAI1 expression and store-operated calcium entry. Upon ORAI1 restoration, steroid-deprived cells transfected with ORAI1 exhibited reestablished channel currents for calcium storage operations, leading to the restoration of normal apoptosis rates. Therefore, irrespective of the stimulus inducing apoptosis, ORAI1 plays a vital role in initiating apoptosis and establishing an anti-apoptotic phenotype in prostate cancer cells[87].

Concurrently, STIM1 and ORAI1 have been demonstrated to hinder cell growth by arresting human prostate cancer cells in the G_0/G_1 phase and promoting cell senescence. Additionally, STIM1 and ORAI1 inhibit the NF-κB signaling pathway and remodel the tumor microenvironment by reducing the formation of M2-type macrophages, potentially creating an unfavorable milieu for tumor growth inhibition. However, STIM1 can also promote cell migration and EMT through the activation of transforming growth factor-beta, Snail, and Wnt/β-Catenin pathways[88]. These findings collectively indicate that STIM1 and ORAI1 play a multifaceted and vital regulatory role in prostate cancer development, encompassing crucial biological processes such as cancer cell growth, apoptosis, and metastasis.

Therefore, this paper discussed prevalent cancer types globally. Table 1 below enumerates the functions and subcellular localizations of these genes during AICD[89-105]. Due to the limited availability of cancer prognosis-related information regarding AICD genes, an extensive analysis was conducted using clinical data from the database provided by the American Cancer Letters and Biology Institute (https://www.aclbi.com/static/index.html/). Table 1, establishes a comprehensive gene prognosis model centered on AICD, aiming to assess the prognostic significance of individual genes across several types of cancer.

AICD AS POTENTIAL CANCER TREATMENT

The elucidation of the AICD mechanism has offered valuable insights into prospective drug investigations, underscoring its promising potential in cancer treatment. In recent years, there has been a notable surge of interest within the scientific community towards harnessing the AICD mechanisms for cancer therapy. This intricate mechanism involves the engagement of eATP with the P2X7R located on the cell membrane's surface, culminating in heightened intracellular calcium ion levels and concurrent activation of the PI3K/Akt signaling cascade, which impacts molecules including NF-KB, toll-like receptor 4, and tumor necrosis factor-alpha (TNF-α), ultimately triggering cell death. This comprehensive exploration into the molecular intricacies furnishes a robust scientific foundation for the future development of novel therapeutics targeting this pathway.

Caffeine exerts its impact by facilitating the degradation of intracellular adenylate (AMP), thereby intensifying the cellular consumption of ATP. In the context of the rat brain, a notable interplay emerged between chronic high-intensity interval training (HIIT) and caffeine consumption, revealing a linkage to the activity of Na^+-K^+-ATPase and antioxidant enzymes within the brain, alongside the manifestation of anti-anxiety behaviors. Notably, caffeine administration was observed to amplify anxiety-related behaviors, while concurrently mitigating alterations induced by HIIT in the antioxidant system and Na^+-K^+-ATPase activity[106]. This implies that caffeine could potentially heighten AMP degradation through the modulation of ATPase activity. Notably, a mitochondrial reverse transport inhibitor, atractyloside, perturbs adenylate transport within mitochondria, thus precipitating intracellular ATP degradation.

Furthermore, recent investigations have revealed a spectrum of novel P2X7R inhibitors, including emodin, which have demonstrated substantial efficacy in suppressing P2X7R-mediated breast cancer invasion, signifying their promising potential for prospective clinical applications[64]. A notable example is brilliant blue G (BBG), a P2X7R inhibitor, crucial in addressing bone cancer pain. Noteworthy findings have indicated that BBG-mediated inhibition of P2X7R or

utilization of small interfering RNA directed against P2X7 in RVM distinctly diminishes spinal cord 5-HT levels and Fos expression[107]. Additionally, it is noteworthy that P2Y12 receptor selective antagonists play a vital role in diverse malignancies. Clopidogrel, for instance, has been identified as an efficacious selective P2Y12 receptor antagonist, pivotal in orchestrating platelet function regulation and eliciting positive effects in the context of cancer[108].

Furthermore, the dose-dependent attenuation of ATP-induced intracellular calcium concentration signaling [$(Ca^{2+})i$] through the phospholipase C inhibitor U73122 underscores its important role. These pharmacological attributes compellingly underscore the functional expression of G-protein-coupled P2Y2 receptors in esophageal squamous cell cells[109]. To encapsulate, P2 receptor-associated inhibitors confer potent suppression of tumor cell proliferation, invasion, immune modulation, angiogenesis, and tumor microenvironment regulation, as well as influencing drug targets and enhancing chemotherapy sensitization. Moreover, these inhibitors may fortify immune cell-mediated tumor assaults, thus augmenting therapeutic outcomes.

Suppression of PANX1 protein levels through shRNA-mediated downregulation or application of channel-blocking agents such as carbenoxolone and probenecid has robustly attenuated cell proliferation and migration, concurrently stimulating melanin synthesis. Intriguingly, cell surface biotin labeling analysis revealed an intracellular reservoir of PANX1 within melanoma cells. Notably, PANX1's potential modulation of signal transduction *via* the Wnt/β-catenin pathway is underscored by the significant reduction in β-catenin levels following PANX1 silencing[110]. Concurrently, berberine (BBR) exhibited notable effects on MDA-MB-231 cell viability, fostering dose-dependent lactate dehydrogenase release, while effectively curtailing colony formation and migratory potential. BBR further exhibited marked suppression of pro-inflammatory cytokine secretion, including IL-1α, IL-1β, IL-6, and TNF-α[111]. Subsequent investigations revealed downregulated expressions of P2X P2X7, NOD-like receptor family pyrin domain containing 3 (NLRP3), pre-Caspase-1, apoptosis-related speckle-like protein (ASC) encompassing caspase activation and recruitment domains, Caspase-1 p20, IL-18, and IL-1β in the NLRP3 inflammatory body pathway. Moreover, decreased mRNA levels of NLRP3, caspase-1, and ASC further corroborated these findings[111].

The concept of AICD mechanism has garnered significant interest within the realm of cancer therapy, emerging as a focal point for exploration within innovative anti-cancer therapeutic avenues. Serving as a fundamental underpinning of cell demise, the AICD mechanism is intrinsically intertwined, either directly or indirectly, with diverse modes of cell death. This interplay holds the potential to reveal intricate associations among distinct cell death modalities. Recent investigations underscore the promise of harnessing AICD as a catalyst for novel therapeutic approaches, potentially encompassing novel drug development and synergistic utilization with established treatments to enhance therapeutic efficacy. Nevertheless, while the appeal of the AICD mechanism is compelling, its practical application necessitates further comprehensive scrutiny, aimed at elucidating intricate molecular underpinnings, refining its applicability spectrum, and addressing safety parameters. Furthermore, this study made use of the ClinicalTrials.gov website (https://clinicaltrials.gov/), a comprehensive repository of clinical trial information, to compile a list of AICD-associated genes that have undergone completed clinical trials (Table 2).

REFLECTIONS ON ATP AND AICD

The intricate interplay between ATP and AICD within the tumor microenvironment, its intersection with anti-tumor immunity, and the nuanced impact of individual variances on cancer progression and therapeutic responsiveness pose an interesting challenge for scientific research. Firstly, while the pivotal role of ATP in instigating apoptotic cascades within neoplastic cells is acknowledged, the precise orchestration of its regulatory mechanisms remains unknown.

Immunological integrity serves as a robust "fortification" to the human body. However, the link between extrinsic factors and unhealthy lifestyles may affect the strength of immune cells over time, leading to gradual weakness and an eventual breach in the body's protective barrier. Consequently, the body becomes susceptible to infections and ailments. AICD has demonstrated its potential to galvanize the immune system. However, the specific recognition and response mechanisms of immune cells against antigens liberated by AICD remain shrouded in mystery. The elevated metabolic activity of tumor cells and their heightened demise in the TME lead to an augmented ATP concentration. Remarkably, ATP undergoes gradual enzymatic transformation into adenosine through the sequential CD39→Ecto-5′-Nucleotidase →androgen receptor pathway. Consequently, the dynamic distribution and concentration of ATP in the tumor microenvironment represents an unsolved conundrum that warrants closer investigation.

The notion of a specific immune response pertains to the targeted immune reaction directed against a particular pathogenic entity. Molecules intricately linked with immunological responses possess the capacity to instigate cell death, often paralleled by the demise of infected cellular hosts. However, the induction of cell death through ATP activation may yield diverse outcomes in distinct immune cell types. Heterogeneous immune cell populations exhibit varying sensitivities to ATP-triggered cell death, thereby influencing the vigor and efficiency of immune functionalities. Interventions targeting the adenosine pathway not only counteract immunosuppression but also amplify ATP accumulation within the tumor microenvironment through the CD39 blockade. Abundant ATP receptors in immune cells, including dendritic cells, macrophages, and neutrophils, foster heightened immune activity upon exposure to eATP.

Furthermore, the intricate role of ATP in modulating immunosuppressive dynamics within the tumor microenvironment remains partially veiled. Often characterized by immunosuppressive traits, the tumor microenvironment's potential for immune subversion, and whether ATP release can serve as a countermeasure to revert this suppressive state, warrant further exploration. Remarkably, individual responsiveness to ATP stimulation may exhibit substantial variation, potentially rendering certain individuals more predisposed to heightened susceptibility to AICD, while others may manifest attenuated responses. Genetic idiosyncrasies among individuals underpin a broad spectrum of cancer treatment

Table 2 Clinical trials for adenosine triphosphate induced cell death

NCT number	Conditions	Drugs	Brief summary
NCT02587819	Carcinoma, basal cell	Treatment with BSCT	This phase 1 clinical trial assesses the safety of BSCT (anti-nf-P2X7) 10% Ointment in basal cell Carcinoma patients
NCT03088644	Healthy	Drug: JNJ-54175446; Drug: 18F-JNJ-64413739	Open-label trial investigates P2X7R occupancy using PET tracer 18F-JNJ-64413739 for P2X7R with JNJ-54175446
NCT03437590	Healthy	Drug: JNJ-55308942; Drug: [18F]-JNJ-64413739	The primary objective of this investigation is to quantify the inhibition of [18F]-JNJ-64413739 uptake in the brain upon achieving peak plasma concentration (Tmax) and at 24 hours after administering a single dose of JNJ-55308942. Additionally, this study aims to establish a comprehensive model for understanding the interplay between JNJ-55308942 exposure and its receptor interactions
NCT01664000	Solid tumors	Drug: Thioureidobutyronitrile	A phase 1 open-label trial with dose escalation is being conducted to explore the safety, pharmacokinetics, and pharmacodynamics of intravenous kevetrin (thioureidobutyronitrile) in advanced solid tumor patients
NCT00899158	Pancreatic cancer	Other: Immunologic techniques; Other: Laboratory biomarker analysis; procedure: Biopsy	The study seeks to clarify how caspase-3, phosphatidylinositol-3 kinase, and 3-methylhistidine contribute to skeletal muscle wasting in weight loss among pancreatic cancer patients
NCT04972188	Healthy	ZYIL1 capsule	This phase I study investigates the safety, tolerability, pharmacokinetics, and pharmacodynamics of orally administered ZYIL1 in healthy adult subjects through a prospective, open-label, multiple-dose approach
NCT04015076	Healthy	Drug: Inzomelid; Drug: Placebo	This phase 1 study aims to assess the safety, tolerability, pharmacokinetics, pharmacodynamics, and food effects of Inzomelid in healthy adults through a randomized, double-blind, placebo-controlled design. An open-label cohort will also verify the safety, pharmacokinetics, and pharmacodynamics of Inzomelid in adult patients with cryopyrin-associated periodic syndromes
NCT04938414	Subarachnoid hemorrhage, aneurysmal	Diagnostic test: Lumbar puncture	Caspase-1 inhibition mitigates pyroptotic neuroinflammation and alleviates cerebrospinal fluid circulation impairment post subarachnoid hemorrhage
NCT02872818	Apoptotic signal pathways in endometrial hyperplasia	Drug: 17β estradiol hemihydrate; Drug: Metformin; Drug: Medroxyprogesterone acetate	This study aims to clarify apoptotic signaling pathways involving Survivin, Bcl-2, Bax, c-Myc, and caspase-9 in a rat model of iatrogenic endometrial hyperplasia treated with metformin and medroxyprogesterone acetate
NCT02466516	Non-alcoholic steato-hepatitis	Drug: SEL; Biological: SIM	This phase 2 randomized, open-label trial evaluates the safety, tolerability, and efficacy of GS-4997 alone or combined with simtuzumab (SIM) in non-alcoholic steatohepatitis subjects with F2-F3 fibrosis stages
NCT00169130	Lymphoma, large-cell, diffuse	Drug: Doxorubicin; Drug: Cyclophosphamide; Procedure: Autologous stem cell transplantation	This prospective study investigates the ACVBP regimen followed by autologous stem cell transplantation in treatment-naive patients aged 60 or below with low-intermediate risk diffuse large B-cell lymphoma and BCL-2 overexpression
NCT02582879	Chronic Lymphocytic Leukemia (CLL)	NA	This multicenter, prospective, observational registry examines CLL/SLL patients initiating approved oral kinase inhibitors, BCL-2 inhibitors, or other anti-CLL therapies. The study aims to comprehensively analyze treatment patterns, including patient characteristics, resource use, clinical outcomes, and patient-reported outcomes
NCT02226965	Lymphoma, diffuse large B-Cell	Drug: PNT2258	A phase II trial investigates PNT2258 in patients with relapsed or refractory diffuse large B-cell lymphoma
NCT00005032	Lung cancer	Biological: Oblimersen sodium; Drug: Paclitaxel	A Phase I/II trial explores the combination of G3139, a BCL-2 antisense oligonucleotide, with paclitaxel for treating recurrent small cell lung cancer
NCT02419560	Lymphoma, mantle-cell recurrent lymphoma, mantle-cell	Drug: ABT-199 and ibrutinib combination	This study aims to determine the optimal dosing regimen for combining ibrutinib with ABT-199 to treat relapsed or refractory mantle cell lymphoma
NCT00085228	Prostate cancer	Biological: Oblimersen sodium; Drug: docetaxel	Docetaxel and similar agents block tumor cell division through diverse mechanisms, while oblimersen may boost docetaxel's impact by sensitizing tumor cells to enhance its efficacy
NCT03255096	Diffuse large B-cell lymphoma high-grade B-cell lymphoma	Drug: RO6870810; Drug: Venetoclax; Drug: Rituximab	An open-label Phase Ib study assessing the safety, pharmacokinetics, and clinical effects of RO6870810 and Venetoclax in patients with relapsed/refractory DLBCL and/or high-grade B-cell lymphoma carrying gene rearrangements (MYC and/or BCL2 and/or BCL6), with or without Rituximab
NCT00001572	B Cell lymphoma follicular lymphoma	Drug: Id-KLH Vaccine; Drug: QS-21 (Stimulation-QS-21) Drug	To evaluate new vaccine formulations for viability and adverse effects, as well as analyze immune responses targeting the patient's lymphoma-specific

	neoplasm		idiotype
NCT00062010	Lung cancer	Biological: Interferon alpha; Drug: 13-cis-retinoic acid; Drug: Paclitaxel	In patients with recurrent small cell lung cancer undergoing interferon alfa, isotretinoin, and paclitaxel treatment, the investigation aims to determine treatment response frequency and duration, evaluate regimen toxicity, assess overall survival duration, and explore potential links between bcl-2 levels in peripheral blood monocytes and treatment outcomes
NCT00039481	Cardiac toxicity; unspecified childhood solid tumor, protocol specific	Biological: Oblimersen sodium; Drug: dexrazoxane hydrochloride; Drug: Doxorubicin hydrochloride	In this phase I trial, oblimersen's effectiveness, combined with chemotherapy and dexrazoxane, is assessed for treating relapsed or refractory solid tumors in youth. Chemotherapeutic agents inhibit tumor cell division through diverse mechanisms, impeding growth or triggering cell death. Oblimersen is anticipated to heighten the potency of doxorubicin and cyclophosphamide by increasing tumor cell sensitivity. Dexrazoxane, a chemoprotective agent, may also shield normal cells from chemotherapy's adverse effects
NCT00666666	Adenocarcinoma of the prostate stage iv prostate cancer	Drug: AT-101; Drug: Bicalutamide; Other: LHRH agent	In this phase II trial, gossypol's potential to hinder tumor cell growth by blocking blood flow is studied when combined with androgen ablation therapy for newly diagnosed metastatic prostate cancer. Androgens stimulate prostate tumor cell proliferation, which can be reduced by luteinizing hormone-releasing hormone agonists and drugs such as bicalutamide. The simultaneous use of gossypol and androgen ablation therapy appears to hold potential as a viable treatment approach for prostate cancer
NCT00003103	Bladder cancer breast cancer colorectal cancer	Biological: Oblimersen sodium; Drug: Docetaxel	This phase I/II trial evaluates oblimersen's effectiveness in treating solid tumors that have not responded to previous therapies, utilizing various mechanisms to halt tumor cell division, leading to growth arrest or cell death
NCT03080311	Small cell lung cancer; solid tumor	Drug: APG-1252	In this Phase I trial, the safety, pharmacokinetic, and pharmacodynamic profiles of intravenously administered APG-1252 are examined in patients with small cell lung cancer or other solid tumors
NCT00016263	Melanoma (skin)	Biological: Oblimersen sodium; Drug: Dacarbazine	This randomized study compares Dacarbazine alone to Dacarbazine combined with G3139 (Bcl-2 Antisense Oligonucleotide) in patients with advanced malignant melanoma
NCT00169000	Metastatic breast cancer	Drug: Capecitabine; Drug: Docetaxel	Phase I study using accelerated titration design to determine MTD of capecitabine (days 1-14) combined with fixed dose docetaxel (75 mg/m2 IV, day 8). Nine patients will be treated at MTD, evaluating pharmacokinetics, Bax: Bcl-2 ratios, and antitumor response
NCT02997423	Glioblastoma		This multi-institutional, consortium-based, non-interventional study aims to assess if high cytochrome c oxidase activity in newly diagnosed primary GBM tumor specimens is linked to reduced overall survival (primary outcome) and progression-free survival (secondary outcome) times
NCT01205503	Breast cancer non-hodgkin's lymphoma	Drug: Mesna; Drug: Saline; Drug: Doxorubicin	This study aims to investigate if mesna can inhibit specific chemical alterations in the blood of doxorubicin-treated patients. Researchers hypothesize that these changes may be associated with "chemobrain," a cognitive impairment reported by some chemotherapy recipients
NCT01037790	Adult solid tumor adenocarcinoma of the colon adenocarcinoma of the rectum	Drug: PD-0332991	PD 0332991 has the potential to hinder tumor cell growth by blocking key enzymes vital for cell proliferation. This phase II trial evaluates PD 0332991's effectiveness and side effects in treating patients with resistant solid tumors
NCT02154490	Recurrent squamous cell lung carcinoma stage iv squamous cell lung carcinoma AJCC v7	Drug: Docetaxel; biological: Durvalumab; Drug: Erlotinib hydrochloride	Create a National Clinical Trials Network for screening sizable yet homogeneous cancer populations, assigning them to a multi-sub-study "Master Protocol." Assess the screen success rate, defined as the percentage of screened patients enrolling in a therapeutic sub-study

outcomes and their efficacy. The profound impact of individual variations in ATP responsiveness on cancer progression and therapeutic response underscores a pressing inquiry, necessitating thorough investigation into the underpinning mechanisms and conceivable implications.

Additionally, the intricate interplay between the complex and diversified tumor microenvironment and individualized patterns of ATP responsiveness can engender pronounced dissimilarities in cell death incidence and severity. Such variances may closely interlink with the tempo of tumor evolution, aggressiveness, and treatment susceptibility. Nonetheless, a comprehensive resolution to this enigma remains elusive, with further research needed to unravel the intricate relationships between ATP responsiveness, individual differences, and the multifaceted intricacies of the tumor microenvironment.

LIMITATIONS AND FUTURE

ATP, an essential extracellular signaling molecule, has been recognized as a cause of cell death induced by high eATP concentrations. It can trigger cell death through diverse mechanisms and directly impact tumor cells to inhibit their

proliferation, invasion, and metastasis. Additionally, ATP can hinder tumor development by activating the immune system. However, the precise mechanisms and occurrence of AICD have been the subject of debate and remain unclear until now, despite preliminary insights into the relationship between AICD and cancer having been gained. Further investigation is warranted to elucidate the intricate mechanisms underlying AICD, particularly at the cellular and molecular levels There also needs to be a comprehensive characterization of the distinctive changes associated with this process. Additionally, a comprehensive understanding of the interplay and relative significance of AICD in relation to other cell death pathways in diverse disease contexts is crucial. Moreover, investigating the varied responses of different cell types to AICD and exploring potential cell-specific mechanisms are important avenues for future research. These endeavors will enhance our understanding of the molecular mechanisms governing AICD, facilitate the identification of novel regulators, and offer new targets and strategies for the development of cancer therapies and other related diseases.

The introduction of the concept of AICD has sparked increasing interest among researchers regarding its association with tumors. Investigations into this relationship have encompassed numerous prevalent cancer types, examining the correlation between AICD and various tumor characteristics. However, due to insufficient biological evidence and experimental verification, these studies have offered indirect evidence of the connection between AICD and cancer. The precise role of genes in the direct or indirect interplay between AICD and tumors remains unclear. Consequently, these studies have been unable to identify the genes and features that may exert a more significant influence on the relationship between AICD and cancer. Consequently, further research is imperative to comprehensively explore and validate the intricate association between AICD and cancer, ultimately identifying the pivotal factors involved in this interplay.

Moving forward, it is crucial to validate the potential of AICD in clinical applications and advance the development of therapeutic strategies that induce AICD with high efficiency and selectivity. Additionally, synergistic combinations with immunotherapy should be further explored. In summary, AICD represents an autonomous and innovative cell death paradigm. However, comprehensive investigations are needed to elucidate the precise mechanisms underlying AICD and establish the intricate connections between AICD and cancer.

CONCLUSION

ATP serves as a vital extracellular signaling molecule for cell survival, yet excessive ATP can induce cell death. With the introduction of the concept of AICD, extensive literature has emerged focusing on its investigation and elucidation. Researchers have made discoveries regarding ATP-activated proteins and provided comprehensive reviews on the topic. However, a comprehensive synthesis of the literature remains lacking, especially an overview of the mechanisms underlying AICD. Further investigation is needed to explore the intricate details of AICD, particularly in terms of its cross-regulation and mutual influence with other cell death pathways, as well as its relative importance in various disease conditions. Moreover, the distinctive changes occurring at the cellular and molecular levels during AICD have yet to be fully described.

This paper provides an in-depth exploration of the multifaceted mechanisms through which AICD. It delineates how ATP serves as a mediator of apoptosis *via* diverse pathways, encompassing the activation of caspases within the cysteine protease family, the regulation of mitochondrial membrane potential, and the modulation of apoptosis-related protein expression. Additionally, ATP exerts a profound impact on cancer cells by instigating various forms of cell necrosis, including necrotic apoptosis and necrotic tumor cell death. The involvement of ATP in orchestrating the delicate balance between cell survival and death is underscored through its regulation of the autophagy process.

In the realm of cancer biology, ATP emerges as a pivotal regulator influencing tumor cell proliferation, invasion, and metastasis. The article underscores ATP's role in impeding tumor growth by activating apoptosis pathways and enhancing immune-mediated tumor clearance through the induction of tumor cell necrosis. Furthermore, ATP's contribution extends to the modulation of the tumor microenvironment, influencing factors such as inflammation and immune responses, thereby exerting a significant impact on tumor development.

On the therapeutic front, the study accentuates the potential of ATP as a therapeutic agent for inducing cell death. By precisely adjusting ATP levels and subsequently activating core pathways involved in cell death, targeted induction of tumor cell death becomes achievable, offering promising prospects for therapeutic intervention. This comprehensive exploration establishes a crucial theoretical foundation for future research endeavors and clinical applications.

ACKNOWLEDGEMENTS

The authors extend their sincere gratitude to all participants who wholeheartedly engaged in this study, offering their invaluable assistance and unwavering support.

FOOTNOTES

Co-corresponding authors: Doblin Sandai and Zhi-Jing Song.

Author contributions: Zhang HL presented the conceptualization and methodology, analyzed the data, and wrote the first draft; Zhang ZW, Song ZJ, Babu D, Tabana Y, Dahham SS, Adam Ahmed Adam M, Wang Y, and Wang W presented the conceptualization and methodology, analyzed the data, and conducted the review and editing; Zhang HL, Zhao R, Barakat K, Harun MSR, Shapudin SNM and

Lok B collected and collated the data and produced a visual atlas; Sandai D presents the conceptualization and methodology, revises manuscripts, conducts reviews and edits, and is responsible for project management; Zhang HL presented the conceptualization and methodology, analyzed the data, and wrote the first draft; Zhang ZW, Song ZJ, Babu D, Tabana Y, Dahham SS, Adam Ahmed Adam M, Wang Y, and Wang W presented the conceptualization and methodology, analyzed the data, and conducted the review and editing; Zhang HL, Zhao R, Barakat K, Harun MSR, Shapudin SNM and Lok B collected and collated the data and produced a visual atlas; Sandai D presents the conceptualization and methodology, revises manuscripts, conducts reviews and edits, and is responsible for project management; both Sandai D and Song ZJ give detailed guidance to this paper, which is of great significance. Therefore, as a co-corresponding author; all authors have read and approve the final manuscript.

Supported by National Natural Science Foundation of China, No. 81960877; University Innovation Fund of Gansu Province, No. 2021A-076; Gansu Province Science and Technology Plan (Innovation Base and Talent Plan), No. 21JR7RA561; Natural Science Foundation of Gansu Province, No. 21JR1RA267 and No. 22JR5RA582; Education Technology Innovation Project of Gansu Province, No. 2022A-067; Innovation Fund of Higher Education of Gansu Province, No. 2023A-088; Gansu Province Science and Technology Plan International Cooperation Field Project, No. 23YFWA0005.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Malaysia

ORCID number: Sandai Doblin 0000-0003-0544-4260; Zhi-Jing Song 0009-0002-3991-2907.

S-Editor: Qu XL
L-Editor: A
P-Editor: Zhang XD

REFERENCES

1 **Jun DJ**, Kim J, Jung SY, Song R, Noh JH, Park YS, Ryu SH, Kim JH, Kong YY, Chung JM, Kim KT. Extracellular ATP mediates necrotic cell swelling in SN4741 dopaminergic neurons through P2X7 receptors. *J Biol Chem* 2007; **282**: 37350-37358 [PMID: 17962183 DOI: 10.1074/jbc.M707915200]

2 **Mello Pde A**, Filippi-Chiela EC, Nascimento J, Beckenkamp A, Santana DB, Kipper F, Casali EA, Nejar Bruno A, Paccez JD, Zerbini LF, Wink MR, Lenz G, Buffon A. Adenosine uptake is the major effector of extracellular ATP toxicity in human cervical cancer cells. *Mol Biol Cell* 2014; **25**: 2905-2918 [PMID: 25103241 DOI: 10.1091/mbc.E14-01-0042]

3 **Song S**, Jacobson KN, McDermott KM, Reddy SP, Cress AE, Tang H, Dudek SM, Black SM, Garcia JG, Makino A, Yuan JX. ATP promotes cell survival *via* regulation of cytosolic [Ca2+] and Bcl-2/Bax ratio in lung cancer cells. *Am J Physiol Cell Physiol* 2016; **310**: C99-114 [PMID: 26491047 DOI: 10.1152/ajpcell.00092.2015]

4 **Prahoveanu E**, Petrescu A, Moisa I. The effect of ATP on lactic dehydrogenase (LDH) activity and influenza B virus multiplication. *Virologie* 1975; **26**: 51-55 [PMID: 1216689]

5 **Hide I**, Tanaka M, Inoue A, Nakajima K, Kohsaka S, Inoue K, Nakata Y. Extracellular ATP triggers tumor necrosis factor-alpha release from rat microglia. *J Neurochem* 2000; **75**: 965-972 [PMID: 10936177 DOI: 10.1046/j.1471-4159.2000.0750965.x]

6 **Grinwald PM**. Positive feedback in the living process: the role of ATP in ischaemic cell death. *Med Hypotheses* 1977; **3**: 138-143 [PMID: 895588 DOI: 10.1016/0306-9877(77)90061-5]

7 **Nagelkerke JF**, Dogterom P, De Bont HJ, Mulder GJ. Prolonged high intracellular free calcium concentrations induced by ATP are not immediately cytotoxic in isolated rat hepatocytes. Changes in biochemical parameters implicated in cell toxicity. *Biochem J* 1989; **263**: 347-353 [PMID: 2597107 DOI: 10.1042/bj2630347]

8 **Zheng LM**, Zychlinsky A, Liu CC, Ojcius DM, Young JD. Extracellular ATP as a trigger for apoptosis or programmed cell death. *J Cell Biol* 1991; **112**: 279-288 [PMID: 1988462 DOI: 10.1083/jcb.112.2.279]

9 **Vandewalle B**, Hornez L, Revillion F, Lefebvre J. Effect of extracellular ATP on breast tumor cell growth, implication of intracellular calcium. *Cancer Lett* 1994; **85**: 47-54 [PMID: 7923101 DOI: 10.1016/0304-3835(94)90237-2]

10 **Ferrari D**, Stroh C, Schulze-Osthoff K. P2X7/P2Z purinoreceptor-mediated activation of transcription factor NFAT in microglial cells. *J Biol Chem* 1999; **274**: 13205-13210 [PMID: 10224077 DOI: 10.1074/jbc.274.19.13205]

11 **Brough D**, Le Feuvre RA, Iwakura Y, Rothwell NJ. Purinergic (P2X7) receptor activation of microglia induces cell death *via* an interleukin-1-independent mechanism. *Mol Cell Neurosci* 2002; **19**: 272-280 [PMID: 11860279 DOI: 10.1006/mcne.2001.1054]

12 **Seki S**, Tsukimoto M, Suzuki A, Hattori F, Takai Erina, Oshima Y. , Kajima S. Anti-angiogenic effect of P2X7 receptor antagonist oxidized ATP as a mechanism of anti-tumor growth. *Pharmaceut Anal Acta* 2012; **3** . Available from: https://www.researchgate.net/publication/290487342_Anti angiogenic_effect_of_P2X7_receptor_antagonist_oxidized_ATP_as_a_mechanism_of_anti-tumor_growth

13 **Wang X**, Li L, Guan R, Zhu D, Song N, Shen L. Emodin Inhibits ATP-Induced Proliferation and Migration by Suppressing P2Y Receptors in Human Lung Adenocarcinoma Cells. *Cell Physiol Biochem* 2017; **44**: 1337-1351 [PMID: 29183030 DOI: 10.1159/000485495]

14 **Trautmann A**. Extracellular ATP in the immune system: more than just a "danger signal". *Sci Signal* 2009; **2**: pe6 [PMID: 19193605 DOI: 10.1126/scisignal.256pe6]

15 **Ryoden Y**, Nagata S. The XK plasma membrane scramblase and the VPS13A cytosolic lipid transporter for ATP-induced cell death. *Bioessays* 2022; **44**: e2200106 [PMID: 35996795 DOI: 10.1002/bies.202200106]

16 **Buttigieg J**, Nurse CA. Detection of hypoxia-evoked ATP release from chemoreceptor cells of the rat carotid body. *Biochem Biophys Res Commun* 2004; **322**: 82-87 [PMID: 15313176 DOI: 10.1016/j.bbrc.2004.07.081]

17 **Davidson AL**, Dassa E, Orelle C, Chen J. Structure, function, and evolution of bacterial ATP-binding cassette systems. *Microbiol Mol Biol Rev* 2008; **72**: 317-364, table of contents [PMID: 18535149 DOI: 10.1128/MMBR.00031-07]

18 **Boyd-Tressler A**, Penuela S, Laird DW, Dubyak GR. Chemotherapeutic drugs induce ATP release *via* caspase-gated pannexin-1 channels and a caspase/pannexin-1-independent mechanism. *J Biol Chem* 2014; **289**: 27246-27263 [PMID: 25112874 DOI: 10.1074/jbc.M114.590240]

19 **Kalsi KK**, González-Alonso J. Temperature-dependent release of ATP from human erythrocytes: mechanism for the control of local tissue perfusion. *Exp Physiol* 2012; **97**: 419-432 [PMID: 22227202 DOI: 10.1113/expphysiol.2011.064238]

20 **Gourine AV**, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, Teschemacher AG, Spyer KM, Deisseroth K, Kasparov S. Astrocytes control breathing through pH-dependent release of ATP. *Science* 2010; **329**: 571-575 [PMID: 20647426 DOI: 10.1126/science.1190721]

21 **Kato Y**, Hiasa M, Ichikawa R, Hasuzawa N, Kadowaki A, Iwatsuki K, Shima K, Endo Y, Kitahara Y, Inoue T, Nomura M, Omote H, Moriyama Y, Miyaji T. Identification of a vesicular ATP release inhibitor for the treatment of neuropathic and inflammatory pain. *Proc Natl Acad Sci U S A* 2017; **114**: E6297-E6305 [PMID: 28720702 DOI: 10.1073/pnas.1704847114]

22 **Li Z**, Gu Y, Wen R, Shen F, Tian HL, Yang GY, Zhang Z. Lysosome exocytosis is involved in astrocyte ATP release after oxidative stress induced by H(2)O(2). *Neurosci Lett* 2019; **705**: 251-258 [PMID: 30928480 DOI: 10.1016/j.neulet.2019.03.046]

23 **Zhang C**, He H, Wang L, Zhang N, Huang H, Xiong Q, Yan Y, Wu N, Ren H, Han H, Liu M, Qian M, Du B. Virus-Triggered ATP Release Limits Viral Replication through Facilitating IFN-β Production in a P2X7-Dependent Manner. *J Immunol* 2017; **199**: 1372-1381 [PMID: 28687662 DOI: 10.4049/jimmunol.1700187]

24 **Skals M**, Bjaelde RG, Reinholdt J, Poulsen K, Vad BS, Otzen DE, Leipziger J, Praetorius HA. Bacterial RTX toxins allow acute ATP release from human erythrocytes directly through the toxin pore. *J Biol Chem* 2014; **289**: 19098-19109 [PMID: 24860098 DOI: 10.1074/jbc.M114.571414]

25 **Yamamoto K**, Imamura H, Ando J. Shear stress augments mitochondrial ATP generation that triggers ATP release and Ca(2+) signaling in vascular endothelial cells. *Am J Physiol Heart Circ Physiol* 2018; **315**: H1477-H1485 [PMID: 30141983 DOI: 10.1152/ajpheart.00204.2018]

26 **Senior AE**, Nadanaciva S, Weber J. The molecular mechanism of ATP synthesis by F1F0-ATP synthase. *Biochim Biophys Acta* 2002; **1553**: 188-211 [PMID: 11997128 DOI: 10.1016/s0005-2728(02)00185-8]

27 **Braunstein GM**, Roman RM, Clancy JP, Kudlow BA, Taylor AL, Shylonsky VG, Jovov B, Peter K, Jilling T, Ismailov II, Benos DJ, Schwiebert LM, Fitz JG, Schwiebert EM. Cystic fibrosis transmembrane conductance regulator facilitates ATP release by stimulating a separate ATP release channel for autocrine control of cell volume regulation. *J Biol Chem* 2001; **276**: 6621-6630 [PMID: 11110786 DOI: 10.1074/jbc.M005893200]

28 **Vander Heiden MG**, Lunt SY, Dayton TL, Fiske BP, Israelsen WJ, Mattaini KR, Vokes NI, Stephanopoulos G, Cantley LC, Metallo CM, Locasale JW. Metabolic pathway alterations that support cell proliferation. *Cold Spring Harb Symp Quant Biol* 2011; **76**: 325-334 [PMID: 22262476 DOI: 10.1101/sqb.2012.76.010900]

29 **Goron A**, Lamarche F, Blanchet S, Delangle P, Schlattner U, Fontaine E, Moinard C. Citrulline stimulates muscle protein synthesis, by reallocating ATP consumption to muscle protein synthesis. *J Cachexia Sarcopenia Muscle* 2019; **10**: 919-928 [PMID: 31070021 DOI: 10.1002/jcsm.12435]

30 **Potma EJ**, Stienen GJ. Increase in ATP consumption during shortening in skinned fibres from rabbit psoas muscle: effects of inorganic phosphate. *J Physiol* 1996; **496** (Pt 1): 1-12 [PMID: 8910191 DOI: 10.1113/jphysiol.1996.sp021660]

31 **Meyer MR**, Wagmann L, Schneider-Daum N, Loretz B, de Souza Carvalho C, Lehr CM, Maurer HH. P-glycoprotein interactions of novel psychoactive substances - stimulation of ATP consumption and transport across Caco-2 monolayers. *Biochem Pharmacol* 2015; **94**: 220-226 [PMID: 25637762 DOI: 10.1016/j.bcp.2015.01.008]

32 **Iwabuchi S**, Kawahara K. Functional significance of the negative-feedback regulation of ATP release *via* pannexin-1 hemichannels under ischemic stress in astrocytes. *Neurochem Int* 2011; **58**: 376-384 [PMID: 21185900 DOI: 10.1016/j.neuint.2010.12.013]

33 **Anselmi F**, Hernandez VH, Crispino G, Seydel A, Ortolano S, Roper SD, Kessaris N, Richardson W, Rickheit G, Filippov MA, Monyer H, Mammano F. ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca2+ signals across the inner ear. *Proc Natl Acad Sci U S A* 2008; **105**: 18770-18775 [PMID: 19047635 DOI: 10.1073/pnas.0800793105]

34 **Johnson ZL**, Chen J. ATP Binding Enables Substrate Release from Multidrug Resistance Protein 1. *Cell* 2018; **172**: 81-89.e10 [PMID: 29290467 DOI: 10.1016/j.cell.2017.12.005]

35 **Zhou Q**, Yan J, Putheti P, Wu Y, Sun X, Toxavidis V, Tigges J, Kassam N, Enjyoji K, Robson SC, Strom TB, Gao W. Isolated CD39 expression on CD4+ T cells denotes both regulatory and memory populations. *Am J Transplant* 2009; **9**: 2303-2311 [PMID: 19656134 DOI: 10.1111/j.1600-6143.2009.02777.x]

36 **Winzer R**, Serracant-Prat A, Brock VJ, Pinto-Espinoza C, Rissiek B, Amadi M, Eich N, Rissiek A, Schneider E, Magnus T, Guse AH, Diercks BP, Koch-Nolte F, Tolosa E. P2X7 is expressed on human innate-like T lymphocytes and mediates susceptibility to ATP-induced cell death. *Eur J Immunol* 2022; **52**: 1805-1818 [PMID: 36178227 DOI: 10.1002/eji.202249932]

37 **Kong H**, Zhao H, Chen T, Song Y, Cui Y. Targeted P2X7/NLRP3 signaling pathway against inflammation, apoptosis, and pyroptosis of retinal endothelial cells in diabetic retinopathy. *Cell Death Dis* 2022; **13**: 336 [PMID: 35410316 DOI: 10.1038/s41419-022-04786-w]

38 **Hanley PJ**, Kronlage M, Kirschning C, Del Rey A, Di Virgilio F, Leipziger J, Chessell IP, Sargin S, Filippov MA, Lindemann O, Mohr S, Königs V, Schillers H, Bähler M, Schwab A. Transient P2X7 receptor activation triggers macrophage death independent of Toll-like receptors 2 and 4, caspase-1, and pannexin-1 proteins. *J Biol Chem* 2012; **287**: 10650-10663 [PMID: 22235111 DOI: 10.1074/jbc.M111.332676]

39 **Xue H**, Lu J, Yuan R, Liu J, Liu Y, Wu K, Wu J, Du J, Shen B. Knockdown of CLIC4 enhances ATP-induced HN4 cell apoptosis through mitochondrial and endoplasmic reticulum pathways. *Cell Biosci* 2016; **6**: 5 [PMID: 26816615 DOI: 10.1186/s13578-016-0070-1]

40 **Lépine S**, Le Stunff H, Lakatos B, Sulpice JC, Giraud F. ATP-induced apoptosis of thymocytes is mediated by activation of P2 X 7 receptor and involves de novo ceramide synthesis and mitochondria. *Biochim Biophys Acta* 2006; **1761**: 73-82 [PMID: 16325464 DOI: 10.1016/j.bbalip.2005.10.001]

41 **Sekar P**, Huang DY, Hsieh SL, Chang SF, Lin WW. AMPK-dependent and independent actions of P2X7 in regulation of mitochondrial and lysosomal functions in microglia. *Cell Commun Signal* 2018; **16**: 83 [PMID: 30458799 DOI: 10.1186/s12964-018-0293-3]

42 **Sarti AC**, Vultaggio-Poma V, Falzoni S, Missiroli S, Giuliani AL, Boldrini P, Bonora M, Faita F, Di Lascio N, Kusmic C, Solini A, Novello S, Morari M, Rossato M, Wieckowski MR, Giorgi C, Pinton P, Di Virgilio F. Mitochondrial P2X7 Receptor Localization Modulates Energy

Metabolism Enhancing Physical Performance. *Function (Oxf)* 2021; **2**: zqab005 [PMID: 35330818 DOI: 10.1093/function/zqab005]
43 **Kerkhofs M**, La Rovere R, Welkenhuysen K, Janssens A, Vandenberghe P, Madesh M, Parys JB, Bultynck G. BIRD-2, a BH4-domain-targeting peptide of Bcl-2, provokes Bax/Bak-independent cell death in B-cell cancers through mitochondrial Ca(2+)-dependent mPTP opening. *Cell Calcium* 2021; **94**: 102333 [PMID: 33450506 DOI: 10.1016/j.ceca.2020.102333]
44 **Wiley JS**, Sluyter R, Gu BJ, Stokes L, Fuller SJ. The human P2X7 receptor and its role in innate immunity. *Tissue Antigens* 2011; **78**: 321-332 [PMID: 21988719 DOI: 10.1111/j.1399-0039.2011.01780.x]
45 **Garrido C**, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ* 2006; **13**: 1423-1433 [PMID: 16676004 DOI: 10.1038/sj.cdd.4401950]
46 **Slee EA**, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, Wang HG, Reed JC, Nicholson DW, Alnemri ES, Green DR, Martin SJ. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J Cell Biol* 1999; **144**: 281-292 [PMID: 9922454 DOI: 10.1083/jcb.144.2.281]
47 **Kuida K**, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P, Flavell RA. Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* 1998; **94**: 325-337 [PMID: 9708735 DOI: 10.1016/s0092-8674(00)81476-2]
48 **Barberà-Cremades M**, Gómez AI, Baroja-Mazo A, Martínez-Alarcón L, Martínez CM, de Torre-Minguela C, Pelegrín P. P2X7 Receptor Induces Tumor Necrosis Factor-α Converting Enzyme Activation and Release to Boost TNF-α Production. *Front Immunol* 2017; **8**: 862 [PMID: 28791020 DOI: 10.3389/fimmu.2017.00862]
49 **Wang Q**, Wang L, Feng YH, Li X, Zeng R, Gorodeski GI. P2X7 receptor-mediated apoptosis of human cervical epithelial cells. *Am J Physiol Cell Physiol* 2004; **287**: C1349-C1358 [PMID: 15269006 DOI: 10.1152/ajpcell.00256.2004]
50 **Harada K**, Hide I, Seki T, Tanaka S, Nakata Y, Sakai N. Extracellular ATP differentially modulates Toll-like receptor 4-mediated cell survival and death of microglia. *J Neurochem* 2011; **116**: 1138-1147 [PMID: 21210814 DOI: 10.1111/j.1471-4159.2011.07170.x]
51 **Schulze-Lohoff E**, Hugo C, Rost S, Arnold S, Gruber A, Brüne B, Sterzel RB. Extracellular ATP causes apoptosis and necrosis of cultured mesangial cells *via* P2Z/P2X7 receptors. *Am J Physiol* 1998; **275**: F962-F971 [PMID: 9843914 DOI: 10.1152/ajprenal.1998.275.6.F962]
52 **Ramírez K T R**, Calzaferri F, Rios C D L, Manuel Vidal-Sanz, Marta Agudo-Barriuso. P2X7R and TNFR1 antagonism neuroprotects rodent retinal ganglion cells in a sepsis model. *Acta Ophthalmologica* 2022; 100 [DOI: 10.1111/j.1755-3768.2022.0676]
53 **Adriouch S**, Dox C, Welge V, Seman M, Koch-Nolte F, Haag F. Cutting edge: a natural P451L mutation in the cytoplasmic domain impairs the function of the mouse P2X7 receptor. *J Immunol* 2002; **169**: 4108-4112 [PMID: 12370338 DOI: 10.4049/jimmunol.169.8.4108]
54 **Li X**, Zhang M, Huang X, Liang W, Li G, Lu X, Li Y, Pan H, Shi L, Zhu H, Qian L, Shan B, Yuan J. Ubiquitination of RIPK1 regulates its activation mediated by TNFR1 and TLRs signaling in distinct manners. *Nat Commun* 2020; **11**: 6364 [PMID: 33311474 DOI: 10.1038/s41467-020-19935-y]
55 **Lu Y**, Yuan T, Min X, Yuan Z, Cai Z. AMPK: Potential Therapeutic Target for Vascular Calcification. *Front Cardiovasc Med* 2021; **8**: 670222 [PMID: 34046440 DOI: 10.3389/fcvm.2021.670222]
56 **Li FJ**, Xu ZS, Soo AD, Lun ZR, He CY. ATP-driven and AMPK-independent autophagy in an early branching eukaryotic parasite. *Autophagy* 2017; **13**: 715-729 [PMID: 28121493 DOI: 10.1080/15548627.2017.1280218]
57 **Schmitt AM**, Crawley CD, Kang S, Raleigh DR, Yu X, Wahlstrom JS, Voce DJ, Darga TE, Weichselbaum RR, Yamini B. p50 (NF-κB1) is an effector protein in the cytotoxic response to DNA methylation damage. *Mol Cell* 2011; **44**: 785-796 [PMID: 22152481 DOI: 10.1016/j.molcel.2011.09.026]
58 **Jacques-Silva MC**, Bernardi A, Rodnight R, Lenz G. ERK, PKC and PI3K/Akt pathways mediate extracellular ATP and adenosine-induced proliferation of U138-MG human glioma cell line. *Oncology* 2004; **67**: 450-459 [PMID: 15714002 DOI: 10.1159/000082930]
59 **Maharati A**, Moghbeli M. PI3K/AKT signaling pathway as a critical regulator of epithelial-mesenchymal transition in colorectal tumor cells. *Cell Commun Signal* 2023; **21**: 201 [PMID: 37580737 DOI: 10.1186/s12964-023-01225-x]
60 **Zhang WJ**, Luo C, Huang C, Pu FQ, Zhu JF, Zhu ZM. PI3K/Akt/GSK-3β signal pathway is involved in P2X7 receptor-induced proliferation and EMT of colorectal cancer cells. *Eur J Pharmacol* 2021; **899**: 174041 [PMID: 33737010 DOI: 10.1016/j.ejphar.2021.174041]
61 **Shiratori M**, Tozaki-Saitoh H, Yoshitake M, Tsuda M, Inoue K. P2X7 receptor activation induces CXCL2 production in microglia through NFAT and PKC/MAPK pathways. *J Neurochem* 2010; **114**: 810-819 [PMID: 20477948 DOI: 10.1111/j.1471-4159.2010.06809.x]
62 **Kitabatake K**, Kaji T, Tsukimoto M. ATP and ADP enhance DNA damage repair in γ-irradiated BEAS-2B human bronchial epithelial cells through activation of P2X7 and P2Y12 receptors. *Toxicol Appl Pharmacol* 2020; **407**: 115240 [PMID: 32941855 DOI: 10.1016/j.taap.2020.115240]
63 **Sakaguchi K**, Herrera JE, Saito S, Miki T, Bustin M, Vassilev A, Anderson CW, Appella E. DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev* 1998; **12**: 2831-2841 [PMID: 9744860 DOI: 10.1101/gad.12.18.2831]
64 **Zhu X**, Li Q, Song W, Peng X, Zhao R. P2X7 receptor: a critical regulator and potential target for breast cancer. *J Mol Med (Berl)* 2021; **99**: 349-358 [PMID: 33486566 DOI: 10.1007/s00109-021-02041-x]
65 **Xia J**, Yu X, Tang L, Li G, He T. P2X7 receptor stimulates breast cancer cell invasion and migration *via* the AKT pathway. *Oncol Rep* 2015; **34**: 103-110 [PMID: 25976617 DOI: 10.3892/or.2015.3979]
66 **Jalaleddine N**, El-Hajjar L, Dakik H, Shaito A, Saliba J, Safi R, Zibara K, El-Sabban M. Pannexin1 Is Associated with Enhanced Epithelial-To-Mesenchymal Transition in Human Patient Breast Cancer Tissues and in Breast Cancer Cell Lines. *Cancers (Basel)* 2019; **11** [PMID: 31817827 DOI: 10.3390/cancers11121967]
67 **McAndrew D**, Grice DM, Peters AA, Davis FM, Stewart T, Rice M, Smart CE, Brown MA, Kenny PA, Roberts-Thomson SJ, Monteith GR. ORAI1-mediated calcium influx in lactation and in breast cancer. *Mol Cancer Ther* 2011; **10**: 448-460 [PMID: 21224390 DOI: 10.1158/1535-7163.MCT-10-0923]
68 **Yang Y**, Jiang Z, Wang B, Chang L, Liu J, Zhang L, Gu L. Expression of STIM1 is associated with tumor aggressiveness and poor prognosis in breast cancer. *Pathol Res Pract* 2017; **213**: 1043-1047 [PMID: 28869106 DOI: 10.1016/j.prp.2017.08.006]
69 **Qiu Y**, Liu Y, Li WH, Zhang HQ, Tian XX, Fang WG. P2Y2 receptor promotes the migration and invasion of breast cancer cells *via* EMT-related genes Snail and E-cadherin. *Oncol Rep* 2018; **39**: 138-150 [PMID: 29115551 DOI: 10.3892/or.2017.6081]
70 **Ma X**, Pan X, Wei Y, Tan B, Yang L, Ren H, Qian M, Du B. Chemotherapy-induced uridine diphosphate release promotes breast cancer metastasis through P2Y6 activation. *Oncotarget* 2016; **7**: 29036-29050 [PMID: 27074554 DOI: 10.18632/oncotarget.8664]
71 **Sarangi S**, Pandey A, Papa AL, Sengupta P, Kopparam J, Dadwal U, Basu S, Sengupta S. P2Y12 receptor inhibition augments cytotoxic effects of cisplatin in breast cancer. *Med Oncol* 2013; **30**: 567 [PMID: 23568163 DOI: 10.1007/s12032-013-0567-y]
72 **Qin J**, Zhang X, Tan B, Zhang S, Yin C, Xue Q, Zhang Z, Ren H, Chen J, Liu M, Qian M, Du B. Blocking P2X7-Mediated Macrophage

Polarization Overcomes Treatment Resistance in Lung Cancer. *Cancer Immunol Res* 2020; **8**: 1426-1439 [PMID: 32933967 DOI: 10.1158/2326-6066.CIR-20-0123]

73 **Wareham K**, Vial C, Wykes RC, Bradding P, Seward EP. Functional evidence for the expression of P2X1, P2X4 and P2X7 receptors in human lung mast cells. *Br J Pharmacol* 2009; **157**: 1215-1224 [PMID: 19552691 DOI: 10.1111/j.1476-5381.2009.00287.x]

74 **Hou MF**, Kuo HC, Li JH, Wang YS, Chang CC, Chen KC, Chen WC, Chiu CC, Yang S, Chang WC. Orai1/CRACM1 overexpression suppresses cell proliferation *via* attenuation of the store-operated calcium influx-mediated signalling pathway in A549 lung cancer cells. *Biochim Biophys Acta* 2011; **1810**: 1278-1284 [PMID: 21782006 DOI: 10.1016/j.bbagen.2011.07.001]

75 **Ge C**, Zeng B, Li R, Li Z, Fu Q, Wang W, Wang Z, Dong S, Lai Z, Wang Y, Xue Y, Guo J, Di T, Song X. Knockdown of STIM1 expression inhibits non-small-cell lung cancer cell proliferation in vitro and in nude mouse xenografts. *Bioengineered* 2019; **10**: 425-436 [PMID: 31564210 DOI: 10.1080/21655979.2019.1669518]

76 **Zhang Y**, Ding J, Wang L. The role of P2X7 receptor in prognosis and metastasis of colorectal cancer. *Adv Med Sci* 2019; **64**: 388-394 [PMID: 31276917 DOI: 10.1016/j.advms.2019.05.002]

77 **Calik I**, Calik M, Turken G, Ozercan IH. A promising independent prognostic biomarker in colorectal cancer: P2X7 receptor. *Int J Clin Exp Pathol* 2020; **13**: 107-121 [PMID: 32211091]

78 **Limami Y**, Pinon A, Leger DY, Pinault E, Delage C, Beneytout JL, Simon A, Liagre B. The P2Y2/Src/p38/COX-2 pathway is involved in the resistance to ursolic acid-induced apoptosis in colorectal and prostate cancer cells. *Biochimie* 2012; **94**: 1754-1763 [PMID: 22521508 DOI: 10.1016/j.biochi.2012.04.006]

79 **Placet M**, Arguin G, Molle CM, Babeu JP, Jones C, Carrier JC, Robaye B, Geha S, Boudreau F, Gendron FP. The G protein-coupled $P2Y_6$ receptor promotes colorectal cancer tumorigenesis by inhibiting apoptosis. *Biochim Biophys Acta Mol Basis Dis* 2018; **1864**: 1539-1551 [PMID: 29454075 DOI: 10.1016/j.bbadis.2018.02.008]

80 **Wang JY**, Sun J, Huang MY, Wang YS, Hou MF, Sun Y, He H, Krishna N, Chiu SJ, Lin S, Yang S, Chang WC. STIM1 overexpression promotes colorectal cancer progression, cell motility and COX-2 expression. *Oncogene* 2015; **34**: 4358-4367 [PMID: 25381814 DOI: 10.1038/onc.2014.366]

81 **Gui L**, Wang Z, Han J, Ma H, Li Z. High Expression of Orai1 Enhances Cell Proliferation and is Associated with Poor Prognosis in Human Colorectal Cancer. *Clin Lab* 2016; **62**: 1689-1698 [PMID: 28164587 DOI: 10.7754/Clin.Lab.2016.160131]

82 **Slater M**, Danieletto S, Gidley-Baird A, Teh LC, Barden JA. Early prostate cancer detected using expression of non-functional cytolytic P2X7 receptors. *Histopathology* 2004; **44**: 206-215 [PMID: 14987223 DOI: 10.1111/j.0309-0167.2004.01798.x]

83 **Qiu Y**, Li WH, Zhang HQ, Liu Y, Tian XX, Fang WG. P2X7 mediates ATP-driven invasiveness in prostate cancer cells. *PLoS One* 2014; **9**: e114371 [PMID: 25486274 DOI: 10.1371/journal.pone.0114371]

84 **Maynard JP**, Lu J, Vidal I, Hicks J, Mummert L, Ali T, Kempski R, Carter AM, Sosa RY, Peiffer LB, Joshu CE, Lotan TL, De Marzo AM, Sfanos KS. P2X4 purinergic receptors offer a therapeutic target for aggressive prostate cancer. *J Pathol* 2022; **256**: 149-163 [PMID: 34652816 DOI: 10.1002/path.5815]

85 **Le HTT**, Murugesan A, Ramesh T, Yli-Harja O, Konda Mani S, Kandhavelu M. Molecular interaction of HIC, an agonist of P2Y1 receptor, and its role in prostate cancer apoptosis. *Int J Biol Macromol* 2021; **189**: 142-150 [PMID: 34425116 DOI: 10.1016/j.ijbiomac.2021.08.103]

86 **Li WH**, Qiu Y, Zhang HQ, Liu Y, You JF, Tian XX, Fang WG. P2Y2 receptor promotes cell invasion and metastasis in prostate cancer cells. *Br J Cancer* 2013; **109**: 1666-1675 [PMID: 23969730 DOI: 10.1038/bjc.2013.484]

87 **Flourakis M**, Lehen'kyi V, Beck B, Raphaël M, Vandenberghe M, Abeele FV, Roudbaraki M, Lepage G, Mauroy B, Romanin C, Shuba Y, Skryma R, Prevarskaya N. Orai1 contributes to the establishment of an apoptosis-resistant phenotype in prostate cancer cells. *Cell Death Dis* 2010; **1**: e75 [PMID: 21364678 DOI: 10.1038/cddis.2010.52]

88 **Xu Y**, Zhang S, Niu H, Ye Y, Hu F, Chen S, Li X, Luo X, Jiang S, Liu Y, Chen Y, Li J, Xiang R, Li N. STIM1 accelerates cell senescence in a remodeled microenvironment but enhances the epithelial-to-mesenchymal transition in prostate cancer. *Sci Rep* 2015; **5**: 11754 [PMID: 26257076 DOI: 10.1038/srep11754]

89 **Tamajusuku AS**, Villodre ES, Paulus R, Coutinho-Silva R, Battasstini AM, Wink MR, Lenz G. Characterization of ATP-induced cell death in the GL261 mouse glioma. *J Cell Biochem* 2010; **109**: 983-991 [PMID: 20069573 DOI: 10.1002/jcb.22478]

90 **Souza CO**, Santoro GF, Figliuolo VR, Nanini HF, de Souza HS, Castelo-Branco MT, Abalo AA, Paiva MM, Coutinho CM, Coutinho-Silva R. Extracellular ATP induces cell death in human intestinal epithelial cells. *Biochim Biophys Acta* 2012; **1820**: 1867-1878 [PMID: 22951220 DOI: 10.1016/j.bbagen.2012.08.013]

91 **Shoji KF**, Sáez PJ, Harcha PA, Aguila HL, Sáez JC. Pannexin1 channels act downstream of P2X 7 receptors in ATP-induced murine T-cell death. *Channels (Austin)* 2014; **8**: 142-156 [PMID: 24590064 DOI: 10.4161/chan.28122]

92 **Sadatomi D**, Nakashioya K, Mamiya S, Honda S, Kameyama Y, Yamamura Y, Tanimura S, Takeda K. Mitochondrial function is required for extracellular ATP-induced NLRP3 inflammasome activation. *J Biochem* 2017; **161**: 503-512 [PMID: 28096454 DOI: 10.1093/jb/mvw098]

93 **Ohishi A**, Keno Y, Marumiya A, Sudo Y, Uda Y, Matsuda K, Morita Y, Furuta T, Nishida K, Nagasawa K. Expression level of P2X7 receptor is a determinant of ATP-induced death of mouse cultured neurons. *Neuroscience* 2016; **319**: 35-45 [PMID: 26812038 DOI: 10.1016/j.neuroscience.2016.01.048]

94 **Yoon MJ**, Lee HJ, Kim JH, Kim DK. Extracellular ATP induces apoptotic signaling in human monocyte leukemic cells, HL-60 and F-36P. *Arch Pharm Res* 2006; **29**: 1032-1041 [PMID: 17146973 DOI: 10.1007/BF02969288]

95 **Peng H**, Hao Y, Mousawi F, Roger S, Li J, Sim JA, Ponnambalam S, Yang X, Jiang LH. Purinergic and Store-Operated Ca(2+) Signaling Mechanisms in Mesenchymal Stem Cells and Their Roles in ATP-Induced Stimulation of Cell Migration. *Stem Cells* 2016; **34**: 2102-2114 [PMID: 27038239 DOI: 10.1002/stem.2370]

96 **Zeng CY**, Li CG, Shu JX, Xu LH, Ouyang DY, Mai FY, Zeng QZ, Zhang CC, Li RM, He XH. ATP induces caspase-3/gasdermin E-mediated pyroptosis in NLRP3 pathway-blocked murine macrophages. *Apoptosis* 2019; **24**: 703-717 [PMID: 31175486 DOI: 10.1007/s10495-019-01551-x]

97 **Zhao LR**, Xing RL, Wang PM, Zhang NS, Yin SJ, Li XC, Zhang L. NLRP1 and NLRP3 inflammasomes mediate LPS/ATP-induced pyroptosis in knee osteoarthritis. *Mol Med Rep* 2018; **17**: 5463-5469 [PMID: 29393464 DOI: 10.3892/mmr.2018.8520]

98 **Humphreys BD**, Rice J, Kertesy SB, Dubyak GR. Stress-activated protein kinase/JNK activation and apoptotic induction by the macrophage P2X7 nucleotide receptor. *J Biol Chem* 2000; **275**: 26792-26798 [PMID: 10854431 DOI: 10.1074/jbc.M002770200]

99 **Noguchi T**, Ishii K, Fukutomi H, Naguro I, Matsuzawa A, Takeda K, Ichijo H. Requirement of reactive oxygen species-dependent activation of ASK1-p38 MAPK pathway for extracellular ATP-induced apoptosis in macrophage. *J Biol Chem* 2008; **283**: 7657-7665 [PMID: 18211888 DOI: 10.1074/jbc.M708402200]

100 **Wen LT**, Caldwell CC, Knowles AF. Poly(ADP-ribose) polymerase activation and changes in Bax protein expression associated with extracellular ATP-mediated apoptosis in human embryonic kidney 293-P2X7 cells. *Mol Pharmacol* 2003; **63**: 706-713 [PMID: 12606781 DOI: 10.1124/mol.63.3.706]

101 **Hwang SM**, Koo NY, Choi SY, Chun GS, Kim JS, Park K. P2X7 Receptor-mediated Membrane Blebbing in Salivary Epithelial Cells. *Korean J Physiol Pharmacol* 2009; **13**: 175-179 [PMID: 19885034 DOI: 10.4196/kjpp.2009.13.3.175]

102 **Tsukimoto M**, Maehata M, Harada H, Ikari A, Takagi K, Degawa M. P2X7 receptor-dependent cell death is modulated during murine T cell maturation and mediated by dual signaling pathways. *J Immunol* 2006; **177**: 2842-2850 [PMID: 16920919 DOI: 10.4049/jimmunol.177.5.2842]

103 **Banfi C**, Ferrario S, De Vincenti O, Ceruti S, Fumagalli M, Mazzola A, D' Ambrosi N, Volontè C, Fratto P, Vitali E, Burnstock G, Beltrami E, Parolari A, Polvani G, Biglioli P, Tremoli E, Abbracchio MP. P2 receptors in human heart: upregulation of P2X6 in patients undergoing heart transplantation, interaction with TNFalpha and potential role in myocardial cell death. *J Mol Cell Cardiol* 2005; **39**: 929-939 [PMID: 16242142 DOI: 10.1016/j.yjmcc.2005.09.002]

104 **Ousingsawat J**, Wanitchakool P, Kmit A, Romao AM, Jantarajit W, Schreiber R, Kunzelmann K. Anoctamin 6 mediates effects essential for innate immunity downstream of P2X7 receptors in macrophages. *Nat Commun* 2015; **6**: 6245 [PMID: 25651887 DOI: 10.1038/ncomms7245]

105 **Wang J**, Liu S, Nie Y, Wu B, Wu Q, Song M, Tang M, Xiao L, Xu P, Tan X, Zhang L, Li G, Liang S, Zhang C. Activation of P2X7 receptors decreases the proliferation of murine luteal cells. *Reprod Fertil Dev* 2015; **27**: 1262-1271 [PMID: 25782073 DOI: 10.1071/RD14381]

106 **Vieira JM**, Carvalho FB, Gutierres JM, Soares MSP, Oliveira PS, Rubin MA, Morsch VM, Schetinger MR, Spanevello RM. Caffeine prevents high-intensity exercise-induced increase in enzymatic antioxidant and Na(+)-K(+)-ATPase activities and reduction of anxiolytic like-behaviour in rats. *Redox Rep* 2017; **22**: 493-500 [PMID: 28482759 DOI: 10.1080/13510002.2017.1322739]

107 **Huang ZX**, Lu ZJ, Ma WQ, Wu FX, Zhang YQ, Yu WF, Zhao ZQ. Involvement of RVM-expressed P2X7 receptor in bone cancer pain: mechanism of descending facilitation. *Pain* 2014; **155**: 783-791 [PMID: 24447511 DOI: 10.1016/j.pain.2014.01.011]

108 **Wang Z**, Zhu S, Tan S, Zeng Y, Zeng H. The P2 purinoceptors in prostate cancer. *Purinergic Signal* 2023; **19**: 255-263 [PMID: 35771310 DOI: 10.1007/s11302-022-09874-2]

109 **Maaser K**, Höpfner M, Kap H, Sutter AP, Barthel B, von Lampe B, Zeitz M, Scherübl H. Extracellular nucleotides inhibit growth of human oesophageal cancer cells *via* P2Y(2)-receptors. *Br J Cancer* 2002; **86**: 636-644 [PMID: 11870549 DOI: 10.1038/sj.bjc.6600100]

110 **Freeman TJ**, Sayedyahossein S, Johnston D, Sanchez-Pupo RE, O'Donnell B, Huang K, Lakhani Z, Nouri-Nejad D, Barr KJ, Harland L, Latosinsky S, Grant A, Dagnino L, Penuela S. Inhibition of Pannexin 1 Reduces the Tumorigenic Properties of Human Melanoma Cells. *Cancers (Basel)* 2019; **11** [PMID: 30654593 DOI: 10.3390/cancers11010102]

111 **Yao M**, Fan X, Yuan B, Takagi N, Liu S, Han X, Ren J, Liu J. Berberine inhibits NLRP3 Inflammasome pathway in human triple-negative breast cancer MDA-MB-231 cell. *BMC Complement Altern Med* 2019; **19**: 216 [PMID: 31412862 DOI: 10.1186/s12906-019-2615-4]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 December 24; 14(12): 570-583

DOI: 10.5306/wjco.v14.i12.570

ISSN 2218-4333 (online)

REVIEW

Update on current diagnosis and management of anaplastic thyroid carcinoma

Efstathios T Pavlidis, Ioannis N Galanis, Theodoros E Pavlidis

Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gluvic Z, Serbia; Shi B, China

Received: October 5, 2023
Peer-review started: October 5, 2023
First decision: November 9, 2023
Revised: November 9, 2023
Accepted: December 1, 2023
Article in press: December 1, 2023
Published online: December 24, 2023

Efstathios T Pavlidis, Ioannis N Galanis, Theodoros E Pavlidis, 2nd Propedeutic Department of Surgery, Hippokration General Hospital, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki 54642, Greece

Corresponding author: Theodoros E Pavlidis, Doctor, PhD, Emeritus Professor, Surgeon, 2nd Propedeutic Department of Surgery, Hippokration General Hospital, School of Medicine, Aristotle University of Thessaloniki, Konstantinoupoleos 49, Thessaloniki 54642, Greece. pavlidth@auth.gr

Abstract

Well-differentiated thyroid carcinoma has a favorable prognosis with a 5-year survival rate of over 95%. However, the undifferentiated or anaplastic type accounting for < 0.2%, usually in elderly individuals, exhibits a dismal prognosis with rapid growth and disappointing outcomes. It is the most aggressive form of thyroid carcinoma, with a median survival of 5 mo and poor quality of life (airway obstruction, dysphagia, hoarseness, persistent pain). Early diagnosis and staging are crucial. Diagnostic tools include biopsy (fine needle aspiration, core needle, open surgery), high-resolution ultrasound, computed tomography, magnetic resonance imaging, [(18)F]fluoro-D-glucose positron emission tomography/computed tomography, liquid biopsy and microRNAs. The *BRAF* gene (*BRAF-V600E* and *BRAF* wild type) is the most often found molecular factor. Others include the genes *RET, KRAS, HRAS,* and *NRAS*. Recent management policy is based on surgery, even debulking, chemotherapy (cisplatin or doxorubicin), radiotherapy (adjuvant or definitive), targeted biological agents and immunotherapy. The last two options constitute novel hopeful management modalities improving the overall survival in these otherwise condemned patients. Anti-programmed death-ligand 1 antibody immunotherapy, stem cell targeted therapies, nanotechnology achievements and artificial intelligence implementation provide novel promising alternatives. Genetic mutations determine molecular pathways, thus indicating novel treatment strategies such as anti-BRAF, anti-vascular endothelial growth factor-A, and anti-epidermal growth factor receptor. Treatment with the combination of the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib has been approved by the Food and Drug Administration in cases with *BRAF-V600E* gene mutations and is currently the standard care. This neoadjuvant treatment followed by surgery ensures a two-year overall survival of 80%. Prognostic factors for improved outcomes have been found to be younger age, earlier tumor stage and radiation therapy. A multidisciplinary approach is necessary, and the therapeutic plan should be individu-

alized based on surveillance and epidemiology end results.

Key Words: Thyroid diseases; Thyroid cancers; Anaplastic carcinoma; Undifferentiated carcinoma; Neck mass; Aggressive malignancies

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Anaplastic thyroid carcinoma is uncommon but one of the most lethal neoplasms. The optimal management remains unclear. The addition of novel targeted therapy and immunotherapy to the traditional management of surgery, radiation and chemotherapy has improved the outcomes. Multimodality management and the emerging use of individualized treatment based on novel therapeutic agents offers promising results. However, further research efforts involving the molecular microenvironment and biological drivers should be made.

Citation: Pavlidis ET, Galanis IN, Pavlidis TE. Update on current diagnosis and management of anaplastic thyroid carcinoma. *World J Clin Oncol* 2023; 14(12): 570-583
URL: https://www.wjgnet.com/2218-4333/full/v14/i12/570.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i12.570

INTRODUCTION

Thyroid carcinoma incidence is increasing, but that of the anaplastic and medullary types remains rather stable. The overall increase is due mainly to the rise of the most commonly occurring papillary carcinoma, which is associated with the best prognosis[1-3]. The incidence of anaplastic carcinoma in Europe has been assessed to be far less than 6 cases per 100000 population, more precisely, 0.1-0.3 cases per 100000 population in Denmark, the Netherlands and Wales[2,4,5] and 0.12-0.2 cases per 100000 population in the United States[3]; thus, it has been characterized as a rare disease[6].

The 5-year survival of a well-differentiated thyroid carcinoma exceeds 95%[7]. In contrast, the undifferentiated form, also called by a revised and better term, anaplastic carcinoma determined from World Health Organization classification [8], accounts for less than 0.2%[9] or as much as 1%-2% of all thyroid malignancies[10,11]. It comes from the follicular epithelium and constitutes one of the most lethal neoplasms related to disappointing outcomes[4,9,11]. Its median survival is restricted to only 4 to 6 mo, accompanied by poor quality of life[4,10].

Rapidly growing neck tumors are often accompanied by devastating and occasionally life-threatening events. They may invade the trachea, causing airway obstruction and asphyxia; the esophagus, causing dysphagia; the recurrent laryngeal nerve, causing paralysis and hoarseness; major vessels, causing manifestations of superior vena cava syndrome or brain intermittent ischemia; and neural plexuses, causing persistent pain. Additionally, at the time of diagnosis, metastases are found in half of cases, mainly pulmonary metastases (40%), followed by brain metastases (10%)[4,9,12-14].

A long existing untreated nodular goiter (30% of cases) or known history of papillary carcinoma is usually found mainly in the elderly with female predominance[3,4]. Preexisting papillary carcinoma may indicate a potent divergent transformation[15,16]. Early diagnosis based mainly on ultrasound (US) and core needle biopsy is crucial[17-21]. The following staging after the initial diagnosis of anaplastic carcinoma is of great importance and can be achieved by computed tomography (CT), magnetic resonance imaging (MRI)[18,19], and preferably positron emission tomography/CT (PET-CT)[17,22,23]. Modern molecular testing by revealing implicated genes, basically the *BRAF* gene (*BRAF-V600E* and *BRAF* wild type), and other molecules[24-27] can contribute to more accurate diagnosis but most importantly determine molecular pathways indicating novel treatment strategies by targeted biological factors, *i.e.,* anti-BRAF, anti-vascular endothelial growth factor (VEGF)-A or anti-epidermal growth factor receptor (EGFR) agents[24].

Nanotechnology achievements may offer either a vehicle for advanced drug delivery systems promoting targeted therapy[28,29] or a core for chemo-photothermal (lenvatinib-laser irradiation) therapy[30]. Additionally, these advances may provide tools for diagnosing disease progression in the form of magnetic or radiolabeled probes[29]. Immunotherapy with an anti-programmed cell death-ligand 1 (PD-L1) monoclonal antibody (atezolizumab) may increase the action of radiotherapy on cancer cells and is a novel innovation[31]. Stem cell-targeted therapies are other novel emerging alternatives with promising perspectives[24,32,33].

Machine learning with deep learning along with artificial intelligence implementation has provided preliminary encouraging results for diagnosis, imaging assessment, treatment and outcome prediction. It now remains to be used in clinical practice[10,34]. A multidisciplinary approach must be followed with an individualized therapeutic plan based on surveillance and epidemiology end results (SEER)[3,9,35-37].

The management policy constitutes the standard treatment, including surgery first of all, even debulking surgery, adjuvant chemotherapy that mainly uses cisplatin or doxorubicin and docetaxel-paclitaxel, and accelerated hyperfunctional external beam radiotherapy, preferably neo-adjuvant and definitive. It can increase the median survival up to 10 mo[24]. The novel hopeful management by targeted biological agents and immunotherapy has further improved the overall survival in these otherwise condemned patients[4,9,24,38,39].

Treatment with the combination of the *BRAF* inhibitor dabrafenib and the *MEK* inhibitor trametinib was approved by the Food and Drug Administration (FDA) of the United States in 2014 for mutated melanoma and in 2018 for mutated anaplastic thyroid carcinoma; thus, it has already been used successfully in cases of metastatic or locally advanced inoperable anaplastic thyroid carcinoma with *BRAF-V600E* gene mutation. This targeted therapy has been recommended as neoadjuvant treatment followed by surgery. It constitutes the standard care and ensures a two-year overall survival of 80%[4,24,40,41].

The main prognostic factors for improved outcomes have been younger age, earlier tumor stage, tumor size, multifocality, radiation therapy and novel targeted therapy[10,42]. This narrative review evaluates the current knowledge on anaplastic thyroid carcinoma with extreme aggressiveness and a dismal prognosis, emphasizing proper diagnosis and management. This study was based on the data of an extensive literature review from PubMed extending to September 2023, focusing particularly on full-text papers published only in the English language over the last five years.

DIAGNOSIS

The diagnostic steps are shown schematically in Figure 1.

Presentation

The anaplastic thyroid carcinoma exhibits a rapid onset with a large, hard, painful neck mass, cough with or without hemoptysis and dyspnea (35%) in cases of trachea invasion, hoarseness (40%) or dysphagia (40%), with local spread in over 50% of cases, lymph node involvement, possible skin invasion, and rapid evolution with dramatic invasion of adjacent structures that may need urgent intervention as mentioned above in the introduction section[4,6,43].

A recent large study from the United States including 5359 patients with anaplastic thyroid carcinoma provides an analysis of several presentation characteristics. The majority of patients were women (58%), non-Hispanic white (80%), with a median age of 70 ± 12 years, a median tumor size of 6.1 cm (range 4.5-8 cm), and distant metastases (29%)[44].

It is important for rapidly growing neck swelling to differentiate anaplastic thyroid carcinoma from thyroid lymphoma by biopsy[45] since they have completely different management strategies. The lymphoma requires no surgical intervention but only R-CHOP chemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine or otherwise called oncovin, and prednisolone) accompanied by immediate extreme volume reduction and long-term excellent outcomes[46].

Imaging

US can be used to detect heterogeneous echogenicity, abnormal shape, calcifications, increased vascularity, diffuse infiltration of adjacent tissues and regional lymph node involvement. US plain or preferably high resolution is the first step of exploration[18,19], followed preferably by core needle biopsy[21]. After establishing the diagnosis of the primary tumor of anaplastic thyroid carcinoma, staging imaging is necessary for local tumor extension assessment and revealing distant metastases.

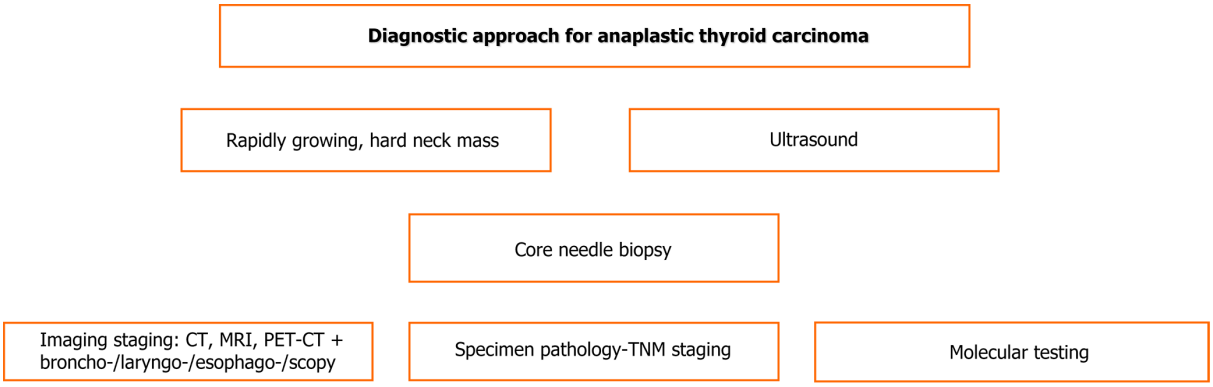
Contrast-enhanced CT or MRI and magnetic resonance angiography can reveal any involvement of major vessels or lymph nodes and any other local involvement or distant metastases. CT can be used to detect heterogeneous tumor appearance, necrosis, calcifications, hypervascularity, and possible infiltration of the trachea and esophagus. CT is preferred over MRI[18,19]. Additionally, fiberoptic laryngoscopy for vocal cord evaluation, bronchoscopy and esophagoscopy are necessary prerequisites[20,43].

However, more precise assessment has been offered by [(18)F]fluoro-D-glucose PET-CT, which ensures better anatomic location with active metabolic uptake detecting occult deposits[17,23,47]. It can be especially valuable in elderly patients for accurate disease setting assessment that can precisely determine the appropriate management strategy[22].

Molecular testing for implicated genes and other molecules

Prompt diagnosis and on-time management without any delay are imperative tasks, particularly in severe life-threatening complications. Molecular testing is indicated to better determine mutations and proper targeted therapy either as neo-adjuvant in unresectable cases or adjuvant after surgical excision[48], especially in cases of refractory carcinoma[49]. The American Thyroid Association (ATA) recent guidelines include recommendations for molecular testing for anaplastic thyroid carcinoma[50].

The *BRAF* gene mutation (*BRAF-V600E* and *BRAF* wild type) is the most important molecular factor found in 40%-50% of patients with anaplastic thyroid carcinoma[4,6,51]. The *MEK* gene has a close connection to the *BRAF* gene. Both are responsible for mitogen-activated protein kinase that promotes cell proliferation, tumor growth and angiogenesis[41,52,53]. Other mutations found involve the following genes: p53 in 63% of cases, *RET*, *RAS* (*KRAS*, *HRAS*, *NRAS*) in 22% of cases, *TERT* promoter in 75% of cases, *PIK3CA* in 18% of cases, *EIF1AX* in 14% of cases, *PTEN* in 14% of cases[6,24,50], and *NESTIN*, *CCND1*, *POU5F1*, *MCL1*, *MYBL2*, *MCL1*, *IQGAP1*, *SOX2*, and *NANOG*[33]. Additionally, epigenetic-related genes, *i.e.*, the chromatin remodeling SWI/SNF complex in 36% of cases, histone methyltransferases in 24% of cases, and DNA mismatch repair pathway genes in 10%-15% of cases, were found[6]. Gong *et al*[54] recognized 10 hub genes for anaplastic thyroid carcinoma: *CXCL8*, *CDH1*, *AURKA*, *CCNA2*, *FN1*, *CDK1*, *ITGAM*, *CDC20*, *MMP9*, and *KIF11*. *RAS* gene mutations have been reported to correlate with increased aggressiveness and increased mortality[55]. Unfortunately, targeted therapy is not yet available[50]. A positive result for mutated neurotrophic tyrosine receptor kinase (*NTRK*) gene testing is valuable to select patients for therapy by tropomyosin receptor kinase inhibitors (larotrectinib, entrectinib)[56]. miRNA-506 downregulation has been found in anaplastic thyroid carcinoma. This molecule normally regulates the WNT and NOTCH signaling pathways to adjust cell proliferation and migration. Thus, in practical view, as a new therapeutic



DOI: 10.5306/wjco.v14.i12.570 **Copyright** ©The Author(s) 2023.

Figure 1 Scheme of diagnostic steps for anaplastic thyroid carcinoma. CT: Computed tomography; MRI: Magnetic resonance imaging; PET-CT: Positron emission tomography/computed tomography; TNM: Tumor-node-metastasis.

targeted biological agent, it could suppress tumor progression and dissemination[25].

In an experimental model, intercellular adhesion molecule-1 (ICAM1) was an attractive target for anaplastic and papillary thyroid carcinoma by a monoclonal antibody; its distribution was explored by MRI[57]. Another molecule, collagen triple helix repeat containing-1 (CTHRC1), has been found to decrease the survival of patients with anaplastic thyroid carcinoma by promoting its progression and invasion *via* the WNT pathway and epithelial-mesenchymal transition. Blocking its action could be particularly useful[58]. Histone lysine lactylation represents a novel epigenetic mark that can boost the proliferation of anaplastic thyroid carcinoma. Blocking this protein may increase the action of *BRAF-V600E* gene inhibitors, thus preventing the progression of the mutated malignancy[51]. PD-L1 expression was found to be positive in a high proportion in papillary 87% but with weaker and patchy expression, and in anaplastic thyroid carcinoma 73%, indicating that the latter can exhibit a better response to immunotherapy[59].

Biopsy

It is obvious, that in cases of rapidly enlarging neck nodules, the necessary first step is an US imaging performance. Advances in the US technology provide precise diagnostic capability by high resolution US. However, then biopsy is fundamental to make the diagnosis. Fine needle aspiration (FNA) cytology using a 21-25 gauge needle under US guidance has been widely used as an initial step in diagnosis by cytologic examination[20,21,34,45,60]. However, due to its high false-negative results, low sensitivity of 54%-61% *vs* 77%-80% of core needle biopsy (CNB), and specificity of 87% *vs* 100% of CNB[20,21] or often inconclusive results, this option tends to be omitted recently in favor of CNB. Because of performance, using it is considered a vain spending of time[21,45].

CNB is performed under US guidance and by local anesthesia using a 16-20 Ga needle to take at least 2-3 tissue samples by separate punctures for histopathologic examination[20,21,45]. In addition, the sample can be immediately used for molecular testing[20]. CNB yields the most accurate diagnostic ability and thus constitutes the method of first choice instead of its application after nondiagnostic FNA, which, in contrast to current guidelines, is advised. There was no patient discomfort or malignant cell seeding or notable complications. A little bleeding requiring simple compression or hematoma formation may occur rarely[20,21,45].

Incision biopsy or open surgery biopsy under local or even general anesthesia and skin incision takes 2-3 cm^3 of tissue, avoiding any necrotic area. However, it has been abandoned and substituted by CNB[21]. Liquid biopsy is a new noninvasive genotyping diagnostic method that can detect malignant cells in serum and tumor DNA or other extracellular parts, providing valuable information. It may contribute to diagnosis, prognosis, and follow-up for assessment of the response to treatment or relapse[26,27]. All the abovementioned diagnostic tools are shown in Table 1.

Pathological staging

By definition, all anaplastic thyroid carcinomas are considered advanced and classified in stage IV by the American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC) according to the tumor-node-metastasis (TNM) system (tumor size and local extension, regional lymph node status and distant metastases). The 8th edition of TNM classification and staging by the AJCC and UICC are shown in Tables 2 and 3[6,8,61].

MANAGEMENT

There have been several management options, including novel targeted therapy and immunotherapy[4,6,24,44,62]. They are presented below. In a recent cohort study including 97 patients, these options were presented in combination as follows: (1) Surgical intervention in 45% of cases; (2) Chemotherapy in 41% of cases; (3) Neoadjuvant or definitive radiotherapy in 35% of cases; and (4) Targeted therapy in 29% of cases. The median overall survival was 6.5 mo, and it was inferior in those who did not undergo surgery. Multivariate analysis showed that stage IVC and lack of radiotherapy

Table 1 Used diagnostic tools for anaplastic thyroid carcinoma

n	Modality
1	Plain ultrasound or preferably high resolution ultrasound
2	Core needle biopsy under ultrasound guidance preferably
3	FNA cytology under ultrasound guidance
4	Staging imaging (CT, MRI-MRA, ^{18}F-FDG PET-CT)
5	Bronchoscopy
6	Esophagoscopy
8	Fiberoptic laryngoscopy
9	Molecular testing (*BRAF, MEK, NTRK, RET, RAS, p53* genes)
10	MicroRNAs
11	PD-L1 expression
12	Liquid biopsy
13	Histopathology
14	Pathological TNM staging

FNA: Fine-needle aspiration; CT: Computed tomography; MRI-MRA: Magnetic resonance imaging/magnetic resonance angiography; ^{18}F-FDG PET-CT: [(18)F]fluoro-D-glucose positron emission tomography/computed tomography; PD-L1: Programmed death-ligand 1; TNM: Tumor-node-metastasis.

Table 2 T classification in tumor-node-metastasis system for anaplastic thyroid carcinoma of American Joint Committee on Cancer and Union for International Cancer Control 8th edition

T	Size	Extension
T1	≤ 2 cm	Limited into thyroid
T1a	≤ 1 cm	Limited into thyroid
T1b	> 1 cm and ≤ 2 cm	Limited into thyroid
T2	> 2 cm and ≤ 4 cm	Limited into thyroid
T3	> 4 cm	Limited into thyroid or extrathyroid macroscopic invasion only of thyroid muscles and subcutaneous tissue
T3a	> 4 cm	Limited into thyroid
T3b	Any size	Extrathyroid macroscopic invasion only of thyroid muscles and subcutaneous tissue
T4	Any size	Macroscopic invasion of major adjacent structures
T4a	Any size	Macroscopic invasion of larynx, trachea, esophagus, recurrent laryngeal nerve
T4b	Any size	Macroscopic invasion of carotid artery, major vessels in mediastinum, prever-tebral fascia

were associated with worse overall survival[42].

This multimodality management, including additional tyrosine kinase inhibitors, could provide a survival of more than one year[63]. Given that in 433 studied patients with advanced metastatic anaplastic thyroid carcinoma (stage IVC), there was a median overall survival of 2 mo and a one-year overall survival of 6.9%[64], better multimodality management including novel therapeutic agents is needed, especially for this most lethal form[65]. The optimal combination of multimodality treatment[63,66] and mainly the new tyrosine kinase inhibitor lenvatinib yields encouraging results[11].

Surgery

Surgery constitutes the cornerstone of treatment despite the existing debate. It may range from palliative debulking intervention to more radical resections, including total or near total thyroidectomy and extended lymphadenectomy, including the central and lateral lymph node level either unilaterally or bilaterally[6,8,36,53,61]. The above curative surgery may be performed in some patients with earlier disease and may provide, accompanied by adjuvant chemotherapy, occasional long survival over 5 years[18,19]. By multivariate analysis in a systematic review and meta-analysis, surgery and radiotherapy were found to be independent factors predicting increased overall survival[36]. Complete surgical excision followed by adjuvant therapy is the optimal opportunity for cure[4,47]. However, in general,

Table 3 Tumor-node-metastasis staging for anaplastic thyroid carcinoma of American Joint Committee on Cancer and Union for International Cancer Control 8th editiona

Stage	IVA	IVB	IVC
Parameters	T1-T3a, N0, M0	T1-T3a, N1, M0	Any T, any N, M1
		T3b, any N, M0	
		T4, any N, M0	

T: Tumor size; N0: Negative regional lymph nodes; N1: Positive regional lymph nodes; M0: Without any distant metastases; M1: Presence of distant metastases.

extreme radical resection, such as laryngectomy, tracheal resection, esophagectomy or complete neck dissection without notable oncological contribution, is not indicated[47]. Tumor removal increases the benefits of the treatment. In combination with radiation, new chemotherapy and novel gene targeted therapy can achieve locoregional disease control and improve survival and quality of life[36,41,53,65,67]. The guidelines of the National Comprehensive Cancer Network and ATA recommend surgical resection by lobectomy or near total thyroidectomy with wide lymphadenectomy in stage IVA and IVB, even in stage IVC when an R0 or at least R1 intervention could be achieved in locally resectable tumors[36,43,68-70]. However, many locally unresectable cases may respond to neoadjuvant external beam radiation, chemotherapy or even targeted therapy (dabrafenib and trametinib) for *BRAF* gene mutation, thus becoming resectable and ensuring surgical excision[43]. Timely detection and proper treatment reduce the number of advanced cases with distant metastases[9].

As shown in Figures 2 and 3, the current best practice for respectable tumors is surgery with adjuvant chemotherapy, radiation therapy and targeted therapy-immunotherapy such as dabrafenib and trametinib but immunotherapy if *BRAF* and *MET* gene mutations exist; for unresectable tumors, current best practice involves palliative surgery and targeted therapy-immunotherapy in combination with chemoradiation therapy[47].

Patients with inoperable disease may undergo palliative surgery to improve morbidity and avoid complications and life-threatening urgent events[48,71]. Unless there is debulking surgery for decompression, it includes the performance of tracheostomy and gastrostomy, preferably percutaneous by endoscopy assistance for feeding in case prior to radiation, which may cause esophageal stricture[36,48]. In cases of esophageal invasion or stenosis, feeding tube placement by an interventional radiologist can ensure enteral feeding[43].

Palliative airway management for symptom relief must be based on multidisciplinary team collaboration by designing the plan carefully. It is well known that tracheostomy may be related to morbidity and problems affecting quality of life [43]. It should be emphasized overall that patients undergoing extended surgical intervention and receiving adjuvant radiation and chemotherapy gain the chance of the best overall survival[9,48]. Aggressive locoregional surgery and radiotherapy must be performed whenever possible, and adding chemotherapy can lead to further improvement; however, in unresectable cases, radiotherapy and chemotherapy must be preferred[9].

Chemotherapy

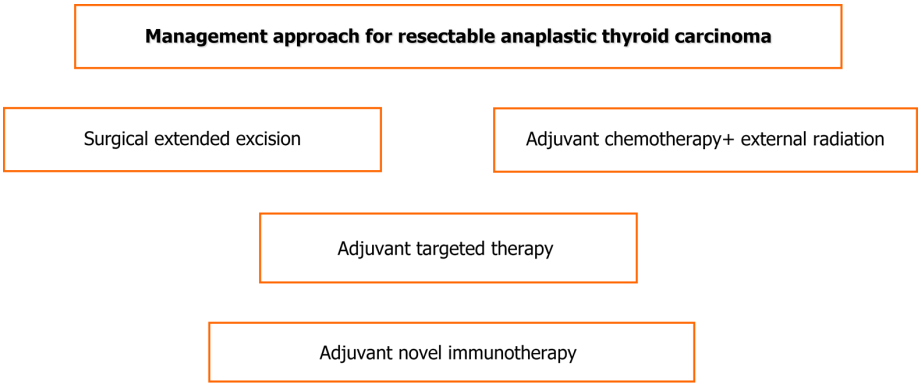
Taxanes (paclitaxel, docetaxel, and cabazitaxel) can be effectively used to treat various forms of cancer by replicating inhibition. Doxorubicin (adriamycin) is an anthracyclin that inhibits cancer cell growth by topoisomerase II activation in the process of DNA repair. Platinum-based chemotherapy (cisplatin, carboplatin, oxaliplatin) is widely used. Attachment to DNA causes destruction of cancer cells by replicating inhibition. These chemotherapeutic drugs have been used in various cancers, including anaplastic thyroid carcinoma[6,53,67].

The ATA guidelines recommend adjuvant or neoadjuvant chemotherapy by combination of: (1) Paclitaxel with carboplatin; (2) Doxorubicin with cisplatin; (3) Doxorubicin with docetaxel; or (4) Paclitaxel alone or doxorubicin alone [6]. Unfortunately, chemoresistance often occurs in anaplastic thyroid carcinoma even in the most effective regimen of paclitaxel[6,72]. Adjuvant chemotherapy increases the median survival and the survival rate[48,67].

Subsequently, other drugs enhancing chemotherapy efficiency have been added. The combination with targeted biological agents such as dabrafenib and trametinib, in cases of mutated *BRAF* and *MEK* genes, respectively, may overcome this resistance[6,12,72]. In unmutated cases, novel immunotherapy (anti-PD-1 and anti-PD-L1) has been a recent revolution[6,12,72].

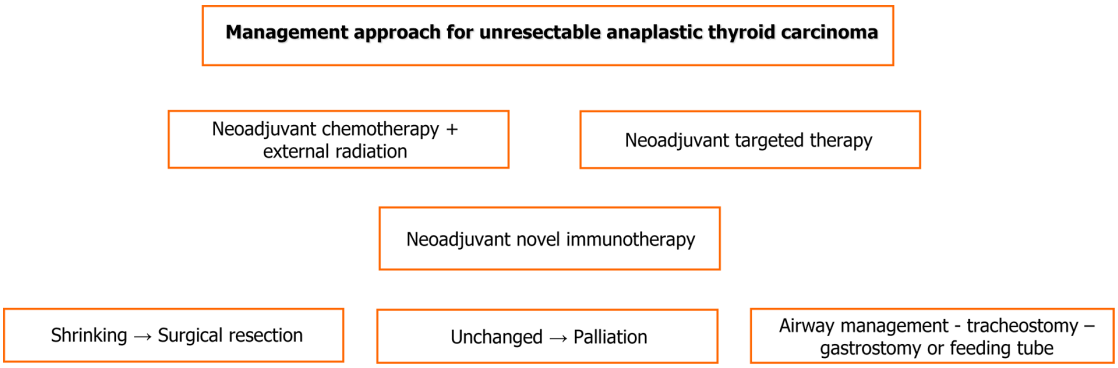
Chemotherapy added to radiation therapy further improves survival compared to radiation alone in resected cases as well as in unresected cases[73]. A randomized controlled phase II trial from 34 centers in the United States including 89 patients showed that the combination of paclitaxel chemotherapy with pazopanib, a multitargeted inhibitor of tyrosine kinase receptors and radiation therapy, was feasible, safe and promising[74].

Anlotinib, another new multitargeted inhibitor of tyrosine kinase receptors [VEGF, FGFR, platelet-derived growth factor receptor (PDGFR), c-kit] approved by the United States FDA, in combination with chemotherapy with paclitaxel, capecitabine or paclitaxel, capecitabine, and carboplatin as first-line therapy is a safe and effective treatment for locally advanced or metastatic thyroid carcinoma. As reported, it had an objective response rate of 60%, a disease control rate of 88%, and provided a progression-free survival of 25.1 wk and a median disease specification survival of 96 wk[75,76].



DOI: 10.5306/wjco.v14.i12.570 **Copyright** ©The Author(s) 2023.

Figure 2 Scheme of treatment steps for resectable anaplastic thyroid carcinoma.



DOI: 10.5306/wjco.v14.i12.570 **Copyright** ©The Author(s) 2023.

Figure 3 Scheme of treatment steps for unresectable anaplastic thyroid carcinoma.

Radiotherapy

Local rapid progression of anaplastic thyroid carcinoma and recurrence are related to the extreme malignancy of the disease. Subsequently, local control is of great importance. Radiation therapy is the main stem of every potent successful management, providing cessation of progression and regression of the tumor extent before surgery as well as prevention of recurrence after attempted surgical resection[6].

Radiotherapy can be applied as a neoadjuvant or definitive adjuvant modality generally by external beam radiation therapy (EBRT), which accelerates hyperfunction and improves median overall survival[24,48,77]. It is a necessary part of the current multimodality treatment combined with surgery, chemotherapy, targeted therapy and novel immunotherapy [9,42,63,73,74].

EBRT was assessed by a multivariate analysis in 433 stage IVC patients with anaplastic thyroid carcinoma as an independent prognostic factor of survival along with surgery and chemotherapy[24]. The optimal dose of hyperfunction EBRT varies between 45-70 G, and a subsequent hypofunction dose > 5 G can prevent local recurrence and death[6]. A retrospective study including 491 patients with anaplastic thyroid carcinoma found that the combination of radiation therapy and chemotherapy provided better overall survival than radiotherapy alone regardless of surgery and distant metastases. Prognostic factors for survival were older age, single marital status, local extension, distal metastases and surgery[73]. Among various management modalities, adjuvant chemoradiation after surgical intervention seems to be the better modality for prolonged survival in stage IVA resectable tumors without negative prognostic factors[67]. Radiotherapy may have synergy with immunotherapy by modulating microenvironmental immunity[62]. Brain metastases account for up to 10% of metastatic cases, with an overall survival of 3 mo[13]. Radiation therapy and lenvatinib targeted therapy have been reported in such cases, but with limited efficacy[14].

Targeted therapies

Targeted therapy by biological agents is based on monoclonal antibodies and is intended to block certain cancer development pathways[56,78-81]. The drugs for mechanisms of some implicated gene mutations include the following: (1) Angiogenesis-lenvatinib, sorafenib, sunitinib, vandetanib, combretastatin; (2) EGFR-docetaxel, gefitinib; (3) BRAF-dabrafenib, vemurafenib, encorafenib; and (4) MEK-trametinib, cobimetinib, binimetinib[24]. The combination of the BRAF inhibitor dabrafenib with the MEK inhibitor trametinib is the most widely used because it is considered more effective than each drug alone[6,24,41,82,83]. Combretastatin targets tumor vascularity. It has been used as adjuvant treatment in combination with paclitaxel - carboplatin but without notable results. Likewise, sorafenib had limited

usefulness; instead, other anti-angiogenetic agents, such as vandetanib, sunitinib, and lenvatinib, exhibited significant anti-neoplastic efficacy[24]. Vandetanib, a multitarget tyrosine inhibitor that acts mainly against EGFR and VEGF receptor (VEGFR), promotes apoptosis (programmed cell death) and inhibits tumor growth, migration and invasion[62,84]. Sunitinib, another multitarget tyrosine inhibitor, acts mainly against VEGFR and PDGFR to inhibit tumor growth by deprivation of its blood supply[62]. Lenvatinib is an inhibitor of kinase that inactivates VEGFRs (VEGFR 1, VEGFR 2, and VEGFR 3) and subsequently prevents angiogenesis and tumor growth. It has been used in anaplastic and well-differentiated thyroid carcinoma as an alternative to radioactive iodine, in inoperable hepatocellular carcinoma, and in advanced renal cell carcinoma in combination with everolimus[11,30,85]. Carfilzomib, a proteasome inhibitor approved by the United States FDA for multiple myeloma, is the most effective such drug for treating anaplastic thyroid carcinoma; by acting on cell proliferation and *p27* gene overexpression, which promotes apoptosis and cell death[62]. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor approved by the United States FDA for skin T-cell lymphoma, causes *p21* gene overexpression that promotes apoptosis and cell death[62].

A recent study from Japan including 36 patients with unresectable anaplastic thyroid carcinoma treated initially with lenvatinib showed an average survival of 5.8 mo, longer than the 2-mo survival from paclitaxel initial treatment, a response rate of 33% and a median overall survival of 5 mo[86].

Glutamin metabolism and subsequent glutaminolysis are biological features that are highly increased in anaplastic thyroid carcinoma and modulate cancer cell survival by sustaining mitochondrial function and oxygen balance. Glutaminolysis inhibition causes cell death. The tyrosine kinase inhibitors lenvatinib and sorafenib affect this signaling pathway of oncogenesis and enhance the efficiency of conventional chemotherapy, such as doxorubicin or taxanes, which otherwise may have minimal influence on patient survival[12]. ICAM1 is an interesting target of monoclonal antibodies with promising results[57]. The polo-like kinase 4 inhibitor has anticancer efficacy and synergy with sorafenib[87]. CTHRC1 promotes the progression of anaplastic thyroid carcinoma and is associated with worse outcomes[58]. The depletion of fibronectin may overcome resistance to *BRAF* gene inhibitor treatment. It can be targeted by MARK (ERK) pathway inhibition (ipilimumab, vemurafenib)[88]. Additionally, diclofenac added to *BRAF* gene inhibitors by targeting metabolism overcomes any resistance and maximizes the treatment effect[89]. Targeting the EZH2 complex promotes anticancer activity and can be a promising strategy[90]. Recent research data showed that one-carbon metabolism had a possible role in metabolic stress, and its targeting would be a valuable promising therapy[12].

Immunotherapy

Immunotherapy has been applied increasingly on a preliminary experimental basis but with promising future perspectives. Thus, it has been included in many research protocols. The existing immunotherapy regimens in combination with targeted therapy (dabrafenib - trametinib) can provide a further potential increase in effectiveness and improved survival benefits[41,91,92]. It is undoubted that the multimodality current treatment plan is accompanied by the best outcomes of this otherwise lethal disease[40]. However, unfortunately, for the mutated *BRAF* gene wild type, there has not yet been effective treatment[4].

Since the recent innovation of targeted PD-1 and PD-L1 interaction by monoclonal antibodies atezolizumab, spartalizumab and pembrolizumab, the application of immunotherapy has been increasing[6,12,93]. It may be a promising therapeutic choice, especially in those with high PD-1 and PD-L1 expression without *BRAF* gene mutations[4,40,48,59,94]. Atezolizumab, a monoclonal antibody against PD-L1, gives encouraging results in combination with radiation therapy[31]. Spartalizumab and pembrolizumab are monoclonal antibodies against PD-1[4]. Spartalizumab has been used in a phase II clinical study in unresectable locally advanced or metastatic cases, showing a one-year survival of 40% and a median overall survival of 5.9 mo. Among the side effects, diarrhea, pruritus, fever, and fatigue have been reported[95]. Pembrolizumab has been used in likewise unresectable cases, showing a one-year survival of 38% and a median overall survival of 4.4 mo[96].

Various tumor experimental models of anaplastic thyroid carcinoma microenvironment in mice have been developed for scientific research and monoclonal antibody or other innovative drug production[30,97]. Novel promising therapies, including immunotherapy, multikinase inhibitors, aurora kinase inhibitors, gene therapy by oncolytic viruses, epigenetic modulators, and apoptosis-inducing agents, have been introduced[40,72,76,98,99].

Prognosis and survival

Early diagnosis and treatment yield the best outcome and prognosis[100-102]. A study from the United States including 719 patients found that racial, ethical and socioeconomic status seems to influence survival and prognosis. Nonwhite patients had a lower likelihood of receiving treatment and poorer survival; those living in high poverty had a worse prognosis[103]. A large cohort study from China including 735 patients with multidisciplinary management of anaplastic thyroid carcinoma found an overall survival of 10.7% at 2 years and 8.1% at 5 years. By stage, survival at 2 years was 36.5% (IVA), 15.6% (IVB), and 1.4% (IVC)[9]. A large single institution 20-year study from the United States including 479 patients with multimodality management of anaplastic thyroid carcinoma found a constantly increasing overall survival among three periods of treatment due to progression improvement by the addition of targeted therapy and immunotherapy. The overall survival for 2000-2013 was 35% at 1 year and 18% at 2 years; for 2014-2016, it was 47% at 1 year and 25% at 2 years; for 2017-2019, it was 59% at 1 year and 42% at 2 years[104]. A nationwide cohort study from the Netherlands including 812 patients with management of anaplastic thyroid carcinoma during the period 1989-2016 found a median overall survival of 2.2 mo, overall one-year survival of 12%, and one-year survival of 21.6% in those without distant metastases. Prognostic factors for better survival were age < 65 years, treatment based on more than two to three modalities, without distant metastases and bilateral lymph node involvement[5]. A recent large study including 5359 patients with anaplastic thyroid carcinoma found a total one-year survival of 23%[44]. A nationwide cohort study from Denmark including 320 patients with management of anaplastic thyroid carcinoma during the period 1980-2014 found a

Table 4 Predictive factors for favorable prognosis of anaplastic thyroid carcinoma

n	Factor
1	Female patients
2	Age ≤ 60 yr
3	Married patients
4	Asymptomatic patients
5	Tumor ≤ 5 cm in size
6	Single primary tumor
7	Without local tissue invasion
8	Without lymph node involvement (N0)
9	Without distant metastases (M0)
10	Kind of therapy
11	Multimodality treatment

1-year survival of 18% and a 5-year survival of 12%[2].

Another recent large study from China including 1080 patients with stage IVA: 6.3%, IVB: 21.9%, IVC: 71.8% anaplastic thyroid carcinoma management found disease specific survival at 1 mo of 83.1%, at 6 mo, 37.5%, and at 12 mo, 21%. The 1-year disease specific survival was 53.3% for stage IVA, 36.5% for IVB and 13.1% for IVC[35]. A recent study from a tertiary academic hospital in the United States including 45 patients found a median survival of 6.1 mo; smaller tumors and chemotherapy were related to better survival[105]. It was found by regression analysis that age, distant metastases and tumor size were independent factors of worse prognosis[106].

It was found by univariate analysis that distant metastases, lymph node involvement, tumor > 5 cm in size, and local infiltration were predictive factors for worse prognosis[107]. Multivariate analysis showed that the absence of both symptoms and distant metastases was related to longer survival. The asymptomatic patients were younger (≤ 60 years) and had smaller tumors (≤ 5 cm) than symptomatic patients with anaplastic thyroid carcinoma[108]. Another study found by multivariate analysis that age, sex, marital status, multiple primary tumors, distant metastases, and therapy type were independent prognostic factors for cancer-specific survival[109]. A recent large study from China including 1140 patients with anaplastic thyroid carcinoma management found an overall survival of 27.6% at 6 mo, 15.1% at 1 year, and 6.2% at 2 years. The age cutoff was 65 years as a significant predictive factor of improved survival[110]. The predictive factors for favorable prognosis are shown in Table 4.

It has been reported that age ≥ 65 years, palliative surgery and white cell count ≥ 10000/mm^3 are predictive factors for worse prognosis[67]. Despite proper management, recurrence with a high incidence may occur. An overall survival of 5-6 mo (specifically 9 mo for stage IVA, 4.8 mo for stage IVB and 3 mo for stage IVC) and a one-year survival of 20% have been reported[43]. Although, the overall prognosis of anaplastic thyroid carcinoma is very poor, in some cases is relatively good. There is evidence that patients with anaplastic carcinoma clearly transformed from papillary thyroid carcinoma, or those with mutated *BRAF* gene had a significantly better prognosis than other patients. Despite, the conflicting aspects for the above mentioned, it could be possibly explained by the higher expression of PD-L1 in papillary than in anaplastic carcinoma and response to immunotherapy[59] and anti-BRAF targeted therapy[24]. A long-term survival reaching above 5 years has been reported in isolated cases after surgical excision and adjuvant chemotherapy[18, 19].

CONCLUSION

Anaplastic thyroid carcinoma is a rare, rapidly growing and extremely aggressive neoplasm with a dismal prognosis. The correct early diagnosis and treatment are important perquisites for the management of an otherwise lethal condition. US and core needle biopsy are used to make the diagnosis. Preoperative imaging, histopathology and molecular testing establish an accurate natural status and determine the therapeutic strategy plan. Complete surgical resection with wide lymphadenectomy whenever possible is the main step followed by chemoradiation therapy, targeted therapy and immunotherapy. Palliation management may improve the quality of an otherwise unbearable life. Novel multimodal treatment that must be personalized offers the best chance to manage this incurable disease.

FOOTNOTES

Author contributions: Pavlidis ET, Galanis IN, and Pavlidis TE analyzed data, and reviewed; Pavlidis TE designed research, contributed new analytic tools; Pavlidis ET performed research and wrote the paper.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Greece

ORCID number: Efstathios T Pavlidis 0000-0002-7282-8101; Ioannis N Galanis 0009-0001-4283-0788; Theodoros E Pavlidis 0000-0002-8141-1412.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **Pereira M**, Williams VL, Hallanger Johnson J, Valderrabano P. Thyroid Cancer Incidence Trends in the United States: Association with Changes in Professional Guideline Recommendations. *Thyroid* 2020; **30**: 1132-1140 [PMID: 32098591 DOI: 10.1089/thy.2019.0415]
2 **Mirian C**, Grønhøj C, Jensen DH, Jakobsen KK, Karnov K, Jensen JS, Hahn CH, Klitmøller TA, Bentzen J, von Buchwald C. Trends in thyroid cancer: Retrospective analysis of incidence and survival in Denmark 1980-2014. *Cancer Epidemiol* 2018; **55**: 81-87 [PMID: 29852396 DOI: 10.1016/j.canep.2018.05.009]
3 **Janz TA**, Neskey DM, Nguyen SA, Lentsch EJ. Is the incidence of anaplastic thyroid cancer increasing: A population based epidemiology study. *World J Otorhinolaryngol Head Neck Surg* 2019; **5**: 34-40 [PMID: 30775700 DOI: 10.1016/j.wjorl.2018.05.006]
4 **Califano I**, Smulever A, Jerkovich F, Pitoia F. Advances in the management of anaplastic thyroid carcinoma: transforming a life-threatening condition into a potentially treatable disease. *Rev Endocr Metab Disord* 2023 [PMID: 37648897 DOI: 10.1007/s11154-023-09833-1]
5 **de Ridder M**, Nieveen van Dijkum E, Engelsman A, Kapiteijn E, Klümpen HJ, Rasch CRN. Anaplastic thyroid carcinoma: a nationwide cohort study on incidence, treatment and survival in the Netherlands over 3 decades. *Eur J Endocrinol* 2020; **183**: 203-209 [PMID: 32460234 DOI: 10.1530/EJE-20-0080]
6 **Jannin A**, Escande A, Al Ghuzlan A, Blanchard P, Hartl D, Chevalier B, Deschamps F, Lamartina L, Lacroix L, Dupuy C, Baudin E, Do Cao C, Hadoux J. Anaplastic Thyroid Carcinoma: An Update. *Cancers (Basel)* 2022; **14** [PMID: 35205809 DOI: 10.3390/cancers14041061]
7 **Pavlidis ET**, Pavlidis TE. Role of prophylactic central neck lymph node dissection for papillary thyroid carcinoma in the era of de-escalation. *World J Clin Oncol* 2023; **14**: 247-258 [PMID: 37583949 DOI: 10.5306/wjco.v14.i7.247]
8 **Abe I**, Lam AK. Anaplastic thyroid carcinoma: Updates on WHO classification, clinicopathological features and staging. *Histol Histopathol* 2021; **36**: 239-248 [PMID: 33170501 DOI: 10.14670/HH-18-277]
9 **Huang NS**, Shi X, Lei BW, Wei WJ, Lu ZW, Yu PC, Wang Y, Ji QH, Wang YL. An Update of the Appropriate Treatment Strategies in Anaplastic Thyroid Cancer: A Population-Based Study of 735 Patients. *Int J Endocrinol* 2019; **2019**: 8428547 [PMID: 30915116 DOI: 10.1155/2019/8428547]
10 **Xu L**, Cai L, Zhu Z, Chen G. Comparison of the cox regression to machine learning in predicting the survival of anaplastic thyroid carcinoma. *BMC Endocr Disord* 2023; **23**: 129 [PMID: 37291551 DOI: 10.1186/s12902-023-01368-5]
11 **Sugitani I**, Onoda N, Ito KI, Suzuki S. Management of Anaplastic Thyroid Carcinoma: the Fruits from the ATC Research Consortium of Japan. *J Nippon Med Sch* 2018; **85**: 18-27 [PMID: 29540641 DOI: 10.1272/jnms.2018_85-3]
12 **Hwang Y**, Yun HJ, Jeong JW, Kim M, Joo S, Lee HK, Chang HS, Kim SM, Fang S. Co-inhibition of glutaminolysis and one-carbon metabolism promotes ROS accumulation leading to enhancement of chemotherapeutic efficacy in anaplastic thyroid cancer. *Cell Death Dis* 2023; **14**: 515 [PMID: 37573361 DOI: 10.1038/s41419-023-06041-2]
13 **Wolff L**, Steindl A, Popov P, Dieckmann K, Gatterbauer B, Widhalm G, Berghoff AS, Preusser M, Raderer M, Kiesewetter B. Clinical characteristics, treatment, and long-term outcome of patients with brain metastases from thyroid cancer. *Clin Exp Metastasis* 2023; **40**: 217-226 [PMID: 37219741 DOI: 10.1007/s10585-023-10208-8]
14 **Obayashi A**, Aoki K, Wada T, Furuie H, Kuraoka K, Hamamoto T, Tatsukawa T. Lenvatinib Administration for Anaplastic Thyroid Carcinoma with Brain Metastasis. *Acta Med Okayama* 2023; **77**: 227-232 [PMID: 37094963 DOI: 10.18926/AMO/65155]
15 **Lu L**, Wang JR, Henderson YC, Bai S, Yang J, Hu M, Shiau CK, Pan T, Yan Y, Tran TM, Li J, Kieser R, Zhao X, Wang J, Nurieva R, Williams MD, Cabanillas ME, Dadu R, Busaidy NL, Zafereo M, Navin N, Lai SY, Gao R. Anaplastic transformation in thyroid cancer revealed by single-cell transcriptomics. *J Clin Invest* 2023; **133** [PMID: 37053016 DOI: 10.1172/JCI169653]
16 **Capdevila J**, Mayor R, Mancuso FM, Iglesias C, Caratù G, Matos I, Zafón C, Hernando J, Petit A, Nuciforo P, Cameselle-Teijeiro JM, Álvarez C, Recio JA, Tabernero J, Matias-Guiu X, Vivancos A, Seoane J. Early evolutionary divergence between papillary and anaplastic thyroid cancers. *Ann Oncol* 2018; **29**: 1454-1460 [PMID: 29648575 DOI: 10.1093/annonc/mdy123]
17 **Roseland ME**, Dewaraja YK, Wong KK. Advanced imaging and theranostics in thyroid cancer. *Curr Opin Endocrinol Diabetes Obes* 2022; **29**: 456-465 [PMID: 36068937 DOI: 10.1097/MED.0000000000000740]
18 **Vithana S**, Udayakumara E, Gunasena M, Mushraf M. Curative surgery for anaplastic thyroid carcinoma: A case report. *SAGE Open Med Case Rep* 2022; **10**: 2050313X221091399 [PMID: 35449526 DOI: 10.1177/2050313X221091399]
19 **Moreno F**, Reyes C, Pineda CA, Castellanos G, Cálix F, Calderón J, Vasquez-Bonilla WO. Anaplastic thyroid carcinoma with unusual long-term survival: a case report. *J Med Case Rep* 2022; **16**: 39 [PMID: 35101107 DOI: 10.1186/s13256-021-03249-8]
20 **Matrone A**, De Napoli L, Torregrossa L, Aghababyan A, Papini P, Ambrosini CE, Cervelli R, Ugolini C, Basolo F, Molinaro E, Elisei R, Materazzi G. Core Needle Biopsy Can Early and Precisely Identify Large Thyroid Masses. *Front Oncol* 2022; **12**: 854755 [PMID: 35463338 DOI: 10.3389/fonc.2022.854755]

21 **Ha EJ**, Baek JH, Lee JH, Kim JK, Song DE, Kim WB, Hong SJ. Core needle biopsy could reduce diagnostic surgery in patients with anaplastic thyroid cancer or thyroid lymphoma. *Eur Radiol* 2016; **26**: 1031-1036 [PMID: 26201291 DOI: 10.1007/s00330-015-3921-y]

22 **Sonavane SN**, Basu S. Role of PET/Computed Tomography in Elderly Thyroid Cancer: Tumor Biology and Clinical Management. *PET Clin* 2023; **18**: 81-101 [PMID: 36718717 DOI: 10.1016/j.cpet.2022.09.005]

23 **Adnan A**, Raju S, Kumar R, Basu S. An Appraisal and Update of Fluorodeoxyglucose and Non-Fluorodeoxyglucose-PET Tracers in Thyroid and Non-Thyroid Endocrine Neoplasms. *PET Clin* 2022; **17**: 343-367 [PMID: 35717097 DOI: 10.1016/j.cpet.2022.03.010]

24 **Ferrari SM**, Elia G, Ragusa F, Ruffilli I, La Motta C, Paparo SR, Patrizio A, Vita R, Benvenga S, Materazzi G, Fallahi P, Antonelli A. Novel treatments for anaplastic thyroid carcinoma. *Gland Surg* 2020; **9**: S28-S42 [PMID: 32055496 DOI: 10.21037/gs.2019.10.18]

25 **Nasrpour Navaei Z**, Taghehchian N, Zangouei AS, Abbaszadegan MR, Moghbeli M. MicroRNA-506 as a tumor suppressor in anaplastic thyroid carcinoma by regulation of WNT and NOTCH signaling pathways. *Iran J Basic Med Sci* 2023; **26**: 594-602 [PMID: 37051101 DOI: 10.22038/IJBMS.2023.69174.15069]

26 **Romano C**, Martorana F, Pennisi MS, Stella S, Massimino M, Tirrò E, Vitale SR, Di Gregorio S, Puma A, Tomarchio C, Manzella L. Opportunities and Challenges of Liquid Biopsy in Thyroid Cancer. *Int J Mol Sci* 2021; **22** [PMID: 34299334 DOI: 10.3390/ijms22147707]

27 **Khatami F**, Tavangar SM. Liquid Biopsy in Thyroid Cancer: New Insight. *Int J Hematol Oncol Stem Cell Res* 2018; **12**: 235-248 [PMID: 30595827]

28 **Huang P**, Tang N, Mao LF, Zhang Y, Tang XF, Zhou RY, Wei B, Tan HL, Shi QM, Lin J, Li ZC, Chang S. Nanoclay Drug-Delivery System Loading Potassium Iodide Promotes Endocytosis and Targeted Therapy in Anaplastic Thyroid Cancer. *Nano Lett* 2023; **23**: 8013-8021 [PMID: 37615624 DOI: 10.1021/acs.nanolett.3c01984]

29 **Uppalapati SS**, Guha L, Kumar H, Mandoli A. Nanotechnological Advancements For The Theranostic Intervention In Anaplastic Thyroid Cancer: Current Perspectives And Future Direction. *Curr Cancer Drug Targets* 2023 [PMID: 37424349 DOI: 10.2174/1568009623666230707155145]

30 **Zhou J**, Ma L, Li Z, Chen B, Wu Y, Meng X. Synthesis of lenvatinib-loaded upconversion@polydopamine nanocomposites for upconversion luminescence imaging-guided chemo-photothermal synergistic therapy of anaplastic thyroid cancer. *RSC Adv* 2023; **13**: 26925-26932 [PMID: 37692340 DOI: 10.1039/d3ra02121a]

31 **Wächter S**, Roth S, Gercke N, Schötz U, Dikomey E, Engenhart-Cabillic R, Maurer E, Bartsch DK, Di Fazio P. Anti-Proliferative Effect of Radiotherapy and Implication of Immunotherapy in Anaplastic Thyroid Cancer Cells. *Life (Basel)* 2023; **13** [PMID: 37374179 DOI: 10.3390/life13061397]

32 **Xu T**, Zhu C, Chen J, Song F, Ren X, Wang S, Yi X, Zhang Y, Zhang W, Hu Q, Qin H, Liu Y, Zhang S, Tan Z, Pan Z, Huang P, Ge M. ISG15 and ISGylation modulates cancer stem cell-like characteristics in promoting tumor growth of anaplastic thyroid carcinoma. *J Exp Clin Cancer Res* 2023; **42**: 182 [PMID: 37501099 DOI: 10.1186/s13046-023-02751-9]

33 **Mahdiannasser M**, Khazaei S, Akhavan Rahnama M, Soufi-Zomorrod M, Soutodeh F, Parichehreh-Dizaji S, Rakhsh-Khorshid H, Samimi H, Haghpanah V. Illuminating the role of lncRNAs ROR and MALAT1 in cancer stemness state of anaplastic thyroid cancer: An exploratory study. *Noncoding RNA Res* 2023; **8**: 451-458 [PMID: 37455764 DOI: 10.1016/j.ncrna.2023.05.006]

34 **Hirokawa M**, Niioka H, Suzuki A, Abe M, Arai Y, Nagahara H, Miyauchi A, Akamizu T. Application of deep learning as an ancillary diagnostic tool for thyroid FNA cytology. *Cancer Cytopathol* 2023; **131**: 217-225 [PMID: 36524985 DOI: 10.1002/cncy.22669]

35 **Dong W**, Okamoto T, Ji X, Xiang J, Zhang D, Zhang P, Zhang H. Conditional Survival Rate Estimates for Anaplastic Thyroid Cancer Beyond the First Year: An Analysis of SEER Data (2004-2019). *Thyroid* 2023; **33**: 523-526 [PMID: 36329668 DOI: 10.1089/thy.2022.0339]

36 **Oliinyk D**, Augustin T, Rauch J, Koehler VF, Belka C, Spitzweg C, Käsmann L. Role of surgery to the primary tumor in metastatic anaplastic thyroid carcinoma: pooled analysis and SEER-based study. *J Cancer Res Clin Oncol* 2023; **149**: 3527-3547 [PMID: 35960373 DOI: 10.1007/s00432-022-04223-7]

37 **Cui H**, Wang R, Zhao X, Wang S, Shi X, Sang J. Development and validation of a nomogram for predicting the early death of anaplastic thyroid cancer: a SEER population-based study. *J Cancer Res Clin Oncol* 2023; **149**: 16001-16013 [PMID: 37689588 DOI: 10.1007/s00432-023-05302-z]

38 **Shaha AR**. Anaplastic Thyroid Cancer: Shifting Paradigms-A Ray of Hope. *Thyroid* 2023; **33**: 402-403 [PMID: 36856459 DOI: 10.1089/thy.2023.29150.sha]

39 **Filetti S**, Durante C, Hartl D, Leboulleux S, Locati LD, Newbold K, Papotti MG, Berruti A; ESMO Guidelines Committee. Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Ann Oncol* 2019; **30**: 1856-1883 [PMID: 31549998 DOI: 10.1093/annonc/mdz400]

40 **Gao X**, Hong C, Xie Y, Zeng X. Immunotherapy or targeted therapy: What will be the future treatment for anaplastic thyroid carcinoma? *Front Oncol* 2023; **13**: 1103147 [PMID: 37007127 DOI: 10.3389/fonc.2023.1103147]

41 **Hamidi S**, Maniakas A. Recent advances in anaplastic thyroid cancer management. *Curr Opin Endocrinol Diabetes Obes* 2023; **30**: 259-264 [PMID: 37410453 DOI: 10.1097/MED.0000000000000823]

42 **Wu SS**, Lamarre ED, Yalamanchali A, Brauer PR, Hong H, Reddy CA, Yilmaz E, Woody N, Ku JA, Prendes B, Burkey B, Nasr C, Skugor M, Heiden K, Chute DJ, Knauf JA, Campbell SR, Koyfman SA, Geiger JL, Scharpf J. Association of Treatment Strategies and Tumor Characteristics With Overall Survival Among Patients With Anaplastic Thyroid Cancer: A Single-Institution 21-Year Experience. *JAMA Otolaryngol Head Neck Surg* 2023; **149**: 300-309 [PMID: 36757708 DOI: 10.1001/jamaoto.2022.5045]

43 **Moyer KF**, Marcadis AR, Shaha AR. Airway management, symptom relief and best supportive care in anaplastic thyroid cancer. *Curr Opin Otolaryngol Head Neck Surg* 2020; **28**: 74-78 [PMID: 32022733 DOI: 10.1097/MOO.0000000000000619]

44 **Ginzberg SP**, Gasior JA, Passman JE, Ballester JMS, Finn CB, Karakousis GC, Kelz RR, Wachtel H. Disparities in Presentation, Treatment, and Survival in Anaplastic Thyroid Cancer. *Ann Surg Oncol* 2023; **30**: 6788-6798 [PMID: 37474696 DOI: 10.1245/s10434-023-13945-y]

45 **Vander Poorten V**, Goedseels N, Triantafyllou A, Sanabria A, Clement PM, Cohen O, Golusinski P, Guntinas-Lichius O, Piazza C, Randolph GW, Rinaldo A, Ronen O, Cabanillas ME, Shaha AR, Teng Y, Tufano RP, Williams MD, Zafereo M, Ferlito A. Effectiveness of core needle biopsy in the diagnosis of thyroid lymphoma and anaplastic thyroid carcinoma: A systematic review and meta-analysis. *Front Endocrinol (Lausanne)* 2022; **13**: 971249 [PMID: 36204100 DOI: 10.3389/fendo.2022.971249]

46 **Pavlidis ET**, Pavlidis TE. A Review of Primary Thyroid Lymphoma: Molecular Factors, Diagnosis and Management. *J Invest Surg* 2019; **32**: 137-142 [PMID: 29058491 DOI: 10.1080/08941939.2017.1383536]

47 **Al-Mulki K**, Smith RV. What is the best treatment for Anaplastic Thyroid Cancer? *Laryngoscope* 2023; **133**: 2044-2045 [PMID: 37561090 DOI: 10.1002/lary.30724]

48 **Alobuia W**, Gillis A, Kebebew E. Contemporary Management of Anaplastic Thyroid Cancer. *Curr Treat Options Oncol* 2020; **21**: 78 [PMID:

32767129 DOI: 10.1007/s11864-020-00776-2]

49 **de la Fouchardière C**, Wassermann J, Calcagno F, Bardet S, Al Ghuzlan A, Borget I, Borson Chazot F, Do Cao C, Buffet C, Zerdoud S, Decaussin-Petrucci M, Godbert Y, Leboulleux S. [Molecular genotyping in refractory thyroid cancers in 2021: When, how and why? A review from the TUTHYREF network]. *Bull Cancer* 2021; **108**: 1044-1056 [PMID: 34593218 DOI: 10.1016/j.bulcan.2021.06.009]

50 **Silver Karcioglu A**, Iwata AJ, Pusztaszeri M, Abdelhamid Ahmed AH, Randolph GW. The American Thyroid Association (ATA) integrates molecular testing into its framework for managing patients with anaplastic thyroid carcinoma (ATC): Update on the 2021 ATA ATC guidelines. *Cancer Cytopathol* 2022; **130**: 174-180 [PMID: 34618407 DOI: 10.1002/cncy.22519]

51 **Wang X**, Ying T, Yuan J, Wang Y, Su X, Chen S, Zhao Y, Sheng J, Teng L, Luo C, Wang W. BRAFV600E restructures cellular lactylation to promote anaplastic thyroid cancer proliferation. *Endocr Relat Cancer* 2023; **30** [PMID: 37184950 DOI: 10.1530/ERC-22-0344]

52 **Papanikolaou V**, Kyrodimos E, Mastronikolis N, Asimakopoulos AD, Papanastasiou G, Tsiambas E, Spyropoulou D, Katsinis S, Manoli A, Papouliakos S, Pantos P, Ragos V, Peschos D, Chrysovergis A. Anti-EGFR/BRAF-Tyrosine Kinase Inhibitors in Thyroid Carcinoma. *Cancer Diagn Progn* 2023; **3**: 151-156 [PMID: 36875315 DOI: 10.21873/cdp.10194]

53 **Cabanillas ME**, Ahmed S, Wang JR. Management of Anaplastic and Recurrent Differentiated Thyroid Cancer: Indications for Surgical Resection, Molecular Testing, and Systemic Therapy. *Neuroimaging Clin N Am* 2021; **31**: 359-366 [PMID: 34243870 DOI: 10.1016/j.nic.2021.04.005]

54 **Gong Y**, Xu F, Deng L, Peng L. Recognition of Key Genes in Human Anaplastic Thyroid Cancer *via* the Weighing Gene Coexpression Network. *Biomed Res Int* 2022; **2022**: 2244228 [PMID: 35782055 DOI: 10.1155/2022/2244228]

55 **Bikas A**, Ahmadi S, Pappa T, Marqusee E, Wong K, Nehs MA, Cho NL, Haase J, Doherty GM, Sehgal K, Barletta JA, Alexander EK, Landa I. Additional Oncogenic Alterations in RAS-Driven Differentiated Thyroid Cancers Associate with Worse Clinicopathologic Outcomes. *Clin Cancer Res* 2023; **29**: 2678-2685 [PMID: 37260297 DOI: 10.1158/1078-0432.CCR-23-0278]

56 **Haddad R**, Elisei R, Hoff AO, Liu Z, Pitoia F, Pruneri G, Sadow PM, Soares F, Turk A, Williams MD, Wirth LJ, Cabanillas ME. Diagnosis and Management of Tropomyosin Receptor Kinase Fusion-Positive Thyroid Carcinomas: A Review. *JAMA Oncol* 2023; **9**: 1132-1141 [PMID: 37289450 DOI: 10.1001/jamaoncol.2023.1379]

57 **Zhang P**, Tao C, Shimura T, Huang AC, Kong N, Dai Y, Yao S, Xi Y, Wang X, Fang J, Moses MA, Guo P. ICAM1 antibody drug conjugates exert potent antitumor activity in papillary and anaplastic thyroid carcinoma. *iScience* 2023; **26**: 107272 [PMID: 37520726 DOI: 10.1016/j.isci.2023.107272]

58 **Chen Y**, Jia L, Zhao K, Chen Z, Han Y, He X. CTHRC1 promotes anaplastic thyroid cancer progression by upregulating the proliferation, migration, and invasion of tumor cells. *PeerJ* 2023; **11**: e15458 [PMID: 37273536 DOI: 10.7717/peerj.15458]

59 **Boruah M**, Gaddam P, Agarwal S, Mir RA, Gupta R, Sharma MC, S Deo SV, Nilima N. PD-L1 expression in rare and aggressive thyroid cancers: A preliminary investigation for a role of immunotherapy. *J Cancer Res Ther* 2023; **19**: 312-320 [PMID: 37006068 DOI: 10.4103/jcrt.jcrt_1471_22]

60 **Manjusha P**, Kandathil JP, Nayanar SK, Jones J, Sajith B. Clinicopathological Spectrum of Anaplastic Carcinoma of Thyroid - 5 Year Experience from a Tertiary Cancer Centre. *Gulf J Oncolog* 2018; **1**: 17-22 [PMID: 30344129]

61 **Onoda N**, Sugitani I, Ito KI, Suzuki A, Higashiyama T, Fukumori T, Suganuma N, Masudo K, Nakayama H, Uno A, Yane K, Yoshimoto S, Ebina A, Kawasaki Y, Maeda S, Iwadate M, Suzuki S. Evaluation of the 8th Edition TNM Classification for Anaplastic Thyroid Carcinoma. *Cancers (Basel)* 2020; **12** [PMID: 32120853 DOI: 10.3390/cancers12030552]

62 **Li W**, Li Y, Li J, Pang H. Combination of Novel Therapies and New Attempts in Anaplastic Thyroid Cancer. *Technol Cancer Res Treat* 2023; **22**: 15330338231169870 [PMID: 37122242 DOI: 10.1177/15330338231169870]

63 **Lee H**, Kim SY, Kim SM, Chang HJ, Lee YS, Park CS, Chang HS. Long-term survival of patients with anaplastic thyroid cancer after multimodal treatment. *Transl Cancer Res* 2020; **9**: 5430-5436 [PMID: 35117908 DOI: 10.21037/tcr-20-1364]

64 **Song T**, Chen L, Zhang H, Lu Y, Yu K, Zhan W, Fang M. Multimodal treatment based on thyroidectomy improves survival in patients with metastatic anaplastic thyroid carcinoma: a SEER analysis from 1998 to 2015. *Gland Surg* 2020; **9**: 1205-1213 [PMID: 33224795 DOI: 10.21037/gs-20-503]

65 **Jonker PKC**, Turchini J, Kruijff S, Lin JF, Gill AJ, Eade T, Aniss A, Clifton-Bligh R, Learoyd D, Robinson B, Tsang V, Glover A, Sidhu S, Sywak M. Multimodality Treatment Improves Locoregional Control, Progression-Free and Overall Survival in Patients with Anaplastic Thyroid Cancer: A Retrospective Cohort Study Comparing Oncological Outcomes and Morbidity between Multimodality Treatment and Limited Treatment. *Ann Surg Oncol* 2021; **28**: 7520-7530 [PMID: 34032961 DOI: 10.1245/s10434-021-10146-3]

66 **Lee JH**, Ahn HK, Seok JY, Lee KC, Chun YS, Chung YS, Lee YD. Optimal combination of treatment modality to increase survival in patients with anaplastic thyroid carcinoma: A STROBE compliant retrospective study. *Medicine (Baltimore)* 2018; **97**: e11037 [PMID: 29923991 DOI: 10.1097/MD.0000000000011037]

67 **Kasemsiri P**, Chaisakgreenon P, Vatanasapt P, Laohasiriwong S, Teeramatwanich W, Thongrong C, Ratanaanekchai T, Suetrong S. Survival Benefit of Intervention Treatment in Advanced Anaplastic Thyroid Cancer. *Int J Surg Oncol* 2021; **2021**: 5545127 [PMID: 34123423 DOI: 10.1155/2021/5545127]

68 **Haddad RI**, Bischoff L, Ball D, Bernet V, Blomain E, Busaidy NL, Campbell M, Dickson P, Duh QY, Ehya H, Goldner WS, Guo T, Haymart M, Holt S, Hunt JP, Iagaru A, Kandeel F, Lamonica DM, Mandel S, Markovina S, McIver B, Raeburn CD, Rezaee R, Ridge JA, Roth MY, Scheri RP, Shah JP, Sipos JA, Sippel R, Sturgeon C, Wang TN, Wirth LJ, Wong RJ, Yeh M, Cassara CJ, Darlow S. Thyroid Carcinoma, Version 2.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2022; **20**: 925-951 [PMID: 35948029 DOI: 10.6004/jnccn.2022.0040]

69 **Bible KC**, Kebebew E, Brierley J, Brito JP, Cabanillas ME, Clark TJ Jr, Di Cristofano A, Foote R, Giordano T, Kasperbauer J, Newbold K, Nikiforov YE, Randolph G, Rosenthal MS, Sawka AM, Shah M, Shaha A, Smallridge R, Wong-Clark CK. 2021 American Thyroid Association Guidelines for Management of Patients with Anaplastic Thyroid Cancer. *Thyroid* 2021; **31**: 337-386 [PMID: 33728999 DOI: 10.1089/thy.2020.0944]

70 **Haddad RI**, Nasr C, Bischoff L, Busaidy NL, Byrd D, Callender G, Dickson P, Duh QY, Ehya H, Goldner W, Haymart M, Hoh C, Hunt JP, Iagaru A, Kandeel F, Kopp P, Lamonica DM, McIver B, Raeburn CD, Ridge JA, Ringel MD, Scheri RP, Shah JP, Sippel R, Smallridge RC, Sturgeon C, Wang TN, Wirth LJ, Wong RJ, Johnson-Chilla A, Hoffmann KG, Gurski LA. NCCN Guidelines Insights: Thyroid Carcinoma, Version 2.2018. *J Natl Compr Canc Netw* 2018; **16**: 1429-1440 [PMID: 30545990 DOI: 10.6004/jnccn.2018.0089]

71 **Koda K**, Katoh M, Yasuhara K. Management of anaplastic thyroid cancer and proposed treatment guidelines-A 5-year case series study. *Cancer Rep (Hoboken)* 2022; **5**: e1727 [PMID: 36196000 DOI: 10.1002/cnr2.1727]

72 **Saini S**, Tulla K, Maker AV, Burman KD, Prabhakar BS. Therapeutic advances in anaplastic thyroid cancer: a current perspective. *Mol Cancer*

2018; **17**: 154 [PMID: 30352606 DOI: 10.1186/s12943-018-0903-0]

73 **Zhou W**, Yue Y, Zhang X. Radiotherapy Plus Chemotherapy Leads to Prolonged Survival in Patients With Anaplastic Thyroid Cancer Compared With Radiotherapy Alone Regardless of Surgical Resection and Distant Metastasis: A Retrospective Population Study. *Front Endocrinol (Lausanne)* 2021; **12**: 748023 [PMID: 34790169 DOI: 10.3389/fendo.2021.748023]

74 **Sherman EJ**, Harris J, Bible KC, Xia P, Ghossein RA, Chung CH, Riaz N, Gunn GB, Foote RL, Yom SS, Wong SJ, Koyfman SA, Dzeda MF, Clump DA, Khan SA, Shah MH, Redmond K, Torres-Saavedra PA, Le QT, Lee NY. Radiotherapy and paclitaxel plus pazopanib or placebo in anaplastic thyroid cancer (NRG/RTOG 0912): a randomised, double-blind, placebo-controlled, multicentre, phase 2 trial. *Lancet Oncol* 2023; **24**: 175-186 [PMID: 36681089 DOI: 10.1016/S1470-2045(22)00763-X]

75 **Zheng X**, Wang J, Ye T, Tang W, Pan X, Wang S, Liu J. Efficacy and safety of anlotinib-based chemotherapy for locally advanced or metastatic anaplastic thyroid carcinoma. *Endocrine* 2023; **81**: 540-546 [PMID: 37219702 DOI: 10.1007/s12020-023-03390-y]

76 **Wu J**, Liang J, Liu R, Lv T, Fu K, Jiang L, Ma W, Pan Y, Tan Z, Liu Q, Qiu W, Ge M, Wang J. Autophagic blockade potentiates anlotinib-mediated ferroptosis in anaplastic thyroid cancer. *Endocr Relat Cancer* 2023; **30** [PMID: 37283515 DOI: 10.1530/ERC-23-0036]

77 **Jacob J**, Vordermark D, Lorenz K, Medenwald D. Prognostic factors in radiotherapy of anaplastic thyroid carcinoma: a single center study over 31 years. *Radiat Oncol* 2023; **18**: 71 [PMID: 37076888 DOI: 10.1186/s13014-023-02249-w]

78 **Zhang L**, Feng Q, Wang J, Tan Z, Li Q, Ge M. Molecular basis and targeted therapy in thyroid cancer: Progress and opportunities. *Biochim Biophys Acta Rev Cancer* 2023; **1878**: 188928 [PMID: 37257629 DOI: 10.1016/j.bbcan.2023.188928]

79 **Miller KC**, Chintakuntlawar AV. Molecular-Driven Therapy in Advanced Thyroid Cancer. *Curr Treat Options Oncol* 2021; **22**: 24 [PMID: 33569661 DOI: 10.1007/s11864-021-00822-7]

80 **Nylén C**, Mechera R, Maréchal-Ross I, Tsang V, Chou A, Gill AJ, Clifton-Bligh RJ, Robinson BG, Sywak MS, Sidhu SB, Glover AR. Molecular Markers Guiding Thyroid Cancer Management. *Cancers (Basel)* 2020; **12** [PMID: 32759760 DOI: 10.3390/cancers12082164]

81 **Ibrahimpasic T**, Ghossein R, Shah JP, Ganly I. Poorly Differentiated Carcinoma of the Thyroid Gland: Current Status and Future Prospects. *Thyroid* 2019; **29**: 311-321 [PMID: 30747050 DOI: 10.1089/thy.2018.0509]

82 **Gouda MA**, Subbiah V. Expanding the Benefit: Dabrafenib/Trametinib as Tissue-Agnostic Therapy for BRAF V600E-Positive Adult and Pediatric Solid Tumors. *Am Soc Clin Oncol Educ Book* 2023; **43**: e404770 [PMID: 37159870 DOI: 10.1200/EDBK_404770]

83 **Subbiah V**, Kreitman RJ, Wainberg ZA, Gazzah A, Lassen U, Stein A, Wen PY, Dietrich S, de Jonge MJA, Blay JY, Italiano A, Yonemori K, Cho DC, de Vos FYFL, Moreau P, Fernandez EE, Schellens JHM, Zielinski CC, Redhu S, Boran A, Passos VQ, Ilankumaran P, Bang YJ. Dabrafenib plus trametinib in BRAFV600E-mutated rare cancers: the phase 2 ROAR trial. *Nat Med* 2023; **29**: 1103-1112 [PMID: 37059834 DOI: 10.1038/s41591-023-02321-8]

84 **Lang M**, Longerich T, Anamaterou C. Targeted therapy with vemurafenib in BRAF(V600E)-mutated anaplastic thyroid cancer. *Thyroid Res* 2023; **16**: 5 [PMID: 36855200 DOI: 10.1186/s13044-023-00147-7]

85 **Iesato A**, Li S, Sadow PM, Abbasian M, Nazarian A, Lawler J, Nucera C. The Tyrosine Kinase Inhibitor Lenvatinib Inhibits Anaplastic Thyroid Carcinoma Growth by Targeting Pericytes in the Tumor Microenvironment. *Thyroid* 2023; **33**: 835-848 [PMID: 37171127 DOI: 10.1089/thy.2022.0597]

86 **Iwasaki H**, Toda S, Takahashi A, Masudo K. Outcome of initial lenvatinib treatment in patients with unresectable anaplastic thyroid cancer. *Oncol Lett* 2023; **26**: 416 [PMID: 37614659 DOI: 10.3892/ol.2023.14002]

87 **Zhu W**, Xie B. PLK4 inhibitor exhibits antitumor effect and synergizes sorafenib *via* arresting cell cycle and inactivating Wnt/β-catenin pathway in anaplastic thyroid cancer. *Cancer Biol Ther* 2023; **24**: 2223383 [PMID: 37351847 DOI: 10.1080/15384047.2023.2223383]

88 **Hicks HM**, Pozdeyev N, Sams SB, Pugazhenthi U, Bales ES, Hofmann MC, McKenna LR, Schweppe RE. Fibronectin Contributes to a BRAF Inhibitor-driven Invasive Phenotype in Thyroid Cancer through EGR1, Which Can Be Blocked by Inhibition of ERK1/2. *Mol Cancer Res* 2023; **21**: 867-880 [PMID: 37219859 DOI: 10.1158/1541-7786.MCR-22-1031]

89 **Aprile M**, Cataldi S, Perfetto C, Federico A, Ciccodicola A, Costa V. Targeting metabolism by B-raf inhibitors and diclofenac restrains the viability of BRAF-mutated thyroid carcinomas with Hif-1α-mediated glycolytic phenotype. *Br J Cancer* 2023; **129**: 249-265 [PMID: 37198319 DOI: 10.1038/s41416-023-02282-2]

90 **de Mello DC**, Saito KC, Cristovão MM, Kimura ET, Fuziwara CS. Modulation of EZH2 Activity Induces an Antitumoral Effect and Cell Redifferentiation in Anaplastic Thyroid Cancer. *Int J Mol Sci* 2023; **24** [PMID: 37175580 DOI: 10.3390/ijms24097872]

91 **Iyer PC**, Dadu R, Gule-Monroe M, Busaidy NL, Ferrarotto R, Habra MA, Zafereo M, Williams MD, Gunn GB, Grosu H, Skinner HD, Sturgis EM, Gross N, Cabanillas ME. Salvage pembrolizumab added to kinase inhibitor therapy for the treatment of anaplastic thyroid carcinoma. *J Immunother Cancer* 2018; **6**: 68 [PMID: 29996921 DOI: 10.1186/s40425-018-0378-y]

92 **Gunda V**, Ghosh C, Hu J, Zhang L, Zhang YQ, Shen M, Kebebew E. Combination BRAF(V600E) Inhibition with the Multitargeting Tyrosine Kinase Inhibitor Axitinib Shows Additive Anticancer Activity in BRAF(V600E)-Mutant Anaplastic Thyroid Cancer. *Thyroid* 2023; **33**: 1201-1214 [PMID: 37675898 DOI: 10.1089/thy.2023.0201]

93 **Sukari A**, Kukreja G, Nagasaka M, Shukairy MK, Yoo G, Lin HS, Hotaling J, Kim H. The role of immune checkpoint inhibitors in anaplastic thyroid cancer (Case Series). *Oral Oncol* 2020; **109**: 104744 [PMID: 32402656 DOI: 10.1016/j.oraloncology.2020.104744]

94 **Chintakuntlawar AV**, Foote RL, Kasperbauer JL, Bible KC. Diagnosis and Management of Anaplastic Thyroid Cancer. *Endocrinol Metab Clin North Am* 2019; **48**: 269-284 [PMID: 30717908 DOI: 10.1016/j.ecl.2018.10.010]

95 **Capdevila J**, Wirth LJ, Ernst T, Ponce Aix S, Lin CC, Ramlau R, Butler MO, Delord JP, Gelderblom H, Ascierto PA, Fasolo A, Führer D, Hütter-Krönke ML, Forde PM, Wrona A, Santoro A, Sadow PM, Szpakowski S, Wu H, Bostel G, Faris J, Cameron S, Varga A, Taylor M. PD-1 Blockade in Anaplastic Thyroid Carcinoma. *J Clin Oncol* 2020; **38**: 2620-2627 [PMID: 32364844 DOI: 10.1200/JCO.19.02727]

96 **Hatashima A**, Archambeau B, Armbruster H, Xu M, Shah M, Konda B, Lott Limbach A, Sukrithan V. An Evaluation of Clinical Efficacy of Immune Checkpoint Inhibitors for Patients with Anaplastic Thyroid Carcinoma. *Thyroid* 2022; **32**: 926-936 [PMID: 35583228 DOI: 10.1089/thy.2022.0073]

97 **Xu Z**, Shin HS, Kim YH, Ha SY, Won JK, Kim SJ, Park YJ, Parangi S, Cho SW, Lee KE. Modeling the tumor microenvironment of anaplastic thyroid cancer: an orthotopic tumor model in C57BL/6 mice. *Front Immunol* 2023; **14**: 1187388 [PMID: 37545523 DOI: 10.3389/fimmu.2023.1187388]

98 **Hu H**, Ng TSC, Kang M, Scott E, Li R, Quintana JM, Matvey D, Vantaku VR, Weissleder R, Parangi S, Miller MA. Thyroid Cancers Exhibit Oncogene-Enhanced Macropinocytosis that Is Restrained by IGF1R and Promote Albumin-Drug Conjugate Response. *Clin Cancer Res* 2023; **29**: 3457-3470 [PMID: 37289199 DOI: 10.1158/1078-0432.CCR-22-2976]

99 **Turkmen E**, Sogutlu F, Erdogan M, Biray Avci C. Evaluation of the anticancer effect of telomerase inhibitor BIBR1532 in anaplastic thyroid

cancer in terms of apoptosis, migration and cell cycle. *Med Oncol* 2023; **40**: 196 [PMID: 37284891 DOI: 10.1007/s12032-023-02063-0]

100 **Bellini MI**, Biffoni M, Patrone R, Borcea MC, Costanzo ML, Garritano T, Melcarne R, Menditto R, Metere A, Scorziello C, Summa M, Ventrone L, D'Andrea V, Giacomelli L. Poorly Differentiated Thyroid Carcinoma: Single Centre Experience and Review of the Literature. *J Clin Med* 2021; **10** [PMID: 34830540 DOI: 10.3390/jcm10225258]

101 **Farahati J**, Mäder U, Gilman E, Görges R, Maric I, Binse I, Hänscheid H, Herrmann K, Buck A, Bockisch A. Changing trends of incidence and prognosis of thyroid carcinoma. *Nuklearmedizin* 2019; **58**: 86-92 [PMID: 30917397 DOI: 10.1055/a-0859-7454]

102 **Walczyk A**, Kopczyński J, Gąsior-Perczak D, Pałyga I, Kowalik A, Chrapek M, Hejnold M, Góźdź S, Kowalska A. Poorly differentiated thyroid cancer in the context of the revised 2015 American Thyroid Association Guidelines and the Updated American Joint Committee on Cancer/Tumor-Node-Metastasis Staging System (eighth edition). *Clin Endocrinol (Oxf)* 2019; **91**: 331-339 [PMID: 30525210 DOI: 10.1111/cen.13910]

103 **Roche AM**, Fedewa SA, Shi LL, Chen AY. Treatment and survival vary by race/ethnicity in patients with anaplastic thyroid cancer. *Cancer* 2018; **124**: 1780-1790 [PMID: 29409119 DOI: 10.1002/cncr.31252]

104 **Maniakas A**, Dadu R, Busaidy NL, Wang JR, Ferrarotto R, Lu C, Williams MD, Gunn GB, Hofmann MC, Cote G, Sperling J, Gross ND, Sturgis EM, Goepfert RP, Lai SY, Cabanillas ME, Zafereo M. Evaluation of Overall Survival in Patients With Anaplastic Thyroid Carcinoma, 2000-2019. *JAMA Oncol* 2020; **6**: 1397-1404 [PMID: 32761153 DOI: 10.1001/jamaoncol.2020.3362]

105 **Yu AC**, Han AY, Cronkite DA, Sajed D, St John MA. Anaplastic Transformation of Differentiated Thyroid Carcinoma. *Laryngoscope* 2023; **133**: 437-442 [PMID: 35779253 DOI: 10.1002/lary.30277]

106 **Marchand-Crety C**, Pascard M, Debreuve-Theresette A, Ettalhaoui L, Schvartz C, Zalzali M, Brugel M, Bellefqih S, Servagi-Vernat S. Prognostic Factors and Survival Score for Patients With Anaplastic Thyroid Carcinoma: A Retrospective Study from a Regional Registry. *Anticancer Res* 2021; **41**: 1555-1561 [PMID: 33788749 DOI: 10.21873/anticanres.14915]

107 **Hussain SA**, Subbiah S, Bharathiraja MS. Anaplastic Carcinoma Thyroid - A Review on the Management of this Aggressive Cancer. *Gulf J Oncolog* 2018; **1**: 37-41 [PMID: 30344132]

108 **Cho S**, Kim H, Oh YL, Hahn SY, Kim TH, Shin JH. Comparison of clinicopathological characteristics and survival between symptomatic and asymptomatic anaplastic thyroid carcinoma. *Sci Rep* 2023; **13**: 3264 [PMID: 36828842 DOI: 10.1038/s41598-023-30162-5]

109 **Jin S**, Liu X, Peng D, Li D, Ye YN. Differences Between Cancer-Specific Survival of Patients With Anaplastic and Primary Squamous Cell Thyroid Carcinoma and Factors Influencing Prognosis: A SEER Database Analysis. *Front Endocrinol (Lausanne)* 2022; **13**: 830760 [PMID: 35360080 DOI: 10.3389/fendo.2022.830760]

110 **Wang M**, Wei T, Gong R, Zhu J, Li Z. Risk stratification in patients with anaplastic thyroid carcinoma: role of age. *Endocrine* 2022; **77**: 305-318 [PMID: 35608773 DOI: 10.1007/s12020-022-03085-w]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 December 24; 14(12): 584-592

DOI: 10.5306/wjco.v14.i12.584

ISSN 2218-4333 (online)

MINIREVIEWS

Current perspectives on the management of lateral pelvic lymph nodes in rectal cancer

Jonathan Yu Jin Chua, James Chi Yong Ngu, Nan Zun Teo

Specialty type: Surgery

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Bustamante-Lopez LA, Brazil; Sano W, Japan

Received: August 28, 2023
Peer-review started: August 28, 2023
First decision: November 1, 2023
Revised: November 7, 2023
Accepted: November 24, 2023
Article in press: November 24, 2023
Published online: December 24, 2023

Jonathan Yu Jin Chua, James Chi Yong Ngu, Nan Zun Teo, Department of General Surgery, Changi General Hospital, Singapore 529889, Singapore

Corresponding author: Nan Zun Teo, Department of General Surgery, Changi General Hospital, 2 Simei Street 3, Singapore 529889, Singapore. teo.nan.zun@singhealth.com.sg

Abstract

Significant controversies exist with regards to the optimal management of lateral pelvic lymph nodes metastases (mLLN) in patients with low rectal cancer. The differing views held by Japanese and Western clinicians on the management of mLLN have been well documented. However, the adequacy of pelvic lymph node dissection (PLND) or neoadjuvant chemoradiation (NACRT) alone in addition to total mesorectal excision (TME) have recently come into question, due to the relatively high incidence of lateral local recurrences following PLND and TME, or NACRT and TME alone. Recently, a more selective approach to PLND has been suggested, involving a combination of neoadjuvant therapy, followed by PLND only to patients in whom the oncological benefit is likely to outweigh the risk of potential adverse events. A number of studies have attempted to retrospectively identify certain nodal characteristics on preoperative imaging, such as nodal size, appearance, and size reduction following neoadjuvant therapy. However, no consensus has been reached regarding the optimal criteria for a selective approach to PLND, partly due to the heterogeneity and retrospective nature of most of these studies. This review aims to provide an overview of recent evidence with regards to the diagnostic challenges, considerations for, and outcomes of the current management strategies for mLLN in rectal cancer patients.

Key Words: Pelvic lymph node dissection; Lateral pelvic lymph nodes; Diagnostic criteria; Short axis diameter; Radiotherapy; Rectal cancer

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The optimal management strategy for lateral pelvic lymph node metastases (mLLN) requires a multimodal approach, involving chemoradiation and pelvic lymph node dissection (PLND), in order to achieve adequate local control in patients with locally advanced low rectal cancer. This selective approach requires careful selection of patients who would benefit most from PLND, using pre-treatment nodal short axis measurements as a surrogate for mLLN risk.

Citation: Chua JYJ, Ngu JCY, Teo NZ. Current perspectives on the management of lateral pelvic lymph nodes in rectal cancer. *World J Clin Oncol* 2023; 14(12): 584-592
URL: https://www.wjgnet.com/2218-4333/full/v14/i12/584.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i12.584

INTRODUCTION

Total mesorectal excision (TME) and the circumferential resection margin have been widely accepted as crucial elements in the surgical treatment of rectal cancer. However, the management of pelvic side wall disease remains controversial, and historically divergent between countries in the West and those in the far East. While the former predominantly recommend the use of radiotherapy (with or without chemotherapy), pelvic lymph node dissection (PLND) is preferred in the latter. This has been reflected in guidelines published by their respective societies[1-3].

Results from the Dutch TME trial[4] (10-year local recurrence (LR) rates of 5% in the irradiated group *vs* 11% in the non-irradiated group, $P < 0.0001$) and the Swedish Rectal Cancer Trial[5] (LR rate of 9% in the irradiated group *vs* 26% in the non-irradiated group, $P < 0.001$) supported the use of neoadjuvant radiotherapy. These rates were comparable to patients who underwent PLND in some Japanese studies. In contrast, early results of PLND in the West[6,7] were discouraging due to high perioperative morbidity and limited reported oncological benefit[8], resulting in its slow uptake.

In Japan, however, lower local failure rates (Dukes B cases 8.4% *vs* 26.1%, $P < 0.01$, Dukes C cases 24.5% *vs* 44.3%, $P < 0.01$) and improved 5-year survival (Dukes B cases 83.2% *vs* 63.7%, $P < 0.05$; Dukes C cases 52.5% *vs* 30.8%, $P < 0.05$) were reported when extended lymphadenectomy was performed[9]. In addition, PLND was only associated with a slight prolongation of operating time (additional 60 min), a modest increase in operative blood loss (additional 150 mL), and no increase in operative mortality[9].

This article aims to elucidate the factors contributing to the contrasting recommendations in the management of lateral pelvic lymph nodes (LLN), and to provide a more contemporary approach to this conundrum. Literature search was performed electronically using PubMed (MEDLINE) and the *Reference Citation Analysis* (https://www.referencecitation-analysis.com) was applied. The search terms were as follows: pelvic lymph node dissection or PLND, lateral lymph node metastasis, and rectal cancer in combination with Boolean operators AND and OR. All studies in English were extracted for review by the authors.

THE SIGNIFICANCE OF THE LATERAL PELVIC LYMPH NODES

The difference in lymphatic drainage of the lower rectum from the upper rectum has been well documented, with Gerota [10] describing how tumours in the mid and lower rectum appear to exhibit lateral lymphatic drainage into the iliac nodes in addition to upward drainage through mesorectal nodes[11,12].

The risk of developing lateral lymph node metastases (mLLN) in rectal cancer has been shown to vary with several factors. Distance from the anal verge has been reported to be inversely related to the risk of mLLN, with rates of up to 33.3% observed in tumours 3.9cm from the anal verge[13]. Locally advanced pT3 and pT4 tumours tend to also be associated with higher rates of mLLN[13]. In particular, it has been demonstrated that mLLN were mostly located in the group of nodes along the internal iliac artery (IIA), being the first draining basin from the lateral rectal ligaments[14-16].

Traditional TNM staging for rectal cancer classifies malignant deposits in the external iliac and obturator nodes as distant metastases[17]. On the other hand, the Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma (3rd edition)[18], includes lymph nodes along the IIA, obturator, external iliac, common iliac (CIA), and median sacral arteries within its definition of regional lymph nodes, in the context of lower rectal cancers. This was based on survival data from the Japanese Nationwide Multi-Institutional Study on Lateral Pelvic Lymph Node Metastasis in Low Rectal Cancer[19]. Patients with metastasis to the above, so-called external lateral pelvic nodes, demonstrated more favourable overall survival and cancer-specific survival if they underwent PLND, than in patients with stage IV disease who underwent R0 resection (overall survival 29% *vs* 24%, $P = 0.0240$, cancer-specific survival 37% *vs* 27%, $P = 0.0117$). In addition, Ogura *et al*[20] determined that LLN enlargement did not appear to influence distant recurrence rate, suggesting that mLLN likely represent locoregional disease.

DIAGNOSTIC DILEMMAS – DIAGNOSTIC CRITERIA, MISDIAGNOSIS AND MISSED DIAGNOSES

However, epidemiological studies on mLLN suffer from the heterogenous methods used in evaluating nodal disease, with incidence rates being reported to range between 8.8% and 34%[13,21]. Studies that do not involve PLND would base their diagnosis on imaging, whereas analyses involving patients who had undergone PLND would report based on pathological confirmation. Most studies that evaluate recurrences in the pelvic side wall do so by means of imaging parameters.

The main challenge in preoperative radiological assessment of LLN lies in not missing occult metastases within the nodes (missed diagnoses), while minimising cases of misdiagnoses. Most imaging modalities have been evaluated for their diagnostic accuracy in detecting suspicious lateral pelvic nodes. Ultrasonography was suggested as a potential imaging modality for this purpose, but failed to adequately examine obturator nodes[22], and has largely been surpassed by other imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI). Even then, the sensitivity of CT and MRI in detecting mLLN varies greatly between studies[23,24]. More recently, the accuracy of F-fluorodeoxyglucose positron-emission tomography (^{18}F-FDG PET) as a diagnostic adjunct in addition to CT or MRI has also been evaluated, although many guidelines do not include ^{18}F-FDG PET scanning as part of the initial staging for rectal cancer patients[2,25]. A study by Ishihara *et al*[26] evaluated the accuracy of ^{18}F-FDG PET scanning in identifying suspicious LLN post neoadjuvant chemotherapy, using a calculated maximum standard uptake value (SUV max) of 1.6, and reported an accuracy, sensitivity, and specificity of 85.7%, 76.5%, and 100% respectively. Metastatic LLN were found to have a significantly higher SUV max when compared to LLN without metastatic deposits (mean ± standard deviation 2.2 ± 1.3 *vs* 1.2 ± 0.3, $P < 0.01$). A similar study by Yukimoto *et al*[27] subsequently reported similar values (accuracy 92.3%, sensitivity 82.4%, specificity 93.4%) with a slightly lower SUVmax cutoff value of 1.5. These studies were mainly limited due to their small cohort size, and the utility of ^{18}F-FDG PET scanning in rectal cancer in most units has been mainly limited to the evaluation of equivocal findings on contrast-enhanced CT, or in patients with a strong contraindication to intravenous contrast[3]. As a result, the European Society for Medical Oncology and the American Society of Colon and Rectal Surgeons still recommend the use of pelvic MRI for locoregional staging[2,25].

Apart from the type of imaging modality, there also exists a lack of consensus in what imaging features constitute a suspicious LLN, or mLLN. Table 1 summarises the various criteria used. Most studies retrospectively identify short (SAD), or long axis diameter (LAD) measurements and nodal features that correlate with pathological nodal metastases and/or oncological outcome. The multi-national Society of Abdominal Radiology – Rectal & Anal Cancer Disease-Focused Panel recently published a consensus statement[28] to promote consistent terminology and reporting standards amongst abdominal radiologists. The consensus statement recommended internal iliac and obturator nodes with SAD > 7 mm be reported as suspicious[28]. The MERCURY[29] study reviewed the preoperative MRI images of patients with biopsy-proven rectal adenocarcinoma within 15cm from the anal verge who underwent TME without PLND. The nodes were considered suspicious based on the presence of mixed signal intensity and/or an irregular nodal capsule border.

Further contributing to the heterogeneity is the inconsistent use of pre- or post-neoadjuvant imaging, or a combination of both sets of imaging (reflecting the response to neoadjuvant treatment). Akiyoshi *et al*[30] showed that the incidence of occult mLLN was as high as 20% even in patients with a post-neoadjuvant nodal size of 5 mm or less, supporting the recommendation of basing further treatment selection on pre-neoadjuvant imaging.

With regards to post-neoadjuvant nodal size, Cribb *et al*[31] found that a SAD of 5 mm on post-treatment MRI was associated with a worse 3-year local recurrence-free survival [hazard ratio (HR) 8.35, $P = 0.001$]. Malakorn *et al*[32] concluded that a post-neoadjuvant nodal size of 5 mm was 100% sensitive for identifying patients with mLLN and as such recommend using a post-neoadjuvant LLN size cutoff of 5 mm for PLND. The high reported sensitivity of a post-treatment nodal SAD of 5 mm is promising and has been recommended as suitable criteria for PLND[32,33]. In addition, the Lateral Node Study Consortium demonstrated that PLND can be safely omitted in patients with LLN measuring 4mm or less on restaging MRI due to the negligible risk of lateral local recurrence at 3 years in this subgroup of patients[14].

Akiyoshi *et al*[30] analysed patients with cT3/4 rectal cancers who underwent either bilateral (15.6%) or unilateral (84.4%) PLND, based on a nodal LAD cutoff of 7 mm on pre-neoadjuvant CT or MRI. Pathological mLLN were found in 40.3% of patients, and persistent LLN on restaging was associated with a higher rate of metastatic deposits when compared with LLN that responded to neoadjuvant chemoradiotherapy (CRT) (75% *vs* 20%, $P < 0.0001$)[30].

In publications reporting pathological results, incidence rates can also be confounded by potential missed diagnoses. The identification of micrometastatic disease or isolated tumour cells may sometimes pose a diagnostic challenge. Miyake *et al*[34] compared the sensitivity of one-step nucleic acid amplification assay results to conventional histological diagnosis, and identified a number of additional histologically-negative nodes with metastatic disease. Limitations in commonly utilised histological processing methods may have resulted in a small proportion of missed diagnoses of mLLN, with failure to pathologically upstage such patients resulting in adverse prognostic implications.

THE ADEQUACY OF PLND OR/AND CHEMORADIATION

While the efficacy of neoadjuvant CRT in reducing LR rates have been well documented[4,5], lateral pelvic recurrences have nonetheless been reported in cases where PLND was omitted after CRT[35]. Kim *et al*[36] reported a 64.6% lateral local recurrence (LLR) rate, out of a 7.2% LR rate following pre or post-operative chemoradiotherapy, after a median follow-up period of 65 mo. Kusters *et al*[37] similarly reported a 64.3% LLR rate and 18.7% LR rate. Both studies concluded that LLN measuring 10 mm were associated with an increased risk of recurrence and poorer overall survival, and that CRT alone in these patients did not confer adequate local control.

Table 1 Summary of diagnostic criteria for suspicious lateral pelvic lymph nodes

Study	Imaging modality	Nodal size	Nodal features
Schaap *et al*[53], 2021	MRI	Pre-treatment: SAD 7 mm	-
Amano *et al*[23], 2020	MRI; CT; PET-CT	(MRI or CT) SAD > 6 mm; (PET/CT) increased FDG uptake	-
Kim *et al*[54], 2020	MRI	Pre-treatment: SAD 7 mm; Post-treatment: SAD 4 mm	-
Lee *et al*[55], 2019	CT or MRI	Pre-treatment: SAD 8 mm	-
Sapci *et al*[56], 2019	MRI	Size > 5 mm	And either heterogeneity or border irregularity
Schaap *et al*[57], 2018	MRI	SAD 10 mm	-
Kim *et al*[58], 2018	MRI	Pre-treatment: SAD 5 mm	Signal intensity homo/heterogenous; Margins irregular or well defined; DWI signal intensity high or low; Size reduction rate
Akiyoshi *et al*[30], 2015	MRI	Pre-treatment: SAD 8 mm	-
Kobayashi *et al*[59], 2015	CT	LAD > 9 mm; SAD > 6 mm	-
Ogawa *et al*[60], 2015	MRI	SAD 10 mm or 5 mm (institution-dependent)	Enlarged LPLN on palpation; Enlarged perirectal node or LPLN 5 mm
Ogawa *et al*[61], 2014	MRI	LAD 5 mm; LAD < 5 mm	-
Shihab *et al*[29], 2011	MRI	No size criteria	Mixed signal intensity or irregular nodal capsule border
Matsuoka *et al*[62], 2007	MRI	LAD 10 mm; SAD 5 mm	Ovoid shape; heterogeneity

CT: Computed tomography; MRI: Magnetic resonance imaging; LAD: Long axis diameter; SAD: Short axis diameter; LPLN: Lateral pelvic lymph node.

On the other hand, the Japanese JCOG0212[38-40] randomised controlled trial illustrated the impact of bilateral prophylactic PLND alone, without the use of CRT, even though adjuvant chemotherapy was prescribed to pathological stage III patients. Only patients without clinically suspicious LLN nodes (SAD 10 mm on CT/MRI) were enrolled. The study reported that the addition of PLND resulted in a statistically significant reduction in LR rates (7.4% *vs* 12.6%, P = 0.024), and a higher local recurrence-free survival of 85.3%, compared to 80.3% with TME alone. The authors therefore concluded that the trial failed to demonstrate the noninferiority of TME alone, even though the significant reduction in LR may have resulted from the SAD cutoff of 10mm being insufficiently sensitive in predicting for mLLN. Nonetheless, the 7% incidence of occult mLLN in this trial suggests that a significant proportion of patients were subjected to the morbidity of PLND without deriving any oncological benefit.

Other studies evaluated the impact of combining the two treatment modalities. Kim *et al*[35] retrospectively analysed 366 patients with cT3/4 tumours within 8 cm from the anal verge who received CRT prior to TME without PLND. They reported a LR rate of 7.9% after a median follow-up duration of 40.1 mo, with 82.7% of these being LLR. Conversely, the addition of PLND to TME significantly reduced LR rates despite prior CRT (CRT+TME 19.5% *vs* CRT+TME+PLND 5.7%, P = 0.042)[20].

A three-armed multinational study by Kusters *et al*[41] compared patients with rectal cancer from the Netherlands and Japan who underwent either: (1) TME alone; (2) TME with (neo)adjuvant radiation; or (3) TME with PLND. Similar overall LR rates were reported between groups (2) and (3) (RT+TME 5.8% *vs* 6.9% PLND+TME, HR 1.0 (0.6-1.8). Only group (1) had a higher 5-year LR rate of 12.1%.

Recently, a multicentre retrospective study by Ogura *et al*[14] found that nodes along the internal iliac artery were less responsive to chemoradiation, and concluded that IIA nodes measuring 7 mm or more on pre-treatment MRI were predictors of lateral local recurrence. The study reported 5-year LLR rates of 52.3% following neoadjuvant chemoradiotherapy and TME surgery but without PLND[14]. When PLND was performed, the 5-year LLR risk was significantly reduced to 8.7% (P = 0.007)[14].

SELECTIVE PLND POST NEOADJUVANT RADIOTHERAPY

The optimal management of mLLN appears to therefore be shifting towards a selective multimodal approach, with selective PLND post neoadjuvant therapy appearing to offer higher rates of local control in several studies. Numerous variables have been proposed as potential indications for PLND due to their reported sensitivities in identifying occult mLLN, and their prognostic implications. In addition to the aforementioned studies, Akiyoshi *et al*[42] reviewed patients with stage II-III low rectal cancer who underwent preoperative CRT prior to surgery. PLND was performed in patients with suspicious LLN on pre-neoadjuvant CT or MRI, using SAD criteria of ≥ 7 mm[42]. Patients with clinically enlarged LLN underwent PLND irrespective of findings on post-treatment restaging[42]. The study observed that no LLR occurred in patients who underwent PLND, while 3.4% of patients who only underwent TME post chemoradiation developed LLR [42]. A similar study by Ishihara *et al*[43] reported similar findings. PLND was again performed based on the presence of suspicious pre-neoadjuvant nodes, irrespective of their response to neoadjuvant treatment[43]. The study reported LLR rates of 0% and 0.9% in patients who underwent TME with PLND and TME only respectively[43], suggesting that the selective addition of PLND is key in achieving local control in the lateral pelvis. Therefore, suspicious internal iliac or obturator nodes with pre-treatment SAD of ≥ 7 mm, or the presence of nodes displaying heterogeneity and/or irregular borders, should form indications for PLND.

TECHNICAL CHALLENGES OF PLND

In the treatment of rectal cancer, PLND typically involves removal of nodes in the internal iliac and obturator compartments[44]. The JCOG0212 trial concluded that the addition of PLND was associated with a significantly longer operative time (median 360 min *vs* 254 min, $P < 0.0001$) when compared to TME alone, and was associated with more intraoperative blood loss (576 mL *vs* 337 mL, $P < 0.0001$)[45]. No statistically significant differences were reported with regards to the incidence of anastomotic leakage ($P = 0.46$), urinary retention ($P = 0.18$), wound infection ($P = 0.81$), pelvic abscess ($P = 0.29$), or bowel obstruction ($P = 1.00$)[45]. A meta-analysis of extended lymphadenectomy *vs* conventional surgery for rectal cancer found similar results, with no significant differences in perioperative mortality ($P = 0.63$) or morbidity ($P = 0.13$)[46].

In a bid to promote the safe implementation of PLND, Ngu *et al*[47] conceptualised the use of origami to convert the pelvic side wall from a 2-dimensional region into a 3-dimensional compartment made up of two triangular pyramids. The authors sought to simplify PLND into a procedure involving three planes, three boundaries, and three steps. The three planes consisted of: (1) The ureterohypogastric nerve fascia (UHNF); (2) the vesicohypogastric fascia; and (3) the external iliac muscular plane. Following medialisation of the UHNF, the proximal boundary is marked by two key landmarks: superficially where the ureter crosses the CIA and, at a deeper plane, the bifurcation of the common iliac vein, where the obturator nerve enters the pelvic sidewall compartment. The distal boundary is delineated superficially by the vas deferens or round ligament, and, at a deeper level, the obturator foramen. The third (deep) boundary is marked by the terminal branches of the internal iliac vessels. The three steps of PLND involve: (1) The separation of these three planes, (2) followed by the delineation of the three boundaries, and (3) finally the dissection of the internal iliac vessels, with en bloc removal of the lympho-fatty tissue.

Tang *et al*[48] compared the short-term outcomes of laparoscopic PLND against open PLND, and concluded that laparoscopic PLND was associated with a shorter operative time (255 min *vs* 300 min, $P = 0.001$), less intraoperative blood loss (50 mL *vs* 300 mL, $P < 0.001$), lower incidence of postoperative complications (32% *vs* 15%, $P = 0.005$), shorter postoperative hospital stay (8 *vs* 14 d, $P < 0.001$), and excision of more lateral pelvic nodes (9 *vs* 7 nodes, $P = 0.025$) when compared to open PLND. Oncological outcomes were similar, with no differences reported in 3-year overall survival ($P = 0.581$) and disease-free survival ($P = 0.745$) rates[48]. Aside from the aforementioned postoperative complications, this study also reported other surgical complications such as chylous ascites and lower limb neuropathy, as well as systemic complications such as renal failure, pneumonia, and arrhythmias[48].

Utilization of the robotic platform in PLND has recently been shown to result in lower blood loss (25 mL *vs* 637 mL, $P < 0.0001$) and less postoperative complications including wound infection, anastomotic leakage, urinary retention, and small bowel obstruction when compared to open PLND, but operative times were longer (455 min *vs* 410 min, $P < 0.007$) [49]. Robotic PLND was also associated with superior 5-year local relapse-free survival rates compared to open PLND (98.6% *vs* 90.9%, $P = 0.029$), with similar overall survival (robotic 95.4% *vs* open 87.8%, $P = 0.106$) and relapse-free survival rates (robotic 79.1% *vs* open 69.9%, $P = 0.157$)[50]. Although PLND is a technically demanding procedure with significant risk of associated morbidity, robotic or laparoscopic assistance may be useful adjuncts, associated with lower postoperative morbidity rates when performed by experienced surgeons.

Although not traditionally a recordable perioperative morbidity, the potential of missed nodes during PLND may result in poorer oncological outcomes. A novel strategy to potentially mitigate the risk of intraoperatively missed nodes during PLND is the utilisation of indocyanine green (ICG) during laparoscopic PLND[51,52]. Ohya *et al*[52] conducted a retrospective study of patients who underwent PLND for tumours cT3 and above with clinically suspicious lateral pelvic nodes on pre-op imaging. The study demonstrated an increased lymph node yield (ICG 14 *vs* no ICG 9, $P < 0.001$), without a substantial difference in post-operative complications ($P = 0.57$), aside from a longer operative time (ICG 426 min *vs* no-ICG 369 min, $P < 0.001$). ICG use was also associated with a significant reduction in intraoperative blood loss (13 mL *vs* 100 mL, $P = 0.001$). The authors recently published their long-term follow-up data, and the higher lymph node yield with ICG translated into a reduction in 3-year cumulative LR rates (ICG 0% *vs* no-ICG 9.3%, $P = 0.048$), although no statistically significant difference was reported in relapse-free survival and overall survival rates[51].

CONCLUSION

The difficulty in reaching a global consensus with regards to the optimal management of LLN in rectal cancer stems from the heterogeneity of available data, mainly consisting of retrospective cohort studies using various parameters to define what constitutes a clinically suspicious LLN, or mLLN. Contemporary data appears to suggest that the optimal strategy may lie somewhere between the traditional views held by Western countries and the far East. Several conclusions can be drawn from the existing data: Firstly, pelvic lymph node dissection in rectal cancer has to offered selectively. The JCOG0212[38-40] study demonstrated that in the absence of radiologically suspicious nodes, the majority of patients would not benefit from PLND, hence justifying a more selective, non-prophylactic approach to PLND. Secondly, the optimal management strategy for mLLN in patients with rectal cancer requires a multimodal approach, involving a combination of neadjuvant chemoradiation and selective PLND. Thirdly, until more robust data is made available, a prudent choice would be to use a SAD of 7 mm, or the presence of suspicious features, as criteria for selective PLND. This assessment should be made based on pre-neoadjuvant MRI.

FOOTNOTES

Author contributions: Chua JYJ drafted the article; Ngu JCY and Teo NZ were involved in the concept and design of the article, critical revision, and final approval.

Conflict-of-interest statement: All the authors report no relevant conflict of interest for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Singapore

ORCID number: Jonathan Yu Jin Chua 0000-0003-1281-0900; James Chi Yong Ngu 0000-0001-5233-457X; Nan Zun Teo 0000-0002-5475-476X.

S-Editor: Liu JH
L-Editor: A
P-Editor: Yu HG

REFERENCES

1 **Hashiguchi Y**, Muro K, Saito Y, Ito Y, Ajioka Y, Hamaguchi T, Hasegawa K, Hotta K, Ishida H, Ishiguro M, Ishihara S, Kanemitsu Y, Kinugasa Y, Murofushi K, Nakajima TE, Oka S, Tanaka T, Taniguchi H, Tsuji A, Uehara K, Ueno H, Yamanaka T, Yamazaki K, Yoshida M, Yoshino T, Itabashi M, Sakamaki K, Sano K, Shimada Y, Tanaka S, Uetake H, Yamaguchi S, Yamaguchi N, Kobayashi H, Matsuda K, Kotake K, Sugihara K; Japanese Society for Cancer of the Colon and Rectum. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2019 for the treatment of colorectal cancer. *Int J Clin Oncol* 2020; **25**: 1-42 [PMID: 31203527 DOI: 10.1007/s10147-019-01485-z]

2 **Glynne-Jones R**, Wyrwicz L, Tiret E, Brown G, Rödel C, Cervantes A, Arnold D; ESMO Guidelines Committee. Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2017; **28**: iv22-iv40 [PMID: 28881920 DOI: 10.1093/annonc/mdx224]

3 **Benson AB**, Venook AP, Al-Hawary MM, Azad N, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Garrido-Laguna I, Grem JL, Gunn A, Hecht JR, Hoffe S, Hubbard J, Hunt S, Jeck W, Johung KL, Kirilcuk N, Krishnamurthi S, Maratt JK, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Nurkin S, Overman MJ, Parikh A, Patel H, Pedersen K, Saltz L, Schneider C, Shibata D, Skibber JM, Sofocleous CT, Stotsky-Himelfarb E, Tavakkoli A, Willett CG, Gregory K, Gurski L. Rectal Cancer, Version 2.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2022; **20**: 1139-1167 [PMID: 36240850 DOI: 10.6004/jnccn.2022.0051]

4 **van Gijn W**, Marijnen CA, Nagtegaal ID, Kranenbarg EM, Putter H, Wiggers T, Rutten HJ, Påhlman L, Glimelius B, van de Velde CJ; Dutch Colorectal Cancer Group. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. *Lancet Oncol* 2011; **12**: 575-582 [PMID: 21596621 DOI: 10.1016/S1470-2045(11)70097-3]

5 **Folkesson J**, Birgisson H, Pahlman L, Cedermark B, Glimelius B, Gunnarsson U. Swedish Rectal Cancer Trial: long lasting benefits from radiotherapy on survival and local recurrence rate. *J Clin Oncol* 2005; **23**: 5644-5650 [PMID: 16110023 DOI: 10.1200/JCO.2005.08.144]

6 **Sauer I**, BACON HE. Influence of lateral spread of cancer of the rectum on radicability of operation and prognosis. *Am J Surg* 1951; **81**: 111-120 [PMID: 14799702 DOI: 10.1016/0002-9610(51)90196-1]

7 **Stearns MW Jr**, DEDDISH MR. Five-year results of abdominopelvic lymph node dissection for carcinoma of the rectum. *Dis Colon Rectum* 1959; **2**: 169-172 [PMID: 13652786 DOI: 10.1007/BF02616711]

8 **Williamson JS**, Quyn AJ, Sagar PM. Rectal cancer lateral pelvic sidewall lymph nodes: a review of controversies and management. *Br J Surg* 2020; **107**: 1562-1569 [PMID: 32770742 DOI: 10.1002/bjs.11925]

9 **Koyama Y**, Moriya Y, Hojo K. Effects of extended systematic lymphadenectomy for adenocarcinoma of the rectum--significant improvement of survival rate and decrease of local recurrence. *Jpn J Clin Oncol* 1984; **14**: 623-632 [PMID: 6520971]

10 **Gerota D**. Die Lymphgefasse des Rectums und des Anus. *Arch Anat Physiol* 1895

11 **Zhou S**, Jiang Y, Pei W, Liang J, Zhou Z. Prognostic significance of lateral pelvic lymph node dissection for middle-low rectal cancer patients

with lateral pelvic lymph node metastasis: a propensity score matching study. *BMC Cancer* 2022; **22**: 136 [PMID: 35109810 DOI: 10.1186/s12885-022-09254-4]

12 **SAUER I**, BACON HE. A new approach for excision of carcinoma of the lower portion of the rectum and anal canal. *Surg Gynecol Obstet* 1952; **95**: 229-242 [PMID: 14950656]

13 **Morikawa E**, Yasutomi M, Shindou K, Matsuda T, Mori N, Hida J, Kubo R, Kitaoka M, Nakamura M, Fujimoto K. Distribution of metastatic lymph nodes in colorectal cancer by the modified clearing method. *Dis Colon Rectum* 1994; **37**: 219-223 [PMID: 8137667 DOI: 10.1007/BF02048158]

14 **Ogura A**, Konishi T, Beets GL, Cunningham C, Garcia-Aguilar J, Iversen H, Toda S, Lee IK, Lee HX, Uehara K, Lee P, Putter H, van de Velde CJH, Rutten HJT, Tuynman JB, Kusters M; Lateral Node Study Consortium. Lateral Nodal Features on Restaging Magnetic Resonance Imaging Associated With Lateral Local Recurrence in Low Rectal Cancer After Neoadjuvant Chemoradiotherapy or Radiotherapy. *JAMA Surg* 2019; **154**: e192172 [PMID: 31268504 DOI: 10.1001/jamasurg.2019.2172]

15 **Kanemitsu Y**, Komori K, Shida D, Ochiai H, Tsukamoto S, Kinoshita T, Moriya Y. Potential impact of lateral lymph node dissection (LLND) for low rectal cancer on prognoses and local control: A comparison of 2 high-volume centers in Japan that employ different policies concerning LLND. *Surgery* 2017; **162**: 303-314 [PMID: 28366499 DOI: 10.1016/j.surg.2017.02.005]

16 **Takahashi T**, Ueno M, Azekura K, Ohta H. Lateral node dissection and total mesorectal excision for rectal cancer. *Dis Colon Rectum* 2000; **43**: S59-S68 [PMID: 11052480 DOI: 10.1007/BF02237228]

17 **Brierley JD**, Gospodarowicz MK, Wittekind C. TNM Classification of Malignant Tumours, 8th Edition. *Wiley-Blackwell* 2016; 73-76

18 **Japanese Society for Cancer of the Colon and Rectum**. Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma: the 3d English Edition [Secondary Publication]. *J Anus Rectum Colon* 2019; **3**: 175-195 [PMID: 31768468 DOI: 10.23922/jarc.2019-018]

19 **Akiyoshi T**, Watanabe T, Miyata S, Kotake K, Muto T, Sugihara K; Japanese Society for Cancer of the Colon and Rectum. Results of a Japanese nationwide multi-institutional study on lateral pelvic lymph node metastasis in low rectal cancer: is it regional or distant disease? *Ann Surg* 2012; **255**: 1129-1134 [PMID: 22549752 DOI: 10.1097/SLA.0b013e3182565d9d]

20 **Ogura A**, Konishi T, Cunningham C, Garcia-Aguilar J, Iversen H, Toda S, Lee IK, Lee HX, Uehara K, Lee P, Putter H, van de Velde CJH, Beets GL, Rutten HJT, Kusters M; Lateral Node Study Consortium. Neoadjuvant (Chemo) radiotherapy With Total Mesorectal Excision Only Is Not Sufficient to Prevent Lateral Local Recurrence in Enlarged Nodes: Results of the Multicenter Lateral Node Study of Patients With Low cT3/4 Rectal Cancer. *J Clin Oncol* 2019; **37**: 33-43 [PMID: 30403572 DOI: 10.1200/JCO.18.00032]

21 **Peacock O**, Manisundaram N, Dibrito SR, Kim Y, Hu CY, Bednarski BK, Konishi T, Stanietzky N, Vikram R, Kaur H, Taggart MW, Dasari A, Holliday EB, You YN, Chang GJ. Magnetic Resonance Imaging Directed Surgical Decision Making for Lateral Pelvic Lymph Node Dissection in Rectal Cancer After Total Neoadjuvant Therapy (TNT). *Ann Surg* 2022; **276**: 654-664 [PMID: 35837891 DOI: 10.1097/SLA.0000000000005589]

22 **Tada M**, Endo M. Ultrasonographic examination for lateral lymphatic spread and local recurrence of rectal cancer. Preoperative detection and evaluation. *Dis Colon Rectum* 1995; **38**: 1047-1052 [PMID: 7555418 DOI: 10.1007/BF02133977]

23 **Amano K**, Fukuchi M, Kumamoto K, Hatano S, Ohno H, Osada H, Ishibashi K, Ishida H. Pre-operative Evaluation of Lateral Pelvic Lymph Node Metastasis in Lower Rectal Cancer: Comparison of Three Different Imaging Modalities. *J Anus Rectum Colon* 2020; **4**: 34-40 [PMID: 32002474 DOI: 10.23922/jarc.2019-022]

24 **Numata K**, Katayama Y, Sawazaki S, Higuchi A, Morinaga S, Rino Y, Masuda M, Shiozawa M. Utility of Preoperative Imaging for Predicting Pelvic Lateral Lymph Node Metastasis in Lower Rectal Cancer. *Indian J Surg Oncol* 2019; **10**: 582-586 [PMID: 31857748 DOI: 10.1007/s13193-019-00947-0]

25 **You YN**, Hardiman KM, Bafford A, Poylin V, Francone TD, Davis K, Paquette IM, Steele SR, Feingold DL; On Behalf of the Clinical Practice Guidelines Committee of the American Society of Colon and Rectal Surgeons. The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Management of Rectal Cancer. *Dis Colon Rectum* 2020; **63**: 1191-1222 [PMID: 33216491 DOI: 10.1097/DCR.0000000000001762]

26 **Ishihara S**, Kawai K, Tanaka T, Kiyomatsu T, Hata K, Nozawa H, Morikawa T, Watanabe T. Diagnostic value of FDG-PET/CT for lateral pelvic lymph node metastasis in rectal cancer treated with preoperative chemoradiotherapy. *Tech Coloproctol* 2018; **22**: 347-354 [PMID: 29623475 DOI: 10.1007/s10151-018-1779-0]

27 **Yukimoto R**, Uemura M, Tsuboyama T, Hata T, Fujino S, Ogino T, Miyoshi N, Takahashi H, Yamamoto H, Mizushima T, Kida A, Furuyashiki M, Doki Y, Eguchi H. Efficacy of positron emission tomography in diagnosis of lateral lymph node metastases in patients with rectal Cancer: a retrospective study. *BMC Cancer* 2021; **21**: 520 [PMID: 33962569 DOI: 10.1186/s12885-021-08278-6]

28 **Lee S**, Kassam Z, Baheti AD, Hope TA, Chang KJ, Korngold EK, Taggart MW, Horvat N. Rectal cancer lexicon 2023 revised and updated consensus statement from the Society of Abdominal Radiology Colorectal and Anal Cancer Disease-Focused Panel. *Abdom Radiol (NY)* 2023; **48**: 2792-2806 [PMID: 37145311 DOI: 10.1007/s00261-023-03893-2]

29 **MERCURY Study Group**, Shihab OC, Taylor F, Bees N, Blake H, Jeyadevan N, Bleehen R, Blomqvist L, Creagh M, George C, Guthrie A, Massouh H, Peppercorn D, Moran BJ, Heald RJ, Quirke P, Tekkis P, Brown G. Relevance of magnetic resonance imaging-detected pelvic sidewall lymph node involvement in rectal cancer. *Br J Surg* 2011; **98**: 1798-1804 [PMID: 21928408 DOI: 10.1002/bjs.7662]

30 **Akiyoshi T**, Matsueda K, Hiratsuka M, Unno T, Nagata J, Nagasaki T, Konishi T, Fujimoto Y, Nagayama S, Fukunaga Y, Ueno M. Indications for Lateral Pelvic Lymph Node Dissection Based on Magnetic Resonance Imaging Before and After Preoperative Chemoradiotherapy in Patients with Advanced Low-Rectal Cancer. *Ann Surg Oncol* 2015; **22** Suppl 3: S614-S620 [PMID: 25896145 DOI: 10.1245/s10434-015-4565-5]

31 **Cribb BI**, Kong JC, Kay JS, Tan TH, Noe GD, Gest B, Lee AB, Oon SF, Warrier SK, Heriot AG. Metabolic and magnetic resonance imaging: complementary modalities for the preoperative assessment of lateral pelvic lymph nodes in rectal cancer. *ANZ J Surg* 2023; **93**: 196-205 [PMID: 36074654 DOI: 10.1111/ans.18020]

32 **Malakorn S**, Yang Y, Bednarski BK, Kaur H, You YN, Holliday EB, Dasari A, Skibber JM, Rodriguez-Bigas MA, Chang GJ. Who Should Get Lateral Pelvic Lymph Node Dissection After Neoadjuvant Chemoradiation? *Dis Colon Rectum* 2019; **62**: 1158-1166 [PMID: 31490825 DOI: 10.1097/DCR.0000000000001465]

33 **Peacock O**, Chang GJ. The Landmark Series: Management of Lateral Lymph Nodes in Locally Advanced Rectal Cancer. *Ann Surg Oncol* 2020; **27**: 2723-2731 [PMID: 32519144 DOI: 10.1245/s10434-020-08639-8]

34 **Miyake Y**, Mizushima T, Hata T, Takahashi H, Hanada H, Shoji H, Nomura M, Haraguchi N, Nishimura J, Matsuda C, Takemasa I, Doki Y, Maeda I, Mori M, Yamamoto H. Inspection of Perirectal Lymph Nodes by One-Step Nucleic Acid Amplification Predicts Lateral Lymph Node Metastasis in Advanced Rectal Cancer. *Ann Surg Oncol* 2017; **24**: 3850-3856 [PMID: 28924845 DOI: 10.1245/s10434-017-6069-y]

35 **Kim TH**, Jeong SY, Choi DH, Kim DY, Jung KH, Moon SH, Chang HJ, Lim SB, Choi HS, Park JG. Lateral lymph node metastasis is a major cause of locoregional recurrence in rectal cancer treated with preoperative chemoradiotherapy and curative resection. *Ann Surg Oncol* 2008; **15**: 729-737 [PMID: 18057989 DOI: 10.1245/s10434-007-9696-x]

36 **Kim MJ**, Kim TH, Kim DY, Kim SY, Baek JY, Chang HJ, Park SC, Park JW, Oh JH. Can chemoradiation allow for omission of lateral pelvic node dissection for locally advanced rectal cancer? *J Surg Oncol* 2015; **111**: 459-464 [PMID: 25559888 DOI: 10.1002/jso.23852]

37 **Kusters M**, Slater A, Muirhead R, Hompes R, Guy RJ, Jones OM, George BD, Lindsey I, Mortensen NJ, Cunningham C. What To Do With Lateral Nodal Disease in Low Locally Advanced Rectal Cancer? A Call for Further Reflection and Research. *Dis Colon Rectum* 2017; **60**: 577-585 [PMID: 28481851 DOI: 10.1097/DCR.0000000000000834]

38 **Fujita S**, Mizusawa J, Kanemitsu Y, Ito M, Kinugasa Y, Komori K, Ohue M, Ota M, Akazai Y, Shiozawa M, Yamaguchi T, Bandou H, Katsumata K, Murata K, Akagi Y, Takiguchi N, Saida Y, Nakamura K, Fukuda H, Akasu T, Moriya Y; Colorectal Cancer Study Group of Japan Clinical Oncology Group. Mesorectal Excision With or Without Lateral Lymph Node Dissection for Clinical Stage II/III Lower Rectal Cancer (JCOG0212): A Multicenter, Randomized Controlled, Noninferiority Trial. *Ann Surg* 2017; **266**: 201-207 [PMID: 28288057 DOI: 10.1097/SLA.0000000000002212]

39 **Tsukamoto S**, Fujita S, Ota M, Mizusawa J, Shida D, Kanemitsu Y, Ito M, Shiomi A, Komori K, Ohue M, Akazai Y, Shiozawa M, Yamaguchi T, Bando H, Tsuchida A, Okamura S, Akagi Y, Takiguchi N, Saida Y, Akasu T, Moriya Y; Colorectal Cancer Study Group of Japan Clinical Oncology Group. Long-term follow-up of the randomized trial of mesorectal excision with or without lateral lymph node dissection in rectal cancer (JCOG0212). *Br J Surg* 2020; **107**: 586-594 [PMID: 32162301 DOI: 10.1002/bjs.11513]

40 **Komori K**, Fujita S, Mizusawa J, Kanemitsu Y, Ito M, Shiomi A, Ohue M, Ota M, Akazai Y, Shiozawa M, Yamaguchi T, Bandou H, Katsumata K, Kinugasa Y, Takii Y, Akasu T, Moriya Y; Colorectal Cancer Study Group of Japan Clinical Oncology Group. Predictive factors of pathological lateral pelvic lymph node metastasis in patients without clinical lateral pelvic lymph node metastasis (clinical stage II/III): The analysis of data from the clinical trial (JCOG0212). *Eur J Surg Oncol* 2019; **45**: 336-340 [PMID: 30477950 DOI: 10.1016/j.ejso.2018.11.016]

41 **Kusters M**, Beets GL, van de Velde CJ, Beets-Tan RG, Marijnen CA, Rutten HJ, Putter H, Moriya Y. A comparison between the treatment of low rectal cancer in Japan and the Netherlands, focusing on the patterns of local recurrence. *Ann Surg* 2009; **249**: 229-235 [PMID: 19212175 DOI: 10.1097/SLA.0b013e318190a664]

42 **Akiyoshi T**, Ueno M, Matsueda K, Konishi T, Fujimoto Y, Nagayama S, Fukunaga Y, Unno T, Kano A, Kuroyanagi H, Oya M, Yamaguchi T, Watanabe T, Muto T. Selective lateral pelvic lymph node dissection in patients with advanced low rectal cancer treated with preoperative chemoradiotherapy based on pretreatment imaging. *Ann Surg Oncol* 2014; **21**: 189-196 [PMID: 23963871 DOI: 10.1245/s10434-013-3216-y]

43 **Ishihara S**, Kawai K, Tanaka T, Kiyomatsu T, Hata K, Nozawa H, Morikawa T, Watanabe T. Oncological Outcomes of Lateral Pelvic Lymph Node Metastasis in Rectal Cancer Treated With Preoperative Chemoradiotherapy. *Dis Colon Rectum* 2017; **60**: 469-476 [PMID: 28383446 DOI: 10.1097/DCR.0000000000000752]

44 **Hazen SJA**, Sluckin TC, Konishi T, Kusters M. Lateral lymph node dissection in rectal cancer: State of the art review. *Eur J Surg Oncol* 2022; **48**: 2315-2322 [PMID: 34802862 DOI: 10.1016/j.ejso.2021.11.003]

45 **Fujita S**, Akasu T, Mizusawa J, Saito N, Kinugasa Y, Kanemitsu Y, Ohue M, Fujii S, Shiozawa M, Yamaguchi T, Moriya Y; Colorectal Cancer Study Group of Japan Clinical Oncology Group. Postoperative morbidity and mortality after mesorectal excision with and without lateral lymph node dissection for clinical stage II or stage III lower rectal cancer (JCOG0212): results from a multicentre, randomised controlled, non-inferiority trial. *Lancet Oncol* 2012; **13**: 616-621 [PMID: 22591948 DOI: 10.1016/S1470-2045(12)70158-4]

46 **Georgiou P**, Tan E, Gouvas N, Antoniou A, Brown G, Nicholls RJ, Tekkis P. Extended lymphadenectomy versus conventional surgery for rectal cancer: a meta-analysis. *Lancet Oncol* 2009; **10**: 1053-1062 [PMID: 19767239 DOI: 10.1016/S1470-2045(09)70224-4]

47 **Ngu JC**, Kuo LJ, Teo NZ, Yusof SB. Teaching pelvic lymph node dissection using origami, planes and boundaries. *Tech Coloproctol* 2020; **24**: 767-769 [PMID: 32185547 DOI: 10.1007/s10151-020-02184-y]

48 **Tang J**, Zhou S, Zhao W, Lou Z, Liang J, Feng B, Yang Y, Wang X, Liu Q; Chinese Lateral Node Collaborative Group. Short- and long-term outcomes of laparoscopic versus open selective lateral pelvic lymph node dissection for locally advanced middle-low rectal cancer: Results of a multicentre lateral node study in China. *Colorectal Dis* 2022; **24**: 1325-1334 [PMID: 35713974 DOI: 10.1111/codi.16223]

49 **Yamaguchi T**, Kinugasa Y, Shiomi A, Tomioka H, Kagawa H. Robotic-assisted laparoscopic versus open lateral lymph node dissection for advanced lower rectal cancer. *Surg Endosc* 2016; **30**: 721-728 [PMID: 26092002 DOI: 10.1007/s00464-015-4266-y]

50 **Yamaguchi T**, Kinugasa Y, Shiomi A, Kagawa H, Yamakawa Y, Furutani A, Manabe S, Yamaoka Y, Hino H. Oncological outcomes of robotic-assisted laparoscopic versus open lateral lymph node dissection for locally advanced low rectal cancer. *Surg Endosc* 2018; **32**: 4498-4505 [PMID: 29721748 DOI: 10.1007/s00464-018-6197-x]

51 **Watanabe J**, Ohya H, Sakai J, Suwa Y, Goto K, Nakagawa K, Ozawa M, Ishibe A, Suwa H, Kunisaki C, Endo I. Long-term outcomes of indocyanine green fluorescence imaging-guided laparoscopic lateral pelvic lymph node dissection for clinical stage II/III middle-lower rectal cancer: a propensity score-matched cohort study. *Tech Coloproctol* 2023; **27**: 759-767 [PMID: 36773172 DOI: 10.1007/s10151-023-02761-x]

52 **Ohya H**, Watanabe J, Suwa H, Suwa Y, Ozawa M, Ishibe A, Kunisaki C, Endo I. Near-Infrared Imaging Using Indocyanine Green for Laparoscopic Lateral Pelvic Lymph Node Dissection for Clinical Stage II/III Middle-Lower Rectal Cancer: A Propensity Score-Matched Cohort Study. *Dis Colon Rectum* 2022; **65**: 885-893 [PMID: 34840301 DOI: 10.1097/DCR.0000000000002156]

53 **Schaap DP**, Boogerd LSF, Konishi T, Cunningham C, Ogura A, Garcia-Aguilar J, Beets GL, Suzuki C, Toda S, Lee IK, Sammour T, Uehara K, Lee P, Tuynman JB, van de Velde CJH, Rutten HJT, Kusters M; Lateral Node Study Consortium. Rectal cancer lateral lymph nodes: multicentre study of the impact of obturator and internal iliac nodes on oncological outcomes. *Br J Surg* 2021; **108**: 205-213 [PMID: 33711144 DOI: 10.1093/bjs/znaa009]

54 **Kim YI**, Jang JK, Park IJ, Park SH, Kim JB, Park JH, Kim TW, Ro JS, Lim SB, Yu CS, Kim JC. Lateral lymph node and its association with distant recurrence in rectal cancer: A clue of systemic disease. *Surg Oncol* 2020; **35**: 174-181 [PMID: 32889250 DOI: 10.1016/j.suronc.2020.08.013]

55 **Lee D**, Matsuda T, Yamashita K, Hasegawa H, Yamamoto M, Kanaji S, Oshikiri T, Nakamura T, Suzuki S, Fukumoto T, Kakeji Y. Significance of Lateral Pelvic Lymph Node Size in Predicting Metastasis and Prognosis in Rectal Cancer. *Anticancer Res* 2019; **39**: 993-998 [PMID: 30711986 DOI: 10.21873/anticanres.13204]

56 **Sapci I**, Delaney CP, Liska D, Amarnath S, Kalady MF, Steele SR, Gorgun E. Oncological Outcomes of Patients with Locally Advanced Rectal Cancer and Lateral Pelvic Lymph Node Involvement. *J Gastrointest Surg* 2019; **23**: 1454-1460 [PMID: 31012043 DOI: 10.1007/s11605-019-04224-x]

57 **Schaap DP**, Ogura A, Nederend J, Maas M, Cnossen JS, Creemers GJ, van Lijnschoten I, Nieuwenhuijzen GAP, Rutten HJT, Kusters M. Prognostic implications of MRI-detected lateral nodal disease and extramural vascular invasion in rectal cancer. *Br J Surg* 2018; **105**: 1844-

1852 [PMID: 30079958 DOI: 10.1002/bjs.10949]

58 **Kim MJ**, Hur BY, Lee ES, Park B, Joo J, Kim MJ, Park SC, Baek JY, Chang HJ, Kim DY, Oh JH. Prediction of lateral pelvic lymph node metastasis in patients with locally advanced rectal cancer with preoperative chemoradiotherapy: Focus on MR imaging findings. *PLoS One* 2018; **13**: e0195815 [PMID: 29649321 DOI: 10.1371/journal.pone.0195815]

59 **Kobayashi H**, Kikuchi A, Okazaki S, Ishiguro M, Ishikawa T, Iida S, Uetake H, Sugihara K. Diagnostic performance of multidetector row computed tomography for assessment of lymph node metastasis in patients with distal rectal cancer. *Ann Surg Oncol* 2015; **22**: 203-208 [PMID: 25124470 DOI: 10.1245/s10434-014-3972-3]

60 **Ogawa S**, Hida J, Ike H, Kinugasa T, Ota M, Shinto E, Itabashi M, Kameoka S, Sugihara K. Selection of Lymph Node-Positive Cases Based on Perirectal and Lateral Pelvic Lymph Nodes Using Magnetic Resonance Imaging: Study of the Japanese Society for Cancer of the Colon and Rectum. *Ann Surg Oncol* 2016; **23**: 1187-1194 [PMID: 26671038 DOI: 10.1245/s10434-015-5021-2]

61 **Ogawa S**, Itabashi M, Hirosawa T, Hashimoto T, Bamba Y, Kameoka S. Lateral pelvic lymph node dissection can be omitted in lower rectal cancer in which the longest lateral pelvic and perirectal lymph node is less than 5 mm on MRI. *J Surg Oncol* 2014; **109**: 227-233 [PMID: 24165955 DOI: 10.1002/jso.23478]

62 **Matsuoka H**, Nakamura A, Masaki T, Sugiyama M, Nitatori T, Ohkura Y, Sakamoto A, Atomi Y. Optimal diagnostic criteria for lateral pelvic lymph node metastasis in rectal carcinoma. *Anticancer Res* 2007; **27**: 3529-3533 [PMID: 17972513]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 December 24; 14(12): 593-605

DOI: 10.5306/wjco.v14.i12.593

ISSN 2218-4333 (online)

MINIREVIEWS

Anti-tumor effect of coix seed based on the theory of medicinal and food homology

Fan-Di Meng, Ling Yuan, Dou-Dou Lu, Ya-Ting Yang, Duo-Jie Xu, Meng-Ying Che, Yi Nan

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ogino S, United States

Received: October 19, 2023
Peer-review started: October 19, 2023
First decision: October 24, 2023
Revised: October 26, 2023
Accepted: November 24, 2023
Article in press: November 24, 2023
Published online: December 24, 2023

Fan-Di Meng, Dou-Dou Lu, Ya-Ting Yang, Duo-Jie Xu, Meng-Ying Che, Traditional Chinese Medicine College, Ningxia Medical University, Yinchuan 750004, Ningxia Hui Autonomous Region, China

Ling Yuan, College of Pharmacy, Ningxia Medical University, Yinchuan 750004, Ningxia Hui Autonomous Region, China

Yi Nan, Key Laboratory of Ningxia Minority Medicine Modernization Ministry of Education, Ningxia Medical University, Yinchuan 750004, Ningxia Hui Autonomous Region, China

Corresponding author: Yi Nan, MD, PhD, Professor, Key Laboratory of Ningxia Minority Medicine Modernization Ministry of Education, Ningxia Medical University, No. 1160 Shengli Street, Yinchuan 750004, Ningxia Hui Autonomous Region, China. 20080011@nxmu.edu.cn

Abstract

Coix seed is a dry and mature seed of *Coix lacryma-jobi L.var.ma-yuen (Roman.) Stapf* in the Gramineae family. Coix seed has a sweet, light taste, and a cool nature. Coix seed enters the spleen, stomach, and lung meridians. It has the effects of promoting diuresis and dampness, strengthening the spleen to prevent diarrhea, removing arthralgia, expelling pus, and detoxifying and dispersing nodules. It is used for the treatment of edema, athlete's foot, poor urination, spleen deficiency and diarrhea, dampness and obstruction, lung carbuncle, intestinal carbuncle, verruca, and cancer. The medicinal and health value is high, and it has been included in the list of medicinal and food sources in China, which has a large development and application space. This article reviews the current research achievements in the processing methods and anti-tumor activities of Coix seed and provides examples of its clinical application in ancient and modern times, aiming to provide reference for further research on Coix seed and contribute to its clinical application and development. Through the analysis of the traditional Chinese patent medicines, and simple preparations and related health food of Coix seed queried by Yaozhi.com, the source, function, and dosage form of Coix seed were comprehensively analyzed, with a view of providing a reference for the development of Coix seed medicine and food.

Key Words: Coix seed; Cancer; Tumor; *Coix lacryma-jobi L.var.ma-yuen (Roman.) Stapf*; Medicinal herbs

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Cancer is a serious disease that causes a huge economic and social burden worldwide. In addition, cancer has become one of the biggest health threats globally. Numerous studies have confirmed that Coix seed has anti-tumor effects. This article will review its preparation, anti-tumor effects, and edible value.

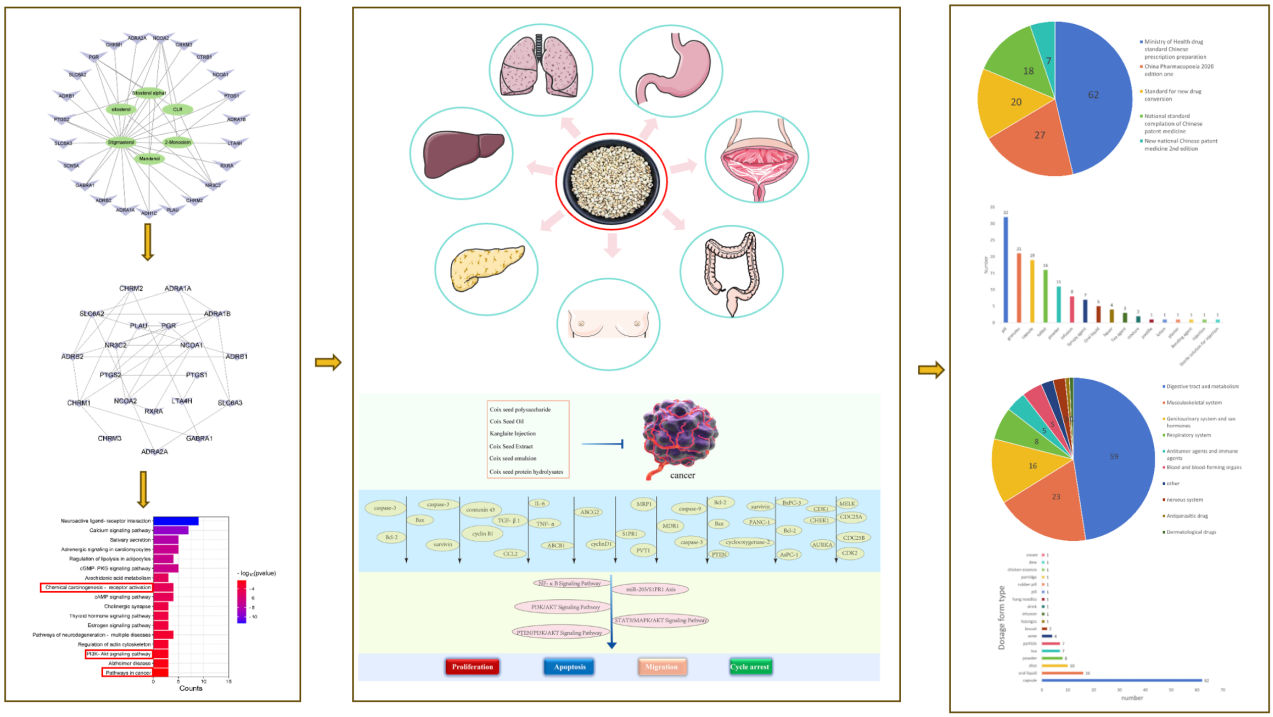
Citation: Meng FD, Yuan L, Lu DD, Yang YT, Xu DJ, Che MY, Nan Y. Anti-tumor effect of coix seed based on the theory of medicinal and food homology. *World J Clin Oncol* 2023; 14(12): 593-605
URL: https://www.wjgnet.com/2218-4333/full/v14/i12/593.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i12.593

INTRODUCTION

Cancer is a serious disease that causes a huge economic and social burden worldwide. An estimated 19.3 million new cancer cases and nearly 10 million cancer deaths were reported in 2020. It is expected that the global cancer burden will reach 28.4 million cases by 2040, an increase of 47% compared to 2020[1]. In addition, cancer has become one of the biggest health threats globally. Therefore, how to effectively prevent and treat cancer has become a global focus of attention[1]. So, exploring effective cancer treatment measures is crucial.

Medicinal and food-dual-use foods can be consumed as both delicious foods and medicinal herbs for treating diseases. They belong to traditional Chinese medicine and have good therapeutic effects. They are also nutritious and delicious foods that people often eat. Coix seed, which can be used as both food[2]and medicine[3], is an important raw material for the development of food or health food. Coix seed is a good medicine and food for dispelling dampness and strengthening the spleen.

At present, Chinese herbal medicine[4] has significant effects in inhibiting cancer proliferation, metastasis, inducing cell apoptosis[5], blocking the cell cycle, alleviating pain[6], improving quality of life[7], and has received widespread attention from researchers[8,9]. Numerous studies have confirmed that Coix seed has anti-tumor effects. This article will review its preparation, anti-tumor effects, and edible value (Figure 1).



DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

Figure 1 Flow chart.

BRIEF INTRODUCTION

Coix seed, also known as Xie Li, Qishi, Ganmi, *etc*, is a dry and mature seed of *Coix lacryma-jobi L. var.ma-yuen (Roman.) Stapf* in the gramineae family[10]. Most regions in China produce it, mainly in Fujian, Hebei, and Liaoning. It is commonly found near houses, in the wilderness, by rivers, in streams, or in damp valleys. Coix seed has a sweet and light taste and a cool nature. It enters the spleen, stomach, and lung meridians. As a drug, it has the effects of promoting diuresis and dampness, strengthening the spleen[11] to stop diarrhea, removing arthralgia, expelling pus, and detoxifying and dispersing nodules. It can treat edema, athlete's foot, poor urination, spleen deficiency and diarrhea, dampness and obstruction, lung carbuncle, intestinal carbuncle, verruca, cancer[12,13], *etc*. As a food, its developed products have functions[14,15] such as increasing bone density, improving sleep, immune regulation[16,17], weight loss [18], regulating blood lipids and blood sugar, improving gastrointestinal function, anti-fatigue, promoting growth and development, improving memory, antioxidant[19], delaying aging, and protecting liver damage[20].

PROCESSING

Coix seed has a long history of and has various methods of processing[21]. Since the Northern and Southern Dynasties, there have been records of two processing methods: Glutinous rice stir frying and salt soup boiling. Subsequently, the Song Dynasty first proposed the stir frying method. Salt frying was added in the Ming Dynasty. During the Qing Dynasty, local stir frying was added. So far, commonly used processing methods such as stir frying, earth frying, bran frying, and sand frying have been recorded in the modern Chinese Pharmacopoeia and national and provincial processing standards (Table 1).

THE ANTI-TUMOR EFFECT OF COIX SEED

Screening of active ingredients and targets in Coix seed

We used Coix seed as a keyword to search on the TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, https://old.tcmsp-e.com/index.php)[22]. The active ingredients and their related action targets were picked according to the criteria of oral bioavailability[23] ≥ 30% and drug-likeness ≥ 0.18. Then, we translated the name into Gene Symbol format to obtain target genes for the main active ingredients of Coix seed *via* Uniprot database (https://www.uniprot.org/). We imported the active ingredients and their targets of Coix seed into Cytoscape 3.9.1 software to draw an "active ingredient-target" network. Next, we imported drug targets into the STRING database (http://string-db.org), species limited to "*Homo sapiens*", to retrieve protein-protein interaction relationships, and imported them into Cytoscape 3.9.1 software to create a network diagram. Through the Metascape database (https://metascape.org/), we conducted the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis on the target. The results of KEGG signal pathways are introduced into the Bioinformatics database (http://www.bioinformatics.com.cn/) and presented in the form of a bar chart and selected pathways related to cancer (Figure 2). The KEGG results indicate that the Coix seed target is associated with multiple cancer pathways and can effectively combat cancer. So, the next main analysis is the anti-tumor effect of Coix seed.

Anti-cancer effect of Coix seed and its components

Coix seed is a commonly used clinical drug with activities such as anti-tumor (Figure 3), immune regulation[24,25], hypoglycemic[26,27], anti-inflammatory[28,29], improving intestinal microbiota[17,30], lowering blood lipids[31,32], and promoting angiogenesis[33]. After KEGG enrichment analysis, we mainly discuss the pharmacological effects of Coix seed on anti-tumor effects (Table 2). Studies have confirmed that Coix seed and its extract can reduce the proliferation, invasion and migration of lung cancer[34], colon cancer, liver cancer, breast cancer, cervical cancer, gastric cancer, pancreatic cancer and other cancers, and can promote their apoptosis (Figure 4).

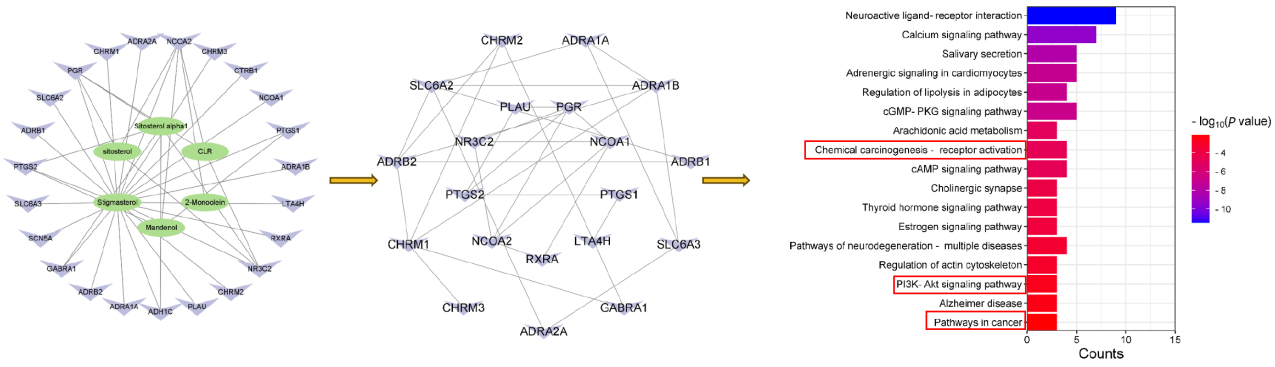
Lung cancer: Coix seed has a prominent inhibitory healing effect on lung cancer metastasis, and can inhibit proliferation and promote apoptosis. Research has shown that Paclitaxel combined with Kanglaite (KLT) can significantly improve patients' physical fitness, reduce bone metastasis area and tumor weight, and have significant effects in clinical treatment [35]. MiRNA-21 is a therapeutic effect indicator for lung cancer. By comparing the changes in indicators before and after treatment with KLT, the expression of miRNA-21 is reduced, indicating that KLT has a significant therapeutic effect on advanced lung cancer[36]. After cell experiments, Coix Polysaccharides can significantly inhibit the proliferation of lung cancer cells, and may induce apoptosis of lung cancer cells by increasing the expression of caspase-3 and caspase-9 genes [37]. KLT has significant anti-tumor activity in Lewis lung cancer mice, and when combined with cisplatin, it can improve chemotherapy efficacy and immune function by reducing TAM levels and improving hypoxia status[38]. Other studies have confirmed that Coix polysaccharides can demonstrably inhibit the migration and invasion of A549 cells *in vitro* cell experiments, and its molecular mechanism may be the down-regulation of S100A4 gene and protein expression levels[39].

Colon cancer: Research has shown that Coix seed performs well in combating colon cancer, blocking cell cycle, promoting apoptosis, and synergistic effects to achieve the effect of inhibiting colon cancer. On the HT-29 colon cancer cell model, the anticancer effect of Coix seed oil is dose-dependent and time-dependent. With the increase of drug concentration and the passage of time, the survival rate of tumor cells will also decrease[40]. The synergistic effect of paclitaxel treatment

Table 1 Processing method of Coix seed

Coix seed	Processing method	Source
Coix seed	Remove imports	Chinese Pharmacopoeia (2020)
Fried coix seeds	Clean the mix seeds and fry them until they are slightly yellow	Processing Standards of TCM Decoction Pieces in Hubei Province (2018)
Fried mix seed with bran	Clean the mix seeds and fry them with bran until they are slightly yellow	Chinese Pharmacopoeia (2020)
Fried coix seed in clay	Take the pure Coix seeds and fry them according to the method of soil frying until the surface benefits burn yellow and bulks up to the degree	Processing standard of TCM detection pieces in Henan Province (2005)
Coix seed powder	Take coix seeds, remove impurities and crush them into fine powder	Processing Standards of TCM Decoction Pieces in Sichuan Province (2015)
Jiao coix seed	Fry until browned	Processing Standards of TCM Decoction Pieces in Tianjin (2018)
Scald coix seed	Take the coix seed, wash it, moisten it thoroughly, steam it, dry it, and press it with the oil and method until it looks like a bubble	Fujian Province Traditional Chinese Medicine Processing Standards (1988)

TCM: Traditional Chinese medicine.



DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

Figure 2 Relationship between active components of Coix seed and cancer.

after pretreatment with KLT is the best, and KLT inhibits nuclear factor NF-κB and upregulates the expression of connexin 43, making cancer cells sensitive to paclitaxel[41], thereby exerting an inhibitory effect on colon cancer cells.

Liver cancer: The components of Coix seed have good therapeutic effects on liver cancer, and an efficient and safe anticancer drug delivery system has been developed. There have been studies on injecting KLT into transplanted liver tumors in rats and evaluating its impact, pros and cons. Research has shown that injecting KLT into implanted hepatocellular carcinoma is more effective than ethanol, and KLT has fewer side effects on liver function than ethanol[42]. In the study, Wang *et al*[43] discovered that the combination of Norcantharidin and Coix seed oil can exert anti-tumor efficacy by regulating the immune system. Coix seed components have an inhibitory effectiveness on the progression of liver tumors in nude mice and have minimal toxicity to the liver and kidneys[44]. Bitargeted microenvironments based on Coix seed receptors can effectively target tumors, enhance their inhibitory effect on tumor proliferation, and induce cancer cell apoptosis, thereby prolonging patient survival time[45].

Breast cancer: Coix seed oil has a large scale anti-cancer effect. Ting F found that Coix seed oil has a great inhibitory effect on triple negative breast cancer, which inhibited the proliferation and growth of triple negative breast cancer[46]. The results of network pharmacology and *in vitro* experiments show that KLT has an inhibitory effect on triple negative breast cancer, which can inhibit cell proliferation and invasion, block cell cycle and induce cell apoptosis. Its mechanism of action may be to block G2/M phase cells and downregulate G2/M phase related genes[47].

Cervical cancer: Microemulsions containing Coix seed components exhibit good anti cervical cancer effects, leading to cell cycle arrest and apoptosis, and to cancer cell death. Dissolving paclitaxel in Coix seed oil, the two synergistically fight cancer, exert stronger *in vitro* cytotoxicity, and induce cell apoptosis, which has a stronger therapeutic effect on cervical cancer[48]. Joint application of Coix Seed Oil and Tripterine can work synergistically on the proliferation of cervical cancer, as well as anti-angiogenesis and induction of cell apoptosis. In mouse models, minimal toxicity to important

Table 2 Anti-cancer effect of Coix seed and its components

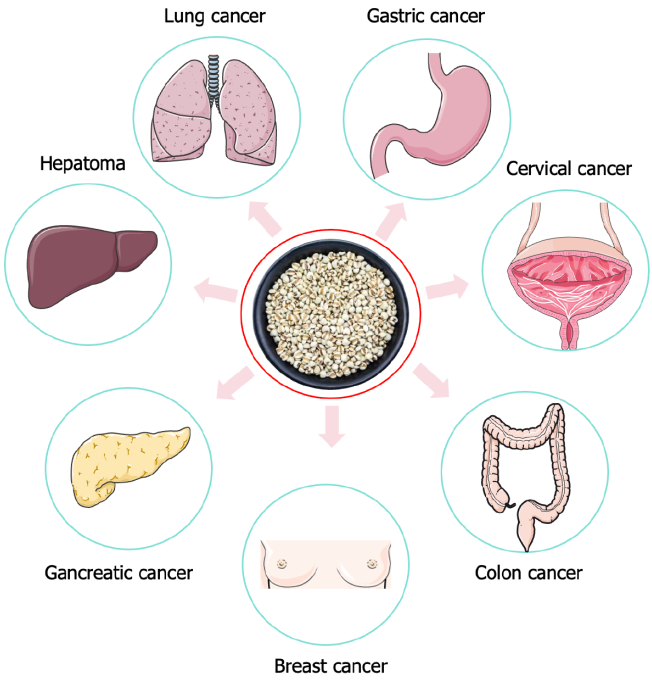
Pharmacological effect	Ingredient	Conclusion
Lung cancer	Kanglaite	Paclitaxel combined with kanglaite is effective in improving bone metastasis of lung cancer
	Kanglaite injection	Kanglaite injection can significantly reduce the expression of miRNA-21 in patients with advanced lung cancer, and has a good thermal effect
	Kanglaite	Kanglaite can achieve benefits by reducing TAM levels and improving hypoxia in mice with Lewis lung cancer
Colon cancer	Coix seed oil	Coix seed oil plays an anti-colon cancer role by inducing G2 rest and topology of HT-29 cells by regulating PI3K/AKT signaling pathway
Colorectal cancer	Kanglaite injection	Kanglaite pretreatment may increase the effect of Taxol on colored cancer
Hepatoma	Coix seed components	Octanoyl galactose ester modified microemulsion system self-assembled by coil seed components to enhance tumor targeting and hepatoma therapy
	Coix seed ingredients	Bitargeted microemissions based on Coix seed ingredients have the effect of enhancing life tube transmission and synergistic therapy
Triple negative breast cancer	Kanglaite injection	Kanglaite injection was confirmed to have anti TNBC effects by arresting cell cycle and inhibiting CDK1 precipitation
	Coix seed oil	Coix seed oil exerts an anti-triple negative breast cancer effect by interrupting miR-205/S1PR1 axis
Clinical cancer	Coix seed oil	Self-enhancing system colored with paclitaxel and Coix seed oil deeply penetrated can enhance efficiency of clinical cancer
	Coix seed oil	Transferrin modified microemulsion carrying Coix seed oil and tripterine (Tf CT MEs) can be used to improve tube specific accumulation and connection to enhance clinical cancer treatment
	Coix seed oil	Coix seed oil and tripterine coated microemissions with a transfer modification (Tf CT MES) could improve the treatment of cervical cancer
Gastric cancer	Kanglaite injection	Kanglaite inhibits the expression of drug resistance genes through suppressing PVT1 in cisplatin-resistant gas cancer cells
Pancreatic cancer	Coix seed oil	Coix seed oil regulations mitochondrial functional image to induce apoptosis of human pancreatic cancer cells *via* the PTEN/PI3K/AKT signaling pathway
	Coix seed extract	Coix seed extract could augment the efficiency of gemcitabine therapy in pancreatic cancer cells
	Coix seed emission	Coix seed emission synergistically enhances the antagonist activity of gemcitabine in pancreatic cancer through inhibition of NF- κ B signaling
Ameliorates cancer cachexia	Coix seed oil	Coix seed oil ameliorates cancer cachexia by counteracting muscle loss and fat lipolysis

TNBC: Triple negative breast cancer.

organs was detected[49,50].

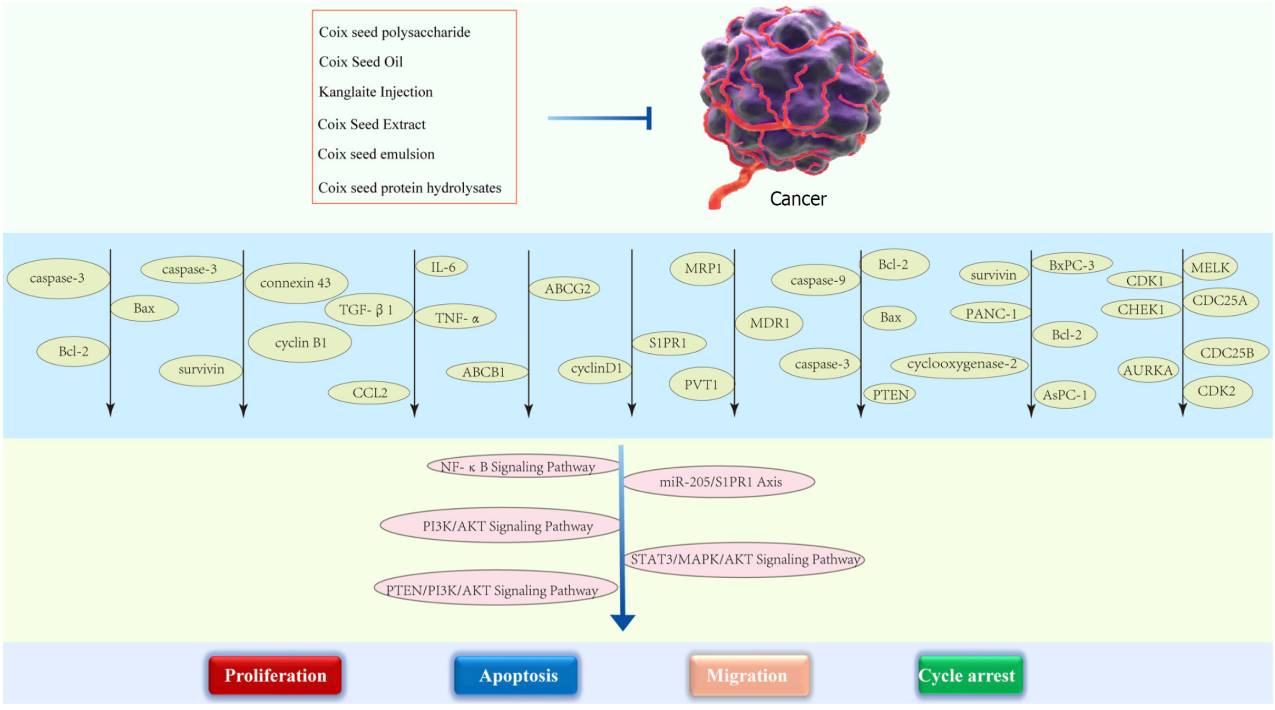
Gastric cancer: Coix seed can reduce the vitality of gastric cancer cells, promote cell apoptosis, and upgrade the quality of life. The reason of KLT regulating chemotherapy resistance in gastric cancer cells may be through regulating expression of MDR1 and MRP1 to inhibit cell viability and promote cell apoptosis. KLT can alleviate the development of multiple drug resistance (MDR) and participate in the potential mechanism of MDR in gastric cancer[51]. Comparing the indicators before and after treatment, the study found that patients with advanced gastric cancer treated with KLT combined with chemotherapy had reduced cancer, reduced chemotherapy side effects, and a further improved quality of life[52].

Pancreatic cancer: Coix seed can promote apoptosis of pancreatic cancer cells, make it sensitive to treatment, and enhance the therapeutic effect. Coix seed oil may adjust mitochondrial dysfunction and induces apoptosis in PANC-1 PC cells through PTEN, which may be related to the down-regulation of p-AKT and p-PI3K protein expression by Coix seed oil [53]. Coix seed extract can synergistically reinforce the anti-pancreatic cancer effect of Gemcitabine, significantly alleviate the up regulation of ABCB1 and ABCG2 proteins caused by the use of Gemcitabine, and detect strong correlation between Bioluminescence pharmacokinetic parameters and pharmacodynamic indicators and anti-tumor efficacy[54]. The anti-tumor effect of Coix seed emission combined with Gemcitabine is superior to that of any drug alone, and its mechanism is that Coix seed emission can eliminate the activation of NF-κB, making pancreatic cancer cells sensitive to gemcitabine therapy[55].



DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

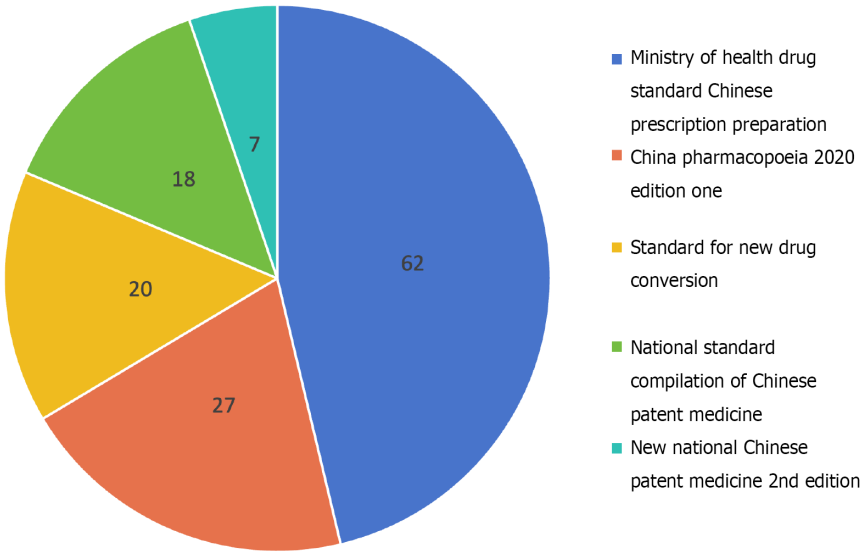
Figure 3 Relationship between Coix seed and cancer.



DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

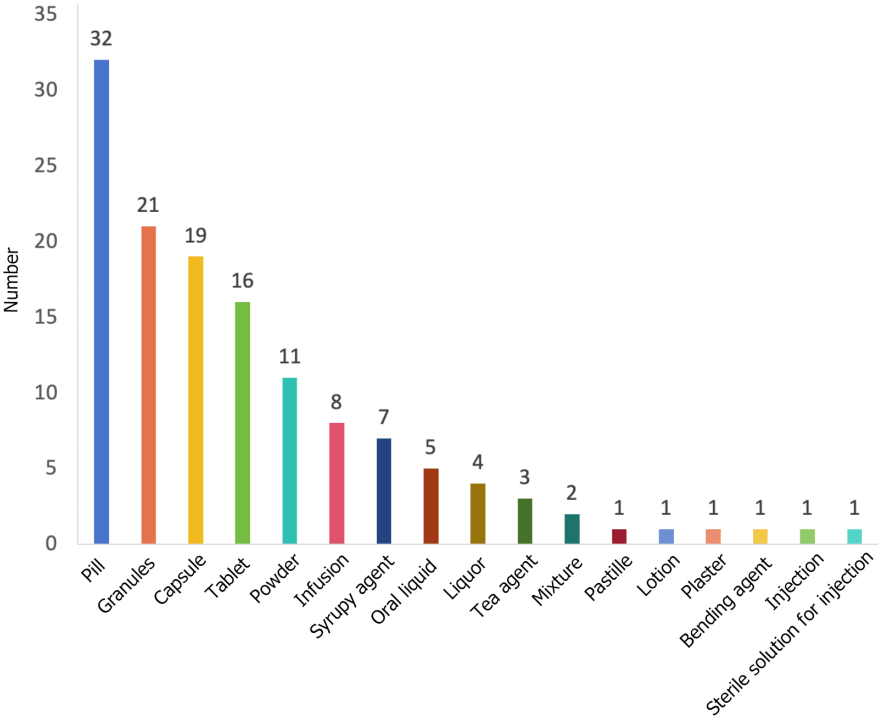
Figure 4 Mechanism of anti-tumor action of Coix seed and its components.

Improving cancer cachexia: Researchers have found that administering Coix seed oil can significantly prevent weight loss and improve systemic inflammation in mice, without affecting food intake and tumor size. The results indicate that Coix seed oil can cause muscle and adipose tissue loss caused by cancer cachexia[56]. The results of clinical research on the injection of Coix seed oil into patients showed that Coix seed oil can effectively control the degree of pain, alleviate adverse reactions such as constipation and nausea, and raise the quality of life[57].



DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

Figure 5 Source and quantity of Chinese patient medicine containing Coix seed.



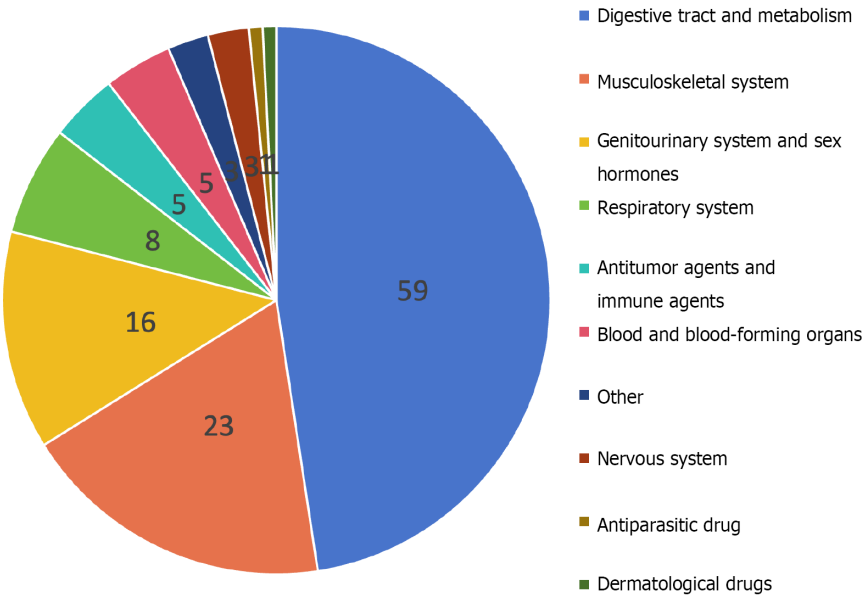
DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

Figure 6 Chinese medicine dosage form containing Coix seed.

APPLICATION OF COIX SEED

The medicinal value of coix seed

Coix seed has been widely applied since ancient times, and formulas containing Coix seed have also been widely used. Yiyi Fuzi Baijiang Powder can slow down the progression of colorectal cancer by simultaneously regulating target genes and related signaling pathways of multiple active ingredients, possibly by regulating cell apoptosis, cell proliferation, and protein and enzyme binding[58], and this has been experimentally validated[59]. Yiyi Fuzi Baijiang Powder has a good effect in treating ulcerative colitis, can inhibit intestinal symptoms in mice, and improve intestinal pathology[60]. According to reports, Qingyi huaji decoction can be applied as a valid method to treat pancreatic cancer, and research has confirmed that it can inhibit the growth and progression of tumor through various mechanisms such as anti-inflammatory and induction of cell apoptosis[61]. Shenling Baizhu Powder inhibits colitis related colorectal cancer by inhibiting epithelial mesenchymal transformation and myelogenous inhibitor infiltration, and reduces mortality by reducing the



DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

Figure 7 Anatomic Therapeutic Chemical classification of Chinese patient medicine.

incidence rate and diversity of colon tumors[62]. Traditional Chinese patent medicines and simple preparations containing coix seed was searched on the website of Yaozhi (http://db.yaozh.com/) with the keyword "coix seed". It was recorded in the Ministry of Health drug standard Chinese prescription preparation, China Pharmacopoeia 2020 edition one, Standard for new drug conversion, National standard competition of Chinese patient medicine, New national Chinese patient medicine 2nd edition. There are 134 kinds of traditional Chinese patent medicines and simple preparations containing Coix seed (Figure 5). From the perspective of dosage forms, there are 17 types of traditional Chinese patent medicines and simple preparations containing *Coix lachryma jobi* seed, in which tablets are the main, followed by granules and capsules (Figure 6). We summarized the efficacy of 134 traditional Chinese patent medicines and simple preparations varieties containing coix seed, which can be roughly divided into 9 categories (Figure 7). According to the efficacy analysis, the traditional Chinese patent medicines and simple preparations that contain *Coix lachryma jobi* seed mainly focuses on the digestive system, musculoskeletal system, urogenital system.

Edible value of Coix seed

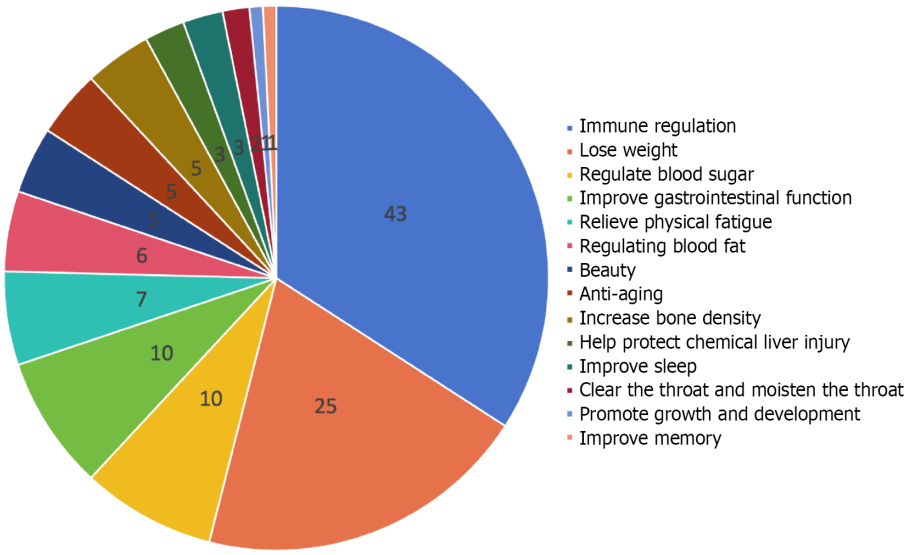
Coix seed is often used in dietary therapy[25]. Gastrointestinal symptoms caused by chemotherapy, such as weakness, vomiting, and nausea, can be alleviated by qi-yin-reinforcing porridge[63]. In recent years, there have also been many health foods mainly made of Coix seed. The keyword "Coix seed" was searched on Yaozhi.com (http://db.yaozh.com/), and a total of 126 Coix seed related health foods approved by the State Food and Drug Administration were obtained, such as mountain medicine Coix seed granules, Coix seed sea buckthorn capsules, healthy Runtong tea, bone strengthening powder, *etc*, which have immune regulation, weight loss, blood lipid and blood glucose regulation properties. To improve gastrointestinal function and other functions, the statistical data of the health functions involved in Coix Seed Health Products are shown in Figure 8. So far, there are mainly 18 types of Coix seed health product formulations used (Figure 9). The development forms of Coix seed health food functions are very diverse, with diverse products and dosage forms that can meet the specific needs of different populations.

Usage of Coix seed

Coix seed has the effect of promoting metabolism and reducing gastrointestinal burden, and can be used as a nourishing food for weak patients during or after illness[64]. It is worth noting that people with spleen deficiency and diarrhea can stir fry Coix seed before consumption, which has a better effect. Due to its ability to remove dampness, Coix seed should be used with caution for those who suffer from body fluid depletion after fever, or for those who are usually Yin deficient or Yin deficient with excessive fire. Pregnant women and those with slippery semen or constipation should not consume it. If these people consume coix seed, it may cause a greater burden on their physical health.

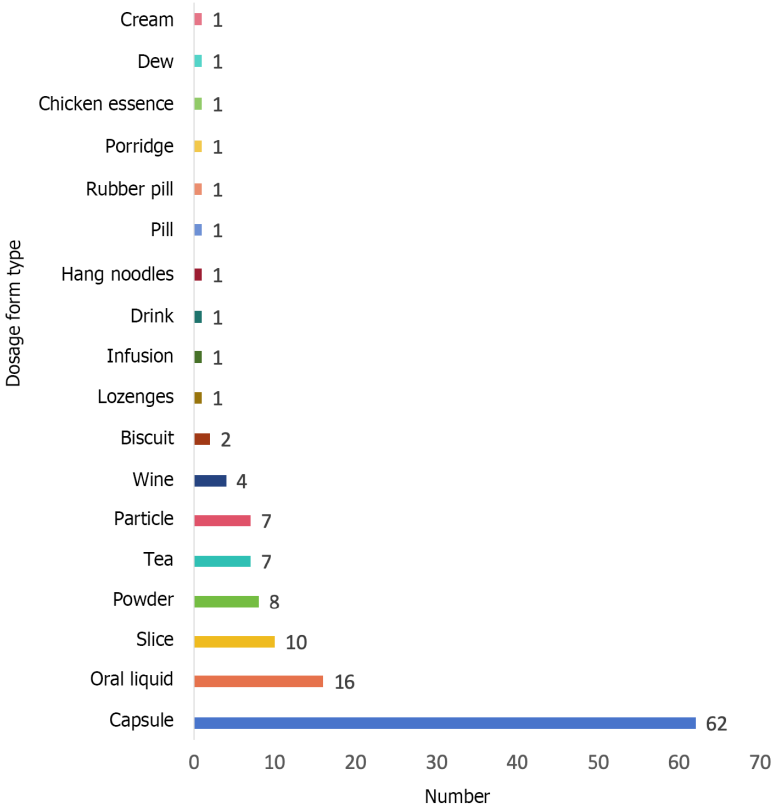
DISCUSSION

We used Coix Seed; Semen coicis; *Coix lacryma jobi L. var. mayen (Roman.) Stapf* and cancer; neoplasm and tumor as keywords to search on PubMed. And four related reviews were found in the past 5 years. Among them, Huang *et al*[12] discussed the chemical composition, anticancer mechanisms, marketed drugs, dosage forms, and clinical applications of fatty oils, including coix seed and other plants. Pan *et al*[65] only discusses the treatment of malignant tumors in the



DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

Figure 8 Function statistics of health care products containing Coix seed.



DOI: 10.5306/wjco.v14.i12.593 **Copyright** ©The Author(s) 2023.

Figure 9 Dosage form containing Coix seed health care products.

female reproductive system with coix seed. This article discusses the anti-tumor effect of coix seed and is not limited to malignant tumors in the female reproductive system. Lu *et al*[66] discussed the anticancer effect of KLT, which is an extract of Coix seed oil. This article also discusses some other components of coix seed. Kim *et al*[67] discussed the anti-pancreatic cancer effect of various natural plants including Coix lachryma seed. In the past 5 years, there has been no specialized review on the anticancer effect of Coix seed and its components, as well as the homology between medicine and food. This article starts from the perspective of homology between medicine and food, and conducts KEGG analysis of the effective ingredient targetscoix seed and its components, as well as the homology between medicine and food. This article starts from the perspective of homology between medicine and food, and conducts KEGG analysis of the effective ingredient targets of Coix seed through bioinformatics methods, proving that Coix seed indeed has anti-tumor effects,

and systematically reviews the anti-tumor effect of Coix seed. The application of Coix lachryma seed in traditional Chinese patent medicines and simple preparations and food was also summarized and sorted out, and the relevant data was displayed through charts. Methods, proving that Coix seed indeed has anti-tumor effects, and systematically reviews the anti-tumor effect of Coix seed. The application of Coix lachryma seed in traditional Chinese patent medicines and simple preparations and food was also summarized and sorted out, and the relevant data was displayed through charts.

CONCLUSION

In recent years, more and more studies have shown that Coix seed has the function of inhibiting the growth and metastasis of cancer cells, reducing the mortality rate of cancer patients. Therefore, Coix seed has become a highly anticipated health product. With the increasing emphasis on healthy diet, the idea of "treating diseases before they occur" has become increasingly popular, and Coix seed has received more and more attention in the field of medicinal and food homology. In the future, coix seed can be used to develop various new health products, such as cosmetics, and pharmaceuticals, to meet people's needs for health and beauty. At the same time, Coix seed can also be used to study new medicinal ingredients and treatment methods in order to further improve its health benefits.

FOOTNOTES

Author contributions: Meng FD, Lu DD, Yang YT, Xu DJ, Che MY and Nan Y designed the research study; Meng FD, Yang YT, Che MY and Yuan L collected the literature, Yuan L, Meng FD and Xu DJ analyzed the data and wrote the manuscript; All authors have read and approved the final manuscript.

Supported by Ningxia Natural Science Foundation, No. 2022AAC02039; Ningxia Key Research and Development Program, No. 2023BEG02015; National Natural Science Foundation of China, No. 82260879.

Conflict-of-interest statement: The authors declare having no conflict of interests for this article.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Fan-Di Meng 0009-0009-9757-5815; Ling Yuan 0000-0003-2838-0976; Dou-Dou Lu 0009-0009-4644-9181; Ya-Ting Yang 0009-0004-4026-7428; Duo-Jie Xu 0009-0000-8992-4688; Meng-Ying Che 0009-0002-7560-1719; Yi Nan 0000-0002-5511-9266.

S-Editor: Lin C
L-Editor: Filipodia
P-Editor: Zhang XD

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Igbokwe IC**, Wei M, Feng Y, Duan Y, Ma H, Zhang H. Coix Seed: A Review of Its Physicochemical Composition, Bioactivity, Processing, Application, Functionality, and Safety Aspects. *Food Rev Int* 2022; **38** Suppl 1: 921-939 [DOI: 10.1080/87559129.2021.1892129]

3 **Nurkolis F**, Gunawan W, Hardinsyah H, Mayulu N, Wijayanti M. Health Benefits of Coix Seed (Coix lacryma-jobi): A Study Review. Curr Dev Nutr 2022; 6 Suppl 1: 524 [DOI: 10.1093/cdn/nzac077.027]

4 **Yang M**, Zhu SJ, Shen C, Zhai R, Li DD, Fang M, Xu JN, Gan YN, Yang L, Ren ZY, Zheng RX, Robinson N, Liu JP. Clinical Application of Chinese Herbal Injection for Cancer Care: Evidence-Mapping of the Systematic Reviews, Meta-analyses, and Randomized Controlled Trials. *Front Pharmacol* 2021; **12**: 666368 [PMID: 34025425 DOI: 10.3389/fphar.2021.666368]

5 **Lu Y**, Li CS, Dong Q. Chinese herb related molecules of cancer-cell-apoptosis: a minireview of progress between Kanglaite injection and related genes. *J Exp Clin Cancer Res* 2008; **27**: 31 [PMID: 18718024 DOI: 10.1186/1756-9966-27-31]

6 **Su P**, Leng Y, Liu J, Yu Y, Wang Z, Dang H. Comparative Analysis of the Efficacy and Safety of Different Traditional Chinese Medicine Injections in the Treatment of Cancer-Related Pain: A Bayesian Network Meta-Analysis. *Front Pharmacol* 2021; **12**: 803676 [PMID: 35197850 DOI: 10.3389/fphar.2021.803676]

7 **Wu X**, Chung VCH, Lu P, Poon SK, Hui EP, Lau AYL, Balneaves LG, Wong SYS, Wu JCY. Chinese Herbal Medicine for Improving Quality of Life Among Nonsmall Cell Lung Cancer Patients: Overview of Systematic Reviews and Network Meta-Analysis. *Medicine (Baltimore)* 2016; **95**: e2410 [PMID: 26735544 DOI: 10.1097/MD.0000000000002410]

8 **Qi F**, Li A, Inagaki Y, Gao J, Li J, Kokudo N, Li XK, Tang W. Chinese herbal medicines as adjuvant treatment during chemo- or radio-therapy for cancer. *Biosci Trends* 2010; **4**: 297-307 [PMID: 21248427]

9 **Ni M**, Wang H, Wang M, Zhou W, Zhang J, Wu J, Zhang D, Jing Z, Liu X, Wu Z, Guo S, Jia S, Zhang X, Sheng X. Investigation on the Efficiency of Chinese Herbal Injections for Treating Non-small Cell Lung Cancer With Vinorelbine and Cisplatin Based on Multidimensional Bayesian Network Meta-Analysis. *Front Pharmacol* 2020; **11**: 631170 [PMID: 33708126 DOI: 10.3389/fphar.2020.631170]

10 **Tajalifar M**. RCoix Seeds: A Mini Review. *J Plant Genet Breed* 2018; **2**

11 **Du W**, Zhu W, Ge W, Li C. Research on the effect of spleen-invigorating and anti-swelling active ingredients in crude and processed coix seed based on Spectrum - Effects relationship combined with chemometrics. *J Pharm Biomed Anal* 2021; **205**: 114350 [PMID: 34507270 DOI: 10.1016/j.jpba.2021.114350]

12 **Huang Y**, Zhu J, Lin X, Hong Y, Feng Y, Shen L. Potential of Fatty Oils from Traditional Chinese Medicine in Cancer Therapy: A Review for Phytochemical, Pharmacological and Clinical Studies. *Am J Chin Med* 2019; **47**: 727-750 [PMID: 31094213 DOI: 10.1142/S0192415X19500381]

13 **Kuo CC**, Chen HH, Chiang W. Adlay (yì yǐ; "soft-shelled job's tears"; the seeds of Coix lachryma-jobi L. var. ma-yuen Stapf) is a Potential Cancer Chemopreventive Agent toward Multistage Carcinogenesis Processes. *J Tradit Complement Med* 2012; **2**: 267-275 [PMID: 24716141 DOI: 10.1016/s2225-4110(16)30112-2]

14 **Weng WF**, Peng Y, Pan X, Yan J, Li XD, Liao ZY, Cheng JP, Gao AJ, Yao X, Ruan JJ, Zhou ML. Adlay, an ancient functional plant with nutritional quality, improves human health. *Front Nutr* 2022; **9**: 1019375 [PMID: 36618703 DOI: 10.3389/fnut.2022.1019375]

15 **Zeng Y**, Yang J, Chen J, Pu X, Li X, Yang X, Yang L, Ding Y, Nong M, Zhang S, He J. Actional Mechanisms of Active Ingredients in Functional Food Adlay for Human Health. *Molecules* 2022; **27** [PMID: 35956759 DOI: 10.3390/molecules27154808]

16 **Suzuki Y**, Miyahara T, Jinnouchi M, Miura Y, Taka H, Kaga N, Ohara-Takada A. A Comprehensive Analysis of Plasma Cytokines and Metabolites Shows an Association between Galectin-9 and Changes in Peripheral Lymphocyte Subset Percentages Following Coix Seed Consumption. *Nutrients* 2022; **14** [PMID: 35565664 DOI: 10.3390/nu14091696]

17 **Jinnouchi M**, Miyahara T, Suzuki Y. Coix Seed Consumption Affects the Gut Microbiota and the Peripheral Lymphocyte Subset Profiles of Healthy Male Adults. *Nutrients* 2021; **13** [PMID: 34836336 DOI: 10.3390/nu13114079]

18 **Jang JW**, Lim DW, Chang JU, Kim JE. The Combination of Ephedrae herba and Coicis semen in Gambihwan Attenuates Obesity and Metabolic Syndrome in High-Fat Diet-Induced Obese Mice. *Evid Based Complement Alternat Med* 2018; **2018**: 5614091 [PMID: 30210573 DOI: 10.1155/2018/5614091]

19 **He C**, Li Z, Liu H, Zhang H, Wang L, Chen H. Chemical compositions and antioxidant activity of adlay seed (Coixlachryma-jobi L.) oil extracted from four main producing areas in China. *J Food Sci* 2020; **85**: 123-131 [PMID: 31872874 DOI: 10.1111/1750-3841.14982]

20 **Chen LC**, Zhang SY, Zi Y, Zhao HM, Wang HY, Zhang Y. Functional coix seed protein hydrolysates as a novel agent with potential hepatoprotective effect. *Food Funct* 2020; **11**: 9495-9502 [PMID: 33078805 DOI: 10.1039/d0fo01658f]

21 **Zhou Y**, Wu MH, Luo SM, Huang ZH, Zhang Y, Ma ZG, Cao H, Xu CL. [Historical evolution of Coicis Semen processing methods]. *Zhongguo Zhong Yao Za Zhi* 2020; **45**: 2694-2701 [PMID: 32627505 DOI: 10.19540/j.cnki.cjcmm.20200328.306]

22 **Ru J**, Li P, Wang J, Zhou W, Li B, Huang C, Guo Z, Tao W, Yang Y, Xu X, Li Y, Wang Y, Yang L. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform* 2014; **6**: 13 [PMID: 24735618 DOI: 10.1186/1758-2946-6-13]

23 **Xu X**, Zhang W, Huang C, Li Y, Yu H, Wang Y, Duan J, Ling Y. A novel chemometric method for the prediction of human oral bioavailability. *Int J Mol Sci* 2012; **13**: 6964-6982 [PMID: 22837674 DOI: 10.3390/ijms13066964]

24 **Suzuki Y**, Konaya Y. Coix Seed May Affect Human Immune Function. *Nat Prod Commun* 2021; **16** [DOI: 10.1177/1934578X211048642]

25 **Yin HM**, Wang SN, Nie SP, Xie MY. Coix polysaccharides: Gut microbiota regulation and immunomodulatory. *Bioactive Carbohydrates and Dietary Fibre* 2018; **16**: 53-61 [DOI: 10.1016/j.bcdf.2018.04.002]

26 **Hameed A**, Hafizur RM, Khan MI, Jawed A, Wang H, Zhao M, Matsunaga K, Izumi T, Siddiqui S, Khan F, Adhikari A, Sharma KR. Coixol amplifies glucose-stimulated insulin secretion *via* cAMP mediated signaling pathway. *Eur J Pharmacol* 2019; **858**: 172514 [PMID: 31265841 DOI: 10.1016/j.ejphar.2019.172514]

27 **Zhang W**, Jia X, Xu Y, Xie Q, Zhu M, Zhang H, Zhao Z, Hao J, Li H, Du J, Liu Y, Liu WH, Ma X, Hung W, Feng H. Effects of Coix Seed Extract, Bifidobacterium BPL1, and Their Combination on the Glycolipid Metabolism in Obese Mice. *Front Nutr* 2022; **9**: 939423 [PMID: 35923203 DOI: 10.3389/fnut.2022.939423]

28 **Yuan HB**, Zhu YD, Wang SS, Meng LN. Anti-Inflammatory Effect of Adlay Seed Protein in Diabetic Mice. *Curr Top Nutraceut R* 2018 [DOI: 10.37290/ctnr2641-452x.17:380-387]

29 **Zhou Q**, Yu R, Liu T, Li Y, Zhong J, Zhang T, Liu Z, Hu Y. Coix Seed Diet Ameliorates Immune Function Disorders in Experimental Colitis Mice. *Nutrients* 2021; **14** [PMID: 35010997 DOI: 10.3390/nu14010123]

30 **Wang H**, Yin H, Zhong Y, Hu J, Xia S, Wang Z, Nie S, Xie M. Polysaccharides from fermented coix seed modulates circulating nitrogen and immune function by altering gut microbiota. *Curr Res Food Sci* 2022; **5**: 1994-2003 [PMID: 36324864 DOI: 10.1016/j.crfs.2022.10.007]

31 **Chen L**, Xue S, Dai B, Zhao H. Effects of Coix Seed Oil on High Fat Diet-Induced Obesity and Dyslipidemia. *Foods* 2022; **11** [PMID: 37431015 DOI: 10.3390/foods11203267]

32 **Kim SO**, Yun SJ, Lee EH. The water extract of adlay seed (Coix lachrymajobi var. mayuen) exhibits anti-obesity effects through neuroendocrine modulation. *Am J Chin Med* 2007; **35**: 297-308 [PMID: 17436369 DOI: 10.1142/S0192415X07004825]

33 **Du J**, Yin G, Hu Y, Shi S, Jiang J, Song X, Zhang Z, Wei Z, Tang C, Lyu H. Coicis semen protects against focal cerebral ischemia-reperfusion injury by inhibiting oxidative stress and promoting angiogenesis *via* the TGFβ/ALK1/Smad1/5 signaling pathway. *Aging (Albany NY)* 2020; **13**: 877-893 [PMID: 33290255 DOI: 10.18632/aging.202194]

34 **Lu T**, Yu J, Gao R, Wang J, Wang H, Wang X, Xu B, Cao L, Wu J, Ni B, Li S, Li J. Chinese patent medicine Kanglaite injection for non-small-cell lung cancer: An overview of systematic reviews. *J Ethnopharmacol* 2023; **302**: 115814 [PMID: 36240975 DOI: 10.1016/j.jep.2022.115814]

35 **Cao L**, Long L, Hu C. Efficacy of Paclitaxel Combined with Kanglaite Injection in Treatment of Bone Metastases of Lung Cancer. *Iran J Public Health* 2019; **48**: 1445-1451 [PMID: 32292727]

36 **Wu Y**, Zhang J, Hong Y, Wang X. Effects of Kanglaite Injection on Serum miRNA-21 in Patients with Advanced Lung Cancer. *Med Sci Monit* 2018; **24**: 2901-2906 [PMID: 29735968 DOI: 10.12659/MSM.909719]

37 **Lu X**, Liu W, Luo C. Apoptotic effect of coix polysaccharides on A549 lung cancer cells in vitro. *Zhongguo Fei Ai Za Zhi* 2012; **15**: 624-629 [PMID: 23164347 DOI: 10.3779/j.issn.1009-3419.2012.11.04]

38 **Duan GC**. The Effects of Combination of Coix Seed Extract and Cisplatin on TAM and Expression of HIF-1α in Vivo in Lewis Lung Carcinoma. *Iran J Public Health* 2018; **47**: 838-843 [PMID: 30087869]

39 **Luo C**, Wang X, An C, Hwang CF, Miao W, Yang L, Xu M, Bai A, Deng S. Molecular inhibition mechanisms of cell migration and invasion by coix polysaccharides in A549 NSCLC cells *via* targeting S100A4. *Mol Med Rep* 2017; **15**: 309-316 [PMID: 27922683 DOI: 10.3892/mmr.2016.5985]

40 **Ni C**, Li B, Ding Y, Wu Y, Wang Q, Wang J, Cheng J. Anti-Cancer Properties of Coix Seed Oil against HT-29 Colon Cells through Regulation of the PI3K/AKT Signaling Pathway. *Foods* 2021; **10** [PMID: 34829119 DOI: 10.3390/foods10112833]

41 **Wang Y**, Zhang C, Zhang S, Zhao Z, Wang J, Song J, Wang Y, Liu J, Hou S. Kanglaite sensitizes colorectal cancer cells to Taxol *via* NF-κB inhibition and connexin 43 upregulation. *Sci Rep* 2017; **7**: 1280 [PMID: 28455529 DOI: 10.1038/s41598-017-01480-2]

42 **Wu LQ**, Lu Y, Lu HJ, Zhao ZG, Yang M. Efficacy of intra-tumor injection of Kang-Lai-Te in treating transplanted hepatoma in rats. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 580-584 [PMID: 15567749]

43 **Wang D**, Yang C, Wang Z, Yang Y, Li D, Ding X, Xu W, Zheng Q. Norcantharidin combined with Coix seed oil synergistically induces apoptosis and inhibits hepatocellular carcinoma growth by downregulating regulatory T cells accumulation. *Sci Rep* 2017; **7**: 9373 [PMID: 28839202 DOI: 10.1038/s41598-017-09668-2]

44 **Qu D**, Liu M, Huang M, Wang L, Chen Y, Liu C, Liu Y. Octanoyl galactose ester-modified microemulsion system self-assembled by coix seed components to enhance tumor targeting and hepatoma therapy. *Int J Nanomedicine* 2017; **12**: 2045-2059 [PMID: 28352174 DOI: 10.2147/IJN.S125293]

45 **Qu D**, Sun W, Liu M, Liu Y, Zhou J, Chen Y. Bitargeted microemulsions based on coix seed ingredients for enhanced hepatic tumor delivery and synergistic therapy. *Int J Pharm* 2016; **503**: 90-101 [PMID: 26947738 DOI: 10.1016/j.ijpharm.2016.03.001]

46 **Fang T**, Jiang YX, Chen L, Huang L, Tian XH, Zhou YD, Nagle DG, Zhang DD. Coix Seed Oil Exerts an Anti-Triple-Negative Breast Cancer Effect by Disrupting miR-205/S1PR1 Axis. *Front Pharmacol* 2020; **11**: 529962 [PMID: 33101013 DOI: 10.3389/fphar.2020.529962]

47 **Zhao M**, Fu L, Xu P, Wang T, Li P. Network Pharmacology and Experimental Validation to Explore the Effect and Mechanism of Kanglaite Injection Against Triple-Negative Breast Cancer. *Drug Des Devel Ther* 2023; **17**: 901-917 [PMID: 36998242 DOI: 10.2147/DDDT.S397969]

48 **Chen Y**, Wang S, Hu Q, Zhou L. Self-emulsifying System Co-loaded with Paclitaxel and Coix Seed Oil Deeply Penetrated to Enhance Efficacy in Cervical Cancer. *Curr Drug Deliv* 2023; **20**: 919-926 [PMID: 35762559 DOI: 10.2174/1567201819666220628094239]

49 **Guo M**, Qu D, Qin Y, Chen Y, Liu Y, Huang M. Transferrin-Functionalized Microemulsions Coloaded with Coix Seed Oil and Tripterine Deeply Penetrate To Improve Cervical Cancer Therapy. *Mol Pharm* 2019; **16**: 4826-4835 [PMID: 31663764 DOI: 10.1021/acs.molpharmaceut.9b00717]

50 **Chen Y**, Qu D, Fu R, Guo M, Qin Y, Guo J, Chen Y. A Tf-modified tripterine-loaded coix seed oil microemulsion enhances anti-cervical cancer treatment. *Int J Nanomedicine* 2018; **13**: 7275-7287 [PMID: 30510417 DOI: 10.2147/IJN.S182475]

51 **Zhang XW**, Liu L, Zhang XZ, Bo P. Kanglaite inhibits the expression of drug resistance genes through suppressing PVT1 in cisplatin-resistant gastric cancer cells. *Exp Ther Med* 2017; **14**: 1789-1794 [PMID: 28810651 DOI: 10.3892/etm.2017.4650]

52 **Zhan YP**, Huang XE, Cao J, Lu YY, Wu XY, Liu J, Xu X, Xiang J, Ye LH. Clinical safety and efficacy of Kanglaite® (Coix Seed Oil) injection combined with chemotherapy in treating patients with gastric cancer. *Asian Pac J Cancer Prev* 2012; **13**: 5319-5321 [PMID: 23244156 DOI: 10.7314/apjcp.2012.13.10.5319]

53 **Yang J**, Liu Y, Lu S, Sun X, Yin Y, Wang K, Liu S. Coix seed oil regulates mitochondrial functional damage to induce apoptosis of human pancreatic cancer cells *via* the PTEN/PI3K/AKT signaling pathway. *Mol Biol Rep* 2022; **49**: 5897-5909 [PMID: 35543827 DOI: 10.1007/s11033-022-07371-8]

54 **Qian Y**, Xiong Y, Feng D, Wu Y, Zhang X, Chen L, Gu M. Coix Seed Extract Enhances the Anti-Pancreatic Cancer Efficacy of Gemcitabine through Regulating ABCB1- and ABCG2-Mediated Drug Efflux: A Bioluminescent Pharmacokinetic and Pharmacodynamic Study. *Int J Mol Sci* 2019; **20** [PMID: 31652737 DOI: 10.3390/ijms20215250]

55 **Qian Y**, Yang B, Xiong Y, Gu M. Coix seed emulsion synergistically enhances the antitumor activity of gemcitabine in pancreatic cancer through abrogation of NF-κB signaling. *Oncol Rep* 2016; **36**: 1517-1525 [PMID: 27459907 DOI: 10.3892/or.2016.4958]

56 **Liu H**, Li L, Zou J, Zhou T, Wang B, Sun H, Yu S. Coix seed oil ameliorates cancer cachexia by counteracting muscle loss and fat lipolysis. *BMC Complement Altern Med* 2019; **19**: 267 [PMID: 31615487 DOI: 10.1186/s12906-019-2684-4]

57 **Zhang P**, Meng X, Tang X, Ren L, Liang J. The effect of a coix seed oil injection on cancer pain relief. *Support Care Cancer* 2019; **27**: 461-465 [PMID: 29971522 DOI: 10.1007/s00520-018-4313-z]

58 **Yan H**, Li Y, Yang B, Long F, Yang Z, Tang D. Exploring the mechanism of action of Yiyi Fuzi Baijiang powder in colorectal cancer based on network pharmacology and molecular docking studies. *Biotechnol Genet Eng Rev* 2023; 1-21 [PMID: 36735641 DOI: 10.1080/02648725.2023.2167765]

59 **Xiang B**, Geng R, Zhang Z, Ji X, Zou J, Chen L, Liu J. Identification of the effect and mechanism of Yiyi Fuzi Baijiang powder against colorectal cancer using network pharmacology and experimental validation. *Front Pharmacol* 2022; **13**: 929836 [PMID: 36353478 DOI: 10.3389/fphar.2022.929836]

60 **Yang J**, Miao L, Xue Y, Wang X. Yiyi Fuzi Baijiang Powder Alleviates Dextran Sulfate Sodium-Induced Ulcerative Colitis in Rats *via* Inhibiting the TLR4/NF-κB/NLRP3 Inflammasome Signaling Pathway to Repair the Intestinal Epithelial Barrier, and Modulating Intestinal Microbiota. *Oxid Med Cell Longev* 2023; **2023**: 3071610 [PMID: 36691639 DOI: 10.1155/2023/3071610]

61 **Yang PW**, Xu PL, Cheng CS, Jiao JY, Wu Y, Dong S, Xie J, Zhu XY. Integrating network pharmacology and experimental models to investigate the efficacy of QYHJ on pancreatic cancer. *J Ethnopharmacol* 2022; **297**: 115516 [PMID: 35817247 DOI: 10.1016/j.jep.2022.115516]

62 **Lin X**, Xu W, Shao M, Fan Q, Wen G, Li C, Jing L, Sun X. Shenling Baizhu San supresses colitis associated colorectal cancer through inhibition of epithelial-mesenchymal transition and myeloid-derived suppressor infiltration. *BMC Complement Altern Med* 2015; **15**: 126 [PMID: 25897964 DOI: 10.1186/s12906-015-0649-9]

63 **Zhao CH**, He B, Yang YF, Liao J. Dietary therapy of qi-yin-reinforcing porridge for the alleviation of chemotherapy related symptoms of gastrointestinal tumors: a single-case randomized controlled study. *Chin J Integr Med* 2013; **19**: 418-423 [PMID: 23784467 DOI: 10.1007/s11655-013-1329-y]

64 **Li H**, Peng L, Yin F, Fang J, Cai L, Zhang C, Xiang Z, Zhao Y, Zhang S, Sheng H, Wang D, Zhang X, Liang Z. Research on Coix seed as a food and medicinal resource, it's chemical components and their pharmacological activities: A review. *J Ethnopharmacol* 2023; **319**: 117309 [PMID: 37858750 DOI: 10.1016/j.jep.2023.117309]

65 **Pan X**, Shen Q, Zhang C, Zhang X, Li Y, Chang Z, Pang B. Coicis Semen for the treatment of malignant tumors of the female reproductive system: A review of traditional Chinese medicinal uses, phytochemistry, pharmacokinetics, and pharmacodynamics. *Front Pharmacol* 2023; **14**: 1129874 [PMID: 36909176 DOI: 10.3389/fphar.2023.1129874]

66 **Lu C**, Wu S, Ke L, Liu F, Shang W, Deng X, Huang Y, Zhang Q, Cui X, Mentis AA, Xie Y, Wang Z. Kanglaite (Coix Seed Extract) as Adjunctive Therapy in Cancer: Evidence Mapping Overview Based on Systematic Reviews With Meta-Analyses. *Front Pharmacol* 2022; **13**: 901875 [PMID: 36034785 DOI: 10.3389/fphar.2022.901875]

67 **Kim A**, Ha J, Kim J, Cho Y, Ahn J, Cheon C, Kim SH, Ko SG, Kim B. Natural Products for Pancreatic Cancer Treatment: From Traditional Medicine to Modern Drug Discovery. *Nutrients* 2021; **13** [PMID: 34836055 DOI: 10.3390/nu13113801]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 December 24; 14(12): 606-619

DOI: 10.5306/wjco.v14.i12.606

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

Clinical outcomes of newly diagnosed primary central nervous system lymphoma treated with zanubrutinib-based combination therapy

Ning Wang, Fei-Li Chen, Lu Pan, Yan Teng, Xiao-Juan Wei, Han-Guo Guo, Xin-Miao Jiang, Ling Huang, Si-Chu Liu, Zhan-Li Liang, Wen-Yu Li

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Mehdipour P, Iran

Received: June 1, 2023
Peer-review started: June 1, 2023
First decision: August 16, 2023
Revised: September 5, 2023
Accepted: November 17, 2023
Article in press: November 17, 2023
Published online: December 24, 2023

Ning Wang, Lu Pan, Yan Teng, Wen-Yu Li, School of Medicine, South China University of Technology, Guangzhou 510006, Guangdong Province, China

Fei-Li Chen, Xiao-Juan Wei, Han-Guo Guo, Xin-Miao Jiang, Ling Huang, Si-Chu Liu, Zhan-Li Liang, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, Guangdong Province, China

Corresponding author: Wen-Yu Li, Doctor, Chief Doctor, School of Medicine, South China University of Technology, No. 123 Huifu West Road, Guangzhou 510006, Guangdong Province, China. lwy80411@163.com

Abstract

BACKGROUND

High-dose methotrexate (HD-MTX) combined with other chemotherapeutic agents is an effective treatment for patients with newly diagnosed primary central nervous system lymphoma (PCNSL); however, some patients have adverse reactions.

AIM

To retrospectively evaluate disease outcomes and mutational profiles in newly diagnosed PCNSL patients treated with a zanubrutinib/HD-MTX combination regimen.

METHODS

Nineteen newly diagnosed PCNSL patients were treated with zanubrutinib/HD-MTX until disease progression, intolerable toxicities, or physician/patient-directed withdrawal. Safety and efficacy were assessed per the CTCAE v5.0 and RECIST v1.1 criteria, respectively. The primary endpoint was the objective response rate (ORR), and the secondary endpoints were progression-free survival, overall survival (OS), and safety.

RESULTS

The median follow-up duration was 14.7 mo (range, 3.9–30 mo). The ORR for all patients was 84.2%, and 2-year progression-free- and OS rates were 75.6% and 94.1%, respectively. All patients completed the induction phase, and nine patients

underwent autologous stem cell transplantation as consolidation therapy, resulting in an ORR of 88.9%. Ten patients received zanubrutinib as maintenance therapy and achieved an ORR of 80%. All patients showed an acceptable safety profile. The sequencing results for cerebrospinal fluid (CSF) and tumor tissue showed that PIM1 mutations were the most frequent genetic alterations. Circulating tumor DNA was correlated with disease relapse and response.

CONCLUSION

Our empirical observations demonstrated that the combination of zanubrutinib with HD-MTX yielded a marked clinical response and tolerability among newly diagnosed PCNSL patients. Non-invasive CSF liquid biopsy profiling may be feasible for evaluating treatment response and tumor burden.

Key Words: Zanubrutinib; High-dose methotrexate; Primary central nervous system lymphoma; Liquid biopsy; Circulating tumor DNA

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Zanubrutinib combined with high-dose methotrexate provided a marked clinical response and tolerance in newly diagnosed primary central nervous system lymphoma patients. Additionally, the detection of circulating tumor DNA in cerebrospinal fluid played a significant part in disease surveillance and treatment response monitoring. However, given the small sample size and retrospective nature of this study, further research is required to validate our findings.

Citation: Wang N, Chen FL, Pan L, Teng Y, Wei XJ, Guo HG, Jiang XM, Huang L, Liu SC, Liang ZL, Li WY. Clinical outcomes of newly diagnosed primary central nervous system lymphoma treated with zanubrutinib-based combination therapy. *World J Clin Oncol* 2023; 14(12): 606-619

URL: https://www.wjgnet.com/2218-4333/full/v14/i12/606.htm

DOI: https://dx.doi.org/10.5306/wjco.v14.i12.606

INTRODUCTION

Primary central nervous system lymphoma (PCNSL) is an aggressive lymphoma that is confined to the brain, leptomeninges, eyes, cerebrospinal fluid (CSF), or spinal cord, without evidence of systemic disease[1,2]. Almost all PCNSLs constitute diffuse large B-cell lymphoma (DLBCL)[3]. However, the treatments for PCNSL and DLBCL differ. High-dose methotrexate (HD-MTX) is the primary treatment for PCNSL. HD-MTX (3.5 g/m^2) combined with other chemotherapeutic agents is effective; however, some patients have adverse reactions[4,5]. Therefore, it is necessary to identify drugs that can be combined with HD-MTX to solve this issue.

Zanubrutinib, a novel oral inhibitor of Bruton's tyrosine kinase (BTK), is a promising therapeutic intervention in B-cell antigen receptor (BCR) and Toll-like receptor (TLR) signaling. This signaling network integrates signals from the BCR and TLR pathways. The key players, BCR-associated protein CD79B and myeloid differentiation primary response 88 (MYD88), act as bridges linking interleukin-1 and TLRs with the potent nuclear factor kappa B pathway[6-9]. Activating mutations were observed in MYD88 and CD79B across various studies of PCNSL[6,10-13]. Studies have shown that BTK inhibitors can cross the blood-brain barrier and effectively modulate signaling cascades downstream of MYD88 and CD79B[14-17], demonstrating their potential efficacy in PCNSL. Recent studies on zanubrutinib-containing therapeutic regimens have highlighted their effectiveness in cases of DLBCL with CNS involvement[18]. However, despite these advancements, a critical gap remains: the absence of concrete clinical evidence supporting the use of zanubrutinib in PCNSL with CNS involvement. The BTK inhibitor, ibrutinib, combined with HD-MTX has demonstrated an objective response rate (ORR) of 80% with an acceptable safety profile in a phase Ib study[19]. Therefore, we retrospectively analyzed the clinicopathological characteristics, treatment outcomes, and adverse events in newly diagnosed PCNSL patients treated with combined HD-MTX and zanubrutinib. We also explored the next-generation sequencing of circulating tumor DNA (ctDNA) in CSF, both before and during treatment, as well as the safety profile, treatment response, and genomic biomarkers.

MATERIALS AND METHODS

Patients

From May 2020 to April 2022, 19 eligible PCNSL patients from XX Hospital, China, were identified for inclusion in this study. The inclusion criteria were as follows: (1) Newly diagnosed pathologically confirmed PCNSL; (2) ≥ 18 years of age; (3) Treatment with HD-MTX and zanubrutinib combination therapy; and (4) Received at least two cycles of chemotherapy. The exclusion criteria were as follows: (1) Non-primary CNS lymphoma; (2) Previous treatment with other BTK

inhibitors; and (3) Patients with incomplete follow-up data, for whom we were unable to evaluate efficacy. The selection criteria are also shown in Figure 1.

This study was approved by the Clinical and Research Ethics Committee of the Guangdong Provincial People's Hospital, Guangzhou, China. All procedures in the present study that involved human participants were performed in accordance with the Declaration of Helsinki. All patients provided written informed consent to participate in this study.

Treatment protocol

The treatment regimen was designed to achieve optimal outcomes through induction therapy with combined HD-MTX and zanubrutinib. HD-MTX was administered at a dose of 3.5 g/m^2, with a total of 4-8 doses planned. Zanubrutinib was prescribed at a dose of 160 mg orally (PO) twice daily (BID). Zanubrutinib administration was paused on the days of HD-MTX infusion to mitigate potential interactions and was resumed once HD-MTX clearance was achieved. Following induction therapy, zanubrutinib was administered as maintenance therapy until specific endpoints were reached, namely disease progression, intolerable toxicity, autologous stem cell transplantation (ASCT), or mortality.

Stem cell assessment and ASCT

Prior to ASCT, a comprehensive evaluation of each patient's stem cell composition was performed. The ASCT process comprised the use of peripheral blood autologous hematopoietic stem cells. To prepare patients for ASCT, a pre-conditioning regimen was administered that comprised either carmustine, etoposide, cytarabine, and melphalan or carmustine, etoposide, cytarabine, and cyclophosphamide. This pre-conditioning aimed to optimize the transplantation environment. Subsequently, granulocyte colony-stimulating factor was administered to mobilize stem cells. The screening process involved monitoring cluster of differentiation 34-positive (CD34+) hematopoietic stem cells in peripheral blood using flow cytometry. The ideal threshold for peripheral blood CD34+ cells was set at ≥ 20 cells/μL. This monitoring enabled prediction of the required collection quantity and duration, with a minimum standard of CD34+ cells not at 2 × 10^6/kg. A desirable transplant condition was generally achieved when the final collection of CD34+ cells exceeded 5 × 10^6/kg.

Response assessment

Therapeutic response was evaluated in accordance with the international PCNSL Collaborative Group guidelines[1]. Response to treatment was assessed using magnetic resonance imaging and CSF evaluation every second cycle. In accordance with the guidelines, each patient's best response to treatment was recorded to evaluate the ORR, including complete response (CR, no contrast enhancement on imaging) and partial response (≥ 50% decrease disease enhancement on imaging). Any new lesions were defined as progressive disease (PD), and any other conditions were defined as stable disease. Progression-free survival (PFS) was calculated from the start of treatment to the time of disease progression or death due to PCNSL. Overall survival (OS) was calculated from the date of diagnosis to the time of death from any cause.

Sample collection and processing

CSF and peripheral blood samples were collected and stored at -80°C. Tumor biopsy specimens were obtained using formalin-fixed, paraffin-embedded tissues. Samples were analyzed using capture-based targeted next-generation sequencing in a central testing laboratory (Nanjing Geneseq Technology, Inc., Nanjing, China). This approach, as previously outlined, targets 102 lymphoma-associated genes, facilitating precise genetic characterization[20,21]. The DLBCL [non-germinal center B-cell (non-GCB) or germinal center B-cell (GCB)] subtype was determined using immuno-histochemical staining in accordance with the Hans classification, in the Department of Pathology of the Guangdong People's Hospital, Guangzhou, China.

Statistical analysis

GraphPad Prism 9 (version 9.0.2; GraphPad Software, Inc., San Diego, CA, United States) was used for the data analysis. Baseline characteristics were described using medians for continuous variables and percentages for categorical variables. PFS and OS were analyzed by the Kaplan–Meier method, P values were calculated using the log-rank test, and $P < 0.05$ indicated a significant difference.

RESULTS

Baseline patients' data

Data for 19 patients with newly diagnosed PCNSL who were treated with HD-MTX plus zanubrutinib were retrospectively analyzed (Figure 1). The patients' clinicopathological characteristics are summarized in Table 1. The patients' median age was 57 years (range, 27-81 years), and five patients had an Eastern Cooperative Oncology Group performance score > 2 (Table 1). Ten patients were women, and 16 patients had lesions in deep areas, namely the periventricular tissue, corpus callosum, brainstem, basal ganglia, and/or cerebellum. Eleven patients had high CSF protein concentrations (> 450 mg/L), and only one patient had a high lactate dehydrogenase serum level (> 250 U/L). The International Extranodal Lymphoma Study Group risk score was low-grade in 3 patients, median-grade in 12 patients, and high-grade in 1 patient.

Table 1 Baseline data of the patients with primary central nervous system lymphoma

Characteristic	*N* = 19
Age, yr	57 (27–81)
Sex, *n* (%)	
Male	9 (47.4)
Female	10 (52.6)
ECOG-PS ≥ 2, *n* (%)	5 (26.3)
Invasion of deep intracranial areas, *n* (%)	16 (84.2)
High CSF protein concentration (> 450 mg/L), *n* (%)	11 (68.75)[1]
High LDH serum concentration (> 250 U/L), *n* (%)	1 (5.3)
IELSG risk score, *n* (%)	
Low	3 (18.75)[1]
Intermediate	12 (75)[1]
High	1 (6.25)[1]
Follow-up time (mo)	14.7 (3.9–30)

[1]Three patients refused lumbar puncture for personal reasons.
ECOG-PS: Eastern Cooperative Oncology Group performance score; CSF: Cerebrospinal fluid; LDH: Lactate dehydrogenase; IELSG: International Extranodal Lymphoma Study Group.

Treatment duration and response

All 19 patients received 120 doses of induction therapy. ASCT was administered as consolidation therapy in nine patients. None of the patients received corticosteroid therapy. HD-MTX therapy was discontinued in one patient due to delayed HD-MTX excretion. Nine patients completed ASCT, with an ORR of 88.9% (CR/PR: 6/2). Eight patients were still in remission at the time of writing (Figure 2 and Table 2). Ten patients received maintenance therapy comprising zanubrutinib with lenalidomide for 6 mo, and zanubrutinib monotherapy was administered continuously until disease progression.

The median follow-up duration was 14.7 mo (range, 3.9-30 mo). All patients were evaluated for treatment response, which revealed CR in 11 patients, partial response in 5 patients, and PD in 3 patients. The ORR was 84.2%, and 2-year PFS and OS rates were 75.6% and 94.1%, respectively. The median PFS and median OS for the entire cohort were not reached (Table 3 and Figure 3).

Safety and adverse events

The prevalent hematological toxicity in patients who received HD-MTX plus zanubrutinib treatment was anemia (100%), followed by lymphocytopenia (84.2%). The leading non-hematological toxicities were hypoalbuminemia (94.7%) and hypokalemia (78.9%) (Table 4). It is noteworthy that no grade 4 non-hematological toxicities were recorded, and the observed adverse effects of therapy were mild and required no additional therapeutic interventions. No treatment-related fatalities were observed.

Clinical response and baseline tumor genomic characteristics

We also explored the association between treatment response and tumor genomic traits. CSF samples were available for eight patients, while six patients had baseline tumor biopsy samples available for genomic analysis (Figure 4). Forty-two genetic alterations were detected (tumor tissue samples: n = 30, CSF samples: n = 30), and 18 alterations were the same in the primary tumor tissue and CSF samples (Figure 5). The most common mutation detected in both CSF and primary tumor samples was PIM1, followed by alterations of B-cell lymphoma 6, MYD88, GNA13, and TBL1XR1.

Among the 19 patients, 9 had non-GCB disease, with 6 (66.7%) responding positively to the zanubrutinib-based regimen. The remaining 10 patients with GCB disease achieved a 100% response rate to the same regimen. Among the patients with MYD88 alterations, four achieved CR, constituting 50% of this subgroup, with the zanubrutinib-based regimen. In the subset of eight patients with alterations in key genes involved in the BCR pathway, such as CD79B and MYD88, the ORR reached 60%. The response rates for patients with alterations in MYD88 and CD79B were 50% (4/8) and 37.5% (3/8), respectively (Table 2). Two patients (P1 and P7) with alterations in both MYD88 and CD79B genes demonstrated a 50% ORR, with one achieving a CR, as shown in Table 2.

The role of CSF ctDNA in disease surveillance

Eight CSF samples were collected during various treatment cycles, *i.e.*, at baseline and just before cycles 3, 5, and 6. One exception was patient 6, who underwent assessments only after cycle 3 and cycle 6 for personal reasons. Six patients

Table 2 Baseline tumor genomic characteristics of the patients with primary central nervous system lymphoma

Patient	ID	COO Subtype	Best response (mo)	MYDBB	CD79B	Ki-67	Cyclin D1	Other IHC results
p973624	1	Non-GCB	CR (22.2)[1]	L265P	Y196D	> 90%+	NA	CD20(+++), CD79a(+++), CD3(−), CD5(−), CD21(−), CD23(−), Bcl6(>90%+), MUM1(>90%+), FOXP1(>90%+), Bcl2(60%+), c-Myc(40%+), CD30(−), ALK(ALK1)(−), CD138(−), P53(+), c-Met(−), PD-L1(22C3)(30%+)
p968283	2	GCB	CR (23)[1]	NA	NA	100%+	−	CD43(−), CD20(+++), CD3(−), CD79a(+++), CD5(−), CD23(−), CD10(95%+), CD21(−), CD30(−), ALK(ALK1)(−), Bcl6(90%+), CD138(−), MUM1(65%+), Bcl2(50%+), c-Myc(20%+), GFAP(−), Olig2(−)
p955842	3	Non-GCB	SD (6.0)	NA	NA	70%+	−	LCA(+++), OCT-2(+++), CD20(+++), CD19(+++), CD10(−), Bcl6(70%+), MUM1(40%+), CD3(−), CD5(+), ALK(ALK1)(-), CD23(−), CD21(−), CD30(−), CD138(−), Bcl2(++), TdT(−), GECT1(+), FOXP1(+++), c-Myc(80%+), c-Met(−), P53(+++), GFAP(−), CK(−), EMA(−)
p241574	4	Non-GCB	CR (27.5)[1]	NA	K159Q, V223R	90%+	−	LCA(++), CD79a(++), CD43(−), CD20(+), CD3(−), CD5(−), CD23(−), CD10(−), CD21(−), CD30(−), ALK(ALK1)(−), Bcl6(90%++), CD138(−), MUM1(70%+), Bcl2(50%+), TdT(−), GECT1(30%+), FOXP1(+), c-Myc(70%+), c-Met(+), LMP-1(−), EBNA2(−), P53(+), PD-L1(22C3)(90%+)
p939668	5	Non-GCB	PR (28.5)[1]	NA	NA	NA	NA	NA
p932230	6	GCB	CR (29.5)[1]	S219C	NA	98%+	NA	CD20(+++), CD79a(+++), CD3(−), CD5(−), ALK(ALK1)(−), CD21(−), CD23(−), Bcl6(90%+), MUM1(20%+), CD10(100%+), CK(−), Vimentin(−), EMA(−), S100(−), GFAP(−), Bcl2(80%+), GECT1(35%+), FOXP1(80%+), c-Myc(45%+), C-MET(50%+), P53(4%+), PD-L1(22C3)(10%+)
p929763	7	Non-GCB	CR (18.8)	L265P	C.553-2A>C	90%+	−	CD43(−), CD20(+++), CD3(−), CD79a(+++), CD5(−), CD23(−), CD10(−), CD19(+++), CD22(++), CD21(−), CD30(−), ALK(ALK1)(−), Bcl6(10%+), CD138(−), MUM1(20%+), Bcl2(70%+), TdT(−), c-Myc(5%+), GFAP(−)
p173185	8	Non-GCB	PR (8.0)	L265P	NA	NA	NA	ERCC1(−), β-tubulin(+++), EGFR(+++), VEGF(+), ALK(−), CD56(−), CgA(−), Syn(−)
p651739	9	GCB	CR (23.0)	NA	NA	90%+	−	CD43(+), CD20(+++), CD3(+), CD79a(++), CD5(−), CD23(−), CD10(+++), CD21(−), CD30(−), ALK(ALK1)(−), Bcl6(60%+), CD138(−), MUM1(++), Bcl2(−), TdT(−), c-Myc(20%+), GFAP(−), Olig2(−)
p1013138	10	GCB	CR (12.5)	NA	NA	90%+	−	CD3(−), CD5(−), CD20(++), CD79a(++), CD30(−), ALK(ALK1)(−), SALL4(−), OCT3/4(−), AFP(−), GFAP(−), Olig2(−), MUM1(+), CD10(++), Bcl6(++), CD23(−), Bcl2(−), GCET-1(+), FOXP1(+), c-Myc(70%+), c-Met(−), P53(95%++), PD-L1(22C3)(TC <1%+, IC 70%+)
p2010722	11	Non-GCB	PR (16.6)	NA	NA	80%+	−	CK(−), CD20 and CD79a(+), CD3(−), CD5(−), Bcl-2(80%+),

								MUM-1(+), CD10(−), Bcl6(−)
p998505	12	Non-GCB	PR (22.2)	NA	NA	70%+	2%+	D3(+), CD5(++), CD20(+++), CD79a(+++), CD30(−), CD10(−), GFAP(−), Olig2(−), CK(−), CD43(++), CD23(−), CD21(−), ALK(ALK1)(−), Bcl6(20%+), CD138(−), MUM1(80%+), Bcl2(90%+++), TdT(−), GCET-1(−), FOXP1(+++), c-Myc(70%+), c-Met(−), P53(1%+), PD-L1(22C3)(70%+)
p1013897	13	GCB	CR (14.2)	NA	NA	85%+	−	CD20(+++), CD3(−), CD79a(+++), CD5(−), CD30(<1%+), ALK(ALK1)(−), CD23(−), CD10(+++), CD21(−), Bcl6(70%+), CD138(+), MUM1(40%+), Bcl2(5%+), TdT(−), Cyclin D1(−), c-Myc(40%+), c-Met(60%+), P53(70%+), PD-L1(22C3)(40%+)
p2020811	14	GCB	CR (10.8)	NA	NA	90%+	NA	CD3(+), CD5(+), CD79a(+), CD10(+), Bcl6(±), CD20(+++), Bcl6(80%+), MUM1(<5%+), CD10(+), Ki67(90%+), CD3(−), AE1/AE3(−), EMA(−), P40(−), CD3(−), GFAP(−)
P2003851	15	GCB	CR (13.1)	NA	NA	80%+	−	EMA(−), S100(−), GFAP(+), Syn(+), CgA(−), Olig2(+), NeuN(+), CD34(+), CD3(+), CD5(+), CD79a(+), CD20(+), CD43(10%+), CD30(−), ALK(ALK1)(−), Bcl6(>90%+), Bcl2(90%+), TdT(−), GCET-1(90%+), FOXP1(>90%+), c-Myc(40%+), c-Met(−), LMP-1(−), EBNA2(−), P53(60%+), PD(−), L1(22C3)(30%+)
p996213	16	GCB	CR (18.3)	NA	NA	NA	NA	NA
P1010986	17	GCB	CR (14.7)	NA	NA	95%+	−	CD43(+), CD20(+++), CD3(−), CD79a(++), CD5(−), CD23(−), CD10(60%+), CD21(−), CD30(−), ALK(ALK1)(−), Bcl6(60%+), CD138(−), MUM1(90%+), Bcl2(15%+), TdT(−), c-Myc(30%+)
P2052819	18	Non-GCB	PR (3.9)	NA	NA	90%+	−	GFAP(−), Olig2(−), CD20(+++), CD79a(++), CD3(−), CD5(−), CD21(−), CD23(−), CD10(−), Bcl6(60%++), MUM1(90%+++), CD138(−), Bcl2(90%+++), c-Myc(70%++), CD30(−), ALK(ALK1)(−)
P2045773	19	GCB	PR (5.2)	NA	NA	90%+	−	CD19(+++), CD20(+++), CD3(−), CD5(−), GFAP(−), Olig2(−), CD23(+), CD10(−), CD21(−), CD30(−), ALK(ALK1)(−), Bcl6(70%+), CD138(−), MUM1(70%+), Bcl-2(95%+), TdT(−), c-Myc(40%+), c-Met(−)

[1]Still in remission.

PCNSL: Primary central nervous system lymphoma; ID: Identification number; COO: Cell of origin; NA: Not available; IHC: Immunohistochemistry; GCB: Germinal center B cell; CR: Complete response; SD: Stable disease; PR: Partial response: CD: Cluster of differentiation; Bcl6: B cell lymphoma 6; MUM-1: Multiple myeloma antigen 1; FOXP1: Forkhead box protein P1; Bcl2: B cell lymphoma 2; ALK: Anaplastic lymphoma kinase; PD-L1: Programmed death-ligand 1; GFAP: Glial fibrillary acidic protein; LCA: Leucocyte common antigen; TdT: Terminal deoxynucleotidyl transferase; GECT: Gene expression in developing tissues with micro computed tomography; CK: Cytokeratin; EMA: Epithelial membrane antigen; LMP-1: Epstein–Barr virus-encoded latent membrane protein 1; EBNA2: Epstein–Barr virus nuclear antigen 2; P53: Tumor protein 53; EGFR: Epidermal growth factor receptor; VEGF: Vascular endothelial growth factor; CgA: Chromogranin A; Syn: Syndecan; SALL4: Sal-like protein 4; AFP: Alpha fetoprotein; Olig2: Oligodendrocyte lineage transcription factor 2; TC: Tumor cells; IC: Immune cells; D3: Cyclin D3; NeuN: Neuronal nuclear antigen.

showed a robust radiographic response to treatment, resulting in a significant reduction in CSF mutant allele frequency. One patient (P3) showed a stable radiographic response, confirmed by magnetic resonance imaging, but the ctDNA levels remained unchanged in the CSF specimen (Figure 6). However, this patient experienced PD following completion of the zanubrutinib-based induction regimen. Therefore, whole-brain radiotherapy (30 GY) with temozolomide and zanubrutinib was initiated. This approach led to a favorable radiographic response, and the patient is presently

Table 3 Efficacy of high-dose methotrexate plus zanubrutinib for newly diagnosed primary central nervous system lymphoma

Parameter	*N* = 19
OS rate (%)	-
24-mo (95%CI)	94.1% (83.6%-100%)
Median PFS	-
24-mo (95%CI)	75.6% (53.4%-100%)
ORR (%)	84.2%
ASCT (consolidation therapy)	88.9%
Zanubrutinib (maintenance therapy)	80%
Median follow-up time (mo)	14.7
95%CI	3.9–30

OS: Overall survival; CI: Confidence interval; PFS: Progression-free survival; ORR: Objective response rate; ASCT: Autologous stem cell transplantation.

Table 4 Adverse events in patients treated with high-dose methotrexate plus zanubrutinib

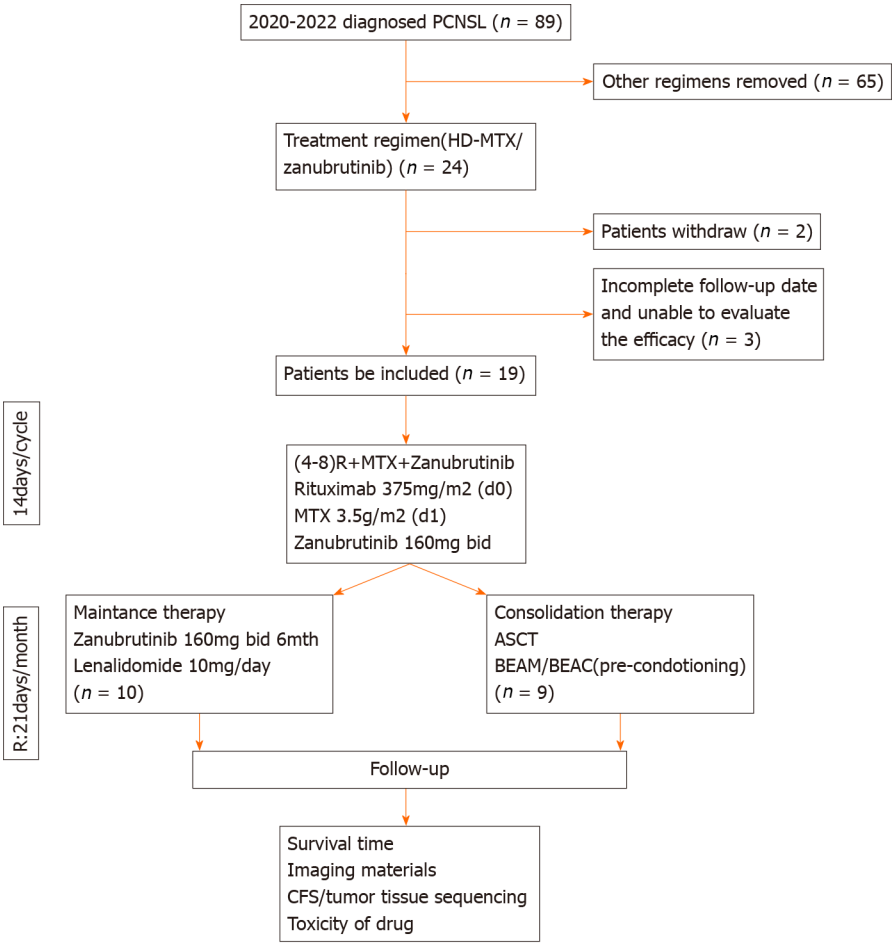
Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Total (%)
Hematological toxicities					
Leukopenia	3	7	1		11 (57.9)
Neutropenia	3	6	2		11 (57.9)
Lymphocytopenia	6	8	2		16 (84.2)
Thrombocytopenia	5			1	6 (31.6)
Anemia	8	10	1		19 (100)
Non-hematological toxicities					
Transaminase increase	4				4 (21.1)
Creatinine increase	2	1			3 (15.8)
Hypoalbuminemia	16	2			18 (94.7)
Hypokalemia	10	4	1		15 (78.9)
Lung infection					NA

NA: Not applicable.

continuing with this treatment. Another patient (P8) demonstrated a partial radiographic response. The mutant allele frequency in the CSF decreased markedly with treatment, excluding the gene fusion of BCR-ABL1 (Figure 6). However, this patient developed PD as peripheral lesions, and subsequently received rituximab, zanubrutinib, and lenalidomide (IR2) as second-line treatment.

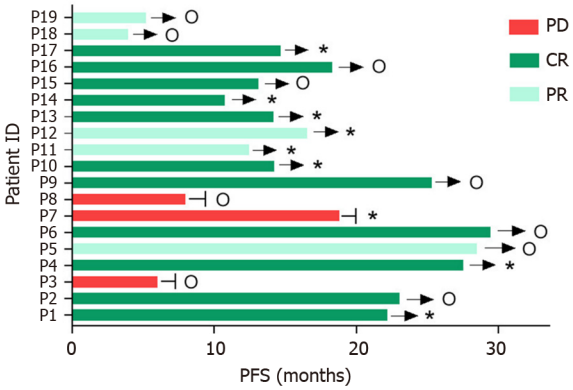
DISCUSSION

The outcomes in our case series showed that combined therapy with zanubrutinib and HD-MTX was well-tolerated as a frontline therapeutic regimen for patients diagnosed with PCNSL. Nine patients transitioned to ASCT following the zanubrutinib and HD-MTX induction phase, while another 10 patients underwent maintenance therapy with zanubrutinib alone. Only three patients developed disease progression. Data for all 19 patients were included in the evaluation of PFS and OS. Only one patient (P6) discontinued HD-MTX therapy, owing to delayed HD-MTX excretion, and the regimen was changed to rituximab, zanubrutinib, and lenalidomide. No instances of treatment-related mortality were recorded throughout the study. However, limitations exist in our study. It is well recognized that the journey from the initiation of the oncogenic event to the point of clinical diagnosis is protracted, spanning approximately a decade. This extended timeline underscores the intricacies inherent in the development of neoplastic disorders, revealing that the characterization of "newly diagnosed" necessitates a more nuanced understanding-one that acknowledges the substantial



DOI: 10.5306/wjco.v14.i12.606 **Copyright** ©The Author(s) 2023.

Figure 1 Patient acquisition flow diagram. PCNSL: Primary central nervous system lymphoma; HD-MTX: High-dose methotrexate; ASCT: Autologous stem cell transplantation; BEAM: Carmustine, etoposide, cytarabine, and melphalan; BEAC: Carmustine, etoposide, cytarabine, and cyclophosphamide; CFS: Cancer Fatigue Scale. R:21 days/month: Lenalidomide for maintenance therapy.

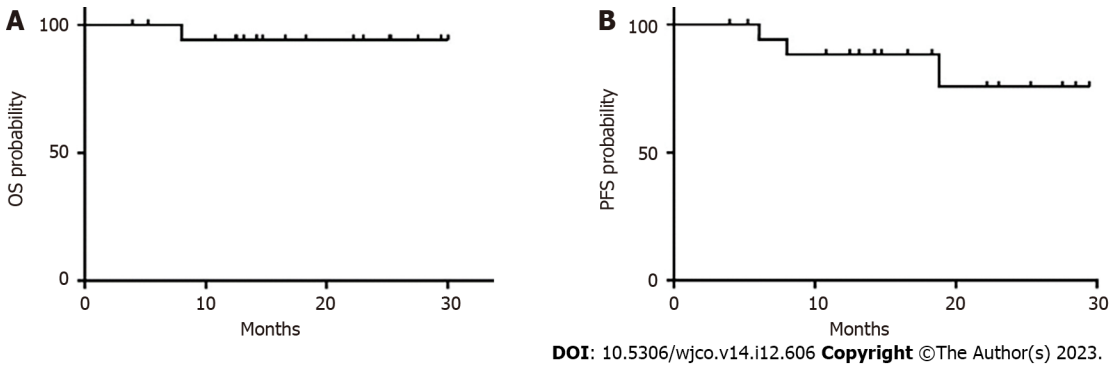


DOI: 10.5306/wjco.v14.i12.606 **Copyright** ©The Author(s) 2023.

Figure 2 Clinical response and progression-free survival of all patients. o: using a zanubrutinib-based maintenance regimen. ∗: Using ASCT as a consolidation regimen; →: Ongoing; ⊣: PD; ID: Identification number; PD: Progressive disease; CR: Complete response; PR: Partial response; PFS: Progression-free survival; ASCT: Autologous stem cell transplantation.

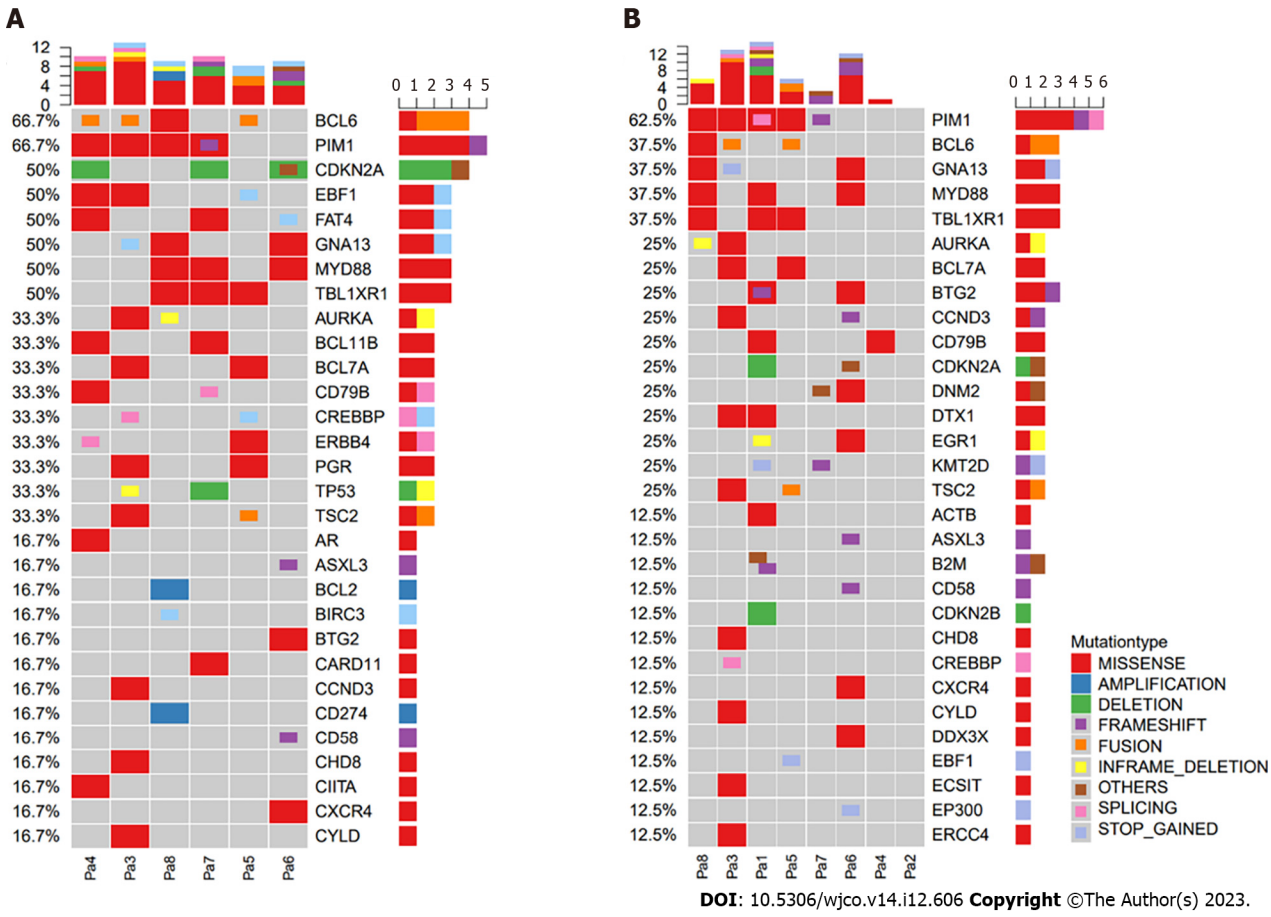
span of disease evolution prior to medical recognition.

Our study emphasizes the importance of adopting novel therapeutic strategies to address the multifaceted nature of PCNSL. Historically, HD-MTX has played a pivotal role, serving as a cornerstone for first-line induction regimens in PCNSL. This is owing to its ability to penetrate the blood-brain barrier and achieve effective anti-tumor concentrations [22-24]. However, even with this treatment, approximately half of the patients experience relapse, and 5-year survival rates remain discouragingly low, at 30%-40%[3]. Studies have shown that HD-MTX-based first-line regimens result in an



DOI: 10.5306/wjco.v14.i12.606 **Copyright** ©The Author(s) 2023.

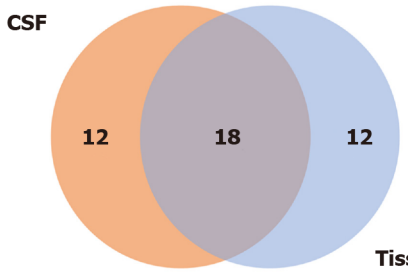
Figure 3 Kaplan-Meier curve for overall survival and progression-free survival. A: overall survival; B: progression-free survival. OS: Overall survival; PFS: Progression-free survival.



DOI: 10.5306/wjco.v14.i12.606 **Copyright** ©The Author(s) 2023.

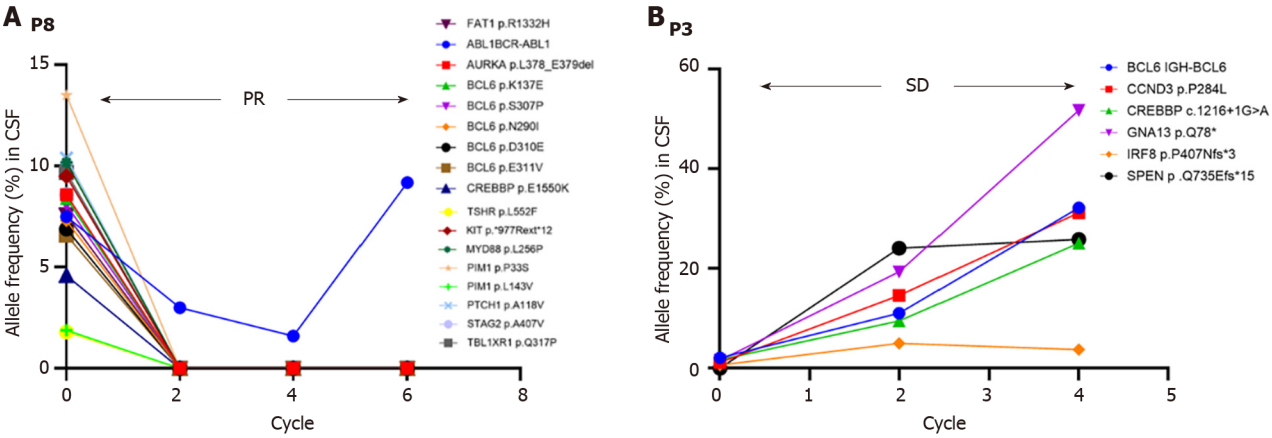
Figure 4 Gene alterations detected in tumor tissue and cerebrospinal fluid. A: Tumor tissue; B: Cerebrospinal fluid. CSF: Cerebrospinal fluid; P: Patient.

ORR of approximately 68% in PCNSL patients over the age of 60 years. In newly diagnosed PCNSL, the median PFS is 35 mo and 8 mo for patients younger and older than 60 years, respectively[22,25]. In this study, the combination of zanubrutinib with HD-MTX demonstrated robust anti-tumor activity, with an ORR of 84.2%, which is higher than that achieved by HD-MTX-based chemotherapy alone. Our results also identified a 2-year PFS of 75.6% and an OS of 94.1%. The median PFS and median OS for the entire cohort were not reached at the time of writing, even after a follow-up of 14.7 mo (range: 3.9-30 mo). Previous studies have shown that zanubrutinib exhibits greater selectivity in inhibiting BTK compared with the off-target effects observed with ibrutinib[26]. The profound BTK inhibition observed with zanubrutinib in both blood and lymph nodes is hypothesized to maximize the potential for deep and sustained remissions in conditions such as chronic lymphocytic leukemia and other hematological disorders. In the phase I BGB-3111-AU-003 study, which evaluated zanubrutinib monotherapy for chronic lymphocytic leukemia/small lymphocytic leukemia, efficacy was assessed in a cohort of 78 patients. This patient group included individuals with high-risk disease features, such as adverse cytogenetics (del(11q), 23.3%; del(17p) and/or TP53 mutation) at a rate of 19.1%[26]. After a



DOI: 10.5306/wjco.v14.i12.606 **Copyright** ©The Author(s) 2023.

Figure 5 Concordance between baseline primary tumor tissue and cerebrospinal fluid samples for mutation detection. CSF: Cerebrospinal fluid.



DOI: 10.5306/wjco.v14.i12.606 **Copyright** ©The Author(s) 2023.

Figure 6 Disease monitoring during therapy by evaluating cerebrospinal fluid circulating tumor DNA. A: P8; B: P3. PR: Partial response; SD: Stable disease; P8: Patient 8; P3: Patient 3.

median follow-up of 13.7 mo (range: 0.4-30.5 mo), the ORR was 96.2% (75/78) (95% confidence interval: 89.2-99.2). This ORR group included two patients (2.6%) who achieved CR, 63 (80.8%) who achieved PR, and 10 (12.8%) with PR with lymphocytosis[26].

In our study, nine patients completed ASCT after the induction phase of zanubrutinib-based combination therapy and achieved an ORR of 88.9% (CR/PR: 6/2), indicating the advantage of ASCT as a consolidation regimen. Furthermore, studies have shown that ASCT is an effective consolidation strategy in PCNSL[27,28]. Therefore, ASCT should be the first choice for suitable patients.

Lenalidomide is an immunomodulatory agent that shows good anti-tumor activity as a BTK inhibitor in DLBCL[27]. Lenalidomide combined with ibrutinib and rituximab shows promising anti-tumor activity in relapsed/refractory DLBCL[29]. In our study, one patient (P6) achieved CR after receiving an IR2-based regimen and zanubrutinib maintenance. In patients who do not tolerate HD-MTX and experience toxicity, IR2 may be a better choice. In our study, 8 of 10 patients achieved a therapeutic response with zanubrutinib maintenance. Therefore, for PCNSL patients who are unsuitable for ASCT, lenalidomide and zanubrutinib as maintenance therapy might be promising.

PCNSL patients are divided into three major molecular subtypes: A GCB subtype, an activated B-cell (ABC) subtype, and a type III subtype, whose cell origin is unidentified. The first two subtypes account for approximately 80% of all cases; ABC DLBCL patients have poorer outcomes[30]. To our knowledge, there are no reports of the results of zanubrutinib therapy for the GCB and ABC subtypes of PCNSL. Nine of the 19 patients in our study had non-GCB disease and 6 (66.7%) responded to the zanubrutinib-based regimen. Ten patients had GCB disease, and all (100%) responded to the zanubrutinib-based regimen. Zanubrutinib may have had a better effect on the ABC subtype in previous studies.

Previous studies have shown that next-generation sequencing may be used as a molecular diagnostic method prior to delivering targeted therapies, particularly BCR inhibitors, in the case of MYD88-mutated tumors[31]. In our study, CSF liquid biopsies were evaluated using next-generation sequencing in eight patients, while XX underwent radiological evaluation. Six patients had dramatically lower CSF mutant allele frequencies compared with patients 3 and 8. Patient 8 achieved a partial radiographic response during the induction treatment, while the CSF mutant allele frequency increased after cycle 4 (Figure 6). This patient developed PD while receiving the maintenance regimen. As shown in Figure 6, patient 3 had a stable radiographic response, with an increased level of ctDNA in the CSF specimen. This patient developed PD after completing the induction regimen.

Performing CSF liquid biopsy profiling with radiologic evaluation is feasible in PCNSL. Studies show frequent MYD88 and CD79B mutations in PCNSL[6,10-13]. On the basis of the genetic analysis of CSF in our study, we found frequent alterations of MYD88 and CD79B involved in the BCR pathway, and zanubrutinib combined with HD-MTX resulted in good anti-tumor activity. Therefore, CSF liquid biopsy profiling might be feasible for evaluating the response to a therapeutic protocol. However, the fleeting presence of ctDNA in the bloodstream poses a challenge to the reliability of the results of CSF liquid biopsy profiling[32].

An extensive safety analysis performed on pooled data from six zanubrutinib monotherapy trials revealed a notable trend toward favorable tolerability among patients diagnosed with various B-cell malignancies[29]. These conditions, which are often associated with symptoms such as diarrhea, thrombocytopenia, bleeding, atrial fibrillation, skin rash, and fatigue, respond well to zanubrutinib treatment[33]. The results of our study highlight the reassuring absence of grade 4 non-hematological toxicities. The reported side effects were characterized as mild and did not require further therapeutic intervention. No treatment-related mortality was observed, indicating a moderate safety profile for zanubrutinib combined with HD-MTX for patients with PCNSL.

While our findings provide valuable insights into the tolerability of zanubrutinib, this study has limitations. First, owing to the retrospective design and the small number of included patients, larger-scale prospective cohort studies and longer follow-up may be warranted to validate our results. Second, generally, regarding cellular origin in PCNSL, zanubrutinib may have a better effect on the ABC subtype. However, in this study, we were able to identify only the GCB and non-GCB phenotypes owing to the limited experimental conditions; ABC genotyping was not performed. Therefore, it is not possible to conduct a more detailed analysis.

CONCLUSION

Zanubrutinib combined with HD-MTX provided a good clinical response and was well tolerated in newly diagnosed PCNSL patients. Additionally, the detection of ctDNA in CSF was very useful in disease surveillance and treatment response monitoring. However, given the small sample size and retrospective study design, further research is required to validate our findings.

ARTICLE HIGHLIGHTS

Research background

Primary central nervous system lymphoma (PCNSL) is an aggressive brain lymphoma with limited treatment options. The current standard treatment involves high-dose methotrexate (HD-MTX), but there is a need for effective combination therapies to address adverse reactions. Zanubrutinib, a Bruton's tyrosine kinase inhibitor, shows promise owing to its potential to modulate B-cell receptor and Toll-like receptor signaling, which are associated with PCNSL.

Research motivation

This study aimed to evaluate the efficacy and safety of combining zanubrutinib with HD-MTX for newly diagnosed PCNSL patients. Additionally, the study explored the use of circulating tumor DNA (ctDNA) in cerebrospinal fluid (CSF) as a monitoring tool for treatment response.

Research objectives

The main objectives were to assess the treatment outcomes, adverse events, and genomic characteristics of PCNSL patients treated with HD-MTX and zanubrutinib combination therapy, and to investigate the potential of CSF ctDNA in disease surveillance.

Research methods

Nineteen eligible PCNSL patients were included in the study and received HD-MTX and zanubrutinib combination therapy. Clinical responses were evaluated, and ctDNA in CSF was analyzed using next-generation sequencing. Safety, treatment duration, and response were assessed.

Research results

The study demonstrated an overall response rate of 84.2% with the combination therapy, including complete and partial responses. Adverse events were mild and manageable. ctDNA levels in CSF were monitored and correlated with treatment response.

Research conclusions

Zanubrutinib combined with HD-MTX resulted in effective clinical responses in newly diagnosed PCNSL patients. The study highlighted the potential of CSF ctDNA for monitoring treatment response and disease surveillance. This combination therapy demonstrated promising safety and efficacy profiles.

Research perspectives

While the study results are promising, further research with larger patient cohorts and longer follow-up periods is needed to confirm the findings. The potential of zanubrutinib in different molecular subtypes of PCNSL and its long-term effects need to be explored. The clinical use of CSF ctDNA requires further investigation.

FOOTNOTES

Author contributions: The study conception and design were performed by Wang N, Chen FL, and Li WY; Data collection was performed by Wang N; All authors contributed to the data analysis and interpretation; Statistical analysis was performed by Wang N and Chen FL; The first draft of the manuscript was written by Wang N; All authors revised the manuscript.

Institutional review board statement: The study was reviewed and approved by the Guangdong Provincial People's Hospital Institutional Review Board.

Informed consent statement: All patients provided written informed consent to participate in this study.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Wen-Yu Li 0000-0002-4462-7513.

S-Editor: Fan JR
L-Editor: Webster JR
P-Editor: Yu HG

REFERENCES

1 **Ferreri AJM**. Therapy of primary CNS lymphoma: role of intensity, radiation, and novel agents. *Hematology Am Soc Hematol Educ Program* 2017; **2017**: 565-577 [PMID: 29222306 DOI: 10.1182/asheducation-2017.1.565]
2 **Batchelor TT**. Primary central nervous system lymphoma: A curable disease. *Hematol Oncol* 2019; **37** Suppl 1: 15-18 [PMID: 31187523 DOI: 10.1002/hon.2598]
3 **Schaff LR**, Grommes C. Primary central nervous system lymphoma. *Blood* 2022; **140**: 971-979 [PMID: 34699590 DOI: 10.1182/blood.2020008377]
4 **Langner-Lemercier S**, Houillier C, Soussain C, Ghesquières H, Chinot O, Taillandier L, Soubeyran P, Lamy T, Morschhauser F, Benouaich-Amiel A, Ahle G, Moles-Moreau MP, Moluçon-Chabrot C, Bourquard P, Damaj G, Jardin F, Larrieu D, Gyan E, Gressin R, Jaccard A, Choquet S, Brion A, Casasnovas O, Colin P, Reman O, Tempescul A, Marolleau JP, Fabbro M, Naudet F, Hoang-Xuan K, Houot R. Primary CNS lymphoma at first relapse/progression: characteristics, management, and outcome of 256 patients from the French LOC network. *Neuro Oncol* 2016; **18**: 1297-1303 [PMID: 26951382 DOI: 10.1093/neuonc/now033]
5 **Ferreri AJ**, Reni M, Foppoli M, Martelli M, Pangalis GA, Frezzato M, Cabras MG, Fabbri A, Corazzelli G, Ilariucci F, Rossi G, Soffietti R, Stelitano C, Vallisa D, Zaja F, Zoppegno L, Aondio GM, Avvisati G, Balzarotti M, Brandes AA, Fajardo J, Gomez H, Guarini A, Pinotti G, Rigacci L, Uhlmann C, Picozzi P, Vezzulli P, Ponzoni M, Zucca E, Caligaris-Cappio F, Cavalli F; International Extranodal Lymphoma Study Group (IELSG). High-dose cytarabine plus high-dose methotrexate versus high-dose methotrexate alone in patients with primary CNS lymphoma: a randomised phase 2 trial. *Lancet* 2009; **374**: 1512-1520 [PMID: 19767089 DOI: 10.1016/S0140-6736(09)61416-1]
6 **Ngo VN**, Young RM, Schmitz R, Jhavar S, Xiao W, Lim KH, Kohlhammer H, Xu W, Yang Y, Zhao H, Shaffer AL, Romesser P, Wright G, Powell J, Rosenwald A, Muller-Hermelink HK, Ott G, Gascoyne RD, Connors JM, Rimsza LM, Campo E, Jaffe ES, Delabie J, Smeland EB, Fisher RI, Braziel RM, Tubbs RR, Cook JR, Weisenburger DD, Chan WC, Staudt LM. Oncogenically active MYD88 mutations in human lymphoma. *Nature* 2011; **470**: 115-119 [PMID: 21179087 DOI: 10.1038/nature09671]
7 **Jost PJ**, Ruland J. Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. *Blood* 2007; **109**: 2700-2707 [PMID: 17119127 DOI: 10.1182/blood-2006-07-025809]
8 **Eluard B**, Nuan-Aliman S, Faumont N, Collares D, Bordereaux D, Montagne A, Martins I, Cagnard N, Caly M, Taoui O, Lordello L, Lehmann-Che J, Tesson B, Martinez-Climent JA, Copie-Bergman C, Haioun C, Tilly H, Bonsang B, Vincent-Salomon A, Jais JP, Jardin F, Leroy K, Maiuri MC, Kroemer G, Molina TJ, Feuillard J, Baud V. The alternative RelB NF-κB subunit is a novel critical player in diffuse large B-cell lymphoma. *Blood* 2022; **139**: 384-398 [PMID: 34232979 DOI: 10.1182/blood.2020010039]
9 **Xinshuang Z**, Lei S, Hailong Y, Haiping LI, Mengheng W, Wanci S, Laichun L, Hezhen WU, Yanfang Y, Junfeng Z, Yanwen L, Hanxiong D, Qiang Y, Pengtao Y. Compound Gaoziban tablet alleviates depression toll-like receptor 4/myeloid differentiation factor 88/nuclear factor-

kappa B pathway. *J Tradit Chin Med* 2022; **42**: 956-964 [PMID: 36378054 DOI: 10.19852/j.cnki.jtcm.20220902.001]

10 **Braggio E**, Van Wier S, Ojha J, McPhail E, Asmann YW, Egan J, da Silva JA, Schiff D, Lopes MB, Decker PA, Valdez R, Tibes R, Eckloff B, Witzig TE, Stewart AK, Fonseca R, O'Neill BP. Genome-Wide Analysis Uncovers Novel Recurrent Alterations in Primary Central Nervous System Lymphomas. *Clin Cancer Res* 2015; **21**: 3986-3994 [PMID: 25991819 DOI: 10.1158/1078-0432.CCR-14-2116]

11 **Schmitz R**, Wright GW, Huang DW, Johnson CA, Phelan JD, Wang JQ, Roulland S, Kasbekar M, Young RM, Shaffer AL, Hodson DJ, Xiao W, Yu X, Yang Y, Zhao H, Xu W, Liu X, Zhou B, Du W, Chan WC, Jaffe ES, Gascoyne RD, Connors JM, Campo E, Lopez-Guillermo A, Rosenwald A, Ott G, Delabie J, Rimsza LM, Tay Kuang Wei K, Zelenetz AD, Leonard JP, Bartlett NL, Tran B, Shetty J, Zhao Y, Soppet DR, Pittaluga S, Wilson WH, Staudt LM. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med* 2018; **378**: 1396-1407 [PMID: 29641966 DOI: 10.1056/NEJMoa1801445]

12 **Roschewski M**, Phelan JD. Sorting biologic subtypes of primary CNS lymphoma. *Blood* 2021; **137**: 1436-1437 [PMID: 33734333 DOI: 10.1182/blood.2020009968]

13 **Nakamura T**, Tateishi K, Niwa T, Matsushita Y, Tamura K, Kinoshita M, Tanaka K, Fukushima S, Takami H, Arita H, Kubo A, Shuto T, Ohno M, Miyakita Y, Kocialkowski S, Sasayama T, Hashimoto N, Maehara T, Shibui S, Ushijima T, Kawahara N, Narita Y, Ichimura K. Recurrent mutations of CD79B and MYD88 are the hallmark of primary central nervous system lymphomas. *Neuropathol Appl Neurobiol* 2016; **42**: 279-290 [PMID: 26111727 DOI: 10.1111/nan.12259]

14 **Soussain C**, Choquet S, Blonski M, Leclercq D, Houillier C, Rezai K, Bijou F, Houot R, Boyle E, Gressin R, Nicolas-Virelizier E, Barrie M, Moluçon-Chabrot C, Lelez ML, Clavert A, Coisy S, Leruez S, Touitou V, Cassoux N, Daniau M, Ertault de la Bretonnière M, El Yamani A, Ghesquières H, Hoang-Xuan K. Ibrutinib monotherapy for relapse or refractory primary CNS lymphoma and primary vitreoretinal lymphoma: Final analysis of the phase II 'proof-of-concept' iLOC study by the Lymphoma study association (LYSA) and the French oculo-cerebral lymphoma (LOC) network. *Eur J Cancer* 2019; **117**: 121-130 [PMID: 31279304 DOI: 10.1016/j.ejca.2019.05.024]

15 **Nayyar N**, White MD, Gill CM, Lastrapes M, Bertalan M, Kaplan A, D'Andrea MR, Bihun I, Kaneb A, Dietrich J, Ferry JA, Martinez-Lage M, Giobbie-Hurder A, Borger DR, Rodriguez FJ, Frosch MP, Batchelor E, Hoang K, Kuter B, Fortin S, Holdhoff M, Cahill DP, Carter S, Brastianos PK, Batchelor TT. MYD88 L265P mutation and CDKN2A loss are early mutational events in primary central nervous system diffuse large B-cell lymphomas. *Blood Adv* 2019; **3**: 375-383 [PMID: 30723112 DOI: 10.1182/bloodadvances.2018027672]

16 **Lionakis MS**, Dunleavy K, Roschewski M, Widemann BC, Butman JA, Schmitz R, Yang Y, Cole DE, Melani C, Higham CS, Desai JV, Ceribelli M, Chen L, Thomas CJ, Little RF, Gea-Banacloche J, Bhaumik S, Stetler-Stevenson M, Pittaluga S, Jaffe ES, Heiss J, Lucas N, Steinberg SM, Staudt LM, Wilson WH. Inhibition of B Cell Receptor Signaling by Ibrutinib in Primary CNS Lymphoma. *Cancer Cell* 2017; **31**: 833-843.e5 [PMID: 28552327 DOI: 10.1016/j.ccell.2017.04.012]

17 **Grommes C**, Pastore A, Palaskas N, Tang SS, Campos C, Schartz D, Codega P, Nichol D, Clark O, Hsieh WY, Rohle D, Rosenblum M, Viale A, Tabar VS, Brennan CW, Gavrilovic IT, Kaley TJ, Nolan CP, Omuro A, Pentsova E, Thomas AA, Tsyvkin E, Noy A, Palomba ML, Hamlin P, Sauter CS, Moskowitz CH, Wolfe J, Dogan A, Won M, Glass J, Peak S, Lallana EC, Hatzoglou V, Reiner AS, Gutin PH, Huse JT, Panageas KS, Graeber TG, Schultz N, DeAngelis LM, Mellinghoff IK. Ibrutinib Unmasks Critical Role of Bruton Tyrosine Kinase in Primary CNS Lymphoma. *Cancer Discov* 2017; **7**: 1018-1029 [PMID: 28619981 DOI: 10.1158/2159-8290.CD-17-0613]

18 **Zhang Y**, Li Y, Zhuang Z, Wang W, Wei C, Zhao D, Zhou D, Zhang W. Preliminary Evaluation of Zanubrutinib-Containing Regimens in DLBCL and the Cerebrospinal Fluid Distribution of Zanubrutinib: A 13-Case Series. *Front Oncol* 2021; **11**: 760405 [PMID: 35004280 DOI: 10.3389/fonc.2021.760405]

19 **Grommes C**, Tang SS, Wolfe J, Kaley TJ, Daras M, Pentsova EI, Piotrowski AF, Stone J, Lin A, Nolan CP, Manne M, Codega P, Campos C, Viale A, Thomas AA, Berger MF, Hatzoglou V, Reiner AS, Panageas KS, DeAngelis LM, Mellinghoff IK. Phase 1b trial of an ibrutinib-based combination therapy in recurrent/refractory CNS lymphoma. *Blood* 2019; **133**: 436-445 [PMID: 30567753 DOI: 10.1182/blood-2018-09-875732]

20 **Shu Y**, Wu X, Tong X, Wang X, Chang Z, Mao Y, Chen X, Sun J, Wang Z, Hong Z, Zhu L, Zhu C, Chen J, Liang Y, Shao H, Shao YW. Circulating Tumor DNA Mutation Profiling by Targeted Next Generation Sequencing Provides Guidance for Personalized Treatments in Multiple Cancer Types. *Sci Rep* 2017; **7**: 583 [PMID: 28373672 DOI: 10.1038/s41598-017-00520-1]

21 **Yang Z**, Yang N, Ou Q, Xiang Y, Jiang T, Wu X, Bao H, Tong X, Wang X, Shao YW, Liu Y, Wang Y, Zhou C. Investigating Novel Resistance Mechanisms to Third-Generation EGFR Tyrosine Kinase Inhibitor Osimertinib in Non-Small Cell Lung Cancer Patients. *Clin Cancer Res* 2018; **24**: 3097-3107 [PMID: 29506987 DOI: 10.1158/1078-0432.CCR-17-2310]

22 **Kasenda B**, Ferreri AJ, Marturano E, Forst D, Bromberg J, Ghesquieres H, Ferlay C, Blay JY, Hoang-Xuan K, Pulczynski EJ, Fosså A, Okoshi Y, Chiba S, Fritsch K, Omuro A, O'Neill BP, Bairey O, Schandelmaier S, Gloy V, Bhatnagar N, Haug S, Rahner S, Batchelor TT, Illerhaus G, Briel M. First-line treatment and outcome of elderly patients with primary central nervous system lymphoma (PCNSL)--a systematic review and individual patient data meta-analysis. *Ann Oncol* 2015; **26**: 1305-1313 [PMID: 25701456 DOI: 10.1093/annonc/mdv076]

23 **Li Y**, Sun Z, Liu B, Shan Y, Zhao L, Jia L. Tumor-suppressive miR-26a and miR-26b inhibit cell aggressiveness by regulating FUT4 in colorectal cancer. *Cell Death Dis* 2017; **8**: e2892 [PMID: 28640257 DOI: 10.1038/cddis.2017.281]

24 **Han CH**, Batchelor TT. Diagnosis and management of primary central nervous system lymphoma. *Cancer* 2017; **123**: 4314-4324 [PMID: 28950405 DOI: 10.1002/cncr.30965]

25 **Mendez JS**, Grommes C. Treatment of Primary Central Nervous System Lymphoma: From Chemotherapy to Small Molecules. *Am Soc Clin Oncol Educ Book* 2018; **38**: 604-615 [PMID: 30231317 DOI: 10.1200/EDBK_200829]

26 **Tam CS**, Trotman J, Opat S, Burger JA, Cull G, Gottlieb D, Harrup R, Johnston PB, Marlton P, Munoz J, Seymour JF, Simpson D, Tedeschi A, Elstrom R, Yu Y, Tang Z, Han L, Huang J, Novotny W, Wang L, Roberts AW. Phase 1 study of the selective BTK inhibitor zanubrutinib in B-cell malignancies and safety and efficacy evaluation in CLL. *Blood* 2019; **134**: 851-859 [PMID: 31340982 DOI: 10.1182/blood.2019001160]

27 **Ferreri AJ**, Illerhaus G. The role of autologous stem cell transplantation in primary central nervous system lymphoma. *Blood* 2016; **127**: 1642-1649 [PMID: 26834241 DOI: 10.1182/blood-2015-10-636340]

28 **Houillier C**, Taillandier L, Dureau S, Lamy T, Laadhari M, Chinot O, Moluçon-Chabrot C, Soubeyran P, Gressin R, Choquet S, Damaj G, Thyss A, Abraham J, Delwail V, Gyan E, Sanhes L, Cornillon J, Garidi R, Delmer A, Tanguy ML, Al Jijakli A, Morel P, Bourquard P, Moles MP, Chauchet A, Gastinne T, Constans JM, Langer A, Martin A, Moisson P, Lacomblez L, Martin-Duverneuil N, Delgadillo D, Turbiez I, Feuvret L, Cassoux N, Touitou V, Ricard D, Hoang-Xuan K, Soussain C; Intergroupe GOELAMS–ANOCEF and the LOC Network for CNS Lymphoma. Radiotherapy or Autologous Stem-Cell Transplantation for Primary CNS Lymphoma in Patients 60 Years of Age and Younger: Results of the Intergroup ANOCEF-GOELAMS Randomized Phase II PRECIS Study. *J Clin Oncol* 2019; **37**: 823-833 [PMID: 30785830 DOI: 10.1200/JCO.18.00306]

29 **Morabito F**, Skafi M, Recchia AG, Kashkeesh A, Hindiyeh M, Sabatleen A, Morabito L, Alijanazreh H, Hamamreh Y, Gentile M.

Lenalidomide for the treatment of mantle cell lymphoma. *Expert Opin Pharmacother* 2019; **20**: 487-494 [PMID: 30608891 DOI: 10.1080/14656566.2018.1561865]

30 **Dunleavy K**, Erdmann T, Lenz G. Targeting the B-cell receptor pathway in diffuse large B-cell lymphoma. *Cancer Treat Rev* 2018; **65**: 41-46 [PMID: 29549872 DOI: 10.1016/j.ctrv.2018.01.002]

31 **Fontanilles M**, Marguet F, Bohers É, Viailly PJ, Dubois S, Bertrand P, Camus V, Mareschal S, Ruminy P, Maingonnat C, Lepretre S, Veresezan EL, Derrey S, Tilly H, Picquenot JM, Laquerrière A, Jardin F. Non-invasive detection of somatic mutations using next-generation sequencing in primary central nervous system lymphoma. *Oncotarget* 2017; **8**: 48157-48168 [PMID: 28636991 DOI: 10.18632/oncotarget.18325]

32 **Pan S**, Sun S, Liu B, Hou Y. Pan-cancer Landscape of the RUNX Protein Family Reveals their Potential as Carcinogenic Biomarkers and the Mechanisms Underlying their Action. *J Transl Int Med* 2022; **10**: 156-174 [PMID: 35959452 DOI: 10.2478/jtim-2022-0013]

33 **Yang Y**, Shaffer AL 3rd, Emre NC, Ceribelli M, Zhang M, Wright G, Xiao W, Powell J, Platig J, Kohlhammer H, Young RM, Zhao H, Yang Y, Xu W, Buggy JJ, Balasubramanian S, Mathews LA, Shinn P, Guha R, Ferrer M, Thomas C, Waldmann TA, Staudt LM. Exploiting synthetic lethality for the therapy of ABC diffuse large B cell lymphoma. *Cancer Cell* 2012; **21**: 723-737 [PMID: 22698399 DOI: 10.1016/j.ccr.2012.05.024]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Clin Oncol 2023 December 24; 14(12): 620-627

DOI: 10.5306/wjco.v14.i12.620

ISSN 2218-4333 (online)

CASE REPORT

Rapid transformation of branched pancreatic duct-derived intraductal tubulopapillary neoplasm into an invasive carcinoma: A case report

Kenta Yamamoto, Yutaka Takada, Takuya Kobayashi, Ryo Ito, Yuka Ikeda, Shogo Ota, Kanna Adachi, Yukari Shimada, Motohito Hayashi, Toshinao Itani, Satsuki Asai, Kojiro Nakamura

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Chen Z, China; Gupta MK, Germany; Sun GH, China

Received: September 5, 2023
Peer-review started: September 5, 2023
First decision: November 14, 2023
Revised: November 22, 2023
Accepted: December 13, 2023
Article in press: December 13, 2023
Published online: December 24, 2023

Kenta Yamamoto, Yutaka Takada, Takuya Kobayashi, Ryo Ito, Yuka Ikeda, Shogo Ota, Kanna Adachi, Yukari Shimada, Motohito Hayashi, Toshinao Itani, Department of Gastroenterology and Hepatology, Kobe City Nishi-Kobe Medical Center, Kobe 651-2273, Hyogo, Japan

Satsuki Asai, Department of Pathology, Kobe City Nishi-Kobe Medical Center, Kobe 651-2273, Hyogo, Japan

Kojiro Nakamura, Department of Surgery and Gastroenterological Surgery, Kobe City Nishi-Kobe Medical Center, Kobe 651-2273, Hyogo, Japan

Corresponding author: Yutaka Takada, MD, PhD, Doctor, Department of Gastroenterology and Hepatology, Kobe City Nishi-Kobe Medical Center, 5-7-1 Koujidai, Kobe 651-2273, Hyogo, Japan. ichigojellylike@gmail.com

Abstract

BACKGROUND

Intraductal tubulopapillary neoplasm (ITPN) is a rare disease accounting for approximately 3% of all intraductal pancreatic tumors, with intraductal papillary mucinous neoplasm (IPMN) being one of the most common differential diagnoses. Both ITPN and IPMN display slow growth. A branched pancreatic duct type is commonly observed in IPMN, whereas ITPN derived from the branched pancreatic duct has been reported in a limited number of cases; hence, its pathogenesis remains unclear.

CASE SUMMARY

Here, we present the case of a patient with ITPN localized in a branched pancreatic duct, with poorly controlled irritable bowel syndrome. A contrast-enhanced computed tomography scan of the abdomen incidentally revealed a 5-mm oligemic nodule-like change in the body of the pancreas. Endoscopic ultrasound (EUS) indicated a 10-mm hypoechoic mass without any cystic structures that had grown within 2 mo. EUS-guided fine needle aspiration was performed for definitive diagnosis, and the findings suggested ductal papillary carcinoma. Distal pancreatectomy was performed, and the tumor was pathologically diagnosed as ITPN with an invasive cancerous component, pT3N1aM0, pStage IIB (International Cancer Control, 8th edition). The patient underwent

treatment with postoperative adjuvant chemotherapy (S-1 monotherapy); however, relapse was observed 1 year and 10 mo after surgical resection, and subsequent treatment involving a combination of chemotherapy and radiotherapy was administered. Maintenance therapy has since facilitated a stable disease state.

CONCLUSION
Regardless of the microscopic size of the neoplasm, early diagnosis of ITPN with EUS-guided fine needle aspiration and surgical resection are crucial.

Key Words: Intraductal tubulopapillary neoplasm; Pancreatic tumors; Neoplasia; Carcinoma; Pancreaticoduodenectomy; Case report

©The Author(s) 2023. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Intraductal tubulopapillary neoplasm (ITPN), a relatively rare intraductal pancreatic cancer, is frequently derived from the main pancreatic duct rather than from the branching ducts. Although tumors with larger diameters are more likely to develop into cancer, we reported a case of small ITPN originating from a bifurcated pancreatic duct rapidly developing into invasive cancer. The tumor was diagnosed and managed with endoscopic ultrasound-guided fine needle aspiration and surgery. However, relapse occurred 1 year and 10 mo postoperatively and was managed using chemotherapy and radiotherapy. Our case suggests the importance of early diagnosis and surgical resection even of a small ITPN.

Citation: Yamamoto K, Takada Y, Kobayashi T, Ito R, Ikeda Y, Ota S, Adachi K, Shimada Y, Hayashi M, Itani T, Asai S, Nakamura K. Rapid transformation of branched pancreatic duct-derived intraductal tubulopapillary neoplasm into an invasive carcinoma: A case report. *World J Clin Oncol* 2023; 14(12): 620-627
URL: https://www.wjgnet.com/2218-4333/full/v14/i12/620.htm
DOI: https://dx.doi.org/10.5306/wjco.v14.i12.620

INTRODUCTION

Intraductal tubulopapillary neoplasm (ITPN) is a recently reported type of intraductal pancreatic tumor[1,2]. ITPN is extremely rare, accounting for approximately 3% of all intraductal tumors; its pathogenesis remains unclear[1]. Most ITPNs have a primary pancreatic ductal origin, while those growing exclusively in the branching duct are rare[3]. Despite ITPN being considered a precursor to invasive ductal adenocarcinoma, with 70% of cases associated with adenocarcinoma at diagnosis[4,5], ITPN progresses more slowly than conventional pancreatic ductal carcinoma. Further, even with invasive lesions, the prognosis is generally better than that for ductal carcinoma.

Herein, we present the case of a 59-year-old male with ITPN localized in a branched pancreatic duct.

CASE PRESENTATION

Chief complaints

A 59-year-old male was admitted to our hospital with a 3-4-year history of diarrhea and abdominal pain.

History of present illness

He had intermittent abdominal pain in the lower abdomen.

History of past illness

He was taking medication for irritable bowel syndrome, which was poorly controlled.

Personal and family history

He had no family history of malignancy.

Physical examination

The abdomen was soft and flat and showed no tenderness.

Laboratory examinations

Blood tests on admission showed an increased alkaline phosphatase level (363 IU/L, normal range: 38–113 IU/L); however, no other hepatobiliary enzyme levels were elevated. The tumor markers carcinoembryonic antigen and carbohydrate antigen 19-9 were within normal limits (Table 1).

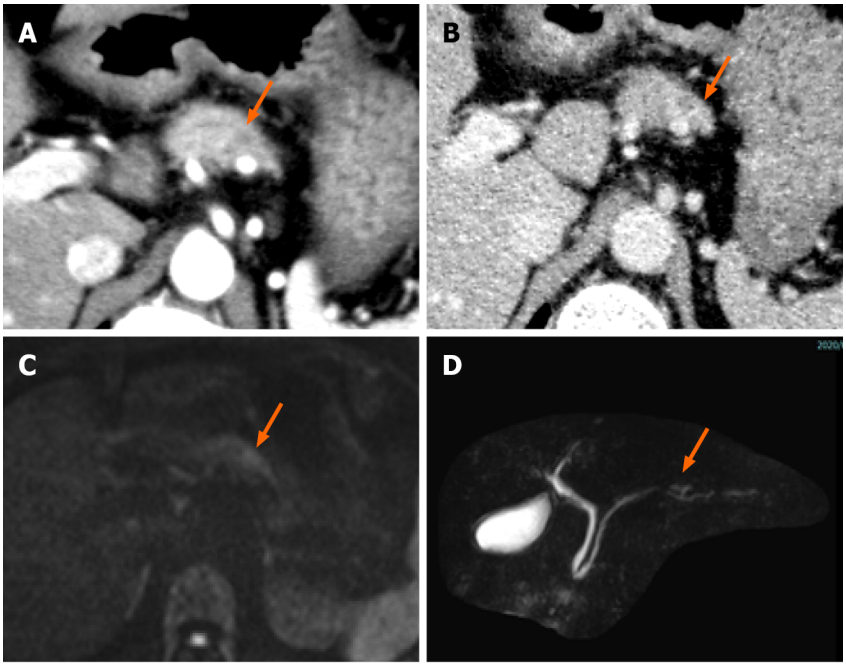
Table 1 Laboratory findings

Test	Result
Hematology	
WBC	6300/μL
RBC	562 × 10^4/μL
Hb	16.7 g/dL
Ht	49.5%
Plt	23.4 × 10^4/μL
PT-INR	0.9
APTT	29.0 S
Biochemistry	
Blood sugar	92 mg/dL
CRP	0.0 mg/dL
TP	6.8 g/dL
Alb	4.4 g/dL
T-Bil	0.1 mg/dL
AST	19 IU/L
ALT	29 IU/L
γ-GTP	35 IU/L
ALP	363 IU/L
LDH	172 IU/L
AMY	60 IU/L
BUN	23 mg/dL
Cre	0.84 mg/dL
Na	139 mEq/L
K	4.5 mEq/L
Cl	105 mEq/L
Tumor maker	
CEA	3.4 ng/mL
CA19-9	< 2.0 ng/dL
DUPAN-2	36 U/mL
Span-1 antigen	< 3 U/mL

Alb: Albumin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AMY: Amylase; APTT: Activated partial thromboplastin time; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen; CA19-9: Carbohydrate antigen 19-9; CEA: Carcinogenic antigen; Cre: Creatinine; CRP: C-reactive protein; DUPAN-2: Duke pancreatic mono-clonal antigen type 2; Hb: Hemoglobin; Ht: Hematocrit; LDH: Lactate dehydrogenase; Plt: Platelet; PT-INR: Prothrombin time-international normalized ratio; RBC: Red blood cell count; T-Bil: Total bilirubin; TP: Total protein; WBC: White blood cell count; γ-GTP: Gamma-glutamyl transpeptidase.

Imaging examinations

Contrast-enhanced computed tomography of the abdomen incidentally revealed a 5-mm nodule-like mass in the pancreatic body. The nodule was not contrast-enhancing in the arterial phase and was faintly contrast-enhancing in the late phase (Figure 1A and B). Magnetic resonance imaging findings indicated a slightly high-intensity signal on diffusion-weighted images in the same area as that identified on computed tomography. However, identification of obvious neoplastic lesions or abnormal signal areas using T1- weighted and T2-weighted images and dynamic studies was difficult, and there was no change in the caliber of the main pancreatic duct (Figure 1C and D). Endoscopic ultrasound (EUS) performed 2 mo after the initial consultation revealed a 10-mm hypoechoic mass without a cystic structure in the body of the pancreas (Figure 2A). Using Sonazoid® (GE Healthcare, Chicago, IL, United States), a contrast agent, the



DOI: 10.5306/wjco.v14.i12.620 **Copyright** ©The Author(s) 2023.

Figure 1 Computed tomography and magnetic resonance imaging. A and B: A 5-mm nodule-like mass was observed in the pancreatic body; the nodule was not contrasted in the arterial phase, arrow (A) and was faintly contrasted in the late phase, arrow (B); C: Diffusion-weighted imaging showed a mild signal increase in the pancreatic body nodule noted on computed tomography, arrow; D: Magnetic resonance pancreatography showed no obvious dilatation or stenosis of the main pancreatic duct, arrow.

hypoechoic mass was identified as a hypovascular mass without contrast effect (Figure 2B).

Further diagnostic work-up

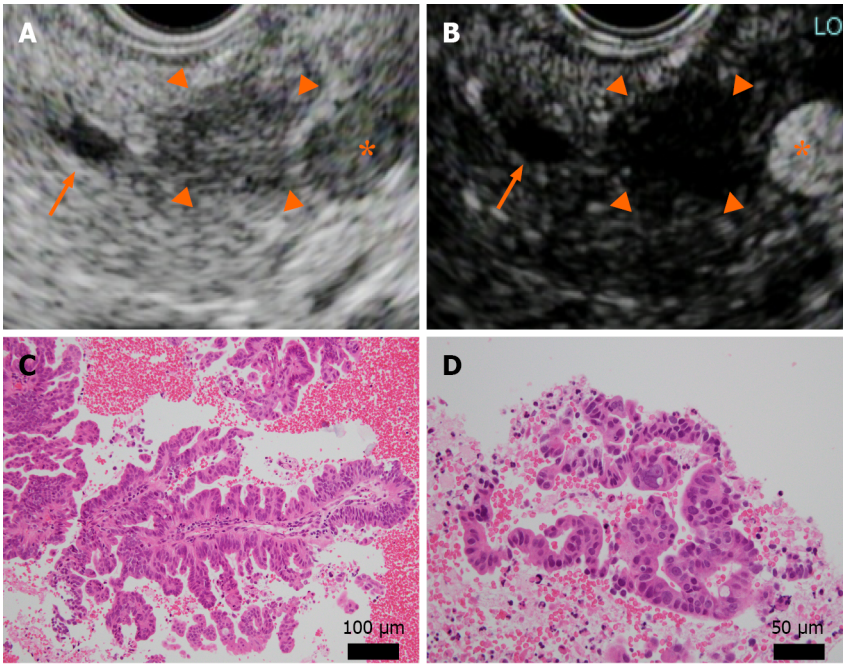
Because of the rapid increase in size within a short period and the possibility of malignancy, we performed EUS-guided fine needle aspiration (EUS-FNA) to obtain a definitive diagnosis. Histopathological examination revealed an atypical epithelium with papillary growth without a mucinous component. Some of the epithelium showed strong nuclear atypia, raising suspicion of an intraductal papillary carcinoma (Figure 2C and D). This was considered an indication for surgery, and a combined distal pancreatectomy and splenic resection were performed 5 mo after the initial detection of the tumor. Macroscopically, a 4 mm × 4 mm borderline brownish nodule and surrounding fibrosis measuring 12 mm × 8 mm × 12 mm were observed at a distance of 3.5 cm from the proximal lateral section of the pancreatic tail. No continuity with the main pancreatic duct was observed, and there was no dilation of the main pancreatic duct or notable changes in the surrounding pancreas (Figure 3). Microscopically, the tumor was located within the branched pancreatic duct and exhibited intraductal tubulopapillary growth with stromal invasion around the branched pancreatic duct (Figure 4A and B). There was no mucus production or cyst formation, and immunohistochemistry was positive for Mucin 1 (MUC1), negative for MUC2, partially positive for MUC5AC, and positive for MUC6 (Figure 4C-F). Mild perineural invasion but no lymphatic or venous invasion with two direct infiltrations in the peripancreatic lymph nodes were observed. Both dissection and pancreatic margins were negative.

FINAL DIAGNOSIS

The pathological diagnosis was ITPN (pT3N1aM0, pStageIIB, according to the eighth edition of the Union for International Cancer Control) with an invasive cancer component. The patient had no postoperative complications and was discharged from the hospital on postoperative day 13.

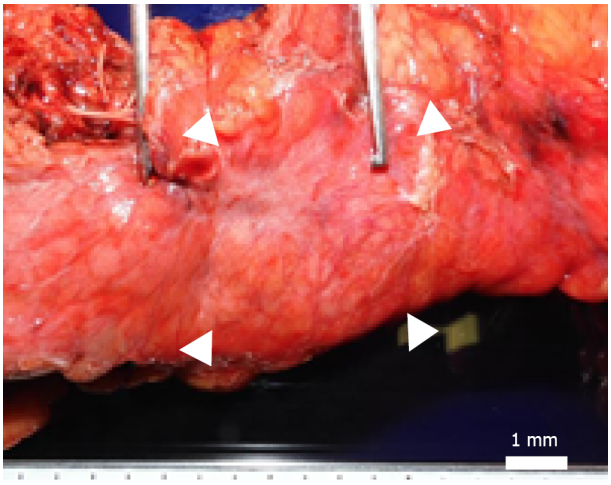
TREATMENT

The patient underwent treatment with postoperative adjuvant chemotherapy [S-1 monotherapy (80 mg/day)] for 6 mo following the treatment protocol for pancreatic cancer in Japan, and recurrence-free survival was maintained for 10 mo.



DOI: 10.5306/wjco.v14.i12.620 **Copyright** ©The Author(s) 2023.

Figure 2 Endoscopic ultrasound and histopathology (hematoxylin-eosin staining) at the time of endoscopic ultrasound-guided fine needle aspiration. A and B: A well-defined hypoechoic mass 10 mm in size was observed in the pancreatic body (A), arrowhead. The main pancreatic duct, arrow; splenic artery, asterisk. The mass was recognized as an oligo-hypoechoic mass with Sonazoid® contrast agent (B), arrowhead; C and D: Atypical epithelium with ductal papillary growth was seen. No intraductal papillary mucinous tumor-like mucus component was present. Original magnification was × 20 (C) and × 40 (D).

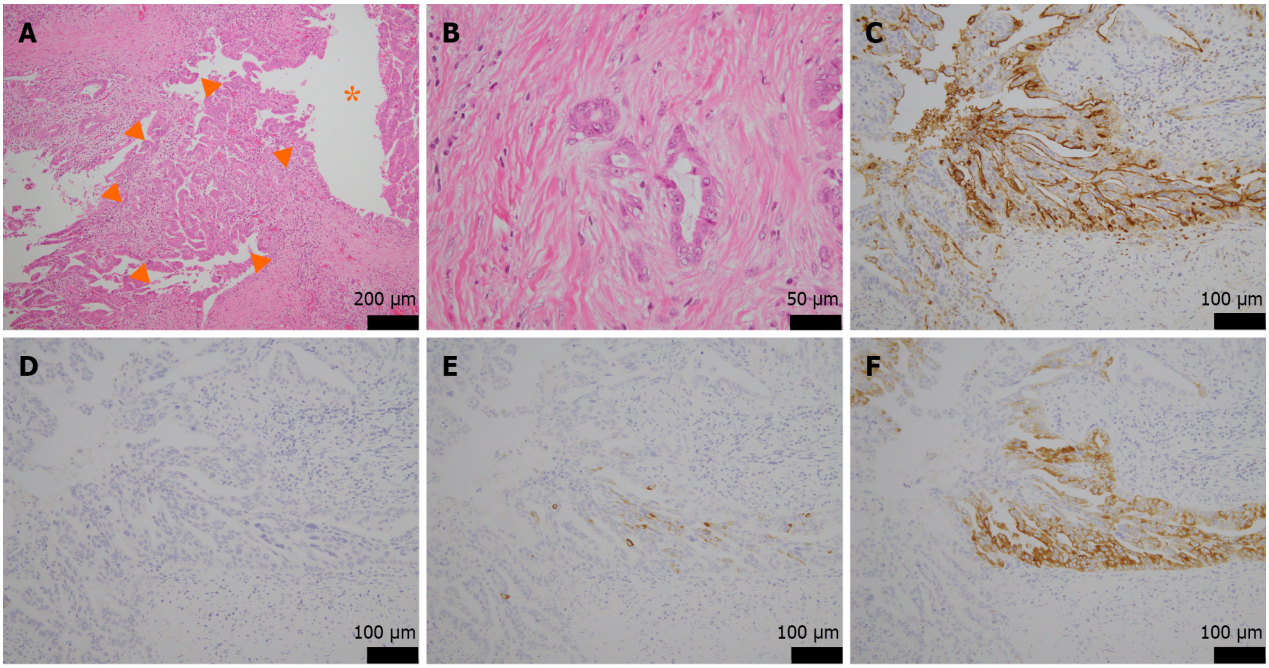


DOI: 10.5306/wjco.v14.i12.620 **Copyright** ©The Author(s) 2023.

Figure 3 Macroscopic findings. A well-defined brownish nodule measuring 4 mm × 4 mm bordering the pancreatic body; fibrosis extending to 8 mm × 12 mm in the periphery, arrowheads.

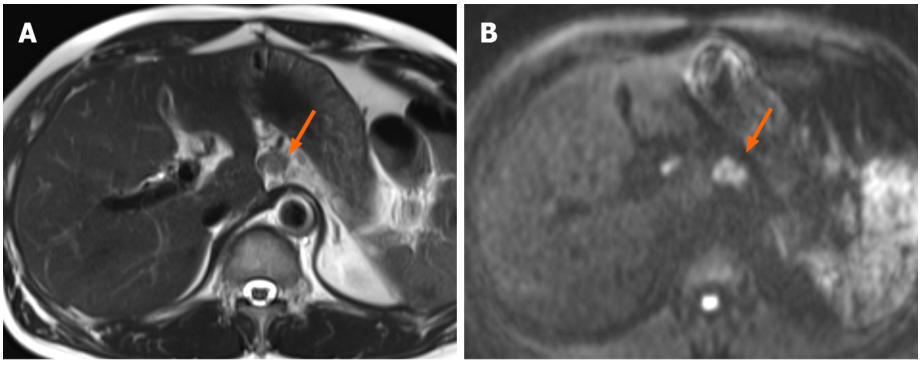
OUTCOME AND FOLLOW-UP

Magnetic resonance imaging performed at 1 year and 10 mo postoperatively revealed recurrence in the retroperitoneal lymph nodes at the surgical site (Figure 5). This postoperative recurrence was treated with oral S-1 in combination with radiation therapy (S-1: 120 mg for 4 wk, 100 mg for 2 wk, and 80 mg for 2 wk), and a reduction in the tumor size at the site of recurrence was observed. Following completion of radiotherapy (total 50 Gy), gemcitabine plus nab-paclitaxel (1000 mg/m^2, 125 mg/m^2) therapy was continued. To date, 2 years and 6 mo postoperatively, the patient's condition has remained in a stable state of disease[6].



DOI: 10.5306/wjco.v14.i12.620 **Copyright** ©The Author(s) 2023.

Figure 4 Microscopic and immunohistological findings. A and B: Original magnification, (× 20, A), and (× 40, B). Tumors with papillary growth with adenoductal structures in the branching pancreatic ducts (asterisk) and some invasive, well-differentiated adenocarcinomas were present, arrowheads. There was no mucus production or cyst formation; C: Mucin 1 was positive; D: Mucin 2 was negative; E: Mucin 5AC was partially positive; F: Mucin 6 was positive. Original magnification was × 40.



DOI: 10.5306/wjco.v14.i12.620 **Copyright** ©The Author(s) 2023.

Figure 5 Magnetic resonance imaging 1 year and 10 mo postoperatively. A: T2-weighted magnetic resonance imaging showed a small nodule in the postoperative area; B: Diffusion-weighted imaging showed a high signal in the same area, indicating recurrence in the retroperitoneal lymph nodes, arrow.

DISCUSSION

ITPN was described for the first time in 2009 by Yamaguchi *et al*[1] and is characterized by papillary growth in the pancreatic duct without mucous production, unlike intraductal papillary mucinous neoplasm (IPMN), which typically involves excessive mucin production. ITPN is a highly rare disease, with a frequency of 0.9% among pancreatic exocrine tumors and 3% among intraductal tumors[1]. The average patient age is 58 (25–82) years, and there are no sex differences [1,7]. The 5-year survival rate of ITPN is 81.5%, which is higher than that of pancreatic ductal carcinoma[3]. This type of tumor tends to grow slowly, and the average tumor diameter at the time of detection is 4.5 (0.5–15.0) cm[4].

ITPN is frequently compared to IPMN. Both are intraductal pancreatic tumors with a common intraductal growth pattern and the potential for malignant transformation; however, ITPN and IPMN differ in their immunohistological features. In the gross view, ITPN is a tumor that fills, expands, and proliferates in the pancreatic duct[1,2]. Although the caudal pancreatic duct may dilate due to its obstruction, the tumor does not have a cystic appearance or mucus deposits as in IPMN[1,2]. The Vaters papilla is not enlarged, and mucus outflow is not as observed in IPMN[1,2].

In IPMN, branched pancreatic duct types are common (88%), whereas in ITPN, this is a relatively rare feature (14%)[8]. Additionally, IPMN is positive for MUC5AC in all subtypes, including the oncocytic subtype. Conversely, ITPN is positive for MUC1 and MUC6 and negative for MUC2 and MUC5AC[9], which is consistent with our findings.

Surgical resection is the recommended treatment for ITPN[1,4,7,10]. Date *et al*[10] reported that among 37 patients with ITPN, the 1-year, 3-year, and 5-year overall survival (OS) rates after surgery were 97.3%, 80.7%, and 80.7%, respectively. Patients with ITPN-associated carcinoma had a favorable 5-year OS of 81.5% compared with the OS of those with ductal adenocarcinoma of the pancreas or IPMN-associated pancreatic cancer. Regarding ITPN prognosis, the recurrence-free survival rate tends to differ according to tumor size (< 4.0 cm *vs* > 4.0 cm) and Ki-67 labeling index (< 20% *vs* > 20%); however, these differences are not statistically significant[11]. Currently, there are no comprehensive reports on the efficacy of chemotherapy for ITPN. In the present case, when ITPN with invasive cancer was finally diagnosed, S-1 was administered as postoperative adjuvant chemotherapy following the standard treatment for conventional pancreatic ductal carcinoma in Japan[12]. It should be noted that the efficacy of S-1 for ITPN treatment is unknown.

Our report described a rare case of ITPN originating from a branched pancreatic duct that was incidentally observed in imaging studies and diagnosed using EUS-FNA. In this case, the tumor was small (3 cm in diameter), and the Ki-67 labeling index was approximately 50%, although it varied depending on the site. Despite being microscopic, the tumor rapidly increased in size, and the resected specimen demonstrated an invasive cancerous component. Thus, despite the general tendency of ITPN to grow slowly, in this case, the tumor rapidly progressed to invasive cancer and recurred 1 year and 10 mo postoperatively. As the occurrence of ITPN in the branched pancreatic duct is extremely rare, the nature of the tumor remains unclear.

CONCLUSION

Based on this case, we suggest that ITPN of the branched pancreatic duct type may have a worse prognosis than that of the main pancreatic duct. Further investigation of such cases is warranted. Moreover, early diagnosis of ITPN through EUS-FNA and subsequent surgical resection of the tumor are crucial. Further studies, especially multicenter studies, are required to validate our findings.

ACKNOWLEDGEMENTS

We would like to express our appreciation to Dr. Yutaka Takada for his thoughtful guidance.

FOOTNOTES

Author contributions: All authors made substantial contributions to the study design and data analysis and interpretation; All authors drafted the manuscript, revised it critically for important intellectual content, and read and approved the final version of the manuscript to be published.

Informed consent statement: Written informed consent has been obtained from the patient to publish this paper.

Conflict-of-interest statement: The authors declare no conflicts of interest.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Japan

ORCID number: Kenta Yamamoto 0009-0008-3457-0067; Yutaka Takada 0000-0002-4937-7859; Shogo Ota 0000-0001-5171-1847.

S-Editor: Lin C
L-Editor: Filipodia
P-Editor: Lin C

REFERENCES

1 **Yamaguchi H**, Shimizu M, Ban S, Koyama I, Hatori T, Fujita I, Yamamoto M, Kawamura S, Kobayashi M, Ishida K, Morikawa T, Motoi F, Unno M, Kanno A, Satoh K, Shimosegawa T, Orikasa H, Watanabe T, Nishimura K, Ebihara Y, Koike N, Furukawa T. Intraductal tubulopapillary neoplasms of the pancreas distinct from pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am J Surg Pathol* 2009; **33**: 1164-1172 [PMID: 19440145 DOI: 10.1097/PAS.0b013e3181a162e5]

2 **Adsay NVKG**, Kloppel G, Fukushima N. WHO Classification for Tumors of the Digestive Systems. In: Intraductal Neoplasms of the Pancreas. Bosman FT, Carneiro F, Hruban RH, Theise ND, editor. Lyon: IARC Publication, 2010: 304-313

3 **Fritz S**, Küper-Steffen R, Feilhauer K, Sommer CM, Richter GM, Bosse A, Hennig R, Köninger J. Intraductal tubular papillary neoplasm (ITPN), a novel entity of pancreatic epithelial neoplasms and precursor of cancer: A case report and review of the literature. *Int J Surg Case Rep* 2019; **55**: 187-191 [PMID: 30753991 DOI: 10.1016/j.ijscr.2019.01.036]

4 **Basturk O**, Adsay V, Askan G, Dhall D, Zamboni G, Shimizu M, Cymes K, Carneiro F, Balci S, Sigel C, Reid MD, Esposito I, Baldaia H, Allen P, Klöppel G, Klimstra DS. Intraductal Tubulopapillary Neoplasm of the Pancreas: A Clinicopathologic and Immunohistochemical Analysis of 33 Cases. *Am J Surg Pathol* 2017; **41**: 313-325 [PMID: 27984235 DOI: 10.1097/PAS.0000000000000782]

5 **Nabrinsky E**, Baste CL, Gonzalez M, James E. Intraductal Tubulopapillary Neoplasm of the Pancreas Presenting as a Localized Pancreatic Tumor in a 52-Year-Old Woman: Focusing on a Rare Pancreatic Malignancy and Contrasting to Intrapapillary Mucinous Neoplasm. *Cureus* 2020; **12**: e8548 [PMID: 32670685 DOI: 10.7759/cureus.8548]

6 **Eisenhauer EA**, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**: 228-247 [PMID: 19097774 DOI: 10.1016/j.ejca.2008.10.026]

7 **Kim H**, Ro JY. Intraductal Tubulopapillary Neoplasm of the Pancreas: An Overview. *Arch Pathol Lab Med* 2018; **142**: 420-423 [PMID: 29494224 DOI: 10.5858/arpa.2016-0405-RSR2]

8 **Fritz S**, Klauss M, Bergmann F, Strobel O, Schneider L, Werner J, Hackert T, Büchler MW. Pancreatic main-duct involvement in branch-duct IPMNs: an underestimated risk. *Ann Surg* 2014; **260**: 848-55; discussion 855 [PMID: 25379856 DOI: 10.1097/SLA.0000000000000980]

9 **Maghrebi H**, Makni A, Rhaeim R, Zehani A, Bensafta Z. Intraductal Tubulopapillary Neoplasm: A New Entity in the Spectrum of Pancreatic Intraductal Neoplasms. *J Clin Diagn Res* 2017; **11**: PD14-PD16 [PMID: 29207779 DOI: 10.7860/JCDR/2017/28596.10663]

10 **Date K**, Okabayashi T, Shima Y, Iwata J, Sumiyoshi T, Kozuki A, Morita S, Hata Y, Noda Y, Nishioka A, Matsumoto M. Clinicopathological features and surgical outcomes of intraductal tubulopapillary neoplasm of the pancreas: a systematic review. *Langenbecks Arch Surg* 2016; **401**: 439-447 [PMID: 27001682 DOI: 10.1007/s00423-016-1391-6]

11 **Kölby D**, Thilén J, Andersson R, Sasor A, Ansari D. Multifocal intraductal tubulopapillary neoplasm of the pancreas with total pancreatectomy: report of a case and review of literature. *Int J Clin Exp Pathol* 2015; **8**: 9672-9680 [PMID: 26464736]

12 **Uesaka K**, Boku N, Fukutomi A, Okamura Y, Konishi M, Matsumoto I, Kaneoka Y, Shimizu Y, Nakamori S, Sakamoto H, Morinaga S, Kainuma O, Imai K, Sata N, Hishinuma S, Ojima H, Yamaguchi R, Hirano S, Sudo T, Ohashi Y; JASPAC 01 Study Group. Adjuvant chemotherapy of S-1 versus gemcitabine for resected pancreatic cancer: a phase 3, open-label, randomised, non-inferiority trial (JASPAC 01). *Lancet* 2016; **388**: 248-257 [PMID: 27265347 DOI: 10.1016/S0140-6736(16)30583-9]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com

© 2023 Baishideng Publishing Group Inc. All rights reserved.