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EDITORIAL

Anaplastic thyroid cancer: Unveiling advances in diagnosis and management

Treshita Dey, Budhi Singh Yadav

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Abstract

The review article by Pavlidis *et al* published in *World J Clin Oncol* provides a meticulous analysis of the intricacies surrounding anaplastic carcinoma of the thyroid. Thyroid carcinoma encompasses a spectrum of diseases, each characterized by distinct behaviors and outcomes. Diagnostic approaches encompass a diverse array of tools. Surgery remains the pivotal treatment for anaplastic thyroid carcinoma. Radiotherapy and chemotherapy offer the best overall survival in aggressive disease. Combinations of immunotherapy with targeted therapies, such as dabrafenib-trametinib, demonstrate potential for enhanced effectiveness and improved survival outcomes. Multifaceted approach fuelled by precision medicine and interdisciplinary collaboration is imperative in charting a course toward improved outcomes in this formidable malignancy.

Key Words: Anaplastic thyroid cancer; Surgery; Radiotherapy; Chemotherapy; Targeted therapy; Immunotherapy

Core Tip: Anaplastic thyroid cancer is an aggressive disease. Surgery is the main treatment. Combination of radiotherapy and chemotherapy help to further improve the outcome of patients with this malignancy. Immunotherapy, targeted therapies, and molecular insights herald a new dawn for a patient cohort hitherto consigned to bleak prognoses.

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INTRODUCTION

The review article by Pavlidis *et al*[1] published in *World J Clin Oncol* provides a meticulous analysis of the intricacies surrounding anaplastic carcinoma of the thyroid[1]. Thyroid carcinoma encompasses a spectrum of diseases, each characterized by distinct behaviors and outcomes. While well-differentiated thyroid carcinomas typically has a favorable prognosis, constituting a 5-year survival rate exceeding 95%, anaplastic thyroid cancer, a rarity accounting for less than 0.2%, stands as an ominous exception[2]. This aggressive variant, often observed among the elderly, presents with rapid growth and lamentable prognosis, resulting in a median survival of 9.5 months, accompanied by a profound deterioration in quality of life[3].

The urgency of early diagnosis and staging cannot be undermined in confronting anaplastic thyroid cancer. Diagnostic approaches encompass a diverse array of tools, ranging from conventional biopsy techniques-fine needle aspiration (FNA), core needle biopsy (CNB), and open surgery-to sophisticated imaging modalities like high-resolution ultrasound (US), computed tomography (CT), magnetic resonance imaging, 18-fluoro-D-glucose positron emission tomography/CT, liquid biopsy, and microRNAs[4]. In cases of rapidly enlarging neck nodules, initial high-resolution US imaging is essential. Although FNA cytology under US guidance has been commonly used, its high false-negative rates do not support its use, and CNB has shown superior accuracy. Contrary to earlier guidelines, CNB is now recommended as the primary diagnostic method, avoiding unnecessary delays caused by inconclusive FNAs[5]. CNB is safe, rarely causing bleeding or hematoma. Incision/open surgery biopsies, once used, are now replaced by CNB. Liquid biopsy, a non-invasive genotyping method detecting malignant cells in serum and tumor DNA, offers valuable diagnostic, prognostic, and treatment response insights. Molecular investigations often unearth the presence of the *BRAF* gene, notably BRAF-V600E and BRAF wild type, alongside other implicated genes like *RET*, *KRAS*, *HRAS*, and *NRAS*, or genes implicated in the WNT and NOTCH signaling pathways, delineating possible options pivotal for personalized therapeutic interventions[6].

MANAGEMENT OF ANAPLASTIC THYROID CARCINOMA

Surgery remains the pivotal treatment for anaplastic thyroid carcinoma, with a spectrum ranging from palliative thyroidectomy to complete thyroidectomy and neck node dissection. Radical surgery, often combined with adjuvant chemotherapy, can yield occasional long-term survival over 5 years, especially in earlier disease stages and can improve locoregional disease control and quality of life. Studies have identified surgery and radiotherapy as independent factors predicting increased overall survival. But extreme radical resections like laryngectomy or extensive neck dissections lack substantial oncological benefits[7,8]. National Comprehensive Cancer Network and American Thyroid Association (ATA) guidelines recommend surgical resection, and lymphadenectomy for stage-IVA and IVB, and even for locally resectable stage-IVC tumors. Locally unresectable cases might respond to neoadjuvant therapies, becoming eligible for surgical excision. For inoperable cases, palliative surgeries aim to alleviate symptoms and prevent life-threatening events[9]. Although aggressive surgery, radiotherapy and chemotherapy offer the best overall survival, their use should be weighed against patient comfort and quality of life, and radiotherapy and chemotherapy are favored for unresectable cases.

Complementary to surgery, chemotherapy with agents like cisplatin or doxorubicin including taxanes (paclitaxel, docetaxel, cabazitaxel), radiotherapy in adjuvant or definitive settings, targeted biological agents, and the promising immunotherapy constitute the pillars of contemporary management paradigms and recommended by ATA guidelines [10]. Adjuvant chemotherapy enhances median survival, and newer strategies combine chemotherapy with targeted biological agents like dabrafenib and trametinib for *BRAF/MEK* gene mutations or immunotherapy for unmutated cases. Combining chemotherapy with radiation improves survival in resected and unresected cases. Food and Drug Administration (FDA)-approved anlotinib, combined with paclitaxel, capecitabine, or carboplatin, demonstrates safety and efficacy as a first-line therapy for advanced thyroid carcinoma[11].

Immunotherapy, specifically employing anti-programmed death-ligand 1 (PD-L1) antibodies, tailored stem cell therapies, advancements in nanotechnology, and the integration of artificial intelligence, have emerged as optimistic alternatives. Combinations of immunotherapy with targeted therapies like dabrafenib-trametinib demonstrate potential for enhanced effectiveness and improved survival outcomes. Recent developments in targeted PD-L1 and programmed cell death 1 (PD-1) interactions *via* monoclonal antibodies including pembrolizumab, atezolizumab and spartalizumab provide increasing adoption, particularly in cases with high PD-1/PD-L1 expression and without BRAF mutations[12]. Atezolizumab, specifically, exhibits encouraging outcomes in combination with radiation therapy. Spartalizumab and pembrolizumab, targeting PD-1, demonstrate promise in phase II studies for locally advanced or metastatic cases, showing notable survival rates but accompanied by side effects like diarrhea, pruritus, fever, and fatigue. These modalities demonstrate potential in reshaping the landscape of treatment outcomes for a patient cohort traditionally consigned to dismal prognoses.

Crucially, the treatment trajectory is increasingly influenced by the genomic profile, delineating molecular pathways, and thereby guiding novel therapeutic strategies. Strategies targeting specific mutations-anti-epidermal growth factor receptor (EGFR), anti-vascular endothelial growth factor-A (VEGF-A), and anti-BRAF-have emerged as a more tailored and effective approach. Notably, the combination therapy of the MEK inhibitor trametinib and the BRAF inhibitor dabrafenib has been approved by the FDA for cases featuring *BRAF-V600E* gene mutations. Drugs targeting various gene mutations include angiogenesis (lenvatinib, sorafenib), BRAF (dabrafenib, vemurafenib), MEK (trametinib, cobimetinib) and EGFR (docetaxel, gefitinib). Dabrafenib combined with trametinib is more effective than individual drugs. Vandetanib, sunitinib, and lenvatinib exhibit potent anti-cancer effects. Carfilzomib and suberoylanilide hydroxamic acid

show promise, affecting cell proliferation and promoting apoptosis. Lenvatinib, targeting VEGFRs, proves effective, extending survival in unresectable cases. Glutaminolysis inhibition by tyrosine kinase inhibitors enhances conventional chemotherapy efficiency. Several targets like ICAM1, CTHRC1, and fibronectin depletion show potential in overcoming resistance to inhibitors. Additionally, targeting EZH2 complex and one-carbon metabolism holds promise, offering potential therapeutic strategies for anaplastic thyroid carcinoma. These diverse approaches represent a broad spectrum of targeted therapies with potential implications for future treatments[13].

Radiation therapy stands as a crucial element in managing this malignancy, halting tumor progression, and preventing recurrence pre- and post-surgery. Utilized as neoadjuvant or adjuvant therapy, external beam radiation therapy (EBRT) significantly improves median survival rates in multimodal treatment, along with surgery, chemotherapy, targeted therapy, and immunotherapy. Optimal EBRT doses (45-70 Gy) and subsequent hypofractionation (> 5 Gy) reduce local recurrence and mortality. Furthermore, radiation therapy may synergize with immunotherapy, although its efficacy remains limited alongside targeted therapy like Lenvatinib[14].

Prognostic factors, such as younger age, earlier tumor stage, and the judicious incorporation of radiation therapy, have been identified as pivotal determinants for improved outcomes. An indispensable facet of confronting anaplastic thyroid cancer lies in adopting a multidisciplinary approach, tailoring therapeutic plans to individualized patient profiles based on insights gleaned from surveillance and epidemiology end results.

CONCLUSION

The contemporary vista of anaplastic thyroid cancer management signifies a departure from the erstwhile despondent landscape, offering rays of hope buoyed by innovative therapeutic avenues. The synergy between conventional interventions and burgeoning advancements in immunotherapy, targeted therapies, and molecular insights heralds a new dawn for a patient cohort hitherto consigned to bleak prognoses. Embracing this multifaceted approach, fuelled by precision medicine and interdisciplinary collaboration, is imperative in charting a course toward improved outcomes and enhanced quality of life for those afflicted by this formidable malignancy.

FOOTNOTES

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REFERENCES

1 **Pavlidis ET**, Galanis IN, Pavlidis TE. Update on current diagnosis and management of anaplastic thyroid carcinoma. *World J Clin Oncol* 2023; **14**: 570-583 [PMID: 38179406 DOI: 10.5306/wjco.v14.i12.570]
2 **Yadav BS**, Sharma SC. Breast and Thyroid Cancer Association. *Int J Radiat Oncol Biol Phys* 2009; **73**: 1604 [DOI: 10.1016/j.ijrobp.2008.11.064]
3 **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]
4 **Chiofalo MG**, Signoriello S, Fulciniti F, Avenia N, Ristagno S, Lombardi CP, Nicolosi A, Pelizzo MR, Perigli G, Polistena A, Panebianco V, Bellantone R, Calò PG, Boschin IM, Badii B, Di Maio M, Gallo C, Perrone F, Pezzullo L. Predictivity of clinical, laboratory and imaging findings in diagnostic definition of palpable thyroid nodules. A multicenter prospective study. *Endocrine* 2018; **61**: 43-50 [PMID: 29569123 DOI: 10.1007/s12020-018-1577-5]
5 **Zhu Y**, Song Y, Xu G, Fan Z, Ren W. Causes of misdiagnoses by thyroid fine-needle aspiration cytology (FNAC): our experience and a systematic review. *Diagn Pathol* 2020; **15**: 1 [PMID: 31900180 DOI: 10.1186/s13000-019-0924-z]
6 **Smallridge RC**, Marlow LA, Copland JA. Anaplastic thyroid cancer: molecular pathogenesis and emerging therapies. *Endocr Relat Cancer*

2009; **16**: 17-44 [PMID: 18987168 DOI: 10.1677/ERC-08-0154]

7 **Harada T**. Surgery for Anaplastic Carcinoma and the Rare Thyroid Tumors. *Prog Surg* [DOI: 10.1159/000415597]

8 **Chang HS**, Nam KH, Chung WY, Park CS. Anaplastic thyroid carcinoma: a therapeutic dilemma. *Yonsei Med J* 2005; **46**: 759-764 [PMID: 16385650 DOI: 10.3349/ymj.2005.46.6.759]

9 **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]

10 **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]

11 **Zhang LY**, Cai SJ, Liang BY, Yan SY, Wang B, Li MY, Zhao WX. Efficacy of anlotinib combined with radioiodine to treat scalp metastasis of papillary thyroid cancer: A case report and review of literature. *World J Clin Cases* 2023; **11**: 2839-2847 [PMID: 37214573 DOI: 10.12998/wjcc.v11.i12.2839]

12 **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]

13 **Yuan J**, Guo Y. Targeted Therapy for Anaplastic Thyroid Carcinoma: Advances and Management. *Cancers (Basel)* 2022; **15** [PMID: 36612173 DOI: 10.3390/cancers15010179]

14 **Goodsell K**, Ermer J, Amjad W, Swisher-McClure S, Wachtel H. External beam radiotherapy for thyroid cancer: Patients, complications, and survival. *Am J Surg* 2023; **225**: 994-999 [PMID: 36707300 DOI: 10.1016/j.amjsurg.2023.01.009]

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EDITORIAL

Neoadjuvant treatment of rectal cancer: Where we are and where we are going

Elísabet González Del Portillo, Felipe Couñago, Fernando López-Campos

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Abstract

Locally advanced rectal cancer requires a multidisciplinary approach based on total neoadjuvant treatment with radiotherapy (RT) and chemotherapy (ChT), followed by deferred surgery. Currently, alternatives to the standard total neoadjuvant therapy (TNT) are being explored, such as new ChT regimens or the introduction of immunotherapy. With standard TNT, up to a third of patients may achieve a complete pathological response (CPR), potentially avoiding surgery. However, as of now, we lack predictive markers of response that would allow us to define criteria for a conservative organ strategy. The presence of mutations, genes, or new imaging tests is helping to define these criteria. An example of this is the diffusion coefficient in the diffusion-weighted sequence of magnetic resonance imaging and the integration of this imaging technique into RT treatment. This allows for the monitoring of the evolution of this coefficient over successive RT sessions, helping to determine which patients will achieve CPR or those who may require intensification of neoadjuvant therapy.

Key Words: Locally advanced rectal cancer; Total neoadyuvant treatment; Radiotherapy; Biomarker; Magnetic resonance imaging; Conservative organ strategy; Watch and wait

Core Tip: The treatment of rectal cancer is well-established, based on total neoadjuvant treatment followed by deferred surgery. However, the development of biomarkers is necessary to predict which patients will achieve a complete pathological response and, therefore, may not require surgical treatment. The advent of new imaging techniques and their morphological, metabolic, and functional information pave the way for defining criteria for patients with locally advanced rectal cancer who are candidates for a conservative strategy.

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INTRODUCTION

Colorectal cancer is the third most common worldwide, accounting for 10% of all diagnosed cancers and standing as the second leading cause of cancer-related deaths globally[1]. Currently, the management of patients with locally advanced rectal cancer (LARC) requires a multidisciplinary approach integrating various treatment modalities such as concurrent and/or sequential neoadjuvant radiotherapy (RT) and chemotherapy (ChT), followed by surgery[2-7].

NEOADJUVANT TREATMENT

The intensification of neoadjuvant treatment, known as total neoadjuvant therapy (TNT), is considered the standard of care for LARC.

TNT is based on ChT and RT. There are two modalities of RT: The short-course and the long-course. Regarding computed tomography (CT), different systemic treatment regimens are being considered[8].

TNT improves compliance rates for ChT treatment compared to adjuvant ChT, with rates of complete pathological responses (CPR) ranging between 10%-30%, depending on the regimen used[2,7,9-13]. Two meta-analyses in recent years have supported that treatment intensification of neoadjuvant TNT therapy in LARC achieved higher CPR rates. One of them is that of Petrelli *et al*[14], that after review of multiple papers and nearly 3000 patients treated with TNT reported CPR rates of 22.4% (95%CI 19.4%-25.7%), concluding that intensification of neoadjuvant LARC treatment achieves higher CPR rates. On the other hand, the meta-analysis by Liu *et al*[15] analyzed the outcome in more than 2000 patients and also concluded that CPR rates were significantly improved with TNT with respect to the standard scheme in LARC. In addition, the CPR variable is considered a prognostic factor that acts as a predictor of local recurrence[3,16,17], possibly impacting overall survival, disease-free survival, and the risk of distant metastasis[15]. Additionally, the interval between RT and surgery becomes crucial in the CPR rates[4,18-20], as indicated by data from the OPRA7 trial[21].

Beyond the combination of RT and ChT in TNT, there is currently debate opening up to consider neoadjuvant monotherapy with ChT or immunotherapy. In this line, the PROSPECT study (NCT01515787) was focused[22]. It compared one arm with the standard treatment of RT and CT against intensified monotherapy CT (FOLFOX). They demonstrated similar short-term oncologic outcomes (disease-free survival, overall survival, and local recurrence). However, they did not conclude that RT could be omitted due to the significantly higher rate of side effects in the experimental arm compared to the standard treatment.

On the other hand, the characterization of the immunological microenvironment tumour may help in the development of new strategies based on immunotherapy. In this regard, tumours with loss of mismatch repair (MMR) protein expression show elevated therapeutic response to PD-1/PD-L1 inhibitors. Nevertheless, those tumours only represent 5%-15% of all colorectal cancers. Although there are no specific publications on rectal cancer, we already have some data on colon cancer, such as the preliminary NICHE study. This study included patients with dMMR or pMMR colon cancers received a single dose of ipilimumab and two doses of nivolumab before surgery, the pMMR group with or without celecoxib. This treatment was safe and feasible, without compromising surgery. Their results showed 100% and 27% pathological response in dMMR and pMMR tumors, respectively[23].

In any case, in the management of LARC, there is a growing interest in the conservative Watch and Wait (WW) strategy, initially proposed by the Habr-Gama *et al*[24] over 20 years ago. The goal is to avoid surgical treatment in selected patients who achieve a complete clinical response after neoadjuvant treatment, opting for close monitoring. The results show survival outcomes similar to those surgically treated with CPR[17,24-27]. Data confirmed in subsequent publications do not show statistically significant differences in terms of local control and overall survival between both treatment approaches. However, differences are observed in quality of life, especially in the physical and cognitive spheres[28], especially in elderly patients and/or those with comorbidities[2,3,7,11] in favour of the conservative strategy However, despite the reported data, prospective studies are still necessary to optimize the management of patients with a WW strategy[7,29-32]. This includes appropriate patient selection, standardization of follow-up strategies, and primarily the identification of applicable biomarkers in clinical practice to help predict which patients would achieve CPR[33]. In this regard, morphofunctional and metabolic imaging, radiomics, and artificial intelligence emerge as elements that open a disruptive and novel research field to define predictive factors for response to RT and ChT treatment.

Some of the biomarkers explored to date include the presence of mutations to assess the response to specific treatments, as mentioned earlier. Additionally, there are groups that have explored the expression of miRNA and found a relationship with resistance to RT[34]. Other studies have examined the expression of specific genes and established a prognostic risk score model to predict the response to ChT[35]. The role of the microbiota has also been analyzed in neoadjuvant treatment response, and the presence of certain bacteria has been associated with a poorer response to treatment[36].

Another area that has been explored as a biomarker is magnetic resonance imaging (MRI). Diffusion-weighted imaging (DWI) sequences characterize the microenvironment and tumour tissues based on the movements of water molecules as a surrogate for the density of the tumour environment, quantified by the apparent diffusion coefficient (ADC). The ADC is inversely proportional to tissue cellularity and has proven capable of distinguishing between tumour recurrence or persistence and inflammation or necrosis with a high level of specificity[2,3,6,37-42].

Several studies propose ADC as a biomarker for CPR in LARC[2,3,9,42-46]. However, to date, there is no well-established relationship between the evolution of this marker and the histopathological response of the surgical specimen. Lambregts *et al*[47] analysed the value of the DWI sequence of MRI in the reevaluation after neoadjuvant treatment to detect patients with a complete response, demonstrating an increase in sensitivity to 52%-64% and a specificity exceeding 90% when adding DWI. This reduced the risk of underestimating residual tumour to below 10%, and these findings were subsequently confirmed[48]. Recently, Azamat *et al*[49] analyzed the response of tumour lesions in patients with LARC after neoadjuvant treatment using the T2 sequence and ADC value by performing an MRI before and after treatment. They concluded that the T2 sequence varied according to whether the response was complete or partial or there was no response, and there is a cut off value of ADC that can be used as a marker for complete response.

A step further has been taken with magnetic resonance-guided RT (MRgRT), allowing for information from morpho-functional imaging and ADC to be obtained throughout successive RT sessions[6]. Cusumano *et al*[50] analyzed data from patients with LARC treated with neoadjuvant MRgRT followed by surgery, studying the early regression index (ERITCP) and correlating it with the rate of pathological responses. They demonstrated that it is a good predictive marker in these patients treated with MRgRT. They obtained combined information on the tumor volume in MRI images at the time of RT treatment planning and the same volume during MRgRT treatment. Moreover, studies suggest that the ADC measured before, during, and after treatment may be useful in predicting pathological response before surgery in LARC, surpassing other classic parameters such as tumor size[2,3]. It positions itself as a potential predictive factor for response in patients undergoing long courses of RT with concurrent CT. In this regard, we will have to await data from ongoing studies like TRIGGER, which will allow us to validate the Magnetic Resonance Tumour Regression Grade as a response marker in the management of LARC with the aim of avoiding surgery, maintaining quality of life in appropriately selected patients without impacting survival rates[13].

CONCLUSION

Taking all of the above into account, we know that the standard TNT has excellent results, but recently, doors have been opened to consider variants based on each patient's characteristics. Predicting the response to TNT remains a challenge. Identifying biomarkers for neoadjuvant treatment response will allow us to determine which patients can safely enter a WW strategy and which patients require an intensification of the treatment.

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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 **Shaverdian N**, Yang Y, Hu P, Hart S, Sheng K, Lamb J, Cao M, Agazaryan N, Thomas D, Steinberg M, Low DA, Lee P. Feasibility evaluation of diffusion-weighted imaging using an integrated MRI-radiotherapy system for response assessment to neoadjuvant therapy in rectal cancer. *Br J Radiol* 2017; **90**: 20160739 [PMID: 28079398 DOI: 10.1259/bjr.20160739]

3 **Lambrecht M**, Vandecaveye V, De Keyzer F, Roels S, Penninckx F, Van Cutsem E, Filip C, Haustermans K. Value of diffusion-weighted magnetic resonance imaging for prediction and early assessment of response to neoadjuvant radiochemotherapy in rectal cancer: preliminary results. *Int J Radiat Oncol Biol Phys* 2012; **82**: 863-870 [PMID: 21398048 DOI: 10.1016/j.ijrobp.2010.12.063]

4 **Oronsky B**, Reid T, Larson C, Knox SJ. Locally advanced rectal cancer: The past, present, and future. *Semin Oncol* 2020; **47**: 85-92 [PMID: 32147127 DOI: 10.1053/j.seminoncol.2020.02.001]

5 **Sauer R**, Liersch T, Merkel S, Fietkau R, Hohenberger W, Hess C, Becker H, Raab HR, Villanueva MT, Witzigmann H, Wittekind C, Beissbarth T, Rödel C. Preoperative versus postoperative chemoradiotherapy for locally advanced rectal cancer: results of the German CAO/ARO/AIO-94 randomized phase III trial after a median follow-up of 11 years. *J Clin Oncol* 2012; **30**: 1926-1933 [PMID: 22529255 DOI: 10.1200/JCO.2011.40.1836]

6 **Chiloiro G**, Boldrini L, Meldolesi E, Re A, Cellini F, Cusumano D, Corvari B, Mantini G, Balducci M, Valentini V, Gambacorta MA. MR-guided radiotherapy in rectal cancer: First clinical experience of an innovative technology. *Clin Transl Radiat Oncol* 2019; **18**: 80-86 [PMID: 31341981 DOI: 10.1016/j.ctro.2019.04.006]

7 **López-Campos F**, Martín-Martín M, Fornell-Pérez R, García-Pérez JC, Die-Trill J, Fuentes-Mateos R, López-Durán S, Domínguez-Rullán J, Ferreiro R, Riquelme-Oliveira A, Hervás-Morón A, Couñago F. Watch and wait approach in rectal cancer: Current controversies and future directions. *World J Gastroenterol* 2020; **26**: 4218-4239 [PMID: 32848330 DOI: 10.3748/wjg.v26.i29.4218]

8 **Dewdney A**, Cunningham D, Tabernero J, Capdevila J, Glimelius B, Cervantes A, Tait D, Brown G, Wotherspoon A, Gonzalez de Castro D, Chua YJ, Wong R, Barbachano Y, Oates J, Chau I. Multicenter randomized phase II clinical trial comparing neoadjuvant oxaliplatin, capecitabine, and preoperative radiotherapy with or without cetuximab followed by total mesorectal excision in patients with high-risk rectal cancer (EXPERT-C). *J Clin Oncol* 2012; **30**: 1620-1627 [PMID: 22473163 DOI: 10.1200/JCO.2011.39.6036]

9 **Boldrini L**, Intven M, Bassetti M, Valentini V, Gani C. MR-Guided Radiotherapy for Rectal Cancer: Current Perspective on Organ Preservation. *Front Oncol* 2021; **11**: 619852 [PMID: 33859937 DOI: 10.3389/fonc.2021.619852]

10 **Kalady MF**, de Campos-Lobato LF, Stocchi L, Geisler DP, Dietz D, Lavery IC, Fazio VW. Predictive factors of pathologic complete response after neoadjuvant chemoradiation for rectal cancer. *Ann Surg* 2009; **250**: 582-589 [PMID: 19710605 DOI: 10.1097/SLA.0b013e3181b91e63]

11 **Wang SJ**, Hathout L, Malhotra U, Maloney-Patel N, Kilic S, Poplin E, Jabbour SK. Decision-Making Strategy for Rectal Cancer Management Using Radiation Therapy for Elderly or Comorbid Patients. *Int J Radiat Oncol Biol Phys* 2018; **100**: 926-944 [PMID: 29485072 DOI: 10.1016/j.ijrobp.2017.12.261]

12 **Maas M**, Beets-Tan RG, Lambregts DM, Lammering G, Nelemans PJ, Engelen SM, van Dam RM, Jansen RL, Sosef M, Leijtens JW, Hulsewé KW, Buijsen J, Beets GL. Wait-and-see policy for clinical complete responders after chemoradiation for rectal cancer. *J Clin Oncol* 2011; **29**: 4633-4640 [PMID: 22067400 DOI: 10.1200/JCO.2011.37.7176]

13 **Battersby NJ**, Dattani M, Rao S, Cunningham D, Tait D, Adams R, Moran BJ, Khakoo S, Tekkis P, Rasheed S, Mirnezami A, Quirke P, West NP, Nagtegaal I, Chong I, Sadanandam A, Valeri N, Thomas K, Frost M, Brown G. A rectal cancer feasibility study with an embedded phase III trial design assessing magnetic resonance tumour regression grade (mrTRG) as a novel biomarker to stratify management by good and poor response to chemoradiotherapy (TRIGGER): study protocol for a randomised controlled trial. *Trials* 2017; **18**: 394 [PMID: 28851403 DOI: 10.1186/s13063-017-2085-2]

14 **Petrelli F**, Trevisan F, Cabiddu M, Sgroi G, Bruschieri L, Rausa E, Ghidini M, Turati L. Total Neoadjuvant Therapy in Rectal Cancer: A Systematic Review and Meta-analysis of Treatment Outcomes. *Ann Surg* 2020; **271**: 440-448 [PMID: 31318794 DOI: 10.1097/SLA.0000000000003471]

15 **Liu S**, Jiang T, Xiao L, Yang S, Liu Q, Gao Y, Chen G, Xiao W. Total Neoadjuvant Therapy (TNT) versus Standard Neoadjuvant Chemoradiotherapy for Locally Advanced Rectal Cancer: A Systematic Review and Meta-Analysis. *Oncologist* 2021; **26**: e1555-e1566 [PMID: 33987952 DOI: 10.1002/onco.13824]

16 **Valentini V**, Coco C, Cellini N, Picciocchi A, Fares MC, Rosetto ME, Mantini G, Morganti AG, Barbaro B, Cogliandolo S, Nuzzo G, Tedesco M, Ambesi-Impiombato F, Cosimelli M, Rotman M. Ten years of preoperative chemoradiation for extraperitoneal T3 rectal cancer: acute toxicity, tumor response, and sphincter preservation in three consecutive studies. *Int J Radiat Oncol Biol Phys* 2001; **51**: 371-383 [PMID: 11567811 DOI: 10.1016/S0360-3016(01)01618-2]

17 **Habr-Gama A**, Perez RO, Nadalin W, Nahas SC, Ribeiro U Jr, Silva E Sousa AH Jr, Campos FG, Kiss DR, Gama-Rodrigues J. Long-term results of preoperative chemoradiation for distal rectal cancer correlation between final stage and survival. *J Gastrointest Surg* 2005; **9**: 90-9; discussion 99 [PMID: 15623449 DOI: 10.1016/j.gassur.2004.10.010]

18 **Ngan SY**, Burmeister B, Fisher RJ, Solomon M, Goldstein D, Joseph D, Ackland SP, Schache D, McClure B, McLachlan SA, McKendrick J, Leong T, Hartopeanu C, Zalcberg J, Mackay J. Randomized trial of short-course radiotherapy versus long-course chemoradiation comparing rates of local recurrence in patients with T3 rectal cancer: Trans-Tasman Radiation Oncology Group trial 01.04. *J Clin Oncol* 2012; **30**: 3827-3833 [PMID: 23008301 DOI: 10.1200/JCO.2012.42.9597]

19 **Valentini V**, Glimelius B, Haustermans K, Marijnen CA, Rödel C, Gambacorta MA, Boelens PG, Aristei C, van de Velde CJ. EURECCA consensus conference highlights about rectal cancer clinical management: the radiation oncologist's expert review. *Radiother Oncol* 2014; **110**: 195-198 [PMID: 24286634 DOI: 10.1016/j.radonc.2013.10.024]

20 **Chapman BC**, Goodman K, Hosokawa P, Gleisner A, Cowan ML, Birnbaum E, Vogel JD. Improved survival in rectal cancer patients who are treated with long-course versus short-course neoadjuvant radiotherapy: A propensity-matched analysis of the NCDB. *J Surg Oncol* 2019; **119**: 518-531 [PMID: 30637750 DOI: 10.1002/jso.25355]

21 **Thompson HM**, Omer DM, Lin S, Kim JK, Yuval JB, Verheij FS, Qin LX, Gollub MJ, Wu AJ, Lee M, Patil S, Hezel AF, Marcet JE, Cataldo PA, Polite BN, Herzig DO, Liska D, Oommen S, Friel CM, Ternent CA, Coveler AL, Hunt SR, Garcia-Aguilar J; OPRA Consortium. Organ Preservation and Survival by Clinical Response Grade in Patients With Rectal Cancer Treated With Total Neoadjuvant Therapy: A Secondary Analysis of the OPRA Randomized Clinical Trial. *JAMA Netw Open* 2024; **7**: e2350903 [PMID: 38194231 DOI: 10.1001/jamanetworkopen.2023.50903]

22 **Schrag D**, Shi Q, Weiser MR, Gollub MJ, Saltz LB, Musher BL, Goldberg J, Al Baghdadi T, Goodman KA, McWilliams RR, Farma JM, George TJ, Kennecke HF, Shergill A, Montemurro M, Nelson GD, Colgrove B, Gordon V, Venook AP, O'Reilly EM, Meyerhardt JA, Dueck AC, Basch E, Chang GJ, Mamon HJ. Preoperative Treatment of Locally Advanced Rectal Cancer. *N Engl J Med* 2023; **389**: 322-334 [PMID: 37272534 DOI: 10.1056/NEJMoa2303269]

23 **Chalabi M**, Fanchi LF, Dijkstra KK, Van den Berg JG, Aalbers AG, Sikorska K, Lopez-Yurda M, Grootscholten C, Beets GL, Snaebjornsson P, Maas M, Mertz M, Veninga V, Bounova G, Broeks A, Beets-Tan RG, de Wijkerslooth TR, van Lent AU, Marsman HA, Nuijten E, Kok NF, Kuiper M, Verbeek WH, Kok M, Van Leerdam ME, Schumacher TN, Voest EE, Haanen JB. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat Med* 2020; **26**: 566-576 [PMID: 32251400 DOI: 10.1038/s41591-020-0805-8]

24 **Habr-Gama A**, Perez RO, Nadalin W, Sabbaga J, Ribeiro U Jr, Silva e Sousa AH Jr, Campos FG, Kiss DR, Gama-Rodrigues J. Operative versus nonoperative treatment for stage 0 distal rectal cancer following chemoradiation therapy: long-term results. Ann Surg 2004; 240: 711-7; discussion 717-8 [PMID: 15383798 DOI: 10.1097/01.sla.0000141194.27992.32]

25 **Habr-Gama A**. Assessment and management of the complete clinical response of rectal cancer to chemoradiotherapy. *Colorectal Dis* 2006; **8** Suppl 3: 21-24 [PMID: 16813588 DOI: 10.1111/j.1463-1318.2006.01066.x]

26 **Habr-Gama A**, Perez RO, Proscurshim I, Campos FG, Nadalin W, Kiss D, Gama-Rodrigues J. Patterns of failure and survival for nonoperative treatment of stage c0 distal rectal cancer following neoadjuvant chemoradiation therapy. *J Gastrointest Surg* 2006; **10**: 1319-28; discussion 1328 [PMID: 17175450 DOI: 10.1016/j.gassur.2006.09.005]

27 **Habr-Gama A**, de Souza PM, Ribeiro U Jr, Nadalin W, Gansl R, Sousa AH Jr, Campos FG, Gama-Rodrigues J. Low rectal cancer: impact of radiation and chemotherapy on surgical treatment. *Dis Colon Rectum* 1998; **41**: 1087-1096 [PMID: 9749491 DOI: 10.1007/BF02239429]

28 **Hupkens BJP**, Martens MH, Stoot JH, Berbee M, Melenhorst J, Beets-Tan RG, Beets GL, Breukink SO. Quality of Life in Rectal Cancer Patients After Chemoradiation: Watch-and-Wait Policy Versus Standard Resection - A Matched-Controlled Study. *Dis Colon Rectum* 2017; **60**: 1032-1040 [PMID: 28891846 DOI: 10.1097/DCR.0000000000000862]

29 **Dossa F**, Chesney TR, Acuna SA, Baxter NN. A watch-and-wait approach for locally advanced rectal cancer after a clinical complete response following neoadjuvant chemoradiation: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2017; **2**: 501-513 [PMID: 28479372 DOI: 10.1016/S2468-1253(17)30074-2]

30 **Goodman KA**. Definitive Chemoradiotherapy ("Watch-and-Wait" Approach). *Semin Radiat Oncol* 2016; **26**: 205-210 [PMID: 27238472 DOI: 10.1016/j.semradonc.2016.02.003]

31 **Huang Y**, Huang SH, Chi P, Wang XJ, Lin HM, Lu XR, Ye DX, Lin Y, Deng Y. [Rectum-preserving surgery after consolidation neoadjuvant therapy or totally neoadjuvant therapy for low rectal cancer: a preliminary report]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2020; **23**: 281-288 [PMID: 32192308 DOI: 10.3760/cma.j.cn.441530-20200228-00096]

32 **Sun TT**, Wang L, Yao YF, Peng YF, Zhao J, Zhan TC, Leng JH, Wang HY, Chen N, Chen PJ, Li YJ, Zhang X, Liu XZ, Zhang Y, Wu AW. ["Watch and wait" strategy after neoadjuvant therapy for rectal cancer: status survey of perceptions, attitudes and treatment selection in Chinese surgeons]. *Zhonghua Wei Chang Wai Ke Za Zhi* 2019; 22: 550-559 [PMID: 31238634 DOI: 10.3760/cma.j.issn.1671-0274.2019.06.008]

33 **Aguiar S Jr**, Rogatto SR. Editorial: Neoadjuvant Therapy in Rectal Cancer: Response Prediction and Organ Preservation Strategies. *Front Oncol* 2022; **12**: 944741 [PMID: 35756614 DOI: 10.3389/fonc.2022.944741]

34 **Wu F**, Wu B, Zhang X, Yang C, Zhou C, Ren S, Wang J, Yang Y, Wang G. Screening of MicroRNA Related to Irradiation Response and the Regulation Mechanism of miRNA-96-5p in Rectal Cancer Cells. *Front Oncol* 2021; **11**: 699475 [PMID: 34458143 DOI: 10.3389/fonc.2021.699475]

35 **Wei FZ**, Mei SW, Wang ZJ, Chen JN, Shen HY, Zhao FQ, Li J, Xiao TX, Liu Q. Development and Validation of a Nomogram and a Comprehensive Prognostic Analysis of an LncRNA-Associated Competitive Endogenous RNA Network Based on Immune-Related Genes for Locally Advanced Rectal Cancer With Neoadjuvant Therapy. *Front Oncol* 2021; **11**: 697948 [PMID: 34350117 DOI: 10.3389/fonc.2021.697948]

36 **Takenaka IKTM**, Bartelli TF, Defelicibus A, Sendoya JM, Golubicki M, Robbio J, Serpa MS, Branco GP, Santos LBC, Claro LCL, Dos Santos GO, Kupper BEC, da Silva IT, Llera AS, de Mello CAL, Riechelmann RP, Dias-Neto E, Iseas S, Aguiar S, Nunes DN. Exome and Tissue-Associated Microbiota as Predictive Markers of Response to Neoadjuvant Treatment in Locally Advanced Rectal Cancer. *Front Oncol* 2022; **12**: 809441 [PMID: 35392220 DOI: 10.3389/fonc.2022.809441]

37 **Vandecaveye V**, De Keyzer F, Nuyts S, Deraedt K, Dirix P, Hamaekers P, Vander Poorten V, Delaere P, Hermans R. Detection of head and neck squamous cell carcinoma with diffusion weighted MRI after (chemo)radiotherapy: correlation between radiologic and histopathologic findings. *Int J Radiat Oncol Biol Phys* 2007; **67**: 960-971 [PMID: 17141979 DOI: 10.1016/j.ijrobp.2006.09.020]

38 **Abdel Razek AA**, Kandeel AY, Soliman N, El-shenshawy HM, Kamel Y, Nada N, Denewar A. Role of diffusion-weighted echo-planar MR imaging in differentiation of residual or recurrent head and neck tumors and posttreatment changes. *AJNR Am J Neuroradiol* 2007; **28**: 1146-1152 [PMID: 17569975 DOI: 10.3174/ajnr.A0491]

39 **Kim SH**, Lee JM, Hong SH, Kim GH, Lee JY, Han JK, Choi BI. Locally advanced rectal cancer: added value of diffusion-weighted MR imaging in the evaluation of tumor response to neoadjuvant chemo- and radiation therapy. *Radiology* 2009; **253**: 116-125 [PMID: 19789256 DOI: 10.1148/radiol.2532090027]

40 **Sun YS**, Zhang XP, Tang L, Ji JF, Gu J, Cai Y, Zhang XY. Locally advanced rectal carcinoma treated with preoperative chemotherapy and radiation therapy: preliminary analysis of diffusion-weighted MR imaging for early detection of tumor histopathologic downstaging. *Radiology* 2010; **254**: 170-178 [PMID: 20019139 DOI: 10.1148/radiol.2541082230]

41 **Beets-Tan RGH**, Lambregts DMJ, Maas M, Bipat S, Barbaro B, Curvo-Semedo L, Fenlon HM, Gollub MJ, Gourtsoyianni S, Halligan S, Hoeffel C, Kim SH, Laghi A, Maier A, Rafaelsen SR, Stoker J, Taylor SA, Torkzad MR, Blomqvist L. Magnetic resonance imaging for clinical management of rectal cancer: Updated recommendations from the 2016 European Society of Gastrointestinal and Abdominal Radiology

(ESGAR) consensus meeting. *Eur Radiol* 2018; **28**: 1465-1475 [PMID: 29043428 DOI: 10.1007/s00330-017-5026-2]

42 **Lambregts DMJ**, Boellaard TN, Beets-Tan RGH. Response evaluation after neoadjuvant treatment for rectal cancer using modern MR imaging: a pictorial review. *Insights Imaging* 2019; **10**: 15 [PMID: 30758688 DOI: 10.1186/s13244-019-0706-x]

43 **Nahas SC**, Rizkallah Nahas CS, Sparapan Marques CF, Ribeiro U Jr, Cotti GC, Imperiale AR, Capareli FC, Chih Chen AT, Hoff PM, Cecconello I. Pathologic Complete Response in Rectal Cancer: Can We Detect It? Lessons Learned From a Proposed Randomized Trial of Watch-and-Wait Treatment of Rectal Cancer. *Dis Colon Rectum* 2016; **59**: 255-263 [PMID: 26953983 DOI: 10.1097/DCR.0000000000000558]

44 **Cai G**, Xu Y, Zhu J, Gu WL, Zhang S, Ma XJ, Cai SJ, Zhang Z. Diffusion-weighted magnetic resonance imaging for predicting the response of rectal cancer to neoadjuvant concurrent chemoradiation. *World J Gastroenterol* 2013; **19**: 5520-5527 [PMID: 24023496 DOI: 10.3748/wjg.v19.i33.5520]

45 **Xie H**, Sun T, Chen M, Wang H, Zhou X, Zhang Y, Zeng H, Wang J, Fu W. Effectiveness of the apparent diffusion coefficient for predicting the response to chemoradiation therapy in locally advanced rectal cancer: a systematic review and meta-analysis. *Medicine (Baltimore)* 2015; **94**: e517 [PMID: 25674749 DOI: 10.1097/MD.0000000000000517]

46 **Kokaine L**, Gardovskis A, Gardovskis J. Evaluation and Predictive Factors of Complete Response in Rectal Cancer after Neoadjuvant Chemoradiation Therapy. *Medicina (Kaunas)* 2021; **57** [PMID: 34684080 DOI: 10.3390/medicina57101044]

47 **Lambregts DM**, Vandecaveye V, Barbaro B, Bakers FC, Lambrecht M, Maas M, Haustermans K, Valentini V, Beets GL, Beets-Tan RG. Diffusion-weighted MRI for selection of complete responders after chemoradiation for locally advanced rectal cancer: a multicenter study. *Ann Surg Oncol* 2011; **18**: 2224-2231 [PMID: 21347783 DOI: 10.1245/s10434-011-1607-5]

48 **Fusco R**, Petrillo M, Granata V, Filice S, Sansone M, Catalano O, Petrillo A. Magnetic Resonance Imaging Evaluation in Neoadjuvant Therapy of Locally Advanced Rectal Cancer: A Systematic Review. *Radiol Oncol* 2017; **51**: 252-262 [PMID: 28959161 DOI: 10.1515/raon-2017-0032]

49 **Azamat S**, Karaman Ş, Azamat IF, Ertaş G, Kulle CB, Keskin M, Sakin RND, Bakır B, Oral EN, Kartal MG. Complete Response Evaluation of Locally Advanced Rectal Cancer to Neoadjuvant Chemoradiotherapy Using Textural Features Obtained from T2 Weighted Imaging and ADC Maps. *Curr Med Imaging* 2022; **18**: 1061-1069 [PMID: 35240976 DOI: 10.2174/1573405618666220303111026]

50 **Cusumano D**, Boldrini L, Yadav P, Yu G, Musurunu B, Chiloiro G, Piras A, Lenkowicz J, Placidi L, Broggi S, Romano A, Mori M, Barbaro B, Azario L, Gambacorta MA, De Spirito M, Bassetti MF, Yang Y, Fiorino C, Valentini V. External Validation of Early Regression Index (ERI(TCP)) as Predictor of Pathologic Complete Response in Rectal Cancer Using Magnetic Resonance-Guided Radiation Therapy. *Int J Radiat Oncol Biol Phys* 2020; **108**: 1347-1356 [PMID: 32758641 DOI: 10.1016/j.ijrobp.2020.07.2323]

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EDITORIAL

Hyoid metastasis an unusual location from lung cancer

Miguel Montijano, Abrahams Ocanto, Felipe Couñago

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Abstract

Bone metastases from lung cancer account for 8.5%, with those located in the hyoid bone being extremely rare. In this editorial, we made a review about Hsu *et al* case report highlighted the importance of palliative radiotherapy, even with an unusual but effective scheme in pain control in a patient with non-small cell lung cancer in stage IV.

Key Words: Lung cancer; Metastases; Radiotherapy; Palliative care; Chemotherapy

Core Tip: Bone metastases from lung cancer account for 8.5%, with those located in the hyoid bone being extremely rare. This editorial remarks the importance of radiotherapy in palliative care, specially in pain control, always accompanied of systemic therapy in oncological patients.

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INTRODUCTION

The treatment of choice in patients with metastatic non-small cell lung cancer (NSCLC) is systemic treatment[1], and around 20%-50% of patients with NSCLC present olig-

ometastases at the time of diagnosis, however approximately around a half of patients will receive radiotherapy (RT) in any moment of the disease[1]. Normally the principal place of lung metastases are brain (35.5%) and contralateral lung (33.6%), followed by adrenal glands (13.0%), bone (8.5%), liver (2.4%), and lymph node (2.4%); within bone metastases the hyoid metastasis are exceptional[2]. In the last number of World Clinical Cancer, Hsu *et al*[3], published a case report 72-year-old, non-smoking woman diagnosed with lung adenocarcinoma with metastasis to the hyoid bone. Treated with a platinum-based chemotherapy regimen and pembrolizumab. Palliative RT was performed on the hyoid metastasis.

UNUSUAL LOCATION OF BONE METASTASES

Bone metastases of pulmonary origin are common in the evolution of the disease, with osteolytic metastases being the most related to lung cancer and produce by cell adhesion molecules, chemokine receptors of tumor cells and cell surface receptors that attach to the bone matrix and establish growth in bone[4], this type of lesions are exceptional in the hyoid bone, in fact in solid tumors only a few case reports have been described in this location, therefore their clinical management is not clearly standardized.

Nowadays in the stage IV lung adenocarcinoma, the targetable oncogenic driver mutations, immune checkpoints (programmed cell death protein 1/programmed cell death ligand 1/cytotoxic T lymphocyte-associated protein 4) and translocations conducted to development of specific drugs (tyrosine kinase or immune checkpoints inhibitors) with impressive response and survival rates[5]. An example is this case report[3], the patient was treated with a platinum-based chemo-immunotherapy along with pembrolizumab, which is recognized as the standard first-line in this stage. The data from KEYNOTE-189[6], published in 2018, suggested that introducing pembrolizumab as a first-line therapy in untreated metastatic non-squamous NSCLC without epidermal growth factor receptor or anaplastic lymphoma kinase mutations improve overall survival across all programmed cell death ligand 1 categories. In this case report epidermal growth factor receptor and anaplastic lymphoma kinase mutations are not evaluated or mentioned, which can change the therapeutic management. For this group of patients, an increase in survival justify local therapies including RT.

There are several phase 2 clinical trials that have shown an increase in progression free survival in oligometastatic patients with NSCLC treated with Stereotactic Body Radiotherapy (SBRT) compared to those undergoing maintenance therapy[7-10]. These trials demonstrated that patients most likely to benefit from RT are those whose disease responds to systemic therapy. Most trials recommend SBRT, either alone, conventionally, hypofractioned RT or chemoradiotherapy to the primary lesion. In fact, the most radical approximation in patients oligometastatic is a combination of primary tumor treatment with metastases directed to therapy.

Palliative RT could achieve a significant pain response in up to 80% of patients with a median response duration of 18-21 months. It is broadly accepted as the standard of care RT in metastatic bone pain, despite the absence of randomized trials comparing RT with pain killing strategies such as opioids or surgical options[11]. Based on all the available evidence, palliative RT as symptom control is similar despite the scheme used[12]. A review of randomized trials determined equivalent outcomes in pain control and toxicity after a single dose of 8 Gy compared to multiple fraction RT in patients with bone metastases[13]. In this case the dose were 36 Gy in 12 fractions, a different palliative scheme compared with traditional but with an excellent local control and pain response. At the moment exists the controversial about the effectivity of SBRT in pain control. Actually, several clinical trials comparing conventional RT with SBRT for patients with non-spinal and spinal bone metastases have been published with conflicting outcomes[14,15], for now, the key is good patient selection and the intent of the RT: Ablative, pain control, *etc.* Another interesting point to note is the reevaluation form, in the clinical practice guidelines is not clear the radiological reevaluation during the treatment, the classic imagen is the computed tomography-scan[16], in this case the use of positron emission tomography-computed tomography helped to identify the clearly effect of palliative RT added to symptom control by the patient.

CONCLUSION

Metastases bone in hyoid are exceptional with a management not clear until the date. In patients oligometastatic the key is the ablative therapy and in palliative stage the RT is the best option with good results in pain control despite the scheme used. RT must to be accompanied of systemic therapy according targetable oncogenic driver mutations/translocations and immune checkpoints.

FOOTNOTES

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REFERENCES

1 **Kong FM**, Zhao J, Wang J, Faivre-Finn C. Radiation dose effect in locally advanced non-small cell lung cancer. *J Thorac Dis* 2014; **6**: 336-347 [PMID: 24688778 DOI: 10.3978/j.issn.2072-1439.2014.01.23]

2 **Ashworth A**, Rodrigues G, Boldt G, Palma D. Is there an oligometastatic state in non-small cell lung cancer? A systematic review of the literature. *Lung Cancer* 2013; **82**: 197-203 [PMID: 24051084 DOI: 10.1016/j.lungcan.2013.07.026]

3 **Hsu J**, Hribar K, Poen J. Radiotherapy for hyoid bone metastasis from lung adenocarcinoma: A case report. *World J Clin Oncol* 2024; **15**: 159-164 [PMID: 38292655 DOI: 10.5306/wjco.v15.i1.159]

4 **Yin JJ**, Pollock CB, Kelly K. Mechanisms of cancer metastasis to the bone. *Cell Res* 2005; **15**: 57-62 [PMID: 15686629 DOI: 10.1038/sj.cr.7290266]

5 **Wujanto C**, Vellayappan B, Siva S, Louie AV, Guckenberger M, Slotman BJ, Onishi H, Nagata Y, Liu M, Lo SS. Stereotactic Body Radiotherapy for Oligometastatic Disease in Non-small Cell Lung Cancer. *Front Oncol* 2019; **9**: 1219 [PMID: 31799188 DOI: 10.3389/fonc.2019.01219]

6 **Gandhi L**, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, Domine M, Clingan P, Hochmair MJ, Powell SF, Cheng SY, Bischoff HG, Peled N, Grossi F, Jennens RR, Reck M, Hui R, Garon EB, Boyer M, Rubio-Viqueira B, Novello S, Kurata T, Gray JE, Vida J, Wei Z, Yang J, Raftopoulos H, Pietanza MC, Garassino MC; KEYNOTE-189 Investigators. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* 2018; **378**: 2078-2092 [PMID: 29658856 DOI: 10.1056/NEJMoa1801005]

7 **Gomez DR**, Tang C, Zhang J, Blumenschein GR Jr, Hernandez M, Lee JJ, Ye R, Palma DA, Louie AV, Camidge DR, Doebele RC, Skoulidis F, Gaspar LE, Welsh JW, Gibbons DL, Karam JA, Kavanagh BD, Tsao AS, Sepesi B, Swisher SG, Heymach JV. Local Consolidative Therapy Vs. Maintenance Therapy or Observation for Patients With Oligometastatic Non-Small-Cell Lung Cancer: Long-Term Results of a Multi-Institutional, Phase II, Randomized Study. *J Clin Oncol* 2019; **37**: 1558-1565 [PMID: 31067138 DOI: 10.1200/JCO.19.00201]

8 **Palma DA**, Olson R, Harrow S, Gaede S, Louie AV, Haasbeek C, Mulroy L, Lock M, Rodrigues GB, Yaremko BP, Schellenberg D, Ahmad B, Senthi S, Swaminath A, Kopek N, Liu M, Moore K, Currie S, Schlijper R, Bauman GS, Laba J, Qu XM, Warner A, Senan S. Stereotactic Ablative Radiotherapy for the Comprehensive Treatment of Oligometastatic Cancers: Long-Term Results of the SABR-COMET Phase II Randomized Trial. *J Clin Oncol* 2020; **38**: 2830-2838 [PMID: 32484754 DOI: 10.1200/JCO.20.00818]

9 **Iyengar P**, Wardak Z, Gerber DE, Tumati V, Ahn C, Hughes RS, Dowell JE, Cheedella N, Nedzi L, Westover KD, Pulipparacharuvil S, Choy H, Timmerman RD. Consolidative Radiotherapy for Limited Metastatic Non-Small-Cell Lung Cancer: A Phase 2 Randomized Clinical Trial. *JAMA Oncol* 2018; **4**: e173501 [PMID: 28973074 DOI: 10.1001/jamaoncol.2017.3501]

10 **Tsai CJ**, Yang JT, Shaverdian N, Patel J, Shepherd AF, Eng J, Guttmann D, Yeh R, Gelblum DY, Namakydoust A, Preeshagul I, Modi S, Seidman A, Traina T, Drullinsky P, Flynn J, Zhang Z, Rimner A, Gillespie EF, Gomez DR, Lee NY, Berger M, Robson ME, Reis-Filho JS, Riaz N, Rudin CM, Powell SN; CURB Study Group. Standard-of-care systemic therapy with or without stereotactic body radiotherapy in patients with oligoprogressive breast cancer or non-small-cell lung cancer (Consolidative Use of Radiotherapy to Block [CURB] oligoprogression): an open-label, randomised, controlled, phase 2 study. *Lancet* 2024; **403**: 171-182 [PMID: 38104577 DOI: 10.1016/S0140-6736(23)01857-3]

11 **Oldenburger E**, Brown S, Willmann J, van der Velden JM, Spałek M, van der Linden YM, Kazmierska J, Menten J, Andratschke N, Hoskin P. ESTRO ACROP guidelines for external beam radiotherapy of patients with complicated bone metastases. *Radiother Oncol* 2022; **173**: 240-253 [PMID: 35688398 DOI: 10.1016/j.radonc.2022.06.002]

12 **Stevens R**, Macbeth F, Toy E, Coles B, Lester JF. Palliative radiotherapy regimens for patients with thoracic symptoms from non-small cell lung cancer. *Cochrane Database Syst Rev* 2015; **1**: CD002143 [PMID: 25586198 DOI: 10.1002/14651858.CD002143.pub4]

13 **Chow E**, Zeng L, Salvo N, Dennis K, Tsao M, Lutz S. Update on the systematic review of palliative radiotherapy trials for bone metastases. *Clin Oncol (R Coll Radiol)* 2012; **24**: 112-124 [PMID: 22130630 DOI: 10.1016/j.clon.2011.11.004]

14 **Pielkenrood BJ**, van der Velden JM, van der Linden YM, Bartels MMT, Kasperts N, Verhoeff JJC, Eppinga WSC, Gal R, Verlaan JJ, Verkooijen HML. Pain Response After Stereotactic Body Radiation Therapy Versus Conventional Radiation Therapy in Patients With Bone Metastases-A Phase 2 Randomized Controlled Trial Within a Prospective Cohort. *Int J Radiat Oncol Biol Phys* 2021; **110**: 358-367 [PMID: 33333200 DOI: 10.1016/j.ijrobp.2020.11.060]

15 **Sahgal A**, Myrehaug SD, Siva S, Masucci GL, Maralani PJ, Brundage M, Butler J, Chow E, Fehlings MG, Foote M, Gabos Z, Greenspoon J, Kerba M, Lee Y, Liu M, Liu SK, Thibault I, Wong RK, Hum M, Ding K, Parulekar WR; trial investigators. Stereotactic body radiotherapy versus conventional external beam radiotherapy in patients with painful spinal metastases: an open-label, multicentre, randomised, controlled, phase 2/3 trial. *Lancet Oncol* 2021; **22**: 1023-1033 [PMID: 34126044 DOI: 10.1016/S1470-2045(21)00196-0]

16 **Ettinger DS**, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, Bruno DS, Chang JY, Chirieac LR, DeCamp M, Dilling TJ, Dowell J, Durm GA, Gettinger S, Grotz TE, Gubens MA, Hegde A, Lackner RP, Lanuti M, Lin J, Loo BW, Lovly CM, Maldonado F, Massarelli E, Morgensztern D, Ng T, Otterson GA, Patel SP, Patil T, Polanco PM, Riely GJ, Riess J, Schild SE, Shapiro TA, Singh AP, Stevenson J, Tam A, Tanvetyanon T, Yanagawa J, Yang SC, Yau E, Gregory KM, Hughes M. NCCN Guidelines® Insights: Non-Small Cell Lung Cancer, Version 2.2023. *J Natl Compr Canc Netw* 2023; **21**: 340-350 [PMID: 37015337 DOI: 10.6004/jnccn.2023.0020]

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EDITORIAL

Screening of colorectal cancer: Methods and strategies

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Abstract

Colorectal cancer (CRC) has high incidence and mortality rates, and the emergence and application of CRC screening have helped us effectively control the occurrence and development of CRC. Currently, common international screening methods include tests based on feces and blood, and examination methods that allow for visualization, such as sigmoidoscopy and colonoscopy. Some methods have been widely used, whereas others such as multi-target stool RNA test are still being explored and developed, and are expected to become front-line screening methods for CRC in the future. The choice of screening method is affected by external conditions and the patients' situation, and the clinician must choose an appropriate strategy according to the actual situation and the patient's wishes. This article introduces various CRC screening methods and analyzes the factors relevant to the screening strategy.

Key Words: Colorectal cancer; Screening; Stool-based test; Endoscopic examination; Computed tomography colonography; Blood-based test

Core Tip: Screening for colorectal cancer have helped us effectively control its occurrence and development. This article presents various colorectal cancer screening methods and analyzes pertinent factors in screening strategies.

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INTRODUCTION

Colorectal cancer (CRC) ranks third globally in terms of cancer incidence (10.0%), and second in terms of mortality (9.4%) [1]. Advanced CRC readily metastasizes to the liver, lungs, bones, brain, and even pancreas[2] and spleen[3], significantly impacting patient prognosis. Hence, early screening for CRC and even intercepting it in the precancerous state are crucial for reducing the incidence and mortality rates of CRC.

In the early 1990s, the American Cancer Society, National Cancer Institute, and American College of Physicians advocated sigmoidoscopy or fecal occult blood tests for CRC screening based on clinical practice, despite the absence of reported randomized controlled trials (RCTs) supporting the safety, efficacy, and practicality of these methods at that time. With medical advancements, colonoscopy, fecal immunochemical tests (FIT), computed tomography colonography (CTC), and FIT-DNA tests have gradually emerged as new screening modalities[4]. Screening plays a pivotal role in reducing mortality by identifying asymptomatic early-stage cancers and preventing diseases by detecting and removing precancerous lesions (adenomas and serrated polyps). Recent data also demonstrate that widespread CRC screening has decreased CRC mortality rates globally[5].

In addition to the benefits of screening methods, judicious application of screening strategies aids in diminishing disease occurrence and progression. Each screening method has its own set of advantages and disadvantages, and is suitable for different demographic groups. Correspondingly, each method has a recommended screening frequency, and the recommended onset and cessation ages for screening are continuously adjusted based on the evolving clinical reality. Therefore, this article presents various CRC screening methods and analyzes pertinent factors in screening strategies.

METHODS OF SCREENING

Stool-based test

Guaiac-based fecal occult blood test: The guaiac-based fecal occult blood test (gFOBT) method leverages the peroxidase activity of hemoglobin in stool to decompose hydrogen peroxide in the reagent, reacting with a guaiac developer to indicate the presence of hemoglobin. This approach is simple to perform, can be conveniently conducted at home, and poses no harm to the patient's body, making it an ideal screening method for early-stage detection. Numerous studies have demonstrated that annual or biennial gFOBT effectively reduced CRC morality rate. Lin *et al*[6] synthesized findings from five RCTs in a comprehensive study. Over a follow-up period ranging from 11-30 years, the study compared CRC mortality rates between the screening and non-screening groups after 2-9 rounds of screening, revealing a lower mortality rate in the screening group [at 19.5 years, risk ratio (RR) = 0.91, 95% confidence interval (CI): 0.84-0.98; at 30 years, RR = 0.78, 95%CI: 0.65-0.93]. Another meta-analysis of 10 RCTs and 47 model studies indicated that gFOBT screening reduced the CRC-specific mortality rate by 21% [RR = 0.79, (95%CI: 0.68-0.91), P =0.001][7]. However, the gFOBT detection method lacks specificity for blood. Dietary substances with peroxidase activity (such as plant peroxidase and red meat) can also yield positive test results, whereas antioxidants (such as ascorbic acid) may inhibit hemoglobin peroxidase activity, leading to false-negative results[8]. Consequently, dietary restrictions are necessary before the gFOBT examination. The sensitivity of gFOBT is notably lower than that of other screening methods and may require multiple samples. The effect of gFOBT on the incidence risk of CRC is limited because the early manifestations of CRC often involves polyps without bleeding symptoms, and only colonoscopy can reliably detect the lesions. The USPSTF recommends annual gFOBT screening.

FIT: The FIT utilizes the high sensitivity of the antigen-antibody response of human hemoglobin to quantitatively detect the level of hemoglobin in feces, measuring the content of human hemoglobin per gram of formed feces. Compared with gFOBT, FIT not only has the advantages of non-invasive and ease of performance but also exhibits high specificity for CRC screening. The FIT remains unaffected by diet or medication, and its sample collection is simpler, thereby increasing population compliance. Automated test reading and quantitative measurement of fecal hemoglobin concentration with FIT enable the adjustment of positively threshold and sensitivity for CRC detection[9]. Findings from an RCT suggests that FIT is significantly more sensitive than gFOBT (sensitivity difference = 18.9%, 95%CI: 10.2-32.6)[10]. Grobbee *et al*[11] analyzed data from 63 studies, revealing sensitivities of 59% for gFOBT and 89% for FIT for CRC detection. In recent years, FIT has progressively supplanted gFOBT as the preferred stool screening method for CRC owing to its relative advantages[12]. However, to date, no randomized trial has assessed the long-term effectiveness of FIT on CRC mortality. Although data modeling has predicted a significant reduction in the CRC-specific mortality rate with FIT compared to no screening[7], actual prospective data as reference and evidence are lacking. The USPSTF recommends annual FIT screening.

Stool DNA test: Currently, the only stool DNA test approved by the US Food and Drug Administration is the multi-target stool DNA (mt-sDNA) test, which integrates a FIT component known as the FIT-DNA test. This test detects cells containing tumor-altered DNA shed from colon cancer by sensitively identifying specific genetic and epigenetic biomarkers, allowing for differentiation between tumor and non-tumor tissues. Leveraging this principle, the stool DNA test identifies DNA biomarkers from cancer cells sloughed off the inner membrane of the colon and rectum in the fecal matter. As a standalone screening test, the stool DNA test surpasses the FIT in terms of sensitivity. The mt-sDNA test boasts a 92% sensitivity for CRC detection, significantly higher than that of FIT (74%)[13]. Nonetheless, the mt-sDNA test exhibits low specificity, leading to false-positive results. Consequently, patients with positive stool screening results are advised to undertake sigmoid colonoscopy or colonoscopy to ascertain the presence of lesions. An increase in false-positive outcomes necessitate more follow-up colonoscopy examinations and associated adverse events. Similar to FIT,

there is no direct evidence evaluating the impact of stool DNA testing on CRC mortality[14]. The USPSTF recommends stool DNA screening every 1-3 years.

Multi-target stool RNA test: The multi-target stool RNA (mt-sRNA) test is not yet available in the market and is still undergoing phase III clinical trials. In a blinded, prospective, cross-sectional study conducted by Barnell *et al*[15] from June 2021 to June 2022, 8920 participants aged > 45 years from 49 states in the United States completed the mt-sRNA test. Stool samples were collected form participants before undergoing colonoscopy at a local endoscopy center. After the study, the results of the mt-sRNA test (positive or negative) were compared with the data obtained from other tests. The findings revealed that, compared to FIT, the mt-sRNA test exhibited significantly improved sensitivity for detecting CRC (94% *vs* 78%, respectively, McNemar $P = 0.01$). We anticipate further reports on the experimental results from the Erica K team to enhance our understanding of the mt-sRNA test. Once this method becomes commercially available, it is imperative to conduct additional research to ascertain the specificity of mt-sRNA testing for CRC screening and its impact on CRC incidence and mortality. Such studies will aid in the informed selection and application of this method in the future.

Endoscopic examination

Sigmoidoscopy: Sigmoidoscopy involves the use of a flexible endoscope to visually assess the internal condition of the rectum and distal colon after intestinal cleansing. This method accurately screens for CRC and identifies and treats precancerous lesions (such as adenomas and serrated polyps) to prevent CRC occurrence. Typically, sedation or anesthesia is unnecessary during sigmoidoscopy. As the earliest endoscopic method employed for CRC screening, sigmoidoscopy, along with gFOBT, is the sole CRC screening method supported by evidence from RCTs. Sigmoidoscopy screening contributes to a reduction in the CRC mortality rate. In an RCT with a median follow-up of 16.8 years, the CRC mortality rate with sigmoidoscopy screening (417 deaths, 3.37 per 10000 people per year) was lower than that of the unscreened group (549 deaths, 4.48 per 10000 people per year, RR = 0.75, 95%CI: 0.66-0.85). Flexible sigmoidoscopy screening continues to exhibit long-term efficacy in reducing CRC mortality rates[16]. A meta-analysis encompassing four large-scale randomized trials of sigmoidoscopy screening conducted in Norway, the United States, the United Kingdom, and Italy, with a 15-year follow-up of the screened population, revealed a difference in CRC mortality of 0.13 deaths per 100 individuals (95%CI: 0.07-0.19) and a mortality ratio of 0.80 (95%CI: 0.72-0.88) between the sigmoidoscopy screening and non-screening groups[17]. In a meta-analysis comprising solely RCTs, patients undergoing sigmoidoscopy, colonoscopy, and gFOBT were followed up and compared with those receiving standard care, revealing that the sole screening test that significantly extended life was sigmoidoscopy (110 d, 95%CI: 0-274). Current evidence does not support the notion that common CRC screening tests prolong life, except for sigmoidoscopy[18]. Nevertheless, sigmoidoscopy has largely been supplanted by colonoscopy, which can examine the entire large intestine and is more effective than sigmoidoscopy[14]. The USPSTF recommends sigmoidoscopy every 5 years, or every 10 years if combined with annual FIT.

Colonoscopy: Before undergoing a colonoscopy examination, patients must adhere to specific standards to ensure that their entire intestines are thoroughly cleansed. Similar to sigmoidoscopy, endoscopists can address precancerous lesions during examination. However, colonoscopy offers the advantage of visualizing the entire colon and rectum, covering a broader area than sigmoidoscopy. Additionally, patients have the option to choose sedation or anesthesia during the examination, which improves the comfort during examination. In an RCT, after a 10-year follow-up, the risk of CRC decreased from 1.22% to 0.84%, and the risk of CRC-related death decreased from 0.30% to 0.15% compared to the conventional care group. Participants invited for colonoscopy in this trial had a lower risk of CRC at the 10-year follow-up than those who were not screened[19]. One possible explanation is that colonoscopy not only screens for CRC but also prevents its occurrence by detecting precancerous lesions. Hence, colonoscopy screening can reduce both the mortality and incidence rates[20]. Further, rectal endoscopic ultrasonography can be used to identify locoregional tumor stage in the rectum with shear wave elastography[21]. Nonetheless, colonoscopy is more invasive and burdensome than stool-based tests or sigmoidoscopies. Colonoscopy examinations necessitate more clinical resources and lack RCT data. Despite being considered the gold standard for CRC screening, colonoscopy is not the most popular screening method for the general population. Therefore, colonoscopy has not replaced sigmoidoscopy in certain regions. Considering these factors, it is crucial to strike a balance between the benefits, harms, and cost-effectiveness of various CRC screening tests[19]. The USPSTF recommends colonoscopy screening every 10 years.

Colon capsule endoscopy: Colon capsule endoscopy (CCE) entails participants swallowing a capsule-sized wireless camera, which captures images of the colorectal mucosa and transmits them to the examiner for evaluation. If the images reveal colorectal polyps or cancer, a colonoscopy is necessary for definitive screening. CCE does not involve radiation exposure, sedation, or gas inhalation during examination[22,23]. The sensitivity of the latest CCE-2 (second generation) for detecting advanced tumors of 10 mm or larger is 76.7% (95%CI: 63.7-86.2), with a specificity of 90.7% (95%CI: 83.6-95.0). The sensitivity of this screening method depends on the percentage of colon surface area displayed in the images and the capsule excretion time. The sensitivity for detecting advanced tumors (> 6 mm) reaches 100% when the transmission time is 3-5 h. Although the USPSTF has not yet approved CCE for average-risk CRC screening, the US Food and Drug Administration has sanctioned its use for CRC screening in individuals with an incomplete history of colonoscopy or a high risk of complications during colonoscopy. Among individuals with incomplete colonoscopy, CCE outperforms CT colonography in detecting tumors > 6 mm and carries a lower risk of serious adverse events than traditional colonoscopy[24].

CTC

Before undergoing CTC, the patients were required to undergo intestinal preparation using laxatives, followed by oral administration of contrast medium and insufflation of gas *via* the rectum to expand the colon and rectum. Subsequently, an abdominal CT examination was conducted. The obtained images were then reconstructed using specialized computer software to generate two-dimensional or three-dimensional images of the large intestine[25], enabling the evaluation of polyps and cancer. In a meta-analysis involving 11151 patients, CTC demonstrated a sensitivity of 96.1% (95%CI: 93.8-97.7) in detecting CRC, which is comparable to that of colonoscopy[26]. However, a guidance statement indicated a sensitivity range of 0.86 to 1 for CTC, with no evidence from feasible studies evaluating its effectiveness in CRC screening [27]. The accuracy of CTC detection is contingent on the experience of the radiologist. Radiologists must undergo specific preliminary training to effectively perform and interpret test results[28]. The USPSTF recommends CTC screening every 5 years.

Blood-based test

Serum methylated septin 9 test: The blood-based SEPT9 methylation test is an inaugural FDA-approved blood test for CRC screening. This method targets the detection of abnormal methylation in the *SEPT9* gene promoter region of substances (circulating tumor DNA or ctDNA) released from CRC cells into the peripheral blood. A meta-analysis comprising 25 independent studies, predominantly case-control or cohort studies, with only one randomized multi-center screening study and two opportunistic screening studies included, demonstrated a sensitivity ranging from 48.2% to 95.6% and specificity ranging from 79.1% to 99.1%[29]. Another study compared the serum methylated septin 9 test (mSEPT9) in peripheral blood to gFOBT in 650 participants, revealing a positive result for mSEPT9 in 73% of patients with CRC, with a specificity of 94.5%. These data suggest that mSEPT9 outperforms gFOBT in CRC screening[30]. However, a positive result for mSEPT9 does not definitively indicate the presence of lesions akin to stool-based tests, necessitating confirmation *via* colonoscopy. A single-tube methylation-specific quantitative polymerase chain reaction assay (mqMSP) was developed, utilizing 10 different methylation markers to quantitatively analyze plasma samples containing tumor DNA at concentrations as low as 0.05%. In a cohort study, mqMSP detection demonstrated a sensitivity of 84.9% and specificity of 83.3%. Multichannel detection entails the simultaneous use of multiple circulating tumor DNA (ctDNA) markers, which may enhance the capability of liquid biopsies[31]. This approach could potentially represent a future development trend for serum mSEPT9 testing.

Cell-free DNA blood-based test: Cell-free DNA (cfDNA) is released by dying and decomposed cancer cells, containing tumor-specific genetic information. Analyzing specific mutations and methylation patterns in these DNA fragments enables the detection of cancer through cfDNA blood-based tests. A recent large-scale study evaluated the performance of cfDNA blood-based tests in asymptomatic and early-stage CRC within a screening-relevant population. The results demonstrated an 83% (95%CI: 72.2-90.3) sensitivity for CRC and 90% (95%CI: 88.8-90.3) specificity for advanced neoplasia, comparable to those of other screening methods. There was no apparent differences among subgroups and no reported serious adverse events. Additionally, blood-based tests are seamlessly integrated into routine healthcare encounters and are relatively simple to complete, making them easier to administer and enhancing patient adherence. The study reported a 3.7% rate of invalid cfDNA blood-based test results, well within the recommended target range (< 5%) for programmatic FIT offering[32]. Thus, cfDNA blood-based tests represent a promising alternative for CRC screening.

STRATEGIES OF SCREENING

Repeated testing

For stool-based screening methods, such as FIT, the CRC detection rate from a single test is low but significantly improves with repeated testing, surpassing the detection rates of other methods. A randomized trial indicated similar CRC detection rates between one-round FIT every two years and once sigmoidoscopy, with even higher rates observed with three-round FIT. Moreover, in detecting advanced adenomas, the detection rate in the one-round FIT group was lower than that in the once-sigmoidoscopy group, while the three-round FIT group exhibited a higher detection rate than the one-round FIT group. The incidence of complications was similar between the repeat FIT and sigmoidoscopy groups [9]. However, repeated testing is contingent on obtaining negative results. If the fecal test result is positive, a colonoscopy should be performed to confirm the presence of lesions rather than repeating the fecal test.

Age

In the past, experts recommended initiating CRC screening in the general population at the age of 50 years, with CRC diagnosed before this age categorized as early onset CRC (eoCRC), which was relatively uncommon. However, studies have found that certain populations, such as black individuals, possess genes predisposing them to a higher risk of CRC, warranting a lower screening age threshold (*e.g.*, 45 years) for more effective early disease detection. Nevertheless, this recommendation did not accurately reflect the situation for the majority of the population and lacked support from realistic data, thus failing to gain widespread acceptance[4]. Before 2018, most international guidelines recommended CRC screening for average-risk individuals aged between 50 and 75[33]. However, with shifting demographic trends, the incidence and mortality rates of eoCRC have increased.

To address the growing burden of eoCRC, the American Cancer Society revised its screening guidelines in 2018, lowering the starting age of average-risk individuals from 50 years to 45 years. Subsequently, in October 2020, the USPSTF issued a draft recommendation statement supporting this adjustment. Notably, in 2020, over 140000 people in the United States were diagnosed with CRC, with one-seventh of these cases occurring in individuals under the age of 50 years[34].

In 2021, the USPSTF released updated CRC screening guidelines, recommending screening for all adults aged 50 years to 75 years (level A recommendation). Additionally, it advised CRC screening for adults aged 45-49 years (level B recommendation). For individuals aged 76 years to 85 years, the USPSTF suggested that clinicians selectively consider CRC screening (level C recommendation), as evidence indicates minimal net benefit from screening in this age group. When determining the appropriateness of CRC screening for individuals, patients and clinicians should consider factors such as overall health status, screening history, and personal preferences[14].

Adjustment of the screening age marks the beginning of a new era, bringing forth both opportunities and challenges for population screening[35].

Compliance

The compliance of participants in various screening methods significantly impacts the experimental results. For instance, in an RCT comparing colonoscopy, gFOBT, and FIT, researchers observed relatively low compliance in the colonoscopy group compared to that in the gFOBT and FIT groups. This discrepancy may be attributed to the invasiveness of colonoscopy, the need for intestinal preparation, and the participants' limited knowledge of CRC screening[36]. In another randomized trial, the compliance rate was 83.6% in the colonoscopy group and 73.1% in the gFOBT group after the first screening round (RR = 1.14, 95%CI: 1.10-1.19, $P \leq 0.001$). However, compliance decreased to 38.3% after four consecutive rounds of gFOBT, indicating unsatisfactory compliance with multiple rounds of gFOBT compared to colonoscopy screening[37]. Approximately 33% of adults aged 50-75 years did not undergo screening as recommended, further highlighting low compliance rates[38]. Such low compliance rates can lead to experimental results failing to meet expectations, resulting in a lower CRC detection rate and impacting the overall judgment of the study.

CONCLUSION

As CRC screening becomes more widespread, numerous studies have consistently demonstrated its crucial role in reducing CRC mortality, although its impact on CRC morbidity varies. Recent data reveal significant declines in the age-standardized incidence of CRC in certain countries, such as the United States, Japan, and France, while other countries, such as Baltic countries, Russia, China, and Brazil, experience an increase in CRC incidence. In high-incidence countries, the decline in CRC incidence is attributed to population-level changes driven by healthier lifestyle choices, such as reduced smoking rates and increased acceptance of screening.

Despite the availability of numerous screening methods, determining the most effective strategy remains challenging owing to limited evidence. The International Agency for Research on Cancer asserts that insufficient evidence exists to rank the effectiveness of screening tests, with no face-to-face studies demonstrating the superiority of one method in reducing CRC mortality or morbidity. However, evidence suggests that in some emerging economies, utilizing more affordable and less invasive methods, such as gFOBT and FIT, for CRC screening may be cost-effective. This approach provides viable options for managing escalating disease burden.

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FOOTNOTES

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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 **Rizza S**, Maldi E, Laudi C, Mellano A, Pisacane A, Staiano T. A pancreatic metastasis from a colon carcinoma mimicking a primary tumor diagnosed by EUS-guided fine-needle biopsy. *Endosc Ultrasound* 2023; **12**: 162-163 [PMID: 35313422 DOI: 10.4103/EUS-D-21-00186]
3 **Deshmukh A**, Elmeligui AM, Okasha HH, Parsa N, Tejedor-Tejada J, Nieto J. EUS-guided fiducial gold marker placement in metastatic colon cancer to the spleen. *Endosc Ultrasound* 2022; **11**: 79-80 [PMID: 34213433 DOI: 10.4103/EUS-D-21-00023]
4 **Lerner BH**, Curtiss-Rowlands G. What Constitutes Evidence? Colorectal Cancer Screening and the U.S. Preventive Services Task Force. *J Gen Intern Med* 2022; **37**: 2855-2860 [PMID: 35428902 DOI: 10.1007/s11606-022-07555-9]
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 **Lin JS**, Perdue LA, Henrikson NB, Bean SI, Blasi PR. Screening for Colorectal Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 2021; **325**: 1978-1998 [PMID: 34003220 DOI: 10.1001/jama.2021.4417]
7 **Zheng S**, Schrijvers JJA, Greuter MJW, Kats-Ugurlu G, Lu W, de Bock GH. Effectiveness of Colorectal Cancer (CRC) Screening on All-Cause and CRC-Specific Mortality Reduction: A Systematic Review and Meta-Analysis. *Cancers (Basel)* 2023; **15** [PMID: 37046609 DOI: 10.3390/cancers15071948]
8 **Sokoro A**, Singh H. Fecal Occult Blood Test for Evaluation of Symptoms or for Diagnostic Testing. *Am J Gastroenterol* 2020; **115**: 679-680 [PMID: 32058343 DOI: 10.14309/ajg.0000000000000560]
9 **Randel KR**, Schult AL, Botteri E, Hoff G, Bretthauer M, Ursin G, Natvig E, Berstad P, Jørgensen A, Sandvei PK, Olsen ME, Frigstad SO, Darre-Næss O, Norvard ER, Bolstad N, Kørner H, Wibe A, Wensaas KA, de Lange T, Holme Ø. Colorectal Cancer Screening With Repeated Fecal Immunochemical Test Versus Sigmoidoscopy: Baseline Results From a Randomized Trial. *Gastroenterology* 2021; **160**: 1085-1096.e5 [PMID: 33227280 DOI: 10.1053/j.gastro.2020.11.037]
10 **Shapiro JA**, Bobo JK, Church TR, Rex DK, Chovnick G, Thompson TD, Zauber AG, Lieberman D, Levin TR, Joseph DA, Nadel MR. A Comparison of Fecal Immunochemical and High-Sensitivity Guaiac Tests for Colorectal Cancer Screening. *Am J Gastroenterol* 2017; **112**: 1728-1735 [PMID: 29016558 DOI: 10.1038/ajg.2017.285]
11 **Grobbee EJ**, Wisse PHA, Schreuders EH, van Roon A, van Dam L, Zauber AG, Lansdorp-Vogelaar I, Bramer W, Berhane S, Deeks JJ, Steyerberg EW, van Leerdam ME, Spaander MC, Kuipers EJ. Guaiac-based faecal occult blood tests versus faecal immunochemical tests for colorectal cancer screening in average-risk individuals. *Cochrane Database Syst Rev* 2022; **6**: CD009276 [PMID: 35665911 DOI: 10.1002/14651858.CD009276.pub2]
12 **Ladabaum U**, Dominitz JA, Kahi C, Schoen RE. Strategies for Colorectal Cancer Screening. *Gastroenterology* 2020; **158**: 418-432 [PMID: 31394083 DOI: 10.1053/j.gastro.2019.06.043]
13 **Carethers JM**. Fecal DNA Testing for Colorectal Cancer Screening. *Annu Rev Med* 2020; **71**: 59-69 [PMID: 31451044 DOI: 10.1146/annurev-med-103018-123125]
14 **US Preventive Services Task Force**, Davidson KW, Barry MJ, Mangione CM, Cabana M, Caughey AB, Davis EM, Donahue KE, Doubeni CA, Krist AH, Kubik M, Li L, Ogedegbe G, Owens DK, Pbert L, Silverstein M, Stevermer J, Tseng CW, Wong JB. Screening for Colorectal Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* 2021; **325**: 1965-1977 [PMID: 34003218 DOI: 10.1001/jama.2021.6238]
15 **Barnell EK**, Wurtzler EM, La Rocca J, Fitzgerald T, Petrone J, Hao Y, Kang Y, Holmes FL, Lieberman DA. Multitarget Stool RNA Test for Colorectal Cancer Screening. *JAMA* 2023; **330**: 1760-1768 [PMID: 37870871 DOI: 10.1001/jama.2023.22231]
16 **Miller EA**, Pinsky PF, Schoen RE, Prorok PC, Church TR. Effect of flexible sigmoidoscopy screening on colorectal cancer incidence and mortality: long-term follow-up of the randomised US PLCO cancer screening trial. *Lancet Gastroenterol Hepatol* 2019; **4**: 101-110 [PMID: 30502933 DOI: 10.1016/S2468-1253(18)30358-3]
17 **Juul FE**, Cross AJ, Schoen RE, Senore C, Pinsky P, Miller E, Segnan N, Wooldrage K, Wieszczy-Szczepanik P, Armaroli P, Garborg KK, Adami HO, Hoff G, Kalager M, Bretthauer M, Løberg M, Holme Ø. 15-Year Benefits of Sigmoidoscopy Screening on Colorectal Cancer Incidence and Mortality : A Pooled Analysis of Randomized Trials. *Ann Intern Med* 2022; **175**: 1525-1533 [PMID: 36215714 DOI: 10.7326/M22-0835]
18 **Bretthauer M**, Wieszczy P, Løberg M, Kaminski MF, Werner TF, Helsingen LM, Mori Y, Holme Ø, Adami HO, Kalager M. Estimated Lifetime Gained With Cancer Screening Tests: A Meta-Analysis of Randomized Clinical Trials. *JAMA Intern Med* 2023; **183**: 1196-1203 [PMID: 37639247 DOI: 10.1001/jamainternmed.2023.3798]
19 **Bretthauer M**, Løberg M, Wieszczy P, Kalager M, Emilsson L, Garborg K, Rupinski M, Dekker E, Spaander M, Bugajski M, Holme Ø, Zauber AG, Pilonis ND, Mroz A, Kuipers EJ, Shi J, Hernán MA, Adami HO, Regula J, Hoff G, Kaminski MF; NordICC Study Group. Effect of Colonoscopy Screening on Risks of Colorectal Cancer and Related Death. *N Engl J Med* 2022; **387**: 1547-1556 [PMID: 36214590 DOI: 10.1056/NEJMoa2208375]
20 **Keum N**, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat Rev Gastroenterol*

Hepatol 2019; **16**: 713-732 [PMID: 31455888 DOI: 10.1038/s41575-019-0189-8]

21 **Cui XW**, Li KN, Yi AJ, Wang B, Wei Q, Wu GG, Dietrich CF. Ultrasound elastography. *Endosc Ultrasound* 2022; **11**: 252-274 [PMID: 35532576 DOI: 10.4103/EUS-D-21-00151]

22 **Shaukat A**, Kahi CJ, Burke CA, Rabeneck L, Sauer BG, Rex DK. ACG Clinical Guidelines: Colorectal Cancer Screening 2021. *Am J Gastroenterol* 2021; **116**: 458-479 [PMID: 33657038 DOI: 10.14309/ajg.0000000000001122]

23 **Shaukat A**, Levin TR. Current and future colorectal cancer screening strategies. *Nat Rev Gastroenterol Hepatol* 2022; **19**: 521-531 [PMID: 35505243 DOI: 10.1038/s41575-022-00612-y]

24 **Pecere S**, Senore C, Hassan C, Riggi E, Segnan N, Pennazio M, Sprujievnik T, Rondonotti E, Baccarin A, Quintero E, Adrian de Ganzo Z, Costamagna G, Spada C. Accuracy of colon capsule endoscopy for advanced neoplasia. *Gastrointest Endosc* 2020; **91**: 406-414.e1 [PMID: 31629719 DOI: 10.1016/j.gie.2019.09.041]

25 **Bryce C**, Bucaj M. CT Colonography for Colorectal Cancer Screening. *Am Fam Physician* 2021; **103**: 55-56 [PMID: 33382558]

26 **Pickhardt PJ**, Hassan C, Halligan S, Marmo R. Colorectal cancer: CT colonography and colonoscopy for detection--systematic review and meta-analysis. *Radiology* 2011; **259**: 393-405 [PMID: 21415247 DOI: 10.1148/radiol.11101887]

27 **Qaseem A**, Harrod CS, Crandall CJ, Wilt TJ; Clinical Guidelines Committee of the American College of Physicians, Balk EM, Cooney TG, Cross JT Jr, Fitterman N, Maroto M, Obley AJ, Tice J, Tufte JE, Shamliyan T, Yost J. Screening for Colorectal Cancer in Asymptomatic Average-Risk Adults: A Guidance Statement From the American College of Physicians (Version 2). *Ann Intern Med* 2023; **176**: 1092-1100 [PMID: 37523709 DOI: 10.7326/M23-0779]

28 **Kroijer R**, Kobaek-Larsen M, Qvist N, Knudsen T, Baatrup G. Colon capsule endoscopy for colonic surveillance. *Colorectal Dis* 2019; **21**: 532-537 [PMID: 30637886 DOI: 10.1111/codi.14557]

29 **Song L**, Jia J, Peng X, Xiao W, Li Y. The performance of the SEPT9 gene methylation assay and a comparison with other CRC screening tests: A meta-analysis. *Sci Rep* 2017; **7**: 3032 [PMID: 28596563 DOI: 10.1038/s41598-017-03321-8]

30 **Sun J**, Fei F, Zhang M, Li Y, Zhang X, Zhu S, Zhang S. The role of (m)SEPT9 in screening, diagnosis, and recurrence monitoring of colorectal cancer. *BMC Cancer* 2019; **19**: 450 [PMID: 31088406 DOI: 10.1186/s12885-019-5663-8]

31 **Jin S**, Zhu D, Shao F, Chen S, Guo Y, Li K, Wang Y, Ding R, Gao L, Ma W, Lu T, Li D, Zhang Z, Cai S, Liang X, Song H, Ji L, Li J, Zheng Z, Jiang F, Wu X, Luan J, Zhang H, Yang Z, Cantor CR, Xu C, Ding C. Efficient detection and post-surgical monitoring of colon cancer with a multi-marker DNA methylation liquid biopsy. *Proc Natl Acad Sci U S A* 2021; **118** [PMID: 33495330 DOI: 10.1073/pnas.2017421118]

32 **Chung DC**, Gray DM 2nd, Singh H, Issaka RB, Raymond VM, Eagle C, Hu S, Chudova DI, Talasaz A, Greenson JK, Sinicrope FA, Gupta S, Grady WM. A Cell-free DNA Blood-Based Test for Colorectal Cancer Screening. *N Engl J Med* 2024; **390**: 973-983 [PMID: 38477985 DOI: 10.1056/NEJMoa2304714]

33 **Bénard F**, Barkun AN, Martel M, von Renteln D. Systematic review of colorectal cancer screening guidelines for average-risk adults: Summarizing the current global recommendations. *World J Gastroenterol* 2018; **24**: 124-138 [PMID: 29358889 DOI: 10.3748/wjg.v24.i1.124]

34 **Siegel RL**, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 145-164 [PMID: 32133645 DOI: 10.3322/caac.21601]

35 **Burnett-Hartman AN**, Lee JK, Demb J, Gupta S. An Update on the Epidemiology, Molecular Characterization, Diagnosis, and Screening Strategies for Early-Onset Colorectal Cancer. *Gastroenterology* 2021; **160**: 1041-1049 [PMID: 33417940 DOI: 10.1053/j.gastro.2020.12.068]

36 **Chen H**, Li N, Ren J, Feng X, Lyu Z, Wei L, Li X, Guo L, Zheng Z, Zou S, Zhang Y, Li J, Zhang K, Chen W, Dai M, He J; group of Cancer Screening Program in Urban China (CanSPUC). Participation and yield of a population-based colorectal cancer screening programme in China. *Gut* 2019; **68**: 1450-1457 [PMID: 30377193 DOI: 10.1136/gutjnl-2018-317124]

37 **Zauber AG**, Winawer SJ, O'Brien MJ, Mills GM, Allen JI, Feld AD, Jordan PA, Fleisher M, Orlow I, Meester RGS, Lansdorp-Vogelaar I, Rutter CM, Knudsen AB, Mandelson M, Shaukat A, Mendelsohn RB, Hahn AI, Lobaugh SM, Soto Palmer B, Serrano V, Kumar JR, Fischer SE, Chen JC, Bayuga-Miller S, Kuk D, O'Connell K, Church TR. Randomized Trial of Facilitated Adherence to Screening Colonoscopy *vs* Sequential Fecal-Based Blood Test. *Gastroenterology* 2023; **165**: 252-266 [PMID: 36948424 DOI: 10.1053/j.gastro.2023.03.206]

38 **Leach KM**, Granzow ME, Popalis ML, Stoltzfus KC, Moss JL. Promoting colorectal cancer screening: A scoping review of screening interventions and resources. *Prev Med* 2021; **147**: 106517 [PMID: 33711351 DOI: 10.1016/j.ypmed.2021.106517]

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EDITORIAL

Poly (ADP-ribose): A double-edged sword governing cancer cell survival and death

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Abstract

Poly (ADP-ribose) (PAR), a polymer of ADP-ribose, is synthesized by PAR polymerase and is crucial for the survival of cancer cells due to its vital functions in DNA repair and post-translational modifications. Beyond its supportive role, PAR also triggers cancer cell death by excessive accumulation of PAR leading to an energy crisis and parthanatos. This phenomenon underscores the potential of targeting PAR regulation as a novel anticancer strategy, and the rationale would present an engaging topic in the field of anticancer research. Therefore, this editorial provides an overview of the mechanisms determining cancer cell fate, emphasizing the central role of PAR. It further introduces promising methods for modulating PAR concentrations that may pave the way for innovative anticancer therapies.

Key Words: ADP-ribose; Poly (ADP-ribose); ADP ribosylation; PARylation; Parthanatos; Anticancer

Core Tip: The biosynthesis of Poly (ADP-ribose) (PAR) by PAR polymerase plays a pivotal role in modulating cellular processes that dictate the survival or death of cancer cells. Considering the dichotomous functions of PAR is instrumental in unveiling novel strategies for cancer treatment.

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INTRODUCTION

Poly (ADP-ribose), or PAR, is a polymer structure in which ADP-ribose is covalently bound to various amino acid residues of target genes or linked to each other *via* ribose-ribose bonds[1]. The polymerization of ADP-ribose from the donor molecule, nicotinamide adenine dinucleotide (NAD)+, is catalyzed by PAR polymerase (PARP). This biochemical action yields a linear or branched conformation, depending on whether the ADP-ribose is attached to the 2′-OH terminus of a growing chain or an ADP-ribose residue adjacent to the PARylation target[2,3]. The formation of PAR enables it to interact non-covalently with other molecules and act as a regulator mediating physiological signals involving potential molecular biological and biochemical reactions[4]. Recent studies have shown that PAR could a significant role in the entire process of carcinogenesis, including initiation, promotion, progression, and metastasis[5]. In other words, PAR can be associated with a major network to understand malignant transformation as a multistep process of cell cycle and signaling regulation, including genomic instability, which represents cancer cell proliferation and differentiation from a functional perspective[6,7]. Despite the involvement of PAR in all major post-translational modification processes in carcinogenesis, the synthesis and hydrolysis of PAR are tightly controlled, with a half-life of only a few minutes[8]. In an environment where the hydrolysis of PAR is delayed, the excessive accumulation of intact PAR freely dissociated from the target can conversely induce cell death[9]. An approach to cancer treatment that leverages this unique entity, which can simultaneously control the survival and death of cancer cells, is considered a promising avenue to explore. In this respect, this editorial briefly introduces the general knowledge about PAR and intriguing methods to induce anticancer effects by regulating PAR.

THE DUAL ROLE OF PAR INVOLVED IN CANCER CELL SURVIVAL AND DEATH

The biosynthesis of PAR is part of various physiological processes that are intrinsically linked to the survival of cancer cells. Therefore, continuous efforts are being made to uncover the relationship between PAR and carcinogenesis. The numerous functions of PAR can be categorized into three main categories based on their roles: overcoming genomic instability in response to DNA damage, regulating proliferation and differentiation, and mitosis[10-17]. Firstly, PAR acts as an epigenetic regulator under conditions of genetic instability caused by external or internal stress[11]. PARylation on histone proteins is associated with an open chromatin structure, crucial for maintaining DNA integrity[10]. At sites of DNA damage, this function is vital for recruiting relevant factors for DNA damage repair, such as X-ray repair cross-complementing and proliferating cell nuclear antigen[16,17]. Secondly, PAR directly regulates the recruitment of transcription factors and cofactors[14]. PARP-1 triggers the synthesis of PAR, which in turn influences the upregulation of oncogenes or the downregulation of tumor suppressor genes. This activity plays a key role in the growth and differentiation of cancer cells[13,14]. Lastly, PAR plays a role in mitosis. PARylation is responsible for the regulation of chromosomal proteins involved in chromosome segregation and interaction with spindle checkpoint components[10,15]. It is also crucial for the formation and maintenance of spindle polarity through covalent modification with spindle pole proteins that cross-link microtubule ends at the spindle poles[15]. This process facilitates the relentless proliferation characteristic of malignancy, ensuring the continuous replication and distribution of oncogenic genetic material necessary for cancer progression[15]. In contrast, the beneficial role of PAR in cancer cell survival may shift significantly under certain stress-inducing conditions. PARylation by PARP-1 is initially linked to DNA repair; however, its overactivation can lead to cell death due to a metabolic catastrophe and represents a caspase-independent cell death mechanism, distinct from conventional apoptosis[9]. This occurs when excessive DNA damage causes PARP-1 to overconsume NAD+, an essential substrate for synthetizing PAR, resulting in an energy crisis[18]. The process involves a complex interplay of cellular mechanisms, including the reversal of mitochondrial ATP synthase activity and the depletion of NAD+ levels[18,19]. Parthanatos represents another form of cell death by PAR accumulation, which leads to PAR translocation from the nucleus to the cytoplasm and alters mitochondrial function[20,21]. It is characterized by the release of apoptosis-inducing factor (AIF) from mitochondria to the nucleus, resulting in chromatin condensation and large-scale DNA fragmentation. Since AIF is known to be a binding protein with a high affinity for PAR, morphologically branched PAR would act as a more potent inducer of AIF release[20,21]. In summary, the functions of PAR within cancer cells are under strict regulation, they simultaneously exhibit dual roles - promoting survival or inducing death, contingent upon the internal conditions of cancer cells. The unique characteristic that the conjugated form of a single molecule can dictate both events of cancer cell fate underscores the potential of PAR as a therapeutic target.

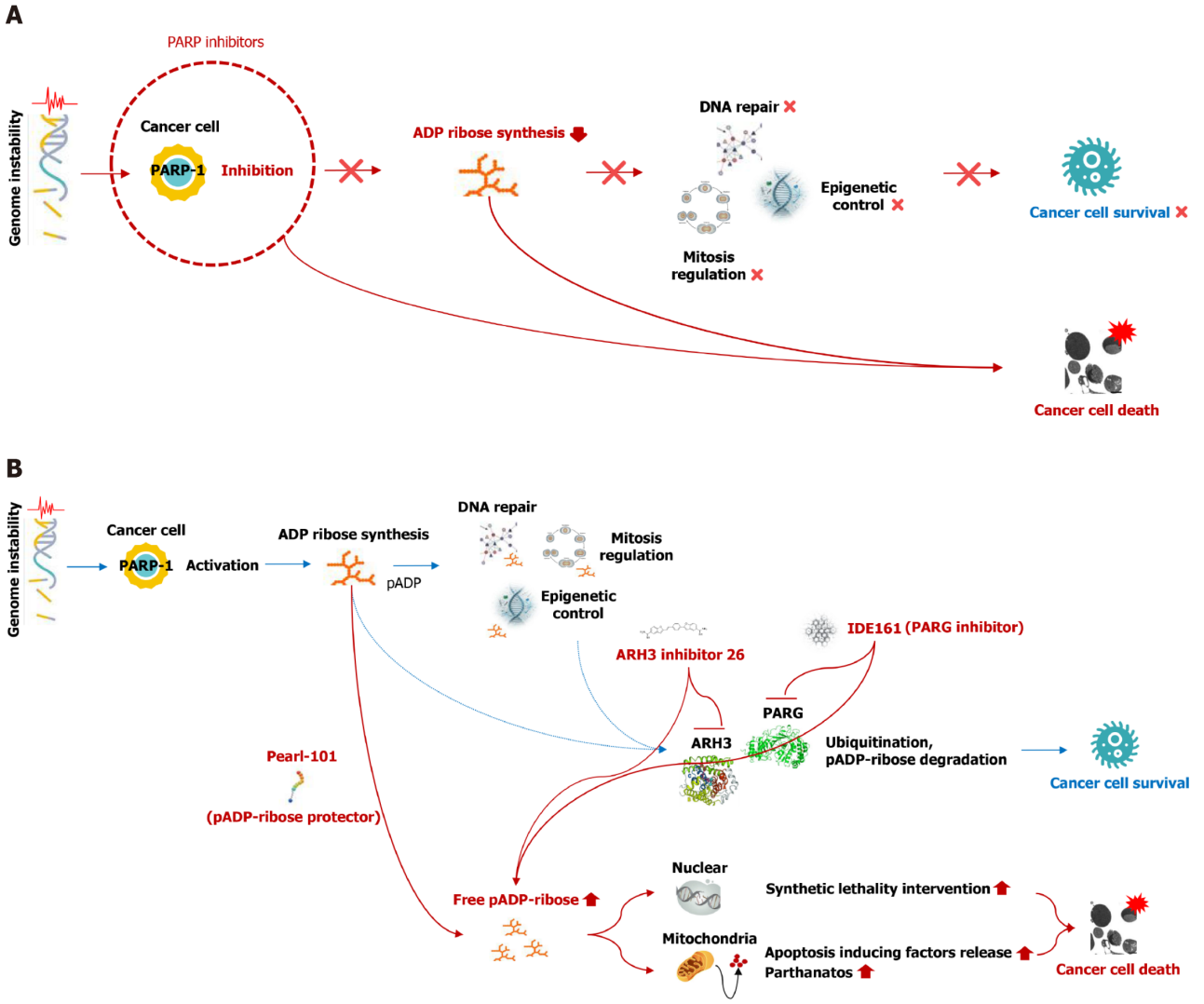

Figure 1 Strategies for inducing cancer cell death through poly (ADP-ribose) regulation. A: The anticancer effect is achieved by diminishing the synthesis of poly (ADP-ribose) (PAR), a process mediated by the inhibition of PAR polymerase-1; B: Promising strategies for the development of innovative anticancer treatments focused on PAR accumulation. The enzymes responsible for PAR degradation, such as PAR glycohydrolase or (ADP-ribosyl)hydrolase 3 (ARH3), are inhibited by IDE161 (IDEAYA Biosciences) or ARH3 inhibitor 26. Pearl-101, a molecule currently under development by PearlsInMires, plays a crucial role in maintaining the structural integrity of the PAR chain. The accumulation of PAR can lead to the death of cancer cells through a process known as synthetic lethality intervention and the release of apoptosis-inducing factors. PAR: Poly (ADP-ribose); PARG: PAR glycohydrolase; ARH3: (ADP-ribosyl)hydrolase 3; PARP-1: Poly (ADP-ribose) polymerase-1; pADP: Poly(ADP-ribose).

HOW TO CONQUER CANCER BY CONTROLLING THE DOUBLE-EDGED SWORD, PAR?

Given the substantial contribution of PAR in promoting cancer cell survival first, strategies that inhibit PAR synthesis could be prioritized in an anticancer area. It has been realized with the development of PARP inhibitors work by impeding the function of PARPs, the enzymes integral to DNA repair processes[22]. PARP inhibitors were designed to treat cancers with breast cancer susceptibility gene mutations, however, one of their key mechanisms of action is the regulation of PARylation[23]. By inhibiting PARylation, PARP inhibitors disrupt the survival of cancer cells, leading to their death (Figure 1A). This mechanism could be harnessed to extend their application in treating several types of cancer. Therefore, the recent research is also focused on the preclinical and clinical advancement of PARP inhibitors as standalone treatments and in combination with other therapies for gastrointestinal cancer[24]. Meanwhile, developing strategies to increase PAR levels could potentially enhance the anticancer effect by considering the mechanisms through which PAR induces cancer cell death. There are two primary strategies for preserving PAR levels within cancer cells: the first involves inhibiting ADP-ribosylhydrolases, such as PAR glycohydrolase (PARG) and (ADP-ribosyl)hydrolase 3 (ARH3), while the second strategy aims to protect PAR itself from these hydrolases (Figure 1B)[25,26]. The most advanced PARG inhibitor, IDE161, is currently under development by IDEAYA Biosciences. IDE161 operates by inhibiting the hydrolysis of PAR chains, leading to an accumulation of PAR and subsequently resulting in synthetic lethality (Figure 1B)[27]. IDE161 is under evaluation in a Phase 1 clinical trial, and this trial is strategically aimed at estrogen receptor-positive, human epidermal growth factor receptor 2-negative breast cancer, as well as other solid tumors with homologous recombination deficiency including colorectal cancer (NCT05787587)[28]. ARH3 inhibitor 26 (AI26) is another recognized

agent for ADP-ribosylhydrolase inhibition[26]. This novel small molecule selectively targets ARH3, binding to its catalytic pocket and inhibiting its enzymatic activity[26]. When cells are pretreated with AI26, there is an accumulation of PAR, leading to defects in DNA damage repair and the induction of synthetic lethality (Figure 1B). This reveals the potential of AI26 as a chemotherapeutic strategy in cancer treatment[26]. However, it is crucial to note that research on AI26 is still in progress, and further studies are required to fully comprehend its therapeutic benefits. In addition to the conventional approach using inhibitory agents, there exists a unique method that promotes intracellular PAR accumulation by protecting the PAR chain. Pearl-101, an anticancer drug currently under non-clinical development by PearlsInMires, employs this unique method (http://eng.pearlsinmires.com/). It recombines an endogenous peptide to specifically bind to PAR, highlighting a novel approach in cancer therapeutics. Pearl-101 directly protects the PAR chain, thereby disrupting the activity of ADP-ribosylhydrolase. This disruption leads to an accumulation of PAR in cancer cells, which subsequently triggers cancer cell death by parthanatos and synthetic lethality intervention with DNA fragmentation (Figure 1B). This approach has the benefit of specifically targeting areas where PAR is excessively synthesized, thereby ensuring precise action within cancer cells while minimizing adverse effects on normal cells. Taken together, inhibitors of PARP and ADP-ribosylhydrolases, and a PAR protector are being developed for cancer treatment as distinct methods to either reduce or accumulate PAR. Considering the distinct targets of these drugs, a synergistic anticancer effect would also be achieved through a combined approach such as a fixed-dose combination in addition to use as a single agent.

CONCLUSION

The dual nature of PAR, which can both support and challenge cancer cell survival, provides a wealth of opportunities for scientific and clinical exploration. As such, ongoing research into the effects of PAR on cancer cell fate is anticipated to be a promising area, and this suggests that PAR could emerge as a significant biomarker in clinical settings and may play a crucial role in anti-cancer strategies.

FOOTNOTES

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REFERENCES

1 **Leung AK**. Poly(ADP-ribose): an organizer of cellular architecture. *J Cell Biol* 2014; **205**: 613-619 [PMID: 24914234 DOI: 10.1083/jcb.201402114]
2 **Kim MY**, Zhang T, Kraus WL. Poly(ADP-ribosyl)ation by PARP-1: 'PAR-laying' NAD+ into a nuclear signal. *Genes Dev* 2005; **19**: 1951-1967 [PMID: 16140981 DOI: 10.1101/gad.1331805]
3 **Rack JGM**, Liu Q, Zorzini V, Voorneveld J, Ariza A, Honarmand Ebrahimi K, Reber JM, Krassnig SC, Ahel D, van der Marel GA, Mangerich A, McCullagh JSO, Filippov DV, Ahel I. Mechanistic insights into the three steps of poly(ADP-ribosylation) reversal. *Nat Commun* 2021; **12**: 4581 [PMID: 34321462 DOI: 10.1038/s41467-021-24723-3]
4 **Beneyton A**, Nonfoux L, Gagné JP, Rodrigue A, Kothari C, Atalay N, Hendzel MJ, Poirier GG, Masson JY. The dynamic process of covalent and non-covalent PARylation in the maintenance of genome integrity: a focus on PARP inhibitors. *NAR Cancer* 2023; **5**: zcad043 [PMID: 37609662 DOI: 10.1093/narcan/zcad043]
5 **Masutani M**, Nakagama H, Sugimura T. Poly(ADP-ribose) and carcinogenesis. *Genes Chromosomes Cancer* 2003; **38**: 339-348 [PMID: 14566854 DOI: 10.1002/gcc.10250]
6 **Masutani M**, Gunji A, Tsutsumi M, Ogawa K, Kamada N, Shirai T, Jishage K-i, Nakagama H, Sugimura T. Role of Poly-ADP-Ribosylation in Cancer Development. Poly(ADP-Ribosyl)ation. Boston, MA: Springer United States, 2006: 203-217
7 **Palazzo L**, Mikolčević P, Mikoč A, Ahel I. ADP-ribosylation signalling and human disease. *Open Biol* 2019; **9**: 190041 [PMID: 30991935

DOI: 10.1098/rsob.190041]
8 **Luo X**, Kraus WL. On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. *Genes Dev* 2012; **26**: 417-432 [PMID: 22391446 DOI: 10.1101/gad.183509.111]
9 **Andrabi SA**, Kim NS, Yu SW, Wang H, Koh DW, Sasaki M, Klaus JA, Otsuka T, Zhang Z, Koehler RC, Hurn PD, Poirier GG, Dawson VL, Dawson TM. Poly(ADP-ribose) (PAR) polymer is a death signal. *Proc Natl Acad Sci USA* 2006; **103**: 18308-18313 [PMID: 17116882 DOI: 10.1073/pnas.0606526103]
10 **Andronikou C**, Rottenberg S. Studying PAR-Dependent Chromatin Remodeling to Tackle PARPi Resistance. *Trends Mol Med* 2021; **27**: 630-642 [PMID: 34030964 DOI: 10.1016/j.molmed.2021.04.010]
11 **Rouleau-Turcotte É**, Pascal JM. ADP-ribose contributions to genome stability and PARP enzyme trapping on sites of DNA damage; paradigm shifts for a coming-of-age modification. *J Biol Chem* 2023; **299**: 105397 [PMID: 37898399 DOI: 10.1016/j.jbc.2023.105397]
12 **Brustel J**, Muramoto T, Fumimoto K, Ellins J, Pears CJ, Lakin ND. Linking DNA repair and cell cycle progression through serine ADP-ribosylation of histones. *Nat Commun* 2022; **13**: 185 [PMID: 35027540 DOI: 10.1038/s41467-021-27867-4]
13 **Jeong KY**, Park MH. The Significance of Targeting Poly (ADP-Ribose) Polymerase-1 in Pancreatic Cancer for Providing a New Therapeutic Paradigm. *Int J Mol Sci* 2021; **22** [PMID: 33805293 DOI: 10.3390/ijms22073509]
14 **Ryu KW**, Kim DS, Kraus WL. New facets in the regulation of gene expression by ADP-ribosylation and poly(ADP-ribose) polymerases. *Chem Rev* 2015; **115**: 2453-2481 [PMID: 25575290 DOI: 10.1021/cr5004248]
15 **Slade D**. Mitotic functions of poly(ADP-ribose) polymerases. *Biochem Pharmacol* 2019; **167**: 33-43 [PMID: 30910692 DOI: 10.1016/j.bcp.2019.03.028]
16 **Wei H**, Yu X. Functions of PARylation in DNA Damage Repair Pathways. *Genomics Proteomics Bioinformatics* 2016; **14**: 131-139 [PMID: 27240471 DOI: 10.1016/j.gpb.2016.05.001]
17 **Verdone L**, La Fortezza M, Ciccarone F, Caiafa P, Zampieri M, Caserta M. Poly(ADP-Ribosyl)ation Affects Histone Acetylation and Transcription. *PLoS One* 2015; **10**: e0144287 [PMID: 26636673 DOI: 10.1371/journal.pone.0144287]
18 **Hurtado-Bagès S**, Knobloch G, Ladurner AG, Buschbeck M. The taming of PARP1 and its impact on NAD(+) metabolism. *Mol Metab* 2020; **38**: 100950 [PMID: 32199820 DOI: 10.1016/j.molmet.2020.01.014]
19 **Hopp AK**, Teloni F, Bisceglie L, Gondrand C, Raith F, Nowak K, Muskalla L, Howald A, Pedrioli PGA, Johnsson K, Altmeyer M, Pedrioli DML, Hottiger MO. Mitochondrial NAD(+) Controls Nuclear ARTD1-Induced ADP-Ribosylation. *Mol Cell* 2021; **81**: 340-354.e5 [PMID: 33450210 DOI: 10.1016/j.molcel.2020.12.034]
20 **Fahrer J**, Kranaster R, Altmeyer M, Marx A, Bürkle A. Quantitative analysis of the binding affinity of poly(ADP-ribose) to specific binding proteins as a function of chain length. *Nucleic Acids Res* 2007; **35**: e143 [PMID: 17991682 DOI: 10.1093/nar/gkm944]
21 **Fatokun AA**, Dawson VL, Dawson TM. Parthanatos: mitochondrial-linked mechanisms and therapeutic opportunities. *Br J Pharmacol* 2014; **171**: 2000-2016 [PMID: 24684389 DOI: 10.1111/bph.12416]
22 **Kassab MA**, Yu LL, Yu X. Targeting dePARylation for cancer therapy. *Cell Biosci* 2020; **10**: 7 [PMID: 32010441 DOI: 10.1186/s13578-020-0375-y]
23 **Min A**, Im SA. PARP Inhibitors as Therapeutics: Beyond Modulation of PARylation. *Cancers (Basel)* 2020; **12** [PMID: 32046300 DOI: 10.3390/cancers12020394]
24 **Chen H**, Hu Y, Zhuang Z, Wang D, Ye Z, Jing J, Cheng X. Advancements and Obstacles of PARP Inhibitors in Gastric Cancer. *Cancers (Basel)* 2023; **15** [PMID: 37958290 DOI: 10.3390/cancers15215114]
25 **Slade D**. PARP and PARG inhibitors in cancer treatment. *Genes Dev* 2020; **34**: 360-394 [PMID: 32029455 DOI: 10.1101/gad.334516.119]
26 **Liu X**, Xie R, Yu LL, Chen SH, Yang X, Singh AK, Li H, Wu C, Yu X. AI26 inhibits the ADP-ribosylhydrolase ARH3 and suppresses DNA damage repair. *J Biol Chem* 2020; **295**: 13838-13849 [PMID: 32753484 DOI: 10.1074/jbc.RA120.012801]
27 **Abed M**, Muñoz D, Seshadri V, Federowicz S, Rao AA, Bhupathi D, Liimatta M, Ousterhout R, Jaipuri F, Neilan C, Lackner M, White M, Mounir Z. Abstract 6093: IDE161, a potential first-in-class clinical candidate PARG inhibitor, selectively targets homologous-recombination-deficient and PARP inhibitor resistant breast and ovarian tumors. *Cancer Res* 2023; **83**: 6093-6093 [DOI: 10.1158/1538-7445.am2023-6093]
28 **IDEAYA Biosciences**. A Study of PARG Inhibitor IDE161 in Participants With Advanced Solid Tumors. [accessed 2024 Jun 27]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://classic.clinicaltrials.gov/show/NCT05787587 ClinicalTrials.gov Identifier: NCT05787587

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EDITORIAL

Barriers in early detection of colorectal cancer and exploring potential solutions

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Abstract

This editorial discusses the literature review article by Tonini and Zanni, the paper was published in January 2024, and the authors provided very interesting conclusions regarding existing barriers to the early diagnosis of colon cancer. Many cancers do not have identifiable precursors, or there are currently no screening tests to find them. Therefore, these cancers do not have preventive screening options. Early detection is crucial for reducing mortality rates by identifying cancer at an earlier stage through screening, as opposed to no screening. Colorectal cancer develops from precancerous lesions, which can be detected early and potentially prevented and cured. Early detection leads to improved survival rates, decreased complications, and reduced healthcare expenses. This editorial provides a brief description of the biology of colon cancer, emphasizing the contrast in outcomes between early detection and late detection. We also describe screening programs around the globe and examine the barriers in each program. Finally, we explore potential future solutions to enhance inclusion in screening programs and improve patient compliance.

Key Words: Colon cancer; Rectal cancer; Early detection

Core Tip: The incidence of colon cancer is on the rise, affecting increasingly younger populations. Contributing factors include dietary changes, sedentary lifestyles, genetic predispositions, and environmental influences. To combat this trend, promoting awareness and encouraging preventive measures is crucial. Early detection of colon cancer is critical for improving survival rates and treatment outcomes. However, several barriers impede effective screening. This editorial article provides a detailed analysis of the obstacles outlined in Tonini and Zanni's publication and investigates potential strategies to enhance screening delivery to diverse populations.

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INTRODUCTION

In 2024, the American Cancer Society projects that there will be approximately 152810 newly diagnosed cases of colorectal cancer (CRC). Of the total number, 106590 (70%) cases will be diagnosed as colon cancer, while 46220 (30%) cases will be identified as rectal cancer. CRC is the second most common cause of cancer-related deaths in the United States. It is estimated that there will be 53010 deaths from CRC in 2024, which is a slight increase compared to the estimated 52550 deaths in 2023. The prevalence of CRC has been steadily increasing at an annual rate of 1% to 2% in individuals under the age of 55, a concerning pattern observed since the mid-1990s. Since the mid-2000s, the mortality rate among young individuals has been steadily increasing by approximately 1% per year. It is currently ranked as the primary cause of cancer-related mortality among males below the age of 50 and as the second most common cause among females in the same age bracket[1].

Currently, there is clear evidence that the mortality rate for patients diagnosed with colon cancer decreases significantly when the cancer is detected at an early stage, as opposed to an advanced stage[2]. The article by Tonini and Zanni [3] focuses on the screening methods currently available in practice. The study examines the barriers, such as technological and patient-related factors, that hinder the early detection process. The authors emphasize the importance of implementing strategies to improve the effectiveness of screening, as this can reduce both the incidence rate and the costs associated with advanced CRC treatment. Their analysis concludes that the ineffectiveness of early detection can be ascribed to the low precision of screening tools, the lack of compliance, the absence of screening programs in specific global regions, and the influence of the coronavirus disease 2019 (COVID-19) pandemic. However, the paper might focus narrowly on certain screening technologies without sufficiently exploring newer, promising methods like liquid biopsies or advanced artificial intelligence (AI) algorithms in patient selection. Their discussion may lack integration of multidisciplinary approaches, such as the role of genetic counseling and personalized medicine in early detection strategies. Moreover, the analysis might be predominantly focused on healthcare systems in high-income countries, without adequately addressing challenges and potential solutions applicable to low- and middle-income countries where CRC incidence is rising.

This review focuses on CRC biology, as comprehending the pathway is essential for early screening programs in various patient groups. Additionally, it is crucial for implementing novel approaches in the future. We also highlight the different methods used for CRC screening in various countries worldwide and the difficulties encountered in each system. In addition, we emphasize the barriers and inequalities in CRC screening among different demographic groups.

BIOLOGY OF COLON CANCER

CRC is a heterogeneous disease. Adenomas, which are precursors to cancer, exhibit different molecular signatures, distinct pathological characteristics, and different natural progressions. CRC can develop through different molecular pathways. The most common pathway, known as chromosomal instability (CIN), is responsible for up to 85% of cases. Another major pathway is the CpG island methylator phenotype (CIMP), which includes sporadic microsatellite instability-high (MSI-H) cancers. Lastly, there is the pure microsatellite instability (MSI) pathway, caused by a genetic mutation in a DNA mismatch repair (*MMR*) gene[4].

For cancer to develop, a series of genetic alterations must occur, regardless of the specific pathway involved. Genetic perturbations lead to the formation of successive clones, with a 'successful cancer' requiring around ten clonal events, each marked by a relative growth advantage[5]. For precancerous cells to progress, they must create an environment that allows for genetic and possibly epigenetic changes, such as genomic and epigenomic instability[6]. Understanding the molecular and genetic alterations in colon cancer allows for the identification of distinct biomarkers that can be detected in blood, stool, or tissue samples. This enables the use of non-invasive screening tests.

The CIN pathway

The majority of CRCs originate from CIN. The first abnormality in this pathway is the dysplastic aberrant crypt focus, a

microscopic mucosal lesion that appears before a polyp forms. At this stage, mutations in the adenomatous polyposis coli gene can lead to the activation of the Wnt signaling pathway. Activating mutations of the proto-oncogene KRAS, mutations in TP53, and loss of heterozygosity at chromosome 18q are necessary for the progression to larger adenomas and early carcinomas. In a small percentage of CRCs, the mutational activation of PIK3CA occurs later in the adenoma-carcinoma sequence[7]. CIN can be seen in benign adenomas and tends to increase as the tumor advances[8]. Colorectal carcinogenesis has been extensively studied through the CIN pathway and its adenoma-carcinoma sequence. This has allowed for the molecular classification of CRC and serves as a benchmark for comparing other molecular profiles. However, research has shown that there are alternative pathways through which CRC can develop.

The MSI pathway

Another form of CRC is characterized by the molecular fingerprint of the deficient *MMR* system, which is present in approximately 15% of cases[9]. MSI develops because of different genetic factors, such as mutations in *MMR* genes or epigenetic changes in the *MLH1* gene. These changes can occur in hereditary conditions such as Lynch syndrome as well as sporadic tumors. In sporadic tumors, there is often methylation of CpG islands in gene promoter regions and frequent hotspot mutations in the BRAF oncogene[6]. Tumors with MSI have unique characteristics and consistently show a more favorable prognosis compared with tumors that are microsatellite-stable[10].

The CIMP pathway

The CIMP pathway is the second most common pathway to sporadic CRCs, accounting for approximately 15% of those cases[11]. The CIMP pathway provides the epigenetic instability necessary for sporadic cancers to methylate the promoter regions of, and thus epigenetically inactivate the expression of, key tumor suppressor genes such as *MLH1*[11]. CIMP-positive CRCs are characterized by a well-defined cluster of clinicopathological features, including proximal location and a gender and age risk bias for development in older women[12]. Classically, CIMP-positive CRCs that are MSI-H share characteristics, specifically a relatively good prognosis, but in the absence of MSI-H, the CIMP-positive phenotype is characterized by more advanced pathology, poorer clinical outcomes, and an absence of tumor-infiltrating lymphocytes [12]. CIMP-positive CRCs differ from the other pathways with respect to their precursor lesions. In contrast to CRCs developing *via* the CIN pathway, and also in hereditary non-polyposis CRC, which originate from adenomatous polyps [4], sessile serrated adenomas are the chief pathological precursor in the CIMP pathway[8].

IMPORTANCE OF EARLY SCREENING IN MORTALITY REDUCTION

Throughout the years, there has been a notable improvement in the 5-year survival rate for CRC. It was reported as 50% in the mid-1970s, compared with 65% from 2012 to 2018 in the United States. This improvement is due to advancements in clinical practice, imaging technology, infection control, surgical techniques, and cancer treatments. These advancements have led to enhanced detection, staging, and treatment of CRC over the long term. The stage at diagnosis remains the crucial factor in determining survival rates. The 5-year relative survival rate reaches up to 91% for stage I disease, while it drops as low as 14% for stage IV disease with distant metastasis[13]. Recent research showed that surveillance-compliant patients with polyps (but no cancer) at screening have a long-term CRC risk following polypectomy similar to that of patients without polyps[2,14]. However, 20%-30% of screen-detected CRCs present with lymph nodes or distant metastasis, detrimentally affecting prognosis[1,13], highlighting the significance of early screening and, above all, the necessity of consistent surveillance. In 2021, the United States Preventive Services Task Force revised their guidelines, lowering the recommended age for CRC screening from 50 to 45 for average-risk individuals [15]. Starting screening at an earlier age suggests a focus on preventing cancer since there will likely be fewer cases of CRC in younger individuals, especially those under 50 years old.

SCREENING PROGRAMS AROUND THE WORLD AND BARRIERS

There are currently two prominent screening models worldwide for CRC: Systematic screening and opportunistic screening. Systematic screening encompasses the entire population in a specific area and necessitates the involvement of specialized institutions and professionals, along with substantial resources. This type of screening is conducted on a population-wide scale. Opportunistic screening focuses on individuals who are seeking medical treatment and aims to screen for specific diseases during their treatment or examination. Many European countries have implemented systematic screening programs that involve fecal occult blood tests (FOBTs) and subsequent colonoscopy. Studies have shown that these programs decrease the mortality of colon cancer; however, the participation rate for these protocols remains low. In addition, these tests often yield a high number of false positive results[16,17]. On the other hand, opportunistic screening eliminates the need for additional examinations, despite a slightly higher cost. A major limitation of opportunistic screening is that it only targets individuals who actively seek medical attention or undergo health examinations, leaving out potential patients who do not seek medical treatment. It is important to note that the screening process may not be able to reach certain high-risk populations, which can reduce its overall effectiveness. A recent review article found that organized screening programs have higher rates of participation and lower rates of non-compliance with follow-up testing after a positive screen compared with opportunistic screening[18]. To successfully implement a systematic screening program, it is essential to identify suitable screening methods that are accessible to the entire

population, cost-effective, and culturally accepted by the target population. Monitoring strategies should be implemented for patients who have tested positive and those who are at higher risk. To date, there is no universally agreed-upon screening consensus on which method to choose or which program is the most effective. In Europe, a fecal immunochemical test (FIT) is a systematic stool sample screening test. Studies have shown that the rate of CRC detection was similar when four rounds of FIT were used in alternating years compared with a single flexible sigmoidoscopy and single colonoscopy. Despite its generally high sensitivity for detecting CRC, FIT may fail to detect approximately one-third of stage I cases. According to the manufacturer's recommended threshold, FIT showed a sensitivity of 52% for detecting T1 cancers, 79% for T2 tumors, 93% for T3 tumors, and 84% for T4 tumors[19]. Research has shown that the effectiveness of FIT in detecting tumors in the proximal colon is lower than in the distal colon[20]. Understanding the limitations of the FIT test is essential when advising patients and ensuring the success of screening programs.

The colonoscopy is the most common method used in areas where opportunistic screening is implemented; however, the compliance rate is lower compared to other test methods. This is due to multiple barriers, including financial, cultural, and resource-related factors. Moreover, over the past decade, there has been growing evidence of significant variability in the proficiency of endoscopists to identify and remove polyps effectively. Patients who undergo colonoscopy performed by an endoscopist with a high adenoma detection rate have a significantly reduced risk of developing CRC compared to those examined by an endoscopist with a low detection rate[21,22]. Consequently, in order to establish colonoscopy as a screening program, it is necessary to implement rigorous quality assurance programs that encompass training, supervision, and auditing.

Surveillance protocols following polyp removal differ across regions; the United States implements more frequent and shorter intervals for surveillance, while Europe opts for less surveillance among a smaller population[23]. It is important to note that existing surveillance recommendations are derived from low-quality evidence relying on surrogate endpoints like recurrent adenomas and expert opinion.

There is potential for practitioners to enhance the visibility of opportunistic screening programs. This can be achieved through increased awareness regarding the importance of screening among both patients and physicians. In one study, 81% of patients who tested positive for FOBT were willing to undergo a subsequent colonoscopy after consulting with physicians and receiving their advice[24]. Currently, the majority of CRC screening efforts are conducted by community physicians and other primary healthcare personnel, who often have limited expertise and training in the field. Research indicates that community health providers in the United States frequently rely on patients' preferences when selecting CRC screening programs, rather than adhering to established national screening guidelines[25]. Thus, dissemination of knowledge among healthcare professionals regarding the significance of screening, coupled with the establishment of dedicated organizations tasked with providing guidance and oversight for CRC screening, could potentially facilitate the implementation and enforcement of screening protocols by clinicians.

BARRIERS AND DISPARITIES OF SCREENING PROGRAM AROUND THE WORLD

The barriers to implementing screening programs are not limited to developing countries, in the United States, multiple disparities and barriers hinder the availability of fair screening and treatment for various groups, including Black Americans, American Indians/Alaska Natives, and underserved Americans. The disease disproportionately impacts these groups. African Americans have a 15% higher likelihood of developing CRC and a 35% higher likelihood of mortality. A lower proportion of Hispanic Americans undergo CRC screening, with just over 50% of eligible individuals being screened. Widespread social disapproval, discomfort with screening techniques, lack of trust in healthcare institutions, and prejudice/discrimination in the healthcare system contribute to these disparities. Individuals from lower socioeconomic backgrounds, regardless of their racial background, face significantly increased risks of receiving inadequate treatment and experiencing delays in care. Financial barriers are significant factors that hinder patients from undergoing screening. Individuals with Medicare and private insurance exhibit a higher likelihood of undergoing screening (76.3%) compared to those with Medicare alone (68.8%) or Medicare and Medicaid (65.2%)[20]. In the United States, Section 4104 of the Patient Protection and Affordable Care Act waived previous cost-sharing requirements for many Medicare-covered preventive services. A study conducted between 2015 and 2023 showed a 20% short-term and 25% long-term increase in the probability of undergoing a mammogram in the four years following the implementation of the 1997 deductible waiver. The potential efficacy of eliminating cost-sharing as a strategy for enhancing the utilization of preventive services is worth considering. Medicare beneficiaries faced unexpected financial charges for a significant period when their screening colonoscopy involved polyp removal categorized as therapeutic. Recent research revealed that 48.2% of patients with commercial insurance and 77.9% of patients with Medicare coverage shared the cost of CRC screening[26]. Cost-sharing contributes to disparities in CRC outcomes based on race, ethnicity, and socioeconomic status. In 2020, the United States Congress took action to address a financial loophole by gradually eliminating coinsurance from 2022 to 2030, potentially enhancing patient access to colonoscopy procedures. The primary obstacle identified in developing countries is the scarcity of resources to implement comprehensive programs, including limited access to colonoscopy procedures and a shortage of trained healthcare professionals[27]. In developing countries where CRC screening methods are established, challenges such as insufficient knowledge about CRC, limitations within the healthcare system, cultural factors, and sociodemographic disparities persist. To address these issues, we recommend adopting more personalized approaches. This includes studying existing barriers in specific populations, providing FITs, offering additional training for healthcare practitioners, and expanding educational campaigns targeting patients, physicians, and community leaders. This comprehensive strategy aims to improve screening uptake and effectiveness by addressing both systemic and individual-level barriers.

THE PREVENTIVE MEASUREMENT CULTURE AND ITS ROLE IN SCREENING ADHERENCE

Despite the proven effectiveness of CRC screening, the level of compliance continues to be relatively low, with rates varying from 19% in Croatia and the Czech Republic to 69% in the Basque region of Spain[28]. Approximately 35% of the eligible population in the United States is not currently undergoing screening[29]. In the early 1950s, the Health Belief Model (HBM) was formulated to comprehend the reasons behind individuals' reluctance to participate in disease prevention strategies or undergo screening tests for early disease detection. It consists of perceived benefits, perceived susceptibility, cues to action, self-efficacy, perceived severity, and perceived barriers. HBMs have been extensively employed in the field of breast cancer screening research. Numerous studies have demonstrated a significant correlation between adherence to mammography in female populations and various factors, including a heightened perception of susceptibility to breast cancer, a greater perception of the benefits of screening, reduced barriers to undergoing screening, and the presence of cues to action such as recommendations from healthcare professionals[30]. A systematic review article on CRC screening, involving a sample of 21010 participants from 18 different countries, proposed that the HBM can provide valuable insights into the factors that promote or hinder CRC screening. The review revealed that cues to action consistently correlated with screening adherence. Notably, the most prevalent cue identified across studies was the presence of a physician's recommendation to screen, as well as advice from family or friends. Perceived obstacles were primarily due to limited healthcare accessibility and concerns regarding the financial implications of screening. Psychosocial factors also exerted a substantial influence, such as feelings of embarrassment associated with undergoing screening and fear of outcomes[31]. Other well-studied factors contributing to poor patient compliance include lack of knowledge, fatalism, and low literacy[31].

EMERGING SCREENING TOOLS AND FUTURE TESTS MIGHT BE THE SOLUTION TO CURRENT BARRIERS

A recently developed multi-target DNA test has been introduced to the market, demonstrating enhanced sensitivity compared to the FIT test for diagnosing both adenoma and CRC. The United States Food and Drug Administration has approved this test only for average-risk patients who are asymptomatic and aged 45 years and older. Patients with ongoing melena or hematochezia, a personal history of colorectal adenoma or inflammatory bowel disease, a family history of colorectal adenoma in a first-degree relative diagnosed at any age, or heritable cancer syndromes should not be offered this test[32]. We agree with the authors that the COVID-19 pandemic has presented various obstacles for prompt CRC screening. These challenges can be effectively addressed by carefully deliberating and engaging in informed conversations with patients regarding all accessible, guideline-endorsed alternatives. Additionally, fostering effective communication between primary and specialty care providers is crucial to ensure proper follow-up for positive screening test outcomes. The home-based test is supported by compelling scientific data and increasing practical experience[33]. Other stool-based studies and liquid biopsies analyzing circulating blood DNA have yet to achieve the necessary sensitivity and specificity standards for effective population-wide screening. Despite this, it is recommended to continue developing these biomarkers as part of a comprehensive panel that includes serum protein biomarkers. Current research efforts are focused on identifying screening markers through the analysis of microRNA in feces and blood, as well as the gut microbiome[34]. The primary objective is to determine the most effective combination of molecular biomarkers to optimize screening sensitivity and specificity. The development of non-invasive and cost-effective methods for CRC screening will be crucial in enhancing early detection and improving patient outcomes.

We also agree with the authors regarding the role of AI in enhancing early detection. Using AI in healthcare practice should promote more preventive measures by tracking and identifying patients at high risk and setting reminders for both clinical providers and patients. Currently, AI is used in colonoscopy to enhance the identification and characterization of polyps, thereby improving patient outcomes[35]. AI models can also serve as a valuable tool for training junior endoscopists, acting as a 'second observer' during colonoscopy procedures. AI-based applications on mobile devices, such as the CRC Awareness Application (ColorAppTM), have the potential to enhance community education and engagement in CRC screening programs[36]. Additionally, the use of AI for reviewing images from second-generation colon capsule endoscopy shows promise.

CONCLUSION

CRC screening remains an unmet need due to various barriers, including insufficient education among healthcare providers, patient-related factors, systemic issues, and limitations in current diagnostic methods. Addressing these challenges is crucial for improving CRC screening rates and outcomes, similar to the success achieved with cervical and breast cancer screenings. Implementing strategies that enhance provider education, increase patient awareness, and improve healthcare systems, particularly within the framework of the Health Promotion Model, could significantly advance CRC early detection efforts. Understanding the biology of CRC is crucial for developing screening methods that surpass the limitations of current tools. Although liquid biopsy has yet to achieve significant sensitivity and specificity, ongoing research in this area is highly encouraged to enhance its effectiveness.

FOOTNOTES

Author contributions: Aleissa M wrote the paper; Drelichman ER contributed in writing the paper; Mittal VK contributed in writing the paper; Bhullar JS design research, wrote the paper. Aleissa M is the primary corresponding author, has undertaken the majority of the research work, including designing the paper, reviewing resources, and manuscript preparation. Aleissa M is responsible for handling all communications with the journal, managing the submission process, and addressing any editorial and reviewer comments. Bhullar JS is the senior corresponding author. He will be available to answer any questions from readers, leveraging their extensive expertise to provide comprehensive responses and further insights. We believe that this dual corresponding author arrangement will enhance the clarity and efficiency of our interactions with both the journal and the broader research community.

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REFERENCES

1 **Siegel RL**, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin* 2024; **74**: 12-49 [PMID: 38230766 DOI: 10.3322/caac.21820]
2 **Robertson DJ**, Greenberg ER, Beach M, Sandler RS, Ahnen D, Haile RW, Burke CA, Snover DC, Bresalier RS, McKeown-Eyssen G, Mandel JS, Bond JH, Van Stolk RU, Summers RW, Rothstein R, Church TR, Cole BF, Byers T, Mott L, Baron JA. Colorectal cancer in patients under close colonoscopic surveillance. *Gastroenterology* 2005; **129**: 34-41 [PMID: 16012932 DOI: 10.1053/j.gastro.2005.05.012]
3 **Tonini V**, Zanni M. Why is early detection of colon cancer still not possible in 2023? *World J Gastroenterol* 2024; **30**: 211-224 [PMID: 38314134 DOI: 10.3748/wjg.v30.i3.211]
4 **Arvelo F**, Sojo F, Cotte C. Biology of colorectal cancer. *Ecancermedicalscience* 2015; **9**: 520 [PMID: 25932044 DOI: 10.3332/ecancer.2015.520]
5 **Rajagopalan H**, Lengauer C. Aneuploidy and cancer. *Nature* 2004; **432**: 338-341 [PMID: 15549096 DOI: 10.1038/nature03099]
6 **de Miranda NF**, Hes FJ, van Wezel T, Morreau H. Role of the microenvironment in the tumourigenesis of microsatellite unstable and MUTYH-associated polyposis colorectal cancers. *Mutagenesis* 2012; **27**: 247-253 [PMID: 22294774 DOI: 10.1093/mutage/ger077]
7 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-i]
8 **Pino MS**, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroenterology* 2010; **138**: 2059-2072 [PMID: 20420946 DOI: 10.1053/j.gastro.2009.12.065]
9 **Sameer AS**, Nissar S, Fatima K. Mismatch repair pathway: molecules, functions, and role in colorectal carcinogenesis. *Eur J Cancer Prev* 2014; **23**: 246-257 [PMID: 24614649 DOI: 10.1097/CEJ.0000000000000019]
10 **Popat S**, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; **23**: 609-618 [PMID: 15659508 DOI: 10.1200/JCO.2005.01.086]
11 **Nazemalhosseini Mojarad E**, Kuppen PJ, Aghdaei HA, Zali MR. The CpG island methylator phenotype (CIMP) in colorectal cancer. *Gastroenterol Hepatol Bed Bench* 2013; **6**: 120-128 [PMID: 24834258]
12 **Advani SM**, Advani P, DeSantis SM, Brown D, VonVille HM, Lam M, Loree JM, Mehrvarz Sarshekeh A, Bressler J, Lopez DS, Daniel CR, Swartz MD, Kopetz S. Clinical, Pathological, and Molecular Characteristics of CpG Island Methylator Phenotype in Colorectal Cancer: A Systematic Review and Meta-analysis. *Transl Oncol* 2018; **11**: 1188-1201 [PMID: 30071442 DOI: 10.1016/j.tranon.2018.07.008]
13 **Siegel RL**, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J Clin* 2023; **73**: 233-254 [PMID: 36856579 DOI: 10.3322/caac.21772]
14 **Jørgensen OD**, Kronborg O, Fenger C. The Funen Adenoma Follow-up Study. Incidence and death from colorectal carcinoma in an adenoma surveillance program. *Scand J Gastroenterol* 1993; **28**: 869-874 [PMID: 8266015 DOI: 10.3109/00365529309103127]
15 **US Preventive Services Task Force**, Davidson KW, Barry MJ, Mangione CM, Cabana M, Caughey AB, Davis EM, Donahue KE, Doubeni CA, Krist AH, Kubik M, Li L, Ogedegbe G, Owens DK, Pbert L, Silverstein M, Stevermer J, Tseng CW, Wong JB. Screening for Colorectal Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* 2021; **325**: 1965-1977 [PMID: 34003218 DOI: 10.1001/jama.2021.6238]
16 **McClements PL**, Madurasinghe V, Thomson CS, Fraser CG, Carey FA, Steele RJ, Lawrence G, Brewster DH. Impact of the UK colorectal cancer screening pilot studies on incidence, stage distribution and mortality trends. *Cancer Epidemiol* 2012; **36**: e232-e242 [PMID: 22425027 DOI: 10.1016/j.canep.2012.02.006]
17 **Zavoral M**, Suchanek S, Zavada F, Dusek L, Muzik J, Seifert B, Fric P. Colorectal cancer screening in Europe. *World J Gastroenterol* 2009; **15**: 5907-5915 [PMID: 20014454 DOI: 10.3748/wjg.15.5907]
18 **Senore C**, Lansdorp-Vogelaar I, de Jonge L, Rabeneck L. Rationale for organized Colorectal cancer screening programs. *Best Pract Res Clin Gastroenterol* 2023; **66**: 101850 [PMID: 37852709 DOI: 10.1016/j.bpg.2023.101850]
19 **Niedermaier T**, Tikk K, Gies A, Bieck S, Brenner H. Sensitivity of Fecal Immunochemical Test for Colorectal Cancer Detection Differs

According to Stage and Location. *Clin Gastroenterol Hepatol* 2020; **18**: 2920-2928.e6 [PMID: 31988043 DOI: 10.1016/j.cgh.2020.01.025]

20 **Chiu HM**, Jen GH, Wang YW, Fann JC, Hsu CY, Jeng YC, Yen AM, Chiu SY, Chen SL, Hsu WF, Lee YC, Wu MS, Wu CY, Jou YY, Chen TH. Long-term effectiveness of faecal immunochemical test screening for proximal and distal colorectal cancers. *Gut* 2021; **70**: 2321-2329 [PMID: 33495268 DOI: 10.1136/gutjnl-2020-322545]

21 **Pohl H**, Anderson JC, Aguilera-Fish A, Calderwood AH, Mackenzie TA, Robertson DJ. Recurrence of Colorectal Neoplastic Polyps After Incomplete Resection. *Ann Intern Med* 2021; **174**: 1377-1384 [PMID: 34370514 DOI: 10.7326/M20-6689]

22 **Corley DA**, Jensen CD, Marks AR, Zhao WK, Lee JK, Doubeni CA, Zauber AG, de Boer J, Fireman BH, Schottinger JE, Quinn VP, Ghai NR, Levin TR, Quesenberry CP. Adenoma detection rate and risk of colorectal cancer and death. *N Engl J Med* 2014; **370**: 1298-1306 [PMID: 24693890 DOI: 10.1056/NEJMoa1309086]

23 **Helsingen LM**, Kalager M. Colorectal Cancer Screening - Approach, Evidence, and Future Directions. *NEJM Evid* 2022; **1**: EVIDra2100035 [PMID: 38319175 DOI: 10.1056/EVIDra2100035]

24 **Mandel JS**, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, Ederer F. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N Engl J Med* 1993; **328**: 1365-1371 [PMID: 8474513 DOI: 10.1056/NEJM199305133281901]

25 **Lin SC**, McKinley D, Sripipatana A, Makaroff L. Colorectal cancer screening at US community health centers: Examination of sociodemographic disparities and association with patient-provider communication. *Cancer* 2017; **123**: 4185-4192 [PMID: 28708933 DOI: 10.1002/cncr.30855]

26 **Fendrick AM**, Princic N, Miller-Wilson LA, Wilson K, Limburg P. Out-of-Pocket Costs for Colonoscopy After Noninvasive Colorectal Cancer Screening Among US Adults With Commercial and Medicare Insurance. *JAMA Netw Open* 2021; **4**: e2136798 [PMID: 34854909 DOI: 10.1001/jamanetworkopen.2021.36798]

27 **Lee R**, Holmes D. Barriers and recommendations for colorectal cancer screening in Africa. *Glob Health Action* 2023; **16**: 2181920 [PMID: 36820646 DOI: 10.1080/16549716.2023.2181920]

28 **Shaukat A**, Levin TR. Current and future colorectal cancer screening strategies. *Nat Rev Gastroenterol Hepatol* 2022; **19**: 521-531 [PMID: 35505243 DOI: 10.1038/s41575-022-00612-y]

29 **Lin JS**, Piper MA, Perdue LA, Rutter CM, Webber EM, O'Connor E, Smith N, Whitlock EP. Screening for Colorectal Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 2016; **315**: 2576-2594 [PMID: 27305422 DOI: 10.1001/jama.2016.3332]

30 **Phillips KA**, Kerlikowske K, Baker LC, Chang SW, Brown ML. Factors associated with women's adherence to mammography screening guidelines. *Health Serv Res* 1998; **33**: 29-53 [PMID: 9566176]

31 **Muthukrishnan M**, Arnold LD, James AS. Patients' self-reported barriers to colon cancer screening in federally qualified health center settings. *Prev Med Rep* 2019; **15**: 100896 [PMID: 31193550 DOI: 10.1016/j.pmedr.2019.100896]

32 **Onieva-García MÁ**, Llanos-Méndez A, Baños-Álvarez E, Isabel-Gómez R. A systematic review of the clinical validity of the Cologuard™ genetic test for screening colorectal cancer. *Rev Clin Esp (Barc)* 2015; **215**: 527-536 [PMID: 26434810 DOI: 10.1016/j.rce.2015.08.002]

33 **Kisiel JB**, Limburg PJ. Colorectal Cancer Screening With the Multitarget Stool DNA Test. *Am J Gastroenterol* 2020; **115**: 1737-1740 [PMID: 33156086 DOI: 10.14309/ajg.0000000000000968]

34 **Zackular JP**, Rogers MA, Ruffin MT 4th, Schloss PD. The human gut microbiome as a screening tool for colorectal cancer. *Cancer Prev Res (Phila)* 2014; **7**: 1112-1121 [PMID: 25104642 DOI: 10.1158/1940-6207.CAPR-14-0129]

35 **Nartowt BJ**, Hart GR, Muhammad W, Liang Y, Stark GF, Deng J. Robust Machine Learning for Colorectal Cancer Risk Prediction and Stratification. *Front Big Data* 2020; **3**: 6 [PMID: 33693381 DOI: 10.3389/fdata.2020.00006]

36 **Mohamad Marzuki MF**, Yaacob NA, Bin Yaacob NM, Abu Hassan MR, Ahmad SB. Usable Mobile App for Community Education on Colorectal Cancer: Development Process and Usability Study. *JMIR Hum Factors* 2019; **6**: e12103 [PMID: 30990454 DOI: 10.2196/12103]

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REVIEW

Circadian rhythm disruption and endocrine-related tumors

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Abstract

This review delved into the intricate relationship between circadian clocks and physiological processes, emphasizing their critical role in maintaining homeostasis. Orchestrated by interlocked clock genes, the circadian timekeeping system regulates fundamental processes like the sleep-wake cycle, energy metabolism, immune function, and cell proliferation. The central oscillator in the hypothalamic suprachiasmatic nucleus synchronizes with light-dark cycles, while peripheral tissue clocks are influenced by cues such as feeding times. Circadian disruption, linked to modern lifestyle factors like night shift work, correlates with adverse health outcomes, including metabolic syndrome, cardiovascular diseases, infections, and cancer. We explored the molecular mechanisms of circadian clock genes and their impact on metabolic disorders and cancer pathogenesis. Specific associations between circadian disruption and endocrine tumors, spanning breast, ovarian, testicular, prostate, thyroid, pituitary, and adrenal gland cancers, are highlighted. Shift work is associated with increased breast cancer risk, with *PER* genes influencing tumor progression and drug resistance. *CLOCK* gene expression correlates with cisplatin resistance in ovarian cancer, while factors like aging and intermittent fasting affect prostate cancer. Our review underscored the intricate interplay between circadian rhythms and cancer, involving the regulation of the cell cycle, DNA repair, metabolism, immune function, and the tumor microenvironment. We advocated for integrating biological timing into clinical considerations for personalized healthcare, proposing that understanding these connections could lead to novel therapeutic approaches. Evidence supports circadian rhythm-focused therapies, particularly chronotherapy, for treating endocrine tumors. Our review called for further research to uncover detailed connections between circadian clocks and cancer, providing essential insights for targeted treatments. We emphasized the importance of public health interventions to mitigate lifestyle-related circadian disruptions and underscored the critical role of circadian rhythms in disease mechanisms and therapeutic interventions.

Key Words: Circadian rhythm; Circadian disruption; Shift work; *CLOCK* gene; Cancer; Endocrine tumors; Chronotherapy

Core Tip: This review explored the pivotal role of circadian clocks in physiological processes and adverse health outcomes, including metabolic syndrome and cancer, linked to their disruption. Orchestrated by interlocked *CLOCK* genes, the circadian system regulates vital functions, from sleep-wake cycles to immune response. Disruptions, often from night shift work, correlate with increased risks, notably in endocrine tumors. Molecular insights highlight potential therapeutic vistas, emphasizing the need for integrating circadian considerations in personalized healthcare. Chronotherapy emerges as a promising approach for endocrine tumor treatment, urging further research into precise mechanisms and public health interventions to mitigate lifestyle-related circadian disruptions.

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INTRODUCTION

Circadian clocks are intrinsic oscillators in cells and organisms, coordinating multiple biological processes within an approximate 24-hour cycle. The circadian timekeeping system comprises a network of clock genes (CGs) interlocked in a complex of autoregulatory transcriptional-translational feedback loops that generate and synchronize circadian rhythms, primarily influenced by light-dark cycles[1]. This self-sustained internal system orchestrates fundamental physiological processes, including the sleep-wake cycle, energy metabolism, immune function, cognitive and physical performance, as well as cell proliferation and tumorigenesis[2,3]. Additionally, it contributes to the adaptation of organisms to environmental changes, thereby maintaining systematic and tissue homeostasis[3,4].

In mammals, the circadian timing system consists of a central oscillator located in the hypothalamic suprachiasmatic nucleus (SCN) and various peripheral tissue clocks. The core pacemaker of these intricate rhythms is the SCN, which is attuned to geophysical time and environmental changes[5]. This synchronization is mediated by light transmitted to the SCN, primarily *via* retinal ganglion cells, and subsequently temporal information is conveyed to peripheral tissue clocks, thereby aligning internal timing with the light-dark cycle[6].

However, it has been proposed that other environmental cues, with feeding times being the most crucial, can also synchronize peripheral oscillators[7]. Circadian homeostasis among central and peripheral clocks is well documented as integral to optimal health status and survival. In contrast, there is growing evidence of the detrimental effects of circadian rhythm disruption due to environmental or behavioral factors, which are often concomitants of modern society. These factors include night shift work, irregular sleep patterns or meal timing, and exposure to artificial light during the night, all of which can perturb the harmonious coordination among clocks, leading to aberrant epigenetic modifications and adverse health outcomes[8,9].

It is worth noting that circadian system dysregulation has been linked to a wide range of adverse health issues, including metabolic syndrome, cardiovascular diseases, susceptibility to infections, and even certain types of cancer, highlighting the pivotal role of biological clock homeostasis in human health[10]. Given this association, it may be that novel therapeutic approaches could be considered for neuropsychiatric diseases and cardiometabolic disorders. A growing body of research into circadian rhythms regarding cancer pathogenesis has shed light on the possible role of chronotherapeutic interventions[11].

The aim of this narrative review was to summarize our understanding of the critical interplay between circadian disruption and its clinical implications in a wide array of related diseases. Additionally, we discussed the latest evidence that connects molecular mechanisms of circadian disruption to tumorigenesis. We aimed in this way to highlight the importance of integrating biological timing into the clinical setting and the need to consider circadian-based approaches in personalized health care. Please note that in this review in murine (mouse and rat) gene nomenclature, gene symbols are typically italicized with only the initial letter capitalized, followed by lowercase letters (*e.g.*, *Gfap*). Corresponding protein symbols maintain the same designation as the gene symbol but are not italicized with only the initial letter capitalized, followed by lowercase letters (*e.g.*, Gfap). In human gene nomenclature, gene symbols are italicized and presented in all uppercase letters (*e.g.*, *TP53*). Protein symbols follow the same naming convention as the gene symbols, except they are not italicized and remain in all uppercase letters, reflecting their human origin (*e.g.*, TP53). mRNAs and cDNAs adopt the same formatting conventions as their respective gene symbols.

CIRCADIAN CLOCK GENES

The CGs constitute a group of genes identified in mammals that collectively bear the responsibility of maintaining independent circadian rhythms in every individual cell[12]. To achieve this, CGs form three major transcriptional-translational feedback loops. The first identified CG, Period (*Per*), was discovered in Drosophila. In humans, *PER* encodes the PER protein, and its mRNA has been demonstrated to oscillate periodically[13,14]. The circadian locomotor output cycles kaput (*Clock*) gene, discovered in mice, encodes a regulatory transcriptional protein with a DNA-binding domain, a PAS dimerization domain, and a Q-rich transactivation domain[15]. While *CLOCK* mRNA does not exhibit periodic oscillation, its cellular localization between the nucleus and cytoplasm varies during the 24-hour cycle[16]. Brain and Muscle ARNT-Like 1 (BMAL1) protein serves as the binding partner of the CLOCK protein[17] and is the primary functional component in the BMAL1-CLOCK complex. Cryptochrome (*CRY1* and *CRY2*) genes, found in the SCN, retina, and ganglion cells[18], exhibit regulatory properties akin to the period genes. CRY1 and CRY2 can form dimers with PER1, PER2, and PER3, and these complexes have the ability to inhibit the CLOCK-BMAL1 heterodimer[19]. The casein kinase 1 epsilon (*CK1e*) gene possesses the capacity to phosphorylate PER proteins, leading to their degradation and disrupting the inhibition of the CLOCK-BMAL1[20].

There are three transcriptional feedback loops governing CGs. The primary loop involves the transcription of *CLOCK* and *BMAL1* genes in the cytoplasm of mammalian cells. These proteins form a complex, CLOCK-BMAL1, which translocates to the nucleus and binds to enhancer box sequences (E-box), thereby regulating transcription[17]. The period (*PER1, PER2, PER3*) and cryptochrome (*CRY1, CRY2*) CGs, when activated by CLOCK-BMAL1, form a PER-CRY protein dimer that is translocated from the cytoplasm to the nucleus. This complex interacts with CLOCK-BMAL1, thereby halting its transcription in a negative feedback loop[21]. Once the protein levels of PER and CRY have decreased, the repression ceases, allowing CLOCK-BMAL1 to initiate gene transcription and commence a new oscillatory cycle[22].

The second loop involves the nuclear receptors *REV-ERBα, REV-ERBβ (REV-ERBs)*, and the retinoic acid orphan receptor *ROR (RORα, RORβ, RORγ)*, which bind to *BMAL1* binding sites[23]. Specifically, *REV-ERBs* nuclear receptors repress the transcription of *BMAL1*, in contrast to the *RORs*, which positively regulate its expression by binding to retinoic acid receptor-related orphan receptor element (*RORE*) on the *BMAL1* gene promoter[23].

The third loop involves CLOCK-BMAL1 and CRY1, which regulate D-box binding protein (DBP) and interleukin-3 regulated protein (NFIL3). These proteins bind to D-box elements, including *RORα* and *RORβ*[24]. NFIL3 can also bind to E-box and repress the transcription of *PER2*[24]. DBP can bind to the *CRY1* promoter, influencing the timing of transcription and playing a role in the PER-CRY negative feedback loop[25]. Figure 1 provides a schematic illustration of the intricate functions of the mammalian circadian clock.

Regulators of the core circadian CGs include certain genes forming minor transcriptional feedback loops in addition to the major transcriptional feedback loops. F-box protein 3 and 21 have been identified as degraders of CRY1[26,27]. Neuronal PAS family member 2 *(NPAS2)* shares homology with the *CLOCK* gene and is the largest member of the family [28]. Differentially expressed in chondrocyte (DEC1) and DEC2 inhibit CLOCK-BMAL1 transactivation by either binding with BMAL1 or E-boxes[29]. Other factors that may intervene in circadian clock rhythms include cAMP signaling, intracellular calcium flux, and membrane depolarization[30,31]. These factors are crucial for the SCN, potentially forming positive feedback loops on the central pacemaker of the circadian clock.

Post-translational regulation of the clock proteins includes phosphorylation, O-GlcNAcylation, and acetylation of the CLOCK-BMAL1 complex. These processes lead to either ubiquitination and degradation of core clock proteins or inhibition of their destruction, thereby promoting stabilization and a prolonged half-life. This delicate balance is crucial for maintaining the period of the molecular clock[32,33]. Molecules responsible for the amplitude of the clock period primarily include casein kinase II, PI3-kinase, and c-Jun N-terminal kinases[34].

CIRCADIAN CLOCK GENES AND METABOLIC DISORDERS

Disruption of circadian rhythms has been increasingly linked to metabolic disorders. Recent guidelines for the management of type 2 diabetes mellitus (T2DM) emphasize the importance of adequate sleep[35]. Circadian misalignments may lead to a notable reduction in muscle insulin sensitivity and an elevated fasting glucose level[36]. Meta-analyses support the idea that shift workers have a greater risk of T2DM[37] and that abdominal obesity is more prevalent among shift workers[38]. Moreover, shift work has been linked to a higher risk of metabolic syndrome[39], and permanent night shift workers tend to have higher levels of total cholesterol and triglycerides and lower levels of high-density lipoprotein cholesterol[40].

In line with epidemiological studies, there is ample evidence that circadian gene variations are associated with metabolic disorders. A *CLOCK* gene single nucleotide polymorphism has been associated with a lower risk of T2DM[41], while polymorphisms in *CLOCK* and *ARNTL* in patients with T2DM have been linked to myocardial infarction[42]. In recent years, research has focused on interventions that could improve metabolic profiles by influencing circadian rhythms. Time-restricted eating between 8 am and 2 pm appears to decrease morning fasting glucose and mean daily glucose levels and affect the expression of circadian CGs, such as *BMAL-1, CRY1, CRY2*[43]. Furthermore, according to a recent cross-sectional study, chronotype-aligned exercise has better outcomes in HbA1c, fasting glucose, and lipidemic profile of individuals with T2DM compared to exercise disregarding chronotype[44]. In addition, a three-meal diet with a rich carbohydrate breakfast appears to optimize HbA1c, reduce body weight and insulin requirements, and concomitantly upregulate the expression of CGs in patients with T2DM compared to a six-meal isocaloric diet[45]. Moreover, a 12-wk warm light exposure can alter the expression of *REV-ERBα* in shift workers[46], suggesting that light therapy could

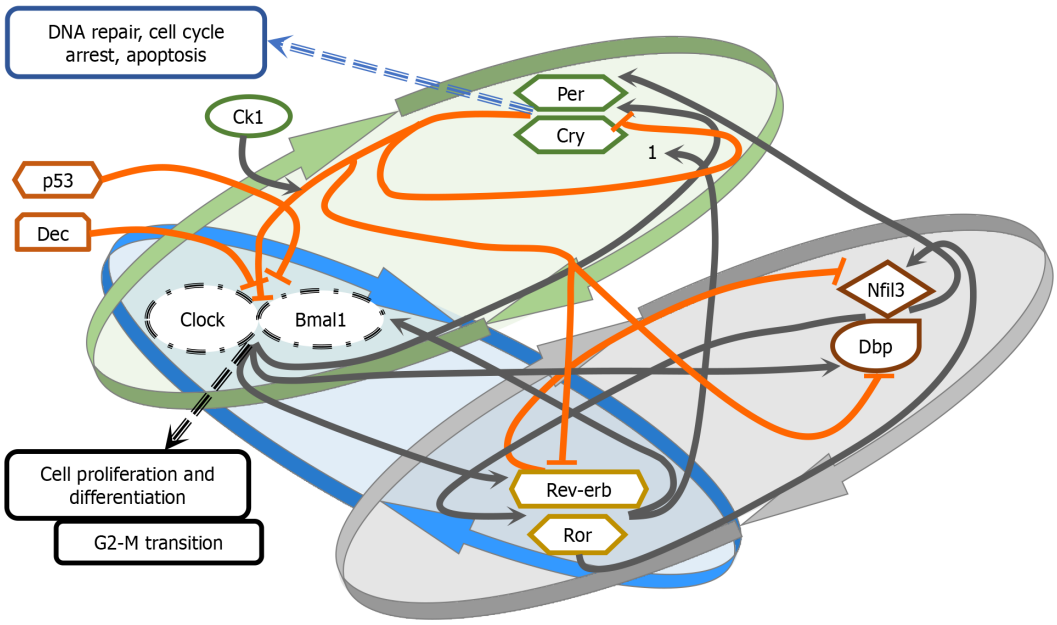

Figure 1 Schematic illustration of the intricate functions of the mammalian circadian clock. Transcriptional-translational feedback loops are highlighted along with the regulatory mechanisms of the clock, interactions of circadian clock genes with cellular processes and regulation of complex biological mechanisms. The circadian clock mechanism primarily relies on a core feedback loop (green area) involving the transcription factors BMAL1 and CLOCK. These factors heterodimerize and bind to the promoters of target genes, such as *PER* and *CRY*. The proteins encoded by these genes form complexes that inhibit their own transcription by interfering with the activity of *BMAL1* and *CLOCK*. A second feedback loop (blue area) features the transcriptional roles of *REV-ERB*. Ck1 and p53 are involved in phosphorylating PER for degradation. ROR/REV-ERB regulates *BMAL1* transcription. The *DEC* gene contributes to circadian gene regulation. A third feedback loop (grey area) features the interrelationship between *ROR/REV-ERB* and *DBP/NFIL3*. There are additional loops and components that contribute to the regulation of the circadian clock. PERs can form a complex (not shown) with the ATM kinase and the checkpoint kinase Chk2, thereby influencing DNA repair, cell cycle arrest, and/or apoptosis. The diagram shows how these circadian components influence critical cellular functions such as cell proliferation and differentiation and the G2-M transition in cell cycle progression (from the G2 phase to the mitotic phase). Each of these processes is essential for the normal functioning of cells and organisms. They work together to ensure that cells divide properly, maintain their genetic integrity, differentiate into various cell types as required, and eliminate cells that are no longer needed or are potentially harmful. Disruptions in any of these processes can lead to serious health issues, including developmental abnormalities, immune system dysfunction, and various forms of cancer. Dark grey lines: Positive regulation of transcription in a loop. Orange lines: Negative regulation of transcription. [1]*CRY1* gene.

be used to rectify circadian disturbances. Based on current data, future research could uncover therapeutic methods for metabolic disorders based on circadian rhythms.

ROLE OF CIRCADIAN CLOCK GENES IN CANCER

Several studies support the notion that disruption of the circadian clock can lead to oncogenesis through various pathways. Interestingly, CGs appear to regulate the expression of the majority (60%-80%) of the proteins of cells. Simultaneously, the tumor itself can influence normal circadian function. Mechanisms contributing to oncogenesis include the disruption of cellular homeostasis, interference with gene expression and DNA repair, as well as alterations in the tumor microenvironment (TME) and the immune response that favor tumor growth, survival, and metastasis[47-50].

The circadian system and the cell cycle are tightly interconnected. CGs control the transcription of key cell cycle regulators, gating the initiation of different phases (G1, S, G2, M)[51]. Transcriptional regulation can be facilitated either through E-boxes in gene promoters (*Cyclins A, B1, D1, p53, c-Myc, and Wee1*)[52,53] or through RORE regions on gene promoters that are targets of *REV-ERBα*[54]. For example, *C-MYC* expression (G0/G1 checkpoint and oncogene) is negatively regulated by BMAL1- CLOCK/NPAS through its E-box[52,55]. Bmal1-Clock controls both cyclin B1, an enhancer of G2/M transition, and Wee1, an inhibitor[56]. This relationship is bidirectional, as both *Per2* and *Rev-Erb* transcription is regulated by genes like *p53* and other tumor suppressor or cyclin kinase genes[57,58]. Therefore, CG disruption could contribute to tumorigenesis by interfering with different phases of the cell cycle[49,51].

A normal part of the cell cycle is the process of ensuring that any damage in the DNA are identified and repaired. Cryptochrome genes seem to be stimulated in states of genotoxic stress, and *Cry*2-deficient cells have been found to sustain damaged DNA[59]. CRYs and PER1 interact with the ATR/CHK1,2 signaling pathway involved in repairing DNA strands destroyed by ionizing radiation[60-62]. An attribute of cancer cells is their infinite replication potential. Telomerase activity, crucial for maintaining telomere length and preventing reproductive senescence[50], seems to be associated with *CLOCK-BMAL* expression[63]. Moreover, the circadian clock controls genes affecting cellular duplication (Ki-67)[64,65], the *MDM-2* oncogene[52,66], cell apoptosis through Bax and Bcl-2[67], invasion and metastasis (MMP9)[68,69], and angiogenesis (VEGF)[64,70,71].

Dysregulation of the circadian clock can cause disruption of cellular metabolism. Chronic jet lag may be associated with the appearance of hepatocellular carcinoma *via* nonalcoholic fatty liver disease[72]. Increased glycolytic metabolism (Warburg effect) is crucial for cancer cell nutrition. For example, PI3-kinase/PdK1/AKT and hypoxia-induced factor 1 (HIF-1) pathways, which increase cell glycolysis, are influenced by circadian clock components[73,74].

Furthermore, the circadian clock controls the immune system and maintains homeostasis during inflammation. Disruption of the circadian clock can lead to immune dysfunction and immunosuppression through interference with antigen presentation, cell trafficking, and the function of T and B lymphocytes, natural killer cells, and monocytes[75,76]. Disruption of the circadian clock is also associated with chronic inflammation *via* cytokines, which have both paracrine and endocrine activity and affect cells locally and in distant tissues[47,48,50,77]. The TME, consisting of many different cells[78], dysfunctional vessel networks, cytokines, and deleterious intercellular signaling, is affected by CGs. This can be through the regulation of lymphocyte TME infiltration, impaired vigilance toward cancer cells, as well as the interaction between CGs and HIFs[71,79]. These processes can contribute to angiogenesis, metastasis, and immune system evasion [80,81].

Animal studies have shown that mutated CGs may act as tumor suppressors or oncogenes in various tissues[82-85]. These mutations might cause different disorders in different tissues. Genetic and genomic studies have shown that variations in the transcription of CGs are more common than mutations. Interestingly, it could be cancer cells themselves that disrupt the circadian clock, enhancing tumorigenesis[50].

Can shift work be solely responsible for the detrimental effects of circadian disruption? Studies have shown that night shift workers have an increased incidence of breast, prostate, and colorectal cancer[86-88]. Environmental factors such as light exposure and food consumption are indeed known circadian modulators. However, the effect of nocturnal activity, be it work or social life, is multifactorial, often mixed with other habits that increase cancer risk, such as alcohol or tobacco use, junk food consumption, and reduced exercise[89-93]. Some are skeptical of the association between cancer and the circadian clock, given certain reports of clockless animals that appear to be resistant to certain cancers[94]. Nevertheless, generalizations should be avoided; research should hone on the specific effects of distinct CGs on distinct forms of cancer and vice versa[94]. More studies that illustrate in detail the connections between the circadian clock and cancer and enable the design of new treatments are necessary.

CIRCADIAN CLOCKS IN CANCER PATIENTS WITH METABOLIC DISORDERS

An interesting but complex issue to address is the effect of circadian disruption in patients with metabolic disorders on the development and progression of cancer. In patients with diabetes, circadian clocks and sirtuins (SIRTs) influence oxidative stress, inflammation, and aging, contributing to organ-specific damages such as those seen in diabetic lung disease[95]. An imbalance of the circadian clock systems can lead to immune dysregulation and chronic inflammation that is linked to diabetes and obesity but additionally promotes cancer cell proliferation and invasion as well as evasion of immune surveillance[96]. Alterations in microbiota that are known to have circadian rhythmicity have been linked to metabolic disorders and could promote carcinogenesis especially in the colon[97]. Further exploration of the interconnections between metabolically-induced circadian disruption and carcinogenesis is beyond the objective of this review albeit being an exciting field.

CIRCADIAN DISRUPTION AND CLOCK GENES IN ENDOCRINE TUMORS

Breast cancer

Breast cancer (BC) is the most common cancer in females. According to a systematic review[98], in which the authors examined the relationship between BC and night shifts among nurses, most studies suggested a relationship between increased BC risk and cumulative years working in night shifts, particularly with three or more nights per month for at least 15 years. Furthermore, long-duration shift work is associated with estrogen and progesterone receptor-positive tumors, especially among young women with intensive shifts (12-h shifts). Night work schedules may shorten telomere length, a potential contributing factor to BC risk, and are associated with irregular menstrual cycles, which are considered a risk factor for BC.

The early onset and high mortality of BC are linked to a heterogeneous TME, consisting of various cell types such as cancer-associated fibroblasts, immune cells, and endothelial cells. Cancer-associated fibroblasts, in particular, contribute to tumor progression, metastasis, and drug resistance. Circadian CGs can modulate the TME, and their disruption can impact the biology of breast tissue, causing metabolic disturbances and immune dysfunction and potentially promoting tumor growth[99]. Additionally, in BC, there is lower expression of *PER* genes compared to normal breast epithelial cells. Loss-of-function mutations in *PER2* have been linked to higher cancer incidence, especially radiation-induced cancer. Loss of *PER2* inhibits programmed cell death, affecting p53 and cell cycle regulation[100]. Single nucleotide polymorphism in circadian rhythm genes are associated with altered expression of cell cycle-regulating genes. For example, *NPAS2* polymorphisms are linked to a higher risk of BC, especially in females[101]. Sirtuin-1 (SIRT1), a histone deacetylase, is involved in many cancer cases, and polymorphisms in the *SIRT1* gene are considered a risk factor[102]. Circadian genes, through the regulation of estrogens, glucocorticoids, and melatonin, as well as estrogen receptor transcription, could be involved in BC development[103].

The role of protein arginine methyltransferase 6 (PRMT6) and poly ADP-ribose polymerase 1 (PARP1) in BC progression and their involvement in circadian rhythm disruption have been recently examined[104]. PRMT6 binds to PARP1, leading to the recruitment of the Cullin4B-Ring E3 Ligase (CRL4B) complex. Together, these components stimulate histone methylation and ubiquitination, which promotes BC progression through the disruption of circadian rhythm and by repressing key DNA repair genes like *PER1* and *PER3*. PRMT6 and PARP1 have individual roles in DNA repair by interacting with different proteins[105]. Furthermore, PRMT6 acts as a TP53 transcriptional repressor and a transcriptional coactivator in different contexts. It enhances BC cell proliferation, invasiveness, metastasis, and epithelial-mesenchymal transition[106]. PARP1 also interacts with PRMT6 and DDB1 in the CRL4B complex for transcriptional regulation. Inhibition of PARP1 enzymatic activity by olaparib disrupts the assembly of the PRMT6/PARP1/CRL4B complex, impacting circadian clock oscillation. As a result, PRMT6 and PARP1 inhibitors are suggested as potential treatments to restore natural circadian rhythms and obstruct tumor progression in BC cells with upregulated PRMT6 expression[104].

Ovarian cancer

Ovarian cancer is a common and deadly gynecologic malignancy, and research has shown that disturbances in circadian rhythms, such as shift work or irregular sleep patterns, might entail an increased risk[107]. Ovaries express circadian genes, controlling hormones during reproductive cycles, and lower expression of *PER1, PER2, CRY2*, and *CLOCK* in ovarian cancer compared to normal ovaries has been demonstrated[108]. Low expression of both *BMAL1* and *CRY1* was connected with a reduced overall survival rate. In contrast, *CRY1*, *PER3*, and *BMAL1* showed higher and antiphasic (opposing) expression in ovarian malignancy.

CLOCK gene expression was linked to cisplatin resistance in ovarian cancer cells, and particularly upregulation of *CLOCK* reduced sensitivity to cisplatin. *CLOCK* knockdown increased the inhibitory effects of cisplatin on cell proliferation and induction of apoptosis[109]. Furthermore, Piwil2 and Pasd1, which are cancer/testis antigens, can interact with CGs like *Bmal1* or *Clock* and interfere with circadian rhythms both in normal testis and cancer cells. Moreover, they negatively regulate the transcriptional activation of clock-controlled genes[110].

Prostate cancer

Prostate cancer (PCa) is a leading cause of cancer-related deaths in males. Studies that included immigrant populations suggested that environmental, lifestyle, and circadian rhythm disruptions may contribute to PCa formation. Both exogenous and endogenous factors might contribute to this process. Aging is closely connected to the circadian clock and changes in the SCN[111]. Sleep patterns, sleep quality, and duration are also factors that may affect PCa risk. Intermittent fasting and its effect on circadian rhythm regulation might offer protection against carcinogenesis, including PCa[112]. The circadian genes *NPAS2*, *PER1*, *PER2*, and *PER3* play roles in controlling DNA damage, cell growth, and cell cycle regulation. Alterations in these genes can influence PCa risk and growth[113-118].

Chronic inflammation is proposed to be implicated in prostate carcinogenesis, and circadian rhythm disruption may contribute to the proinflammatory environment in PCa. The downregulation of *PER2* is associated with the induction of epithelial–mesenchymal transition, which plays a role in cancer dissemination[119]. Increased melatonin is associated with protection against PCa progression[120], while inverse relationship of cortisol with the melatonin/cortisol ratio is linked to PCa emergence and stage[121]. The role of osteoblastic protein kinase D1 (PKD1) in promoting dormancy in PCa cells has been highlighted[122-124]. The results indicated that osteoblastic PKD1 induced dormancy in co-cultured PCa cells by activating cAMP responsive element binding protein 1 and increasing growth arrest specific 6 secretion. This osteoblastic PKD1-induced dormancy also enhanced the expression of core circadian clock molecules in PCa cells, which was linked to recurrence-free survival in metastatic PCa patients[122].

Thyroid and parathyroid glands

The rapid increase in the detection rate of thyroid cancer (TC) recently has been largely attributed to screening[125]. TC accounts for 3.4% of all cancers diagnosed annually worldwide[126], and recent data suggest that TC is increasing globally faster than other malignant lesions[127,128]. Several risk factors for TC have been identified by epidemiological research, namely female sex, advanced age[129], ionizing radiation[130,131], non-Hispanic white race[132], alterations in thyroid stimulating hormone (TSH) levels[133,134], obesity[135,136], and last but not least iodine deficiency[137]. However, even though the above-mentioned risk factors have been studied thoroughly, they cannot fully explain the variation in TC risk.

Growing evidence suggests that the disruption of circadian rhythm is a potential factor in thyroid tumorigenesis[138]. An epidemiological study in post-menopausal non-obese women proposed an association between insomnia and a higher incidence of TC[139], supporting the role of circadian dysregulation in TC development caused by sleep disorders. Furthermore, night shift work has been an established circadian disruptor and related to altered TSH plasma levels[140]. Artificial light at night suppresses melatonin, a fundamental regulator of circadian rhythms, causing circadian disruption [141]. A study in the United States[142] evaluated the hypothesis of a positive association between higher exposure to light at night and the risk of TC, especially papillary thyroid carcinoma. The same study suggested potential sex differences with a stronger association observed in females and a higher risk of anaplastic thyroid carcinoma for higher exposure to artificial light at night.

Based on current evidence, the SCN exerts control over the endocrine system. In humans, thyrotropin-releasing hormone and TSH show a nocturnal peak around 2 am to 4 am, while free thyroxine 4 has a less prominent circadian profile. The abovementioned hypothalamic nucleus directs neuronal outputs into the paraventricular hypothalamic nucleus and may be responsible for the circadian pattern of thyrotropin-releasing hormone[143].

At the genomic level, alterations in the characteristics of CGs were observed recently[144] in thyroid follicular malignancies. The cells were *in vitro* synchronized primary thyrocytes and cells recuperated by tissue biopsies. *BMAL1* gene expression was 13-fold upregulated, while *CRY2* expression was about 2-fold downregulated in papillary thyroid carcinoma nodules compared with benign thyroid nodules. In follicular thyroid carcinoma, *BMAL1* showed 2-fold upregulated levels, with *CRY2* downregulated equally by 2-fold. It is worth mentioning that upregulated levels of tissue inhibitor of metalloproteinase 1 were found, in contrast to downregulated levels of growth arrest and DNA damage inducible gene 153 transcripts in papillary thyroid carcinomas, a finding that was in agreement with previously published studies[145,146].

The transformation of normal thyroid tissue to nodular thyroid tissue with benign characteristics does not alter the circadian oscillator function[144]. However, malignant transformation of thyrocytes might implicate a change in circadian oscillator properties, namely the alteration of *PER2* profile, especially in papillary thyroid carcinoma. PER2 can act as a tumor suppressor and has been found to play a key role by regulating responsive DNA damage pathways[52]. A recent cohort study evaluated the circadian rhythm genes involved in anaplastic thyroid carcinoma, a rare and extremely malignant type of endocrine cancer[147]. This work demonstrated that the *NPAS2* gene promoted malignant phenotypes of anaplastic thyroid carcinoma by modulating the cell cycle as well as focal adhesion signals. This finding could be useful in the development of new therapeutic agents for this challenging type of endocrine cancer.

Dysregulation of various CGs including *CRYs*, *PER1-2-3*, *REV-ERBs*, and *ROR α-β-γ* have been associated with a higher risk of TC development[101,148]. In addition, increased levels of the circadian clock factor DEC1 have been found to promote the induction of several cell cycle-related genes, potentially leading to the development of TC[149].

As far as parathyroid tumors are concerned, available data is scarce. In parathyroid adenomas, mRNA of *NFIL3*, a gene responsible for repression of *PER1* and *PER2* expression, was downregulated. Moreover, *NFIL3* and *CRY2* mRNA levels were following the same pattern of downregulation. It is interesting though that no statistical difference was found between parathyroid adenomas and parathyroid hyperplasia concerning the core CGs[107]. Nevertheless, the mechanisms implicated in the interaction between circadian clock gene abnormalities and malignant transformation of thyroid tissue remain to be clarified and elucidated by more clinical studies, providing new insights that could contribute to malignant nodule preoperative diagnosis and new treatment prospects.

Adrenal gland

Adrenal masses are encountered in 2% of the general population, and less than one-third of them are hormone-producing adenomas[150,151]. Adrenal carcinomas are scarce, with an incidence of 0.5-2.0 cases per million population per year[152,153]. Adrenal tumorigenesis is currently perceived as a gradual process of transformation from normal tissue to adenomatous tissue and consequently to a cancerous lesion[154]. Possible interactions between CGs and adrenal function, as well as the effect of circadian disruption on the development and progression of adrenal masses, are being investigated.

Cortisol-secreting adenomas

Cortisol-secreting adenomas (CSAs) were found to have reduced expression of *PER1*, *CRY1*, and *REV-ERB* mRNAs compared with adjacent normal adrenal tissue in humans[155]. The rhythmicity of glucocorticoid secretion is multifactorial and complex and has been extensively reviewed previously[156,157]. The various ways by which centrally or adrenal-expressed CGs regulate glucocorticoid secretion in humans remain to be elucidated.

Studies in mice show that the loss of core CGs like *PER1* leads to deranged rhythmicity of glucocorticoid production [158]. Lack of *Per1* or *Per2* in mice causes higher circulating levels of glucocorticoids[158], whereas the lack of both *Per2/Cry1* leads to the complete loss of the circadian rhythm of both adrenocorticotropic hormone and corticosterone, as well as other CGs[159]. Conversely, adrenal-specific knockout (KO) of *Bmal1* did not have an effect on basal secretion of glucocorticoids in mice, but an increased glucocorticoid response to acute stress was observed[160]. Therefore, *BMAL1* expressed in the adrenal glands has a local regulatory role.

The effect of circadian disruption on cortisol secretion is another interesting domain. Indicatively, shift work causes a partial adaptation in cortisol secretion in humans, as demonstrated by Koshy *et al*[161], who studied police officers after seven night shifts[161]. This was accompanied by a loss of morning/evening difference in *CLOCK* gene expression in both the oral mucosa and peripheral blood cells. Undoubtedly, more evidence is required, taking into consideration that work-related stress might be an additional driver of glucocorticoid production, independent of circadian rhythm disruption itself.

Aldosterone-producing adenoma

In human tissues from aldosterone-producing adenomas (APAs), all CGs were found to be upregulated compared to normal tissue but not with statistical significance[155]. Interestingly, *PER1* and *BMAL1* were more downregulated in APAs compared with CSAs[155]. Aldosterone stimulates *Per* gene transcription in mice[162]. *CRY1* was found to be upregulated in human tissues of APAs, while *CRY2* was downregulated[163]. Moreover, treatment with angiotensin II caused a significant upregulation of *CRY1* and downregulation of *CRY2*. These studies imply that high levels of circulating aldosterone or angiotensin II are affecting the expression of CGs with consequent circadian disruption but cannot prove the opposite effect.

Further studies have revealed increased aldosterone levels in male mice, with global *Per1* KO and kidney-specific *Per1* KO on a normal salt diet[164-166]. This was accompanied by altered Na+ handling and non-dipping hypertension after high-salt and mineralocorticoid treatment. *Per1* KO also caused an increase in *Cry2* expression in the adrenals[165]. Adrenal-specific *Bmal1* ablation in mice also led to changes in diurnal aldosterone levels and Na+ handling by the kidney [167]. These results would imply that *Per1* and *Bmal1* regulate the expression of aldosterone, but it is unknown if these

sex-specific findings could translate similarly into human tissues.

Pheochromocytoma

In the murine adrenal medulla, *Bmal*, *Per1*, and *Per3* seem to have a distinct circadian rhythm, while other CGs show weak expression[159]. There is yet no evidence of dysregulated CGs in human pheochromocytoma (PCC) tissues. However, research on PCCs and genes implicated in their development has revealed an interesting association between the hypoxia signaling pathway and the circadian clock; *BMAL1*, *CLOCK*, and *PERIOD* are heterodimerizing transcription factors that belong to the same family (bHLH-PAS) as *HIF1α*, *1β*, *2α*[168]. It is well known that mutations in *CLUSTER 1* and *2* genes in PCC cells lead to stabilized and therefore increased hypoxia-inducible factors. As a result, there is increased hypoxia signaling in the environment of PCC cells and consequent overexpression of genes that control apoptosis, cell growth and proliferation, as well as VEGF, which leads to angiogenesis[169-171].

As members of the same family, BMAL1 might form a heterodimer with CLOCK or HIF-1β and HIF-1β a heterodimer with HIF-α or CLOCK, and they can bind to DNA regions that modulate gene expression of either the circadian or the hypoxia pathway. This leads to a bidirectional interaction. Animal studies have demonstrated how VEGF expression can be controlled by *Bmal1* and *Per2*, causing angiogenesis driven by the circadian pathway[71,79]. Rutter *et al*[172] described an association between the redox state within the cell and the binding of CLOCK2-BMAL1 on E-box sites, indicating that circadian clock disruption can occur in states of oxidative stress[172].

One known clock-controlled gene in PCC cells is *Atf5*, which was found to be negatively regulated by Clock-Bmal1 in PC12 cells[173]. In the absence of Bmal, this gene is overexpressed[174], having a negative effect on neuronal differentiation in PC cells. On this occasion, cell differentiation is disrupted by a clock gene.

Adrenocortical carcinoma

Recent studies on human adrenal tissue proclaim that there are different patterns of clock gene disruption in benign compared to malignant adrenal tumors. *CLOCK*, *CRY1*, and *PER1* gene expression were amplified in adrenocortical carcinomas (ACCs) compared to CSAs. On the contrary, *BMAL1*, *RORa*, and *REV-ERB* expression were decreased[155]. Differences have been observed between ACC cells and normal tissue: Upregulation of *CRY1* and *PER1* and downregulation of *BMAL1*, *RORa*, and *REV-ERB* genes in ACC cells were noted[155]. The exact effects of clock gene disruption on tumorigenesis are not yet known.

Pituitary gland tumors

Pituitary adenomas consist of typically monoclonal cell populations that can be either sporadic or familial. Four to five percent of pituitary adenomas are associated with clinical syndromes like multiple endocrine neoplasia 1 (Carney complex) and familial isolated pituitary adenoma, whose diverse genetic profiles are still under investigation[175]. A recent work has revealed the role of *PER2*, a key clock gene, in the pathogenesis of pituitary adenomas[176]. In human tissues obtained from prolactinomas and growth hormone-producing adenomas, the expression of several CGs was dysregulated compared to controls, with *PER2* being consistently overexpressed. This overexpression of *PER2* was confirmed in TSH-producing and adrenocorticotropic hormone-producing adenomas as well as non-functioning pituitary tumors.

Jet-lagged mice that had been inoculated with GH3 cells exhibited a faster rate of tumor growth that had a time-specific pattern[176]. The timing of higher cell proliferation was different compared to mice with intact circadian rhythm and correlated with elevated *Per2* expression. In other groups of mice, one with estrogen-induced prolactinomas and one with xenograft GH3 tumors, KO of *Per2* led to slower progression of the tumors as indicated by decreased Ki67 or smaller tumor mass and volume[176]. Apparently, *Per2* plays an important role in cell cycle regulation, as its loss halts cells in the G2/M phase and negatively affects the number of pituitary cells in mitosis and positively those in apoptosis. More specifically, *Per2* seems to act by upregulating key cell cycle genes (*Ccnb2*, *Cdc20*, *Espl1*) through HIF-1α[176].

Furthermore, a REV-ERBα antagonist administered to mice with GH3 tumors was found to counteract tumor growth by inhibiting *Per2* expression. Interestingly, this agent was injected at a specific time of day that correlated with peak Per2 levels[176]. Patterns of *PER2* expression in pituitary adenoma cells that might reflect times of higher cell proliferation could be useful guides for the timing of pharmacotherapeutic interventions in the future.

Neuroendocrine tumors

The expression of CGs has also been studied recently in gastric neuroendocrine tumors. In human gastric neuroendocrine tumor cells, *CLOCK* and *BMAL1* were significantly upregulated, and *REV-ERB* was downregulated compared to normal tissue. On the contrary, the expression of *PER2* and *CRY1* was not altered significantly[177].

CLOCK GENES AND CANCER THERAPY

During the last decades, an increasing number of studies have investigated the interaction between circadian clocks and cancer therapy as well as the clinical utility of chronotherapy, which consists of three approaches[178,179].

The first approach is to promote and preserve an ideal circadian rhythm by entraining the circadian clock using daytime exposure to LED light[180], administering melatonin (apart from its antiproliferative, antioxidant, and immunological effects)[181], following intermittent fasting[182], doing exercise at different times of the day[183], or administering glucocorticoids[184] (the latter can alter the expression of CGs and display time-dependent pharmacokinetics)[185].

The second approach is to optimize chemotherapy and/or radiotherapy administration to maximize therapeutic results and reduce adverse effects by selecting a specific dosing time during the day[11,186,187]. This is based on the time-dependent expression of CGs, which affects the sensitivity of tumors to anticancer drugs[188] as well as the improvement of radiotherapy outcomes based on chronomodulation[189].

The third approach is to use small molecules that alter a circadian clock gene and can improve efficacy in combination with other anticancer treatments[178,179]. Almost all tumors are characterized by a disruption in the expression of CGs [190], and a number of small molecule modulators have been studied in several types of cancer. Additionally, chemotherapeutic agents have been shown to exhibit rhythmic cytotoxic activity. However, there is a lack of data concerning the use of these molecules and the specific time-of-day administration of chemotherapy in endocrine cancer, and assumptions can be made about their utility in that field.

Some small molecules have been shown to be effective against certain types of cancer. SR9009 and SR9011 are agonists of nuclear receptors Rev-Erbα/β, which reduce *Bmal1* transcription[179], and appear to be specifically lethal to glioma cells without affecting normal cells. KL001, a stabilizer of Cry1/2, reduces glioma stem cell proliferation[191]. These molecules could potentially be useful in endocrine cancers, considering the significant upregulation of *BMAL1* and downregulation of *CRY2* in follicular and papillary TC[144], the synergistic interaction between *Bmal1* and *Hif-1a* at the genomic level in PCC[73], as well as the significant increase of *Bmal1* and reduced expression of *REV-Erbs* in gastric neuroendocrine cells compared to adjoining enterochromaffin-like tissue[177].

Another small molecule, LYC-55716, a RORγ agonist involved in the regulation of circadian rhythms and the immune system, has been successfully tested in a phase 1 open-label multicenter study in advanced tumors[192]. In addition, SR8278, a REV-ERB antagonist leading to the downregulation of pituitary expression of *Per-2*, reduces pituitary tumorigenesis[176]. Nevertheless, CGs display differential expression in different types of cancer[190]. Therefore, further clinical studies are required to elucidate the possible effects of chronomodulating small molecules in accordance with the diverse expression of CGs in cancerous tissues, including endocrine cancer. On the other hand, core clock proteins could be used as tumor biomarkers[193] or diagnostic preoperative markers[144].

PROGNOSTIC VALUE FOR CIRCADIAN CLOCK GENES IN CANCER DIAGNOSIS

Circadian CGs, particularly the PER gene family, play a crucial role in cancer progression and patient prognosis[194]. Mutations in these genes can affect cell function, metabolism, immunity, and response to treatment, contributing to varying cancer outcomes[194,195]. Studies have shown that the expression levels of circadian genes are significantly altered in cancers such as colorectal carcinoma, HER2-positive advanced gastric cancer, BC, pancreatic cancer, and obesity-related cancers[196-200]. These alterations could serve as biomarkers for cancer prognosis.

In colorectal cancer, the variable expression of circadian genes correlates with tumor progression and patient survival [197,201,202]. In pancreatic cancer, targeting circadian genes may optimize treatment and improve prognosis[199]. In BC, disruptions in circadian rhythms and alterations in circadian gene expression have been linked to different BC subtypes, tumor progression, and response to chemotherapy and radiotherapy[196,203-205]. Notably, lower expression of *PER* genes has been correlated with more aggressive tumor phenotypes and poorer survival rates in some cases[194,202], whereas in adrenocortical carcinoma *PER1* expression is enhanced[155] and in pituitary adenomas there is marked overexpression of *Per2*[176]. It is evident that different types of tumors display distinct alterations in CGs that could be associated with prognosis and survival rates. Therefore, there is no single pattern that fits all. Further studies assessing the expression of circadian genes, including the PER family, can provide valuable prognostic information and guide personalized treatment strategies.

CONCLUSION

Disruptions in circadian rhythms, often a result of modern lifestyle factors such as shift work, irregular sleep patterns, and exposure to artificial light, lead to aberrant epigenetic modifications and adverse health outcomes. These disruptions have been linked to a range of diseases, including metabolic syndrome, cardiovascular diseases, and various types of cancer, emphasizing the critical role of biological clock homeostasis in human health. Circadian misalignments may lead to reductions in muscle insulin sensitivity, elevated fasting glucose levels, and an increased risk of T2DM among shift workers. Moreover, circadian gene variations have been associated with different metabolic disorders, highlighting the potential for interventions that could influence circadian rhythms to improve metabolic profiles.

The connection between circadian disruption and cancer involves multiple pathways, including interference with gene expression, DNA repair, and immune response. CGs control key cell cycle regulators and discuss the bidirectional relationship between the circadian system and the TME, which affects tumor growth, survival, and metastasis. An association between night shift work and increased BC risk, particularly with long-duration shift work, has been observed. Table 1 provides a comprehensive overview of how circadian disruption and CGs influence various endocrine tumors along with the related outcomes and interventions, highlighting the complex interplay between circadian rhythms and the pathology of endocrine tumors.

The evidence presented supports the potential for circadian rhythm-focused therapies, including chronotherapy, to treat endocrine tumors. Understanding the rhythmic patterns of drug efficacy and toxicity could optimize treatment schedules and minimize side effects. We call for further research to elucidate the precise mechanisms through which circadian genes influence tumor biology. Investigating specific pathways and molecular mechanisms involved can help

Table 1 The role of circadian disruption and clock genes in different types of endocrine tumors

Endocrine tumor	Circadian gene influence	Associated findings/interventions
Breast cancer	*PER* genes, *PRMT6*, *PARP1*	Night shifts linked to increased risk, *PER* genes involved in tumor progression, *PRMT6* and *PARP1* implicated in cancer progression
Ovarian cancer	*PER1, PER2, CRY2, CLOCK*	Shift work/irregular patterns linked to risk, gene expression alterations, *CLOCK* gene linked to cisplatin resistance
Prostate cancer	*NPAS2, PER1-3*	Environmental/lifestyle factors contribute, aging and sleep patterns, *PER2* linked to cancer dissemination, intermittent fasting as a protective factor
Thyroid cancer	Various clock genes	Disruption as a potential contributing factor, alterations in gene expressions in follicular malignancies, *NPAS2* gene promotes malignant phenotypes
Parathyroid tumors	*NFIL3, CRY2*	Downregulation observed in adenomas, further research needed
Adrenal tumors	*PER1, BMAL1, CRY1*	Altered expression in cortisol-secreting and aldosterone-producing adenomas, adrenal-specific gene influences, implications in glucocorticoid secretion and regulation
Pituitary tumors	*PER2*	Overexpression linked to tumor growth, potential timing for pharmacotherapeutic interventions

Clock genes abbreviations: BMAL1: Basic Helix-Loop-Helix ARNT Like 1; CLOCK: Circadian locomotor output cycles kaput; CRY2: Cryptochrome Circadian Regulator 2; NFIL3: Nuclear Factor, Interleukin 3 Regulated; NPAS2: Neuronal PAS Domain Protein 2; PARP1: Poly(ADP-Ribose) Polymerase 1; Per: Period; PER1: Period Circadian Regulator 1; PER2: Period Circadian Regulator 2; PER3: Period Circadian Regulator 3; PRMT6: Protein arginine N-methyltransferase 6.

identify new therapeutic targets and improve prognostic tools for various cancers. Given the associations between lifestyle factors like shift work and increased cancer risk, there is a significant opportunity for public health interventions. The importance of circadian rhythms in human health and disease is critical, and the need to consider the circadian dimension in understanding disease mechanisms and developing more effective, time-based therapeutic interventions should be emphasized.

FOOTNOTES

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REFERENCES

1 **Pilorz V**, Helfrich-Förster C, Oster H. The role of the circadian clock system in physiology. *Pflugers Arch* 2018; **470**: 227-239 [PMID: 29302752 DOI: 10.1007/s00424-017-2103-y]
2 **Zhou L**, Zhang Z, Nice E, Huang C, Zhang W, Tang Y. Circadian rhythms and cancers: the intrinsic links and therapeutic potentials. *J Hematol Oncol* 2022; **15**: 21 [PMID: 35246220 DOI: 10.1186/s13045-022-01238-y]
3 **Ayyar VS**, Sukumaran S. Circadian rhythms: influence on physiology, pharmacology, and therapeutic interventions. *J Pharmacokinet Pharmacodyn* 2021; **48**: 321-338 [PMID: 33797011 DOI: 10.1007/s10928-021-09751-2]
4 **Qu M**. Molecular crosstalk between circadian clock and cancer and therapeutic implications. *Front Nutr* 2023; **10**: 1143001 [PMID: 36937362 DOI: 10.3389/fnut.2023.1143001]

5 **Oosterman JE**, Wopereis S, Kalsbeek A. The Circadian Clock, Shift Work, and Tissue-Specific Insulin Resistance. *Endocrinology* 2020; **161** [PMID: 33142318 DOI: 10.1210/endocr/bqaa180]

6 **Koronowski KB**, Sassone-Corsi P. Communicating clocks shape circadian homeostasis. *Science* 2021; **371** [PMID: 33574181 DOI: 10.1126/science.abd0951]

7 **Lane JM**, Qian J, Mignot E, Redline S, Scheer FAJL, Saxena R. Genetics of circadian rhythms and sleep in human health and disease. *Nat Rev Genet* 2023; **24**: 4-20 [PMID: 36028773 DOI: 10.1038/s41576-022-00519-z]

8 **Giudice A**, Crispo A, Grimaldi M, Polo A, Bimonte S, Capunzo M, Amore A, D'Arena G, Cerino P, Budillon A, Botti G, Costantini S, Montella M. The Effect of Light Exposure at Night (LAN) on Carcinogenesis *via* Decreased Nocturnal Melatonin Synthesis. *Molecules* 2018; **23** [PMID: 29844288 DOI: 10.3390/molecules23061308]

9 **Meyer N**, Harvey AG, Lockley SW, Dijk DJ. Circadian rhythms and disorders of the timing of sleep. *Lancet* 2022; **400**: 1061-1078 [PMID: 36115370 DOI: 10.1016/S0140-6736(22)00877-7]

10 **Rijo-Ferreira F**, Takahashi JS. Genomics of circadian rhythms in health and disease. *Genome Med* 2019; **11**: 82 [PMID: 31847894 DOI: 10.1186/s13073-019-0704-0]

11 **Lee Y**. Roles of circadian clocks in cancer pathogenesis and treatment. *Exp Mol Med* 2021; **53**: 1529-1538 [PMID: 34615982 DOI: 10.1038/s12276-021-00681-0]

12 **Bell-Pedersen D**, Cassone VM, Earnest DJ, Golden SS, Hardin PE, Thomas TL, Zoran MJ. Circadian rhythms from multiple oscillators: lessons from diverse organisms. *Nat Rev Genet* 2005; **6**: 544-556 [PMID: 15951747 DOI: 10.1038/nrg1633]

13 **Sun ZS**, Albrecht U, Zhuchenko O, Bailey J, Eichele G, Lee CC. RIGUI, a putative mammalian ortholog of the Drosophila period gene. *Cell* 1997; **90**: 1003-1011 [PMID: 9323128 DOI: 10.1016/s0092-8674(00)80366-9]

14 **Tei H**, Okamura H, Shigeyoshi Y, Fukuhara C, Ozawa R, Hirose M, Sakaki Y. Circadian oscillation of a mammalian homologue of the Drosophila period gene. *Nature* 1997; **389**: 512-516 [PMID: 9333243 DOI: 10.1038/39086]

15 **Vitaterna MH**, Shimomura K, Jiang P. Genetics of Circadian Rhythms. *Neurol Clin* 2019; **37**: 487-504 [PMID: 31256785 DOI: 10.1016/j.ncl.2019.05.002]

16 **Kondratov RV**, Chernov MV, Kondratova AA, Gorbacheva VY, Gudkov AV, Antoch MP. BMAL1-dependent circadian oscillation of nuclear CLOCK: posttranslational events induced by dimerization of transcriptional activators of the mammalian clock system. *Genes Dev* 2003; **17**: 1921-1932 [PMID: 12897057 DOI: 10.1101/gad.1099503]

17 **Gekakis N**, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, Takahashi JS, Weitz CJ. Role of the CLOCK protein in the mammalian circadian mechanism. *Science* 1998; **280**: 1564-1569 [PMID: 9616112 DOI: 10.1126/science.280.5369.1564]

18 **Miyamoto Y**, Sancar A. Vitamin B2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. *Proc Natl Acad Sci U S A* 1998; **95**: 6097-6102 [PMID: 9600923 DOI: 10.1073/pnas.95.11.6097]

19 **Kume K**, Zylka MJ, Sriram S, Shearman LP, Weaver DR, Jin X, Maywood ES, Hastings MH, Reppert SM. mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. *Cell* 1999; **98**: 193-205 [PMID: 10428031 DOI: 10.1016/s0092-8674(00)81014-4]

20 **Lee H**, Chen R, Lee Y, Yoo S, Lee C. Essential roles of CKIdelta and CKIepsilon in the mammalian circadian clock. *Proc Natl Acad Sci U S A* 2009; **106**: 21359-21364 [PMID: 19948962 DOI: 10.1073/pnas.0906651106]

21 **Michael AK**, Fribourgh JL, Chelliah Y, Sandate CR, Hura GL, Schneidman-Duhovny D, Tripathi SM, Takahashi JS, Partch CL. Formation of a repressive complex in the mammalian circadian clock is mediated by the secondary pocket of CRY1. *Proc Natl Acad Sci U S A* 2017; **114**: 1560-1565 [PMID: 28143926 DOI: 10.1073/pnas.1615310114]

22 **Takahashi JS**. Transcriptional architecture of the mammalian circadian clock. *Nat Rev Genet* 2017; **18**: 164-179 [PMID: 27990019 DOI: 10.1038/nrg.2016.150]

23 **Sato TK**, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P, Naik KA, FitzGerald GA, Kay SA, Hogenesch JB. A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron* 2004; **43**: 527-537 [PMID: 15312651 DOI: 10.1016/j.neuron.2004.07.018]

24 **Ueda HR**, Hayashi S, Chen W, Sano M, Machida M, Shigeyoshi Y, Iino M, Hashimoto S. System-level identification of transcriptional circuits underlying mammalian circadian clocks. *Nat Genet* 2005; **37**: 187-192 [PMID: 15665827 DOI: 10.1038/ng1504]

25 **Ukai-Tadenuma M**, Yamada RG, Xu H, Ripperger JA, Liu AC, Ueda HR. Delay in feedback repression by cryptochrome 1 is required for circadian clock function. *Cell* 2011; **144**: 268-281 [PMID: 21236481 DOI: 10.1016/j.cell.2010.12.019]

26 **Siepka SM**, Yoo SH, Park J, Song W, Kumar V, Hu Y, Lee C, Takahashi JS. Circadian mutant Overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. *Cell* 2007; **129**: 1011-1023 [PMID: 17462724 DOI: 10.1016/j.cell.2007.04.030]

27 **Dardente H**, Mendoza J, Fustin JM, Challet E, Hazlerigg DG. Implication of the F-Box Protein FBXL21 in circadian pacemaker function in mammals. *PLoS One* 2008; **3**: e3530 [PMID: 18953409 DOI: 10.1371/journal.pone.0003530]

28 **DeBruyne JP**, Weaver DR, Reppert SM. CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock. *Nat Neurosci* 2007; **10**: 543-545 [PMID: 17417633 DOI: 10.1038/nn1884]

29 **Li Y**, Song X, Ma Y, Liu J, Yang D, Yan B. DNA binding, but not interaction with Bmal1, is responsible for DEC1-mediated transcription regulation of the circadian gene mPer1. *Biochem J* 2004; **382**: 895-904 [PMID: 15193144 DOI: 10.1042/BJ20040592]

30 **Lundkvist GB**, Kwak Y, Davis EK, Tei H, Block GD. A calcium flux is required for circadian rhythm generation in mammalian pacemaker neurons. *J Neurosci* 2005; **25**: 7682-7686 [PMID: 16107654 DOI: 10.1523/JNEUROSCI.2211-05.2005]

31 **de Jeu M**, Hermes M, Pennartz C. Circadian modulation of membrane properties in slices of rat suprachiasmatic nucleus. *Neuroreport* 1998; **9**: 3725-3729 [PMID: 9858386 DOI: 10.1097/00001756-199811160-00028]

32 **Spengler ML**, Kuropatwinski KK, Schumer M, Antoch MP. A serine cluster mediates BMAL1-dependent CLOCK phosphorylation and degradation. *Cell Cycle* 2009; **8**: 4138-4146 [PMID: 19946213 DOI: 10.4161/cc.8.24.10273]

33 **Yoshitane H**, Takao T, Satomi Y, Du NH, Okano T, Fukada Y. Roles of CLOCK phosphorylation in suppression of E-box-dependent transcription. *Mol Cell Biol* 2009; **29**: 3675-3686 [PMID: 19414601 DOI: 10.1128/MCB.01864-08]

34 **Yagita K**, Yamanaka I, Koinuma S, Shigeyoshi Y, Uchiyama Y. Mini screening of kinase inhibitors affecting period-length of mammalian cellular circadian clock. *Acta Histochem Cytochem* 2009; **42**: 89-93 [PMID: 19617956 DOI: 10.1267/ahc.09015]

35 **Samson SL**, Vellanki P, Blonde L, Christofides EA, Galindo RJ, Hirsch IB, Isaacs SD, Izuora KE, Low Wang CC, Twining CL, Umpierrez GE, Valencia WM. American Association of Clinical Endocrinology Consensus Statement: Comprehensive Type 2 Diabetes Management Algorithm - 2023 Update. *Endocr Pract* 2023; **29**: 305-340 [PMID: 37150579 DOI: 10.1016/j.eprac.2023.02.001]

36 **Wefers J**, van Moorsel D, Hansen J, Connell NJ, Havekes B, Hoeks J, van Marken Lichtenbelt WD, Duez H, Phielix E, Kalsbeek A,

Boekschoten MV, Hooiveld GJ, Hesselink MKC, Kersten S, Staels B, Scheer FAJL, Schrauwen P. Circadian misalignment induces fatty acid metabolism gene profiles and compromises insulin sensitivity in human skeletal muscle. *Proc Natl Acad Sci U S A* 2018; **115**: 7789-7794 [PMID: 29987027 DOI: 10.1073/pnas.1722295115]

37 **Gan Y**, Yang C, Tong X, Sun H, Cong Y, Yin X, Li L, Cao S, Dong X, Gong Y, Shi O, Deng J, Bi H, Lu Z. Shift work and diabetes mellitus: a meta-analysis of observational studies. *Occup Environ Med* 2015; **72**: 72-78 [PMID: 25030030 DOI: 10.1136/oemed-2014-102150]

38 **Sun M**, Feng W, Wang F, Li P, Li Z, Li M, Tse G, Vlaanderen J, Vermeulen R, Tse LA. Meta-analysis on shift work and risks of specific obesity types. *Obes Rev* 2018; **19**: 28-40 [PMID: 28975706 DOI: 10.1111/obr.12621]

39 **Khosravipour M**, Khanlari P, Khazaie S, Khosravipour H, Khazaie H. A systematic review and meta-analysis of the association between shift work and metabolic syndrome: The roles of sleep, gender, and type of shift work. *Sleep Med Rev* 2021; **57**: 101427 [PMID: 33556868 DOI: 10.1016/j.smrv.2021.101427]

40 **Dutheil F**, Baker JS, Mermillod M, De Cesare M, Vidal A, Moustafa F, Pereira B, Navel V. Shift work, and particularly permanent night shifts, promote dyslipidaemia: A systematic review and meta-analysis. *Atherosclerosis* 2020; **313**: 156-169 [PMID: 33069952 DOI: 10.1016/j.atherosclerosis.2020.08.015]

41 **Corella D**, Asensio EM, Coltell O, Sorlí JV, Estruch R, Martínez-González MÁ, Salas-Salvadó J, Castañer O, Arós F, Lapetra J, Serra-Majem L, Gómez-Gracia E, Ortega-Azorín C, Fiol M, Espino JD, Díaz-López A, Fitó M, Ros E, Ordovás JM. CLOCK gene variation is associated with incidence of type-2 diabetes and cardiovascular diseases in type-2 diabetic subjects: dietary modulation in the PREDIMED randomized trial. *Cardiovasc Diabetol* 2016; **15**: 4 [PMID: 26739996 DOI: 10.1186/s12933-015-0327-8]

42 **Škrlec I**, Milić J, Heffer M, Wagner J, Peterlin B. Circadian clock genes and circadian phenotypes in patients with myocardial infarction. *Adv Med Sci* 2019; **64**: 224-229 [PMID: 30818221 DOI: 10.1016/j.advms.2018.12.003]

43 **Jamshed H**, Beyl RA, Della Manna DL, Yang ES, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves 24-Hour Glucose Levels and Affects Markers of the Circadian Clock, Aging, and Autophagy in Humans. *Nutrients* 2019; **11** [PMID: 31151228 DOI: 10.3390/nu11061234]

44 **Menek MY**, Budak M. Effect of exercises according to the circadian rhythm in type 2 diabetes: Parallel-group, single-blind, crossover study. *Nutr Metab Cardiovasc Dis* 2022; **32**: 1742-1752 [PMID: 35606229 DOI: 10.1016/j.numecd.2022.04.017]

45 **Jakubowicz D**, Landau Z, Tsameret S, Wainstein J, Raz I, Ahren B, Chapnik N, Barnea M, Ganz T, Menaged M, Mor N, Bar-Dayan Y, Froy O. Reduction in Glycated Hemoglobin and Daily Insulin Dose Alongside Circadian Clock Upregulation in Patients With Type 2 Diabetes Consuming a Three-Meal Diet: A Randomized Clinical Trial. *Diabetes Care* 2019; **42**: 2171-2180 [PMID: 31548244 DOI: 10.2337/dc19-1142]

46 **Rizza S**, Luzi A, Mavilio M, Ballanti M, Massimi A, Porzio O, Magrini A, Hannemann J, Menghini R, Cridland J, Staels B, Grant PJ, Boger RH, Marx N, Federici M. Impact of light therapy on rotating night shift workers: the EuRhythDia study. *Acta Diabetol* 2022; **59**: 1589-1596 [PMID: 36044097 DOI: 10.1007/s00592-022-01956-2]

47 **Hojo H**, Enya S, Arai M, Suzuki Y, Nojiri T, Kangawa K, Koyama S, Kawaoka S. Remote reprogramming of hepatic circadian transcriptome by breast cancer. *Oncotarget* 2017; **8**: 34128-34140 [PMID: 28388556 DOI: 10.18632/oncotarget.16699]

48 **de Assis LVM**, Moraes MN, Magalhães-Marques KK, Kinker GS, da Silveira Cruz-Machado S, Castrucci AML. Non-Metastatic Cutaneous Melanoma Induces Chronodisruption in Central and Peripheral Circadian Clocks. *Int J Mol Sci* 2018; **19** [PMID: 29614021 DOI: 10.3390/ijms19041065]

49 **Masri S**, Kinouchi K, Sassone-Corsi P. Circadian clocks, epigenetics, and cancer. *Curr Opin Oncol* 2015; **27**: 50-56 [PMID: 25405464 DOI: 10.1097/CCO.0000000000000153]

50 **Sulli G**, Lam MTY, Panda S. Interplay between Circadian Clock and Cancer: New Frontiers for Cancer Treatment. *Trends Cancer* 2019; **5**: 475-494 [PMID: 31421905 DOI: 10.1016/j.trecan.2019.07.002]

51 **Soták M**, Sumová A, Pácha J. Cross-talk between the circadian clock and the cell cycle in cancer. *Ann Med* 2014; **46**: 221-232 [PMID: 24779962 DOI: 10.3109/07853890.2014.892296]

52 **Fu L**, Pelicano H, Liu J, Huang P, Lee C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell* 2002; **111**: 41-50 [PMID: 12372299 DOI: 10.1016/s0092-8674(02)00961-3]

53 **Tan XM**, Ye H, Yang K, Chen D, Wang QQ, Tang H, Zhao NB. Circadian variations of clock gene Per2 and cell cycle genes in different stages of carcinogenesis in golden hamster buccal mucosa. *Sci Rep* 2015; **5**: 9997 [PMID: 25950458 DOI: 10.1038/srep09997]

54 **Preitner N**, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U, Schibler U. The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 2002; **110**: 251-260 [PMID: 12150932 DOI: 10.1016/s0092-8674(02)00825-5]

55 **Dang CV**. MYC on the path to cancer. *Cell* 2012; **149**: 22-35 [PMID: 22464321 DOI: 10.1016/j.cell.2012.03.003]

56 **Farshadi E**, Yan J, Leclere P, Goldbeter A, Chaves I, van der Horst GTJ. The positive circadian regulators CLOCK and BMAL1 control G2/M cell cycle transition through Cyclin B1. *Cell Cycle* 2019; **18**: 16-33 [PMID: 30558467 DOI: 10.1080/15384101.2018.1558638]

57 **Miki T**, Matsumoto T, Zhao Z, Lee CC. p53 regulates Period2 expression and the circadian clock. *Nat Commun* 2013; **4**: 2444 [PMID: 24051492 DOI: 10.1038/ncomms3444]

58 **Zhao X**, Hirota T, Han X, Cho H, Chong LW, Lamia K, Liu S, Atkins AR, Banayo E, Liddle C, Yu RT, Yates JR 3rd, Kay SA, Downes M, Evans RM. Circadian Amplitude Regulation *via* FBXW7-Targeted REV-ERBα Degradation. *Cell* 2016; **165**: 1644-1657 [PMID: 27238018 DOI: 10.1016/j.cell.2016.05.012]

59 **Papp SJ**, Huber AL, Jordan SD, Kriebs A, Nguyen M, Moresco JJ, Yates JR, Lamia KA. DNA damage shifts circadian clock time *via* Hausp-dependent Cry1 stabilization. *Elife* 2015; **4** [PMID: 25756610 DOI: 10.7554/eLife.04883]

60 **Sancar A**, Lindsey-Boltz LA, Kang TH, Reardon JT, Lee JH, Ozturk N. Circadian clock control of the cellular response to DNA damage. *FEBS Lett* 2010; **584**: 2618-2625 [PMID: 20227409 DOI: 10.1016/j.febslet.2010.03.017]

61 **Kang TH**, Leem SH. Modulation of ATR-mediated DNA damage checkpoint response by cryptochrome 1. *Nucleic Acids Res* 2014; **42**: 4427-4434 [PMID: 24489120 DOI: 10.1093/nar/gku094]

62 **Unsal-Kaçmaz K**, Mullen TE, Kaufmann WK, Sancar A. Coupling of human circadian and cell cycles by the timeless protein. *Mol Cell Biol* 2005; **25**: 3109-3116 [PMID: 15798197 DOI: 10.1128/MCB.25.8.3109-3116.2005]

63 **Chen WD**, Wen MS, Shie SS, Lo YL, Wo HT, Wang CC, Hsieh IC, Lee TH, Wang CY. The circadian rhythm controls telomeres and telomerase activity. *Biochem Biophys Res Commun* 2014; **451**: 408-414 [PMID: 25109806 DOI: 10.1016/j.bbrc.2014.07.138]

64 **Ye H**, Yang K, Tan XM, Fu XJ, Li HX. Daily rhythm variations of the clock gene PER1 and cancer-related genes during various stages of carcinogenesis in a golden hamster model of buccal mucosa carcinoma. *Onco Targets Ther* 2015; **8**: 1419-1426 [PMID: 26089690 DOI:

10.2147/OTT.S83710]
65 **Bjarnason GA**, Jordan RC, Sothern RB. Circadian variation in the expression of cell-cycle proteins in human oral epithelium. *Am J Pathol* 1999; **154**: 613-622 [PMID: 10027418 DOI: 10.1016/S0002-9440(10)65306-0]
66 **Hua H**, Wang Y, Wan C, Liu Y, Zhu B, Yang C, Wang X, Wang Z, Cornelissen-Guillaume G, Halberg F. Circadian gene mPer2 overexpression induces cancer cell apoptosis. *Cancer Sci* 2006; **97**: 589-596 [PMID: 16827798 DOI: 10.1111/j.1349-7006.2006.00225.x]
67 **Granda TG**, Liu XH, Smaaland R, Cermakian N, Filipski E, Sassone-Corsi P, Lévi F. Circadian regulation of cell cycle and apoptosis proteins in mouse bone marrow and tumor. *FASEB J* 2005; **19**: 304-306 [PMID: 15545298 DOI: 10.1096/fj.04-2665fje]
68 **Anea CB**, Ali MI, Osmond JM, Sullivan JC, Stepp DW, Merloiu AM, Rudic RD. Matrix metalloproteinase 2 and 9 dysfunction underlie vascular stiffness in circadian clock mutant mice. *Arterioscler Thromb Vasc Biol* 2010; **30**: 2535-2543 [PMID: 20829506 DOI: 10.1161/ATVBAHA.110.214379]
69 **Zieker D**, Jenne I, Koenigsrainer I, Zdichavsky M, Nieselt K, Buck K, Zieker J, Beckert S, Glatzle J, Spanagel R, Koenigsrainer A, Northoff H, Loeffler M. Circadian expression of clock- and tumor suppressor genes in human oral mucosa. *Cell Physiol Biochem* 2010; **26**: 155-166 [PMID: 20798499 DOI: 10.1159/000320547]
70 **Koyanagi S**, Kuramoto Y, Nakagawa H, Aramaki H, Ohdo S, Soeda S, Shimeno H. A molecular mechanism regulating circadian expression of vascular endothelial growth factor in tumor cells. *Cancer Res* 2003; **63**: 7277-7283 [PMID: 14612524]
71 **Jensen LD**, Cao Z, Nakamura M, Yang Y, Bräutigam L, Andersson P, Zhang Y, Wahlberg E, Länne T, Hosaka K, Cao Y. Opposing effects of circadian clock genes bmal1 and period2 in regulation of VEGF-dependent angiogenesis in developing zebrafish. *Cell Rep* 2012; **2**: 231-241 [PMID: 22884368 DOI: 10.1016/j.celrep.2012.07.005]
72 **Kettner NM**, Voicu H, Finegold MJ, Coarfa C, Sreekumar A, Putluri N, Katchy CA, Lee C, Moore DD, Fu L. Circadian Homeostasis of Liver Metabolism Suppresses Hepatocarcinogenesis. *Cancer Cell* 2016; **30**: 909-924 [PMID: 27889186 DOI: 10.1016/j.ccell.2016.10.007]
73 **Wu Y**, Tang D, Liu N, Xiong W, Huang H, Li Y, Ma Z, Zhao H, Chen P, Qi X, Zhang EE. Reciprocal Regulation between the Circadian Clock and Hypoxia Signaling at the Genome Level in Mammals. *Cell Metab* 2017; **25**: 73-85 [PMID: 27773697 DOI: 10.1016/j.cmet.2016.09.009]
74 **Peek CB**, Levine DC, Cedernaes J, Taguchi A, Kobayashi Y, Tsai SJ, Bonar NA, McNulty MR, Ramsey KM, Bass J. Circadian Clock Interaction with HIF1α Mediates Oxygenic Metabolism and Anaerobic Glycolysis in Skeletal Muscle. *Cell Metab* 2017; **25**: 86-92 [PMID: 27773696 DOI: 10.1016/j.cmet.2016.09.010]
75 **Cao Q**, Zhao X, Bai J, Gery S, Sun H, Lin DC, Chen Q, Chen Z, Mack L, Yang H, Deng R, Shi X, Chong LW, Cho H, Xie J, Li QZ, Müschen M, Atkins AR, Liddle C, Yu RT, Alkan S, Said JW, Zheng Y, Downes M, Evans RM, Koeffler HP. Circadian clock cryptochrome proteins regulate autoimmunity. *Proc Natl Acad Sci U S A* 2017; **114**: 12548-12553 [PMID: 29109286 DOI: 10.1073/pnas.1619119114]
76 **Cermakian N**, Lange T, Golombek D, Sarkar D, Nakao A, Shibata S, Mazzoccoli G. Crosstalk between the circadian clock circuitry and the immune system. *Chronobiol Int* 2013; **30**: 870-888 [PMID: 23697902 DOI: 10.3109/07420528.2013.782315]
77 **Masri S**, Papagiannakopoulos T, Kinouchi K, Liu Y, Cervantes M, Baldi P, Jacks T, Sassone-Corsi P. Lung Adenocarcinoma Distally Rewires Hepatic Circadian Homeostasis. *Cell* 2016; **165**: 896-909 [PMID: 27153497 DOI: 10.1016/j.cell.2016.04.039]
78 **Park H**, Saravanakumar G, Kim J, Lim J, Kim WJ. Tumor Microenvironment Sensitive Nanocarriers for Bioimaging and Therapeutics. *Adv Healthc Mater* 2021; **10**: e2000834 [PMID: 33073497 DOI: 10.1002/adhm.202000834]
79 **Jensen LD**, Cao Y. Clock controls angiogenesis. *Cell Cycle* 2013; **12**: 405-408 [PMID: 23324349 DOI: 10.4161/cc.23596]
80 **Scheiermann C**, Gibbs J, Ince L, Loudon A. Clocking in to immunity. *Nat Rev Immunol* 2018; **18**: 423-437 [PMID: 29662121 DOI: 10.1038/s41577-018-0008-4]
81 **Shalapour S**, Karin M. Immunity, inflammation, and cancer: an eternal fight between good and evil. *J Clin Invest* 2015; **125**: 3347-3355 [PMID: 26325032 DOI: 10.1172/JCI80007]
82 **Janich P**, Pascual G, Merlos-Suárez A, Batlle E, Ripperger J, Albrecht U, Cheng HY, Obrietan K, Di Croce L, Benitah SA. The circadian molecular clock creates epidermal stem cell heterogeneity. *Nature* 2011; **480**: 209-214 [PMID: 22080954 DOI: 10.1038/nature10649]
83 **Janich P**, Toufighi K, Solanas G, Luis NM, Minkwitz S, Serrano L, Lehner B, Benitah SA. Human epidermal stem cell function is regulated by circadian oscillations. *Cell Stem Cell* 2013; **13**: 745-753 [PMID: 24120744 DOI: 10.1016/j.stem.2013.09.004]
84 **Puram RV**, Kowalczyk MS, de Boer CG, Schneider RK, Miller PG, McConkey M, Tothova Z, Tejero H, Heckl D, Järås M, Chen MC, Li H, Tamayo A, Cowley GS, Rozenblatt-Rosen O, Al-Shahrour F, Regev A, Ebert BL. Core Circadian Clock Genes Regulate Leukemia Stem Cells in AML. *Cell* 2016; **165**: 303-316 [PMID: 27058663 DOI: 10.1016/j.cell.2016.03.015]
85 **Matsu-Ura T**, Dovzhenok A, Aihara E, Rood J, Le H, Ren Y, Rosselot AE, Zhang T, Lee C, Obrietan K, Montrose MH, Lim S, Moore SR, Hong CI. Intercellular Coupling of the Cell Cycle and Circadian Clock in Adult Stem Cell Culture. *Mol Cell* 2016; **64**: 900-912 [PMID: 27867006 DOI: 10.1016/j.molcel.2016.10.015]
86 **Salamanca-Fernández E**, Rodríguez-Barranco M, Guevara M, Ardanaz E, Olry de Labry Lima A, Sánchez MJ. Night-shift work and breast and prostate cancer risk: updating the evidence from epidemiological studies. *An Sist Sanit Navar* 2018; **41**: 211-226 [PMID: 30063040 DOI: 10.23938/ASSN.0307]
87 **Papantoniou K**, Castaño-Vinyals G, Espinosa A, Turner MC, Alonso-Aguado MH, Martin V, Aragonés N, Pérez-Gómez B, Pozo BM, Gómez-Acebo I, Ardanaz E, Altzibar JM, Peiro R, Tardon A, Lorca JA, Chirlaque MD, García-Palomo A, Jimenez-Moleon JJ, Dierssen T, Ederra M, Amiano P, Pollan M, Moreno V, Kogevinas M. Shift work and colorectal cancer risk in the MCC-Spain case-control study. *Scand J Work Environ Health* 2017; **43**: 250-259 [PMID: 28251241 DOI: 10.5271/sjweh.3626]
88 **Papantoniou K**, Devore EE, Massa J, Strohmaier S, Vetter C, Yang L, Shi Y, Giovannucci E, Speizer F, Schernhammer ES. Rotating night shift work and colorectal cancer risk in the nurses' health studies. *Int J Cancer* 2018; **143**: 2709-2717 [PMID: 29978466 DOI: 10.1002/ijc.31655]
89 **Leung L**, Grundy A, Siemiatycki J, Arseneau J, Gilbert L, Gotlieb WH, Provencher DM, Aronson KJ, Koushik A. Shift Work Patterns, Chronotype, and Epithelial Ovarian Cancer Risk. *Cancer Epidemiol Biomarkers Prev* 2019; **28**: 987-995 [PMID: 30842128 DOI: 10.1158/1055-9965.EPI-18-1112]
90 **Bhatti P**, Cushing-Haugen KL, Wicklund KG, Doherty JA, Rossing MA. Nightshift work and risk of ovarian cancer. *Occup Environ Med* 2013; **70**: 231-237 [PMID: 23343856 DOI: 10.1136/oemed-2012-101146]
91 **Carter BD**, Diver WR, Hildebrand JS, Patel AV, Gapstur SM. Circadian disruption and fatal ovarian cancer. *Am J Prev Med* 2014; **46**: S34-S41 [PMID: 24512929 DOI: 10.1016/j.amepre.2013.10.032]
92 **Wendeu-Foyet MG**, Bayon V, Cénée S, Trétarre B, Rébillard X, Cancel-Tassin G, Cussenot O, Lamy PJ, Faraut B, Ben Khedher S, Léger D, Menegaux F. Night work and prostate cancer risk: results from the EPICAP Study. *Occup Environ Med* 2018; **75**: 573-581 [PMID: 29921728 DOI: 10.1136/oemed-2018-105009]

93 **Cordina-Duverger E**, Menegaux F, Popa A, Rabstein S, Harth V, Pesch B, Brüning T, Fritschi L, Glass DC, Heyworth JS, Erren TC, Castaño-Vinyals G, Papantoniou K, Espinosa A, Kogevinas M, Grundy A, Spinelli JJ, Aronson KJ, Guénel P. Night shift work and breast cancer: a pooled analysis of population-based case-control studies with complete work history. *Eur J Epidemiol* 2018; **33**: 369-379 [PMID: 29464445 DOI: 10.1007/s10654-018-0368-x]

94 **Sancar A**, Van Gelder RN. Clocks, cancer, and chronochemotherapy. *Science* 2021; **371** [PMID: 33384351 DOI: 10.1126/science.abb0738]

95 **Zhou S**, Dai YM, Zeng XF, Chen HZ. Circadian Clock and Sirtuins in Diabetic Lung: A Mechanistic Perspective. *Front Endocrinol (Lausanne)* 2020; **11**: 173 [PMID: 32308644 DOI: 10.3389/fendo.2020.00173]

96 **Shivshankar P**, Fekry B, Eckel-Mahan K, Wetsel RA. Circadian Clock and Complement Immune System-Complementary Control of Physiology and Pathology? *Front Cell Infect Microbiol* 2020; **10**: 418 [PMID: 32923410 DOI: 10.3389/fcimb.2020.00418]

97 **Bishehsari F**, Voigt RM, Keshavarzian A. Circadian rhythms and the gut microbiota: from the metabolic syndrome to cancer. *Nat Rev Endocrinol* 2020; **16**: 731-739 [PMID: 33106657 DOI: 10.1038/s41574-020-00427-4]

98 **Fagundo-Rivera J**, Gómez-Salgado J, García-Iglesias JJ, Gómez-Salgado C, Camacho-Martín S, Ruiz-Frutos C. Relationship between Night Shifts and Risk of Breast Cancer among Nurses: A Systematic Review. *Medicina (Kaunas)* 2020; **56** [PMID: 33321692 DOI: 10.3390/medicina56120680]

99 **Malla RR**, Padmaraju V, Amajala KC, Chalikonda G, Nagaraju GP. Association between the Circadian Clock and the Tumor Microenvironment in Breast Cancer. *Crit Rev Oncog* 2021; **26**: 43-51 [PMID: 35377985 DOI: 10.1615/CritRevOncog.2021040504]

100 **Magni M**, Buscemi G, Zannini L. Cell cycle and apoptosis regulator 2 at the interface between DNA damage response and cell physiology. *Mutat Res Rev Mutat Res* 2018; **776**: 1-9 [PMID: 29807573 DOI: 10.1016/j.mrrev.2018.03.004]

101 **Morales-Santana S**, Morell S, Leon J, Carazo-Gallego A, Jimenez-Lopez JC, Morell M. An Overview of the Polymorphisms of Circadian Genes Associated With Endocrine Cancer. *Front Endocrinol (Lausanne)* 2019; **10**: 104 [PMID: 30873119 DOI: 10.3389/fendo.2019.00104]

102 **Rizk SM**, Shahin NN, Shaker OG. Association between SIRT1 Gene Polymorphisms and Breast Cancer in Egyptians. *PLoS One* 2016; **11**: e0151901 [PMID: 26999517 DOI: 10.1371/journal.pone.0151901]

103 **Ball LJ**, Palesh O, Kriegsfeld LJ. The Pathophysiologic Role of Disrupted Circadian and Neuroendocrine Rhythms in Breast Carcinogenesis. *Endocr Rev* 2016; **37**: 450-466 [PMID: 27712099 DOI: 10.1210/er.2015-1133]

104 **Yang T**, Huang W, Ma T, Yin X, Zhang J, Huo M, Hu T, Gao T, Liu W, Zhang D, Yu H, Teng X, Zhang M, Qin H, Yang Y, Yuan B, Wang Y. The PRMT6/PARP1/CRL4B Complex Regulates the Circadian Clock and Promotes Breast Tumorigenesis. *Adv Sci (Weinh)* 2023; **10**: e2202737 [PMID: 36941223 DOI: 10.1002/advs.202202737]

105 **El-Andaloussi N**, Valovka T, Toueille M, Steinacher R, Focke F, Gehrig P, Covic M, Hassa PO, Schär P, Hübscher U, Hottiger MO. Arginine methylation regulates DNA polymerase beta. *Mol Cell* 2006; **22**: 51-62 [PMID: 16600869 DOI: 10.1016/j.molcel.2006.02.013]

106 **Gupta S**, Kadumuri RV, Singh AK, Chavali S, Dhayalan A. Structure, Activity and Function of the Protein Arginine Methyltransferase 6. *Life (Basel)* 2021; **11** [PMID: 34575100 DOI: 10.3390/life11090951]

107 **Angelousi A**, Kassi E, Ansari-Nasiri N, Randeva H, Kaltsas G, Chrousos G. Clock genes and cancer development in particular in endocrine tissues. *Endocr Relat Cancer* 2019; **26**: R305-R317 [PMID: 30959483 DOI: 10.1530/ERC-19-0094]

108 **Tokunaga H**, Takebayashi Y, Utsunomiya H, Akahira J, Higashimoto M, Mashiko M, Ito K, Niikura H, Takenoshita S, Yaegashi N. Clinicopathological significance of circadian rhythm-related gene expression levels in patients with epithelial ovarian cancer. *Acta Obstet Gynecol Scand* 2008; **87**: 1060-1070 [PMID: 18720043 DOI: 10.1080/00016340802348286]

109 **Xu H**, Wang Z, Mo G, Chen H. Association between circadian gene CLOCK and cisplatin resistance in ovarian cancer cells: A preliminary study. *Oncol Lett* 2018; **15**: 8945-8950 [PMID: 29844814 DOI: 10.3892/ol.2018.8488]

110 **Lu Y**, Zheng X, Hu W, Bian S, Zhang Z, Tao D, Liu Y, Ma Y. Cancer/testis antigen PIWIL2 suppresses circadian rhythms by regulating the stability and activity of BMAL1 and CLOCK. *Oncotarget* 2017; **8**: 54913-54924 [PMID: 28903391 DOI: 10.18632/oncotarget.18973]

111 **Zhu WZ**, He QY, Feng DC, Wei Q, Yang L. Circadian rhythm in prostate cancer: time to take notice of the clock. *Asian J Androl* 2023; **25**: 184-191 [PMID: 36073562 DOI: 10.4103/aja202255]

112 **Kinouchi K**, Magnan C, Ceglia N, Liu Y, Cervantes M, Pastore N, Huynh T, Ballabio A, Baldi P, Masri S, Sassone-Corsi P. Fasting Imparts a Switch to Alternative Daily Pathways in Liver and Muscle. *Cell Rep* 2018; **25**: 3299-3314.e6 [PMID: 30566858 DOI: 10.1016/j.celrep.2018.11.077]

113 **Chu LW**, Zhu Y, Yu K, Zheng T, Yu H, Zhang Y, Sesterhenn I, Chokkalingam AP, Danforth KN, Shen MC, Stanczyk FZ, Gao YT, Hsing AW. Variants in circadian genes and prostate cancer risk: a population-based study in China. *Prostate Cancer Prostatic Dis* 2008; **11**: 342-348 [PMID: 17984998 DOI: 10.1038/sj.pcan.4501024]

114 **Zhu Y**, Stevens RG, Hoffman AE, Fitzgerald LM, Kwon EM, Ostrander EA, Davis S, Zheng T, Stanford JL. Testing the circadian gene hypothesis in prostate cancer: a population-based case-control study. *Cancer Res* 2009; **69**: 9315-9322 [PMID: 19934327 DOI: 10.1158/0008-5472.CAN-09-0648]

115 **Markt SC**, Valdimarsdottir UA, Shui IM, Sigurdardottir LG, Rider JR, Tamimi RM, Batista JL, Haneuse S, Flynn-Evans E, Lockley SW, Czeisler CA, Stampfer MJ, Launer L, Harris T, Smith AV, Gudnason V, Lindstrom S, Kraft P, Mucci LA. Circadian clock genes and risk of fatal prostate cancer. *Cancer Causes Control* 2015; **26**: 25-33 [PMID: 25388799 DOI: 10.1007/s10552-014-0478-z]

116 **Lin DW**, FitzGerald LM, Fu R, Kwon EM, Zheng SL, Kolb S, Wiklund F, Stattin P, Isaacs WB, Xu J, Ostrander EA, Feng Z, Grönberg H, Stanford JL. Genetic variants in the LEPR, CRY1, RNASEL, IL4, and ARVCF genes are prognostic markers of prostate cancer-specific mortality. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 1928-1936 [PMID: 21846818 DOI: 10.1158/1055-9965.EPI-11-0236]

117 **Mocellin S**, Tropea S, Benna C, Rossi CR. Circadian pathway genetic variation and cancer risk: evidence from genome-wide association studies. *BMC Med* 2018; **16**: 20 [PMID: 29455641 DOI: 10.1186/s12916-018-1010-1]

118 **Yu CC**, Chen LC, Chiou CY, Chang YJ, Lin VC, Huang CY, Lin IL, Chang TY, Lu TL, Lee CH, Huang SP, Bao BY. Genetic variants in the circadian rhythm pathway as indicators of prostate cancer progression. *Cancer Cell Int* 2019; **19**: 87 [PMID: 30996687 DOI: 10.1186/s12935-019-0811-4]

119 **Hadadi E**, Acloque H. Role of circadian rhythm disorders on EMT and tumour-immune interactions in endocrine-related cancers. *Endocr Relat Cancer* 2021; **28**: R67-R80 [PMID: 33446614 DOI: 10.1530/ERC-20-0390]

120 **Bartsch C**, Bartsch H, Schmidt A, Ilg S, Bichler KH, Flüchter SH. Melatonin and 6-sulfatoxymelatonin circadian rhythms in serum and urine of primary prostate cancer patients: evidence for reduced pineal activity and relevance of urinary determinations. *Clin Chim Acta* 1992; **209**: 153-167 [PMID: 1395046 DOI: 10.1016/0009-8981(92)90164-l]

121 **Tai SY**, Huang SP, Bao BY, Wu MT. Urinary melatonin-sulfate/cortisol ratio and the presence of prostate cancer: A case-control study. *Sci*

Rep 2016; **6**: 29606 [PMID: 27387675 DOI: 10.1038/srep29606]

122 **Li G**, Fan M, Zheng Z, Zhang Y, Zhang Z, Huang Z, Luo W, Zhao W, Lai X, Chen H, Zeng F, Deng F. Osteoblastic protein kinase D1 contributes to the prostate cancer cells dormancy *via* GAS6-circadian clock signaling. *Biochim Biophys Acta Mol Cell Res* 2022; **1869**: 119296 [PMID: 35595103 DOI: 10.1016/j.bbamcr.2022.119296]

123 **Chen J**, Deng F, Singh SV, Wang QJ. Protein kinase D3 (PKD3) contributes to prostate cancer cell growth and survival through a PKCepsilon/PKD3 pathway downstream of Akt and ERK 1/2. *Cancer Res* 2008; **68**: 3844-3853 [PMID: 18483269 DOI: 10.1158/0008-5472.CAN-07-5156]

124 **Zou Z**, Zeng F, Xu W, Wang C, Ke Z, Wang QJ, Deng F. PKD2 and PKD3 promote prostate cancer cell invasion by modulating NF-κB- and HDAC1-mediated expression and activation of uPA. *J Cell Sci* 2012; **125**: 4800-4811 [PMID: 22797919 DOI: 10.1242/jcs.106542]

125 **Grodski S**, Brown T, Sidhu S, Gill A, Robinson B, Learoyd D, Sywak M, Reeve T, Delbridge L. Increasing incidence of thyroid cancer is due to increased pathologic detection. *Surgery* 2008; **144**: 1038-43; discussion 1043 [PMID: 19041015 DOI: 10.1016/j.surg.2008.08.023]

126 **Chmielik E**, Rusinek D, Oczko-Wojciechowska M, Jarzab M, Krajewska J, Czarniecka A, Jarzab B. Heterogeneity of Thyroid Cancer. *Pathobiology* 2018; **85**: 117-129 [PMID: 29408820 DOI: 10.1159/000486422]

127 **Lim H**, Devesa SS, Sosa JA, Check D, Kitahara CM. Trends in Thyroid Cancer Incidence and Mortality in the United States, 1974-2013. *JAMA* 2017; **317**: 1338-1348 [PMID: 28362912 DOI: 10.1001/jama.2017.2719]

128 **Haymart MR**, Banerjee M, Reyes-Gastelum D, Caoili E, Norton EC. Thyroid Ultrasound and the Increase in Diagnosis of Low-Risk Thyroid Cancer. *J Clin Endocrinol Metab* 2019; **104**: 785-792 [PMID: 30329071 DOI: 10.1210/jc.2018-01933]

129 **Belfiore A**, La Rosa GL, La Porta GA, Giuffrida D, Milazzo G, Lupo L, Regalbuto C, Vigneri R. Cancer risk in patients with cold thyroid nodules: relevance of iodine intake, sex, age, and multinodularity. *Am J Med* 1992; **93**: 363-369 [PMID: 1415299 DOI: 10.1016/0002-9343(92)90164-7]

130 **Brenner D**, Elliston C, Hall E, Berdon W. Estimated risks of radiation-induced fatal cancer from pediatric CT. *AJR Am J Roentgenol* 2001; **176**: 289-296 [PMID: 11159059 DOI: 10.2214/ajr.176.2.1760289]

131 **Brenner DJ**, Doll R, Goodhead DT, Hall EJ, Land CE, Little JB, Lubin JH, Preston DL, Preston RJ, Puskin JS, Ron E, Sachs RK, Samet JM, Setlow RB, Zaider M. Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. *Proc Natl Acad Sci U S A* 2003; **100**: 13761-13766 [PMID: 14610281 DOI: 10.1073/pnas.2235592100]

132 **Yu GP**, Li JC, Branovan D, McCormick S, Schantz SP. Thyroid cancer incidence and survival in the national cancer institute surveillance, epidemiology, and end results race/ethnicity groups. *Thyroid* 2010; **20**: 465-473 [PMID: 20384488 DOI: 10.1089/thy.2008.0281]

133 **Haymart MR**, Repplinger DJ, Leverson GE, Elson DF, Sippel RS, Jaume JC, Chen H. Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. *J Clin Endocrinol Metab* 2008; **93**: 809-814 [PMID: 18160464 DOI: 10.1210/jc.2007-2215]

134 **Fiore E**, Rago T, Provenzale MA, Scutari M, Ugolini C, Basolo F, Di Coscio G, Berti P, Grasso L, Elisei R, Pinchera A, Vitti P. Lower levels of TSH are associated with a lower risk of papillary thyroid cancer in patients with thyroid nodular disease: thyroid autonomy may play a protective role. *Endocr Relat Cancer* 2009; **16**: 1251-1260 [PMID: 19528244 DOI: 10.1677/ERC-09-0036]

135 **Kitahara CM**, Platz EA, Freeman LE, Hsing AW, Linet MS, Park Y, Schairer C, Schatzkin A, Shikany JM, Berrington de González A. Obesity and thyroid cancer risk among U.S. men and women: a pooled analysis of five prospective studies. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 464-472 [PMID: 21266520 DOI: 10.1158/1055-9965.EPI-10-1220]

136 **Zhao ZG**, Guo XG, Ba CX, Wang W, Yang YY, Wang J, Cao HY. Overweight, obesity and thyroid cancer risk: a meta-analysis of cohort studies. *J Int Med Res* 2012; **40**: 2041-2050 [PMID: 23321160 DOI: 10.1177/03000605120400601]

137 **Zimmermann MB**, Boelaert K. Iodine deficiency and thyroid disorders. *Lancet Diabetes Endocrinol* 2015; **3**: 286-295 [PMID: 25591468 DOI: 10.1016/S2213-8587(14)70225-6]

138 **Ikegami K**, Refetoff S, Van Cauter E, Yoshimura T. Interconnection between circadian clocks and thyroid function. *Nat Rev Endocrinol* 2019; **15**: 590-600 [PMID: 31406343 DOI: 10.1038/s41574-019-0237-z]

139 **Luo J**, Sands M, Wactawski-Wende J, Song Y, Margolis KL. Sleep disturbance and incidence of thyroid cancer in postmenopausal women the Women's Health Initiative. *Am J Epidemiol* 2013; **177**: 42-49 [PMID: 23221728 DOI: 10.1093/aje/kws193]

140 **Moon SH**, Lee BJ, Kim SJ, Kim HC. Relationship between thyroid stimulating hormone and night shift work. *Ann Occup Environ Med* 2016; **28**: 53 [PMID: 27761265 DOI: 10.1186/s40557-016-0141-0]

141 **Reiter RJ**, Tan DX, Korkmaz A, Erren TC, Piekarski C, Tamura H, Manchester LC. Light at night, chronodisruption, melatonin suppression, and cancer risk: a review. *Crit Rev Oncog* 2007; **13**: 303-328 [PMID: 18540832 DOI: 10.1615/critrevoncog.v13.i4.30]

142 **Zhang D**, Jones RR, James P, Kitahara CM, Xiao Q. Associations between artificial light at night and risk for thyroid cancer: A large US cohort study. *Cancer* 2021; **127**: 1448-1458 [PMID: 33554351 DOI: 10.1002/cncr.33392]

143 **Kalsbeek A**, Fliers E. Daily regulation of hormone profiles. *Handb Exp Pharmacol* 2013; 185-226 [PMID: 23604480 DOI: 10.1007/978-3-642-25950-0_8]

144 **Mannic T**, Meyer P, Triponez F, Pusztaszeri M, Le Martelot G, Mariani O, Schmitter D, Sage D, Philippe J, Dibner C. Circadian clock characteristics are altered in human thyroid malignant nodules. *J Clin Endocrinol Metab* 2013; **98**: 4446-4456 [PMID: 23979949 DOI: 10.1210/jc.2013-2568]

145 **Wasenius VM**, Hemmer S, Kettunen E, Knuutila S, Franssila K, Joensuu H. Hepatocyte growth factor receptor, matrix metalloproteinase-11, tissue inhibitor of metalloproteinase-1, and fibronectin are up-regulated in papillary thyroid carcinoma: a cDNA and tissue microarray study. *Clin Cancer Res* 2003; **9**: 68-75 [PMID: 12538453]

146 **Hawthorn L**, Stein L, Varma R, Wiseman S, Loree T, Tan D. TIMP1 and SERPIN-A overexpression and TFF3 and CRABP1 underexpression as biomarkers for papillary thyroid carcinoma. *Head Neck* 2004; **26**: 1069-1083 [PMID: 15515157 DOI: 10.1002/hed.20099]

147 **Xu T**, Jin T, Lu X, Pan Z, Tan Z, Zheng C, Liu Y, Hu X, Ba L, Ren H, Chen J, Zhu C, Ge M, Huang P. A signature of circadian rhythm genes in driving anaplastic thyroid carcinoma malignant progression. *Cell Signal* 2022; **95**: 110332 [PMID: 35430345 DOI: 10.1016/j.cellsig.2022.110332]

148 **Kettner NM**, Katchy CA, Fu L. Circadian gene variants in cancer. *Ann Med* 2014; **46**: 208-220 [PMID: 24901356 DOI: 10.3109/07853890.2014.914808]

149 **Gallo C**, Fragliasso V, Donati B, Torricelli F, Tameni A, Piana S, Ciarrocchi A. The bHLH transcription factor DEC1 promotes thyroid cancer aggressiveness by the interplay with NOTCH1. *Cell Death Dis* 2018; **9**: 871 [PMID: 30158530 DOI: 10.1038/s41419-018-0933-y]

150 **Fassnacht M**, Tsagarakis S, Terzolo M, Tabarin A, Sahdev A, Newell-Price J, Pelsma I, Marina L, Lorenz K, Bancos I, Arlt W, Dekkers OM. European Society of Endocrinology clinical practice guidelines on the management of adrenal incidentalomas, in collaboration with the

European Network for the Study of Adrenal Tumors. *Eur J Endocrinol* 2023; **189**: G1-G42 [PMID: 37318239 DOI: 10.1093/ejendo/lvad066]

151 **Arlt W**, Biehl M, Taylor AE, Hahner S, Libé R, Hughes BA, Schneider P, Smith DJ, Stiekema H, Krone N, Porfiri E, Opocher G, Bertherat J, Mantero F, Allolio B, Terzolo M, Nightingale P, Shackleton CH, Bertagna X, Fassnacht M, Stewart PM. Urine steroid metabolomics as a biomarker tool for detecting malignancy in adrenal tumors. *J Clin Endocrinol Metab* 2011; **96**: 3775-3784 [PMID: 21917861 DOI: 10.1210/jc.2011-1565]

152 **Wooten MD**, King DK. Adrenal cortical carcinoma. Epidemiology and treatment with mitotane and a review of the literature. *Cancer* 1993; **72**: 3145-3155 [PMID: 8242539 DOI: 10.1002/1097-0142(19931201)72:11<3145::aid-cncr2820721105>3.0.co;2-n]

153 **Kebebew E**, Reiff E, Duh QY, Clark OH, McMillan A. Extent of disease at presentation and outcome for adrenocortical carcinoma: have we made progress? *World J Surg* 2006; **30**: 872-878 [PMID: 16680602 DOI: 10.1007/s00268-005-0329-x]

154 **Gicquel C**, Baudin E, Lebouc Y, Schlumberger M. Adrenocortical carcinoma. *Ann Oncol* 1997; **8**: 423-427 [PMID: 9233519 DOI: 10.1023/a:1008257530919]

155 **Angelousi A**, Nasiri-Ansari N, Karapanagioti A, Kyriakopoulos G, Aggeli C, Zografos G, Choreftaki T, Parianos C, Kounadi T, Alexandraki K, Randeva HS, Kaltsas G, Papavassiliou AG, Kassi E. Expression of clock-related genes in benign and malignant adrenal tumors. *Endocrine* 2020; **68**: 650-659 [PMID: 32147772 DOI: 10.1007/s12020-020-02246-z]

156 **Oster H**, Challet E, Ott V, Arvat E, de Kloet ER, Dijk DJ, Lightman S, Vgontzas A, Van Cauter E. The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids. *Endocr Rev* 2017; **38**: 3-45 [PMID: 27749086 DOI: 10.1210/er.2015-1080]

157 **Minnetti M**, Hasenmajer V, Pofi R, Venneri MA, Alexandraki KI, Isidori AM. Fixing the broken clock in adrenal disorders: focus on glucocorticoids and chronotherapy. *J Endocrinol* 2020; **246**: R13-R31 [PMID: 32380472 DOI: 10.1530/JOE-20-0066]

158 **Dallmann R**, Touma C, Palme R, Albrecht U, Steinlechner S. Impaired daily glucocorticoid rhythm in Per1 (Brd) mice. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2006; **192**: 769-775 [PMID: 16505983 DOI: 10.1007/s00359-006-0114-9]

159 **Oster H**, Damerow S, Kiessling S, Jakubcakova V, Abraham D, Tian J, Hoffmann MW, Eichele G. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab* 2006; **4**: 163-173 [PMID: 16890544 DOI: 10.1016/j.cmet.2006.07.002]

160 **Engeland WC**, Massman L, Miller L, Leng S, Pignatti E, Pantano L, Carlone DL, Kofuji P, Breault DT. Sex Differences in Adrenal Bmal1 Deletion-Induced Augmentation of Glucocorticoid Responses to Stress and ACTH in Mice. *Endocrinology* 2019; **160**: 2215-2229 [PMID: 31398249 DOI: 10.1210/en.2019-00357]

161 **Koshy A**, Cuesta M, Boudreau P, Cermakian N, Boivin DB. Disruption of central and peripheral circadian clocks in police officers working at night. *FASEB J* 2019; **33**: 6789-6800 [PMID: 30811213 DOI: 10.1096/fj.201801889R]

162 **Gumz ML**, Popp MP, Wingo CS, Cain BD. Early transcriptional effects of aldosterone in a mouse inner medullary collecting duct cell line. *Am J Physiol Renal Physiol* 2003; **285**: F664-F673 [PMID: 12770840 DOI: 10.1152/ajprenal.00353.2002]

163 **Tetti M**, Castellano I, Venziano F, Magnino C, Veglio F, Mulatero P, Monticone S. Role of Cryptochrome-1 and Cryptochrome-2 in Aldosterone-Producing Adenomas and Adrenocortical Cells. *Int J Mol Sci* 2018; **19** [PMID: 29874863 DOI: 10.3390/ijms19061675]

164 **Solocinski K**, Holzworth M, Wen X, Cheng KY, Lynch IJ, Cain BD, Wingo CS, Gumz ML. Desoxycorticosterone pivalate-salt treatment leads to non-dipping hypertension in Per1 knockout mice. *Acta Physiol (Oxf)* 2017; **220**: 72-82 [PMID: 27636900 DOI: 10.1111/apha.12804]

165 **Douma LG**, Costello HM, Crislip GR, Cheng KY, Lynch IJ, Juffre A, Barral D, Masten S, Roig E, Beguiristain K, Li W, Bratanatawira P, Wingo CS, Gumz ML. Kidney-specific KO of the circadian clock protein PER1 alters renal Na(+) handling, aldosterone levels, and kidney/adrenal gene expression. *Am J Physiol Renal Physiol* 2022; **322**: F449-F459 [PMID: 35129370 DOI: 10.1152/ajprenal.00385.2021]

166 **Douma LG**, Crislip GR, Cheng KY, Barral D, Masten S, Holzworth M, Roig E, Glasford K, Beguiristain K, Li W, Bratanatawira P, Lynch IJ, Cain BD, Wingo CS, Gumz ML. Knockout of the circadian clock protein PER1 results in sex-dependent alterations of ET-1 production in mice in response to a high-salt diet plus mineralocorticoid treatment. *Can J Physiol Pharmacol* 2020; **98**: 579-586 [PMID: 32437627 DOI: 10.1139/cjpp-2019-0688]

167 **Costello HM**, Mckee A, Bratanatawira P, Crislip GR, Juffre A, Cheng K, Wingo CS, Gumz ML. A Role for the Adrenal Clock in Aldosterone Regulation and Renal Excretory Function. *FASEB J* 2022; **36** [DOI: 10.1096/fasebj.2022.36.s1.r1911]

168 **Bersten DC**, Sullivan AE, Peet DJ, Whitelaw ML. bHLH-PAS proteins in cancer. *Nat Rev Cancer* 2013; **13**: 827-841 [PMID: 24263188 DOI: 10.1038/nrc3621]

169 **Kaelin WG Jr**. Cancer and altered metabolism: potential importance of hypoxia-inducible factor and 2-oxoglutarate-dependent dioxygenases. *Cold Spring Harb Symp Quant Biol* 2011; **76**: 335-345 [PMID: 22089927 DOI: 10.1101/sqb.2011.76.010975]

170 **Favier J**, Gimenez-Roqueplo AP. Pheochromocytomas: the (pseudo)-hypoxia hypothesis. *Best Pract Res Clin Endocrinol Metab* 2010; **24**: 957-968 [PMID: 21115164 DOI: 10.1016/j.beem.2010.10.004]

171 **Gimenez-Roqueplo AP**, Favier J, Rustin P, Rieubland C, Kerlan V, Plouin PF, Rötig A, Jeunemaitre X. Functional consequences of a SDHB gene mutation in an apparently sporadic pheochromocytoma. *J Clin Endocrinol Metab* 2002; **87**: 4771-4774 [PMID: 12364472 DOI: 10.1210/jc.2002-020525]

172 **Rutter J**, Reick M, Wu LC, McKnight SL. Regulation of clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 2001; **293**: 510-514 [PMID: 11441146 DOI: 10.1126/science.1060698]

173 **Lemos DR**, Goodspeed L, Tonelli L, Antoch MP, Ojeda SR, Urbanski HF. Evidence for circadian regulation of activating transcription factor 5 but not tyrosine hydroxylase by the chromaffin cell clock. *Endocrinology* 2007; **148**: 5811-5821 [PMID: 17823250 DOI: 10.1210/en.2007-0610]

174 **Greene LA**, Lee HY, Angelastro JM. The transcription factor ATF5: role in neurodevelopment and neural tumors. *J Neurochem* 2009; **108**: 11-22 [PMID: 19046351 DOI: 10.1111/j.1471-4159.2008.05749.x]

175 **Daly AF**, Tichomirowa MA, Beckers A. The epidemiology and genetics of pituitary adenomas. *Best Pract Res Clin Endocrinol Metab* 2009; **23**: 543-554 [PMID: 19945022 DOI: 10.1016/j.beem.2009.05.008]

176 **Guo L**, Cen H, Weng J, He Y, Guo X, He D, Liu K, Duan S, Yang J, Zhang X, Qin Z, Wan Y, Chen Z, Wu B. PER2 integrates circadian disruption and pituitary tumorigenesis. *Theranostics* 2023; **13**: 2657-2672 [PMID: 37215573 DOI: 10.7150/thno.82995]

177 **Schmoldt A**, Benthe HF, Haberland G. Digitoxin metabolism by rat liver microsomes. *Biochem Pharmacol* 1975; **24**: 1639-1641 [PMID: 10 DOI: 10.1530/endoabs.81.P422]

178 **Sulli G**, Manoogian ENC, Taub PR, Panda S. Training the Circadian Clock, Clocking the Drugs, and Drugging the Clock to Prevent, Manage, and Treat Chronic Diseases. *Trends Pharmacol Sci* 2018; **39**: 812-827 [PMID: 30060890 DOI: 10.1016/j.tips.2018.07.003]

179 **Sulli G**, Rommel A, Wang X, Kolar MJ, Puca F, Saghatelian A, Plikus MV, Verma IM, Panda S. Pharmacological activation of REV-ERBs is lethal in cancer and oncogene-induced senescence. *Nature* 2018; **553**: 351-355 [PMID: 29320480 DOI: 10.1038/nature25170]

180 **Dauchy RT**, Wren-Dail MA, Dupepe LM, Hill SM, Xiang S, Anbalagan M, Belancio VP, Dauchy EM, Blask DE. Effect of Daytime Blue-enriched LED Light on the Nighttime Circadian Melatonin Inhibition of Hepatoma 7288CTC Warburg Effect and Progression. *Comp Med* 2018; **68**: 269-279 [PMID: 29875029 DOI: 10.30802/AALAS-CM-17-000107]

181 **Bhattacharya S**, Patel KK, Dehari D, Agrawal AK, Singh S. Melatonin and its ubiquitous anticancer effects. *Mol Cell Biochem* 2019; **462**: 133-155 [PMID: 31451998 DOI: 10.1007/s11010-019-03617-5]

182 **Zhao X**, Yang J, Huang R, Guo M, Zhou Y, Xu L. The role and its mechanism of intermittent fasting in tumors: friend or foe? *Cancer Biol Med* 2021; **18**: 63-73 [PMID: 33628585 DOI: 10.20892/j.issn.2095-3941.2020.0250]

183 **Weitzer J**, Castaño-Vinyals G, Aragonés N, Gómez-Acebo I, Guevara M, Amiano P, Martín V, Molina-Barceló A, Alguacil J, Moreno V, Suarez-Calleja C, Jiménez-Moleón JJ, Marcos-Gragera R, Papantoniou K, Pérez-Gómez B, Llorca J, Ascunce N, Gil L, Gracia-Lavedan E, Casabonne D, Lope V, Pollán M, Kogevinas M. Effect of time of day of recreational and household physical activity on prostate and breast cancer risk (MCC-Spain study). *Int J Cancer* 2021; **148**: 1360-1371 [PMID: 32976649 DOI: 10.1002/ijc.33310]

184 **Damato AR**, Herzog ED. Circadian clock synchrony and chronotherapy opportunities in cancer treatment. *Semin Cell Dev Biol* 2022; **126**: 27-36 [PMID: 34362656 DOI: 10.1016/j.semcdb.2021.07.017]

185 **Li J**, Chen R, Yao QY, Liu SJ, Tian XY, Hao CY, Lu W, Zhou TY. Time-dependent pharmacokinetics of dexamethasone and its efficacy in human breast cancer xenograft mice: a semi-mechanism-based pharmacokinetic/pharmacodynamic model. *Acta Pharmacol Sin* 2018; **39**: 472-481 [PMID: 29119968 DOI: 10.1038/aps.2017.153]

186 **Dakup PP**, Porter KI, Little AA, Gajula RP, Zhang H, Skornyakov E, Kemp MG, Van Dongen HPA, Gaddameedhi S. The circadian clock regulates cisplatin-induced toxicity and tumor regression in melanoma mouse and human models. *Oncotarget* 2018; **9**: 14524-14538 [PMID: 29581861 DOI: 10.18632/oncotarget.24539]

187 **Lee Y**, Fong SY, Shon J, Zhang SL, Brooks R, Lahens NF, Chen D, Dang CV, Field JM, Sehgal A. Time-of-day specificity of anticancer drugs may be mediated by circadian regulation of the cell cycle. *Sci Adv* 2021; **7** [PMID: 33579708 DOI: 10.1126/sciadv.abd2645]

188 **Slat EA**, Sponagel J, Marpegan L, Simon T, Kfoury N, Kim A, Binz A, Herzog ED, Rubin JB. Cell-intrinsic, Bmal1-dependent Circadian Regulation of Temozolomide Sensitivity in Glioblastoma. *J Biol Rhythms* 2017; **32**: 121-129 [PMID: 28470120 DOI: 10.1177/0748730417696788]

189 **Harper E**, Talbot CJ. Is it Time to Change Radiotherapy: The Dawning of Chronoradiotherapy? *Clin Oncol (R Coll Radiol)* 2019; **31**: 326-335 [PMID: 30902558 DOI: 10.1016/j.clon.2019.02.010]

190 **Ye Y**, Xiang Y, Ozguc FM, Kim Y, Liu CJ, Park PK, Hu Q, Diao L, Lou Y, Lin C, Guo AY, Zhou B, Wang L, Chen Z, Takahashi JS, Mills GB, Yoo SH, Han L. The Genomic Landscape and Pharmacogenomic Interactions of Clock Genes in Cancer Chronotherapy. *Cell Syst* 2018; **6**: 314-328.e2 [PMID: 29525205 DOI: 10.1016/j.cels.2018.01.013]

191 **Dong Z**, Zhang G, Qu M, Gimple RC, Wu Q, Qiu Z, Prager BC, Wang X, Kim LJY, Morton AR, Dixit D, Zhou W, Huang H, Li B, Zhu Z, Bao S, Mack SC, Chavez L, Kay SA, Rich JN. Targeting Glioblastoma Stem Cells through Disruption of the Circadian Clock. *Cancer Discov* 2019; **9**: 1556-1573 [PMID: 31455674 DOI: 10.1158/2159-8290.CD-19-0215]

192 **Mahalingam D**, Wang JS, Hamilton EP, Sarantopoulos J, Nemunaitis J, Weems G, Carter L, Hu X, Schreeder M, Wilkins HJ. Phase 1 Open-Label, Multicenter Study of First-in-Class RORγ Agonist LYC-55716 (Cintirorgon): Safety, Tolerability, and Preliminary Evidence of Antitumor Activity. *Clin Cancer Res* 2019; **25**: 3508-3516 [PMID: 30819679 DOI: 10.1158/1078-0432.CCR-18-3185]

193 **Aroca-Siendones MI**, Moreno-SanJuan S, Puentes-Pardo JD, Verbeni M, Arnedo J, Escudero-Feliu J, García-Costela M, García-Robles A, Carazo Á, León J. Core Circadian Clock Proteins as Biomarkers of Progression in Colorectal Cancer. *Biomedicines* 2021; **9** [PMID: 34440171 DOI: 10.3390/biomedicines9080967]

194 **Chen K**, Wang Y, Li D, Wu R, Wang J, Wei W, Zhu W, Xie W, Feng D, He Y. Biological clock regulation by the PER gene family: a new perspective on tumor development. *Front Cell Dev Biol* 2024; **12**: 1332506 [PMID: 38813085 DOI: 10.3389/fcell.2024.1332506]

195 **Li HX**. The role of circadian clock genes in tumors. *Onco Targets Ther* 2019; **12**: 3645-3660 [PMID: 31190867 DOI: 10.2147/OTT.S203144]

196 **Cadenas C**, van de Sandt L, Edlund K, Lohr M, Hellwig B, Marchan R, Schmidt M, Rahnenführer J, Oster H, Hengstler JG. Loss of circadian clock gene expression is associated with tumor progression in breast cancer. *Cell Cycle* 2014; **13**: 3282-3291 [PMID: 25485508 DOI: 10.4161/15384101.2014.954454]

197 **Zhu H**, Chen J, Wen Z, Li J, Yu Q, Liao W, Luo X. The role of circadian clock genes in colorectal carcinoma: Novel insights into regulatory mechanism and implications in clinical therapy. *Life Sci* 2023; **333**: 122145 [PMID: 37797685 DOI: 10.1016/j.lfs.2023.122145]

198 **Wang S**, Khan S, Nabi G, Li HY. Circadian rhythm as a key player in cancer progression as well as a therapeutic target in HER2-positive advanced gastric cancer treatment. *Front Oncol* 2023; **13**: 1240676 [PMID: 37503323 DOI: 10.3389/fonc.2023.1240676]

199 **Pourali G**, Ahmadzade AM, Arastonejad M, Pourali R, Kazemi D, Ghasemirad H, Khazaei M, Fiuji H, Nassiri M, Hassanian SM, Ferns GA, Avan A. The circadian clock as a potential biomarker and therapeutic target in pancreatic cancer. *Mol Cell Biochem* 2024; **479**: 1243-1255 [PMID: 37405534 DOI: 10.1007/s11010-023-04790-4]

200 **Miro C**, Docimo A, Barrea L, Verde L, Cernea S, Sojat AS, Marina LV, Docimo G, Colao A, Dentice M, Muscogiuri G. "Time" for obesity-related cancer: The role of the circadian rhythm in cancer pathogenesis and treatment. *Semin Cancer Biol* 2023; **91**: 99-109 [PMID: 36893964 DOI: 10.1016/j.semcancer.2023.03.003]

201 **Fonnes S**, Donatsky AM, Gögenur I. Expression of core clock genes in colorectal tumour cells compared with normal mucosa: a systematic review of clinical trials. *Colorectal Dis* 2015; **17**: 290-297 [PMID: 25418520 DOI: 10.1111/codi.12847]

202 **Karantanos T**, Theodoropoulos G, Pektasides D, Gazouli M. Clock genes: their role in colorectal cancer. *World J Gastroenterol* 2014; **20**: 1986-1992 [PMID: 24587674 DOI: 10.3748/wjg.v20.i8.1986]

203 **Rida P**, Syed MI, Aneja R. Time will tell: Circadian clock dysregulation in triple negative breast cancer. *Front Biosci (Schol Ed)* 2019; **11**: 178-192 [PMID: 30844743 DOI: 10.2741/S533]

204 **Tsuchiya Y**, Umemura Y, Yagita K. Circadian clock and cancer: From a viewpoint of cellular differentiation. *Int J Urol* 2020; **27**: 518-524 [PMID: 32223039 DOI: 10.1111/iju.14231]

205 **Reszka E**, Przybek M. Circadian Genes in Breast Cancer. *Adv Clin Chem* 2016; **75**: 53-70 [PMID: 27346616 DOI: 10.1016/bs.acc.2016.03.005]

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MINIREVIEWS

Histologic subtypes of non-muscle invasive bladder cancer

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Abstract

The majority of bladder cancers (BCs) are non-muscle invasive BCs (NMIBCs) and show the morphology of a conventional urothelial carcinoma (UC). Aberrant morphology is rare but can be observed. The classification and characterization of histologic subtypes (HS) in UC in BC have mainly been described in muscle invasive bladder cancer (MIBC). However, the currently used classification is applied for invasive urothelial neoplasm and therefore, also valid for a subset of NMIBC. The standard transurethral diagnostic work-up misses the presence of HS in NMIBC in a considerable percentage of patients and the real prevalence is not known. HS in NMIBC are associated with an aggressive phenotype. Consequently, clinical guidelines categorize HS of NMIBC as "(very) high-risk" tumors and recommend offering radical cystectomy to these patients. Alternative strategies for bladder preservation can only be offered to highly selected patients and ideally within clinical trials. Novel treatment strategies and biomarkers have been established MIBC and NMIBC but have not been comprehensively investigated in the context of HS in NMIBC. Further evaluation prior to implementation into clinical practice is needed.

Key Words: Urothelial carcinoma; Non-muscle invasive bladder cancer; Muscle invasive bladder cancer; Histologic subtypes; Histologic variants

Core Tip: The currently used classification for histologic subtypes (HS) in urothelial carcinoma has mainly been described in muscle invasive bladder cancer. However, a subset of non-muscle invasive bladder cancer presents HS, and their presence is clinically relevant. In this minireview, we discuss the epidemiology, classification, characterization and the clinical relevance of HS in non-muscle invasive bladder cancer.

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INTRODUCTION

The majority (75%) of bladder cancers (BCs) are non-muscle invasive BCs (NMIBCs) and are confined to the mucosa or the submucosa. While most NMIBC show the morphology of conventional urothelial carcinoma (UC), aberrant morphology can be observed. These so-called histologic subtypes (HS) were first described in the literature in the 1990s in small case series. More recently, an increasing interest in the biological and clinical characteristics of HS has emerged. In the literature, HS are mainly investigated in radical cystectomy (RC) specimens from patients with muscle-invasive bladder cancer (MIBC). Only for selected specific HS, have aggressive features in NMIBC been identified. However, HS have raised the interest of scientists, urologists and oncologists due to emerging novel diagnostic and therapeutic options. The purpose of this mini-review is to summarize the current literature on HS in NMIBC. Further evaluation prior to implementation into clinical practice is needed.

HS IN NMIBC

Classification

According to the fifth and new edition of the 2022 World Health Organization (WHO) Classification, histologic characteristics are still considered the gold standard for the classification. Due to the recent considerable advances in understanding the genomic landscape of UC and definition of intrinsic molecular subtypes, their future potential clinical impact is acknowledged in the new 2022 WHO Classification. Regarding HS in UC, investigations have mainly been conducted in MIBC and a separate classification of HS in NMIBC has not been described. However, the classification still includes the category "invasive urothelial carcinoma", which includes a subset of NMIBC.

Table 1 indicates the current classification of tumors of the urinary tract. Further categories listed in this table, such as noninvasive urothelial neoplasms and nonurothelial tumors (metastatic, hematolymphoid, mesenchymal, neuroendocrine, and genetic syndrome-related tumors), are not further discussed in this article[1].

The real incidence of HS in NMIBC is unknown and is not comprehensively investigated in the literature. In cystectomy series, pure UC is present in two-thirds of the patients and is the most common histologic entity in BC. Cystectomy series of patients with NMIBC likely overestimate the incidence of HS because these are associated with more aggressive tumor characteristics and are more frequently treated with RC[2].

Accuracy of transurethral resection of the bladder tumor in detecting HS

NMIBC is treated by transurethral resection of the bladder tumor (TURBT) to confirm the diagnosis, define tumor grading, and ideally remove the entire tumor. Pathological evaluation of TURBT specimens has several limitations. In the context of this article, we are focusing on the diagnostic accuracy and potential limitations of TURBT in evaluating the presence of HS. Several studies have shown low concordance between the presence of HS in TURBT and RC[3]. By contrast, other retrospective studies have reported a relatively high rate of detecting HS in TURBT[4]. The reasons for these conflicting results are likely related to the heterogeneity of patient populations, resection techniques, and pathological work-up.

Another critical aspect is the missed diagnosis of HS in the initial pathological reporting, as shown by Kamat *et al*[5]. after reviewing specimens of 100 patients with micropapillary NMIBC. This last aspect is not only related to the experience of the pathologist in uropathology, as interobserver variability between experienced uropathologists is also a critical issue[6].

In summary, TURBT alone likely misses the presence of HS in NMIBC in a considerable percentage of patients, and a comprehensive investigation of this clinically relevant issue has not yet been published.

TREATMENT OPTIONS

According to the current American Urological Association and European Association of Urology guidelines, NMIBC with HS should be considered" (very) high-risk" tumors in the risk stratification for primary UC. This recommendation is based on the association of HS with more advanced TNM stage, worse outcomes, and increased risk of treatment failure after bladder-sparing therapy in T1 disease. Moreover, the risk of progression is significantly higher in patients with HS (16% after 1 year, 40% after 5 years) and therefore, clinical guidelines suggest offering primary RC in these patients[7]. Due to the diagnostic challenges and limitations, and considering the limited data, an assessment of prognostic differences among HS in NMIBC cannot be made.

Table 1 Classification of tumors of the urinary tract

Category	Features
Urothelial tumors	Invasive urothelial neoplasms: (1) Conventional urothelial carcinoma; (2) Urothelial carcinoma with squamous differentiation; (3) Urothelial carcinoma with glandular differentiation; (4) Urothelial carcinoma with trophoblastic differentiation; (5) Nested urothelial carcinoma; (6) Large nested urothelial carcinoma; (7) Tubular and microcystic urothelial carcinomas; (8) Micropapillary urothelial carcinoma; (9) Lymphoepithelioma-like urothelial carcinoma; (10) Plasmacytoid urothelial carcinoma; (11) Giant cell urothelial carcinoma; (12) Lipid-rich urothelial carcinoma; (13) Clear cell (glycogen-rich) urothelial carcinoma; (14) Sarcomatoid urothelial carcinoma; and (15) Poorly differentiated urothelial carcinoma Noninvasive urothelial neoplasms: (1) Urothelial papilloma; (2) Urothelial papilloma, inverted; (3) Papillary urothelial neoplasm of low malignant potential; (4) Inverted papillary urothelial neoplasm of low malignant potential; (5) Noninvasive papillary urothelial carcinoma, low grade; (6) Low-grade papillary urothelial carcinoma with an inverted growth pattern; (7) Noninvasive papillary urothelial carcinoma, high grade; (8) Noninvasive high-grade papillary urothelial carcinoma with an inverted growth pattern; and (9) Urothelial carcinoma in situ
Nonurothelial tumors	(1) Squamous cell neoplasms of the urinary tract; (2) Glandular neoplasms; (3) Adenocarcinomas; (4) Urachal and diverticular neoplasms; (5) Urethral neoplasms; and (6) Tumors of Mullerian type

Instillation therapy

In a retrospective series of 44 patients with micropapillary NMIBC treated with bacillus Calmette-Guérin (BCG), 67% of these patients experienced tumor progression, 22% developed metastasis, and two-thirds ended up with secondary RC [5]. Another retrospective series of 36 patients with micropapillary NMIBC, 21 of whom underwent primary conservative therapy (BCG, surveillance, deferred RC), showed a slightly lower tumor progression rate (10%), a similar rate of metastasis (19%), and a 5-year cancer-specific mortality of 25% (*vs* 17% in the subcohort undergoing early RC; $P = 0.8$)[8]. In 2015, Willis *et al*[9] analyzed 72 patients with micropapillary UC staged as cT1N0M0. Of the 40 patients who received primary BCG therapy, 75% had recurrence, 45% showed progression, and 35% developed metastasis. Five-year disease-specific survival was 60% (*vs* 100% in the subgroup with upfront RC; $P = 0.006$). In 2020, Prado *et al*[10] reviewed 347 patients with NMIBC (59 with HS, 288 with pure UC) who underwent intravesical treatment with BCG. Surprisingly, recurrence-free survival was greater in the HS group compared to the pure UC group (62.1% *vs* 38.0%; $P < 0.05$). The authors concluded that a selected subpopulation may be treated with BCG. However, these results were presented as an abstract in 2020 and a final publication is still pending.

More recently, a systematic review analyzed 16 studies from 2011 to 2020 on NMIBC with HS. According to their analysis, TURBT and BCG seem to be feasible in NMIBC with squamous and/or glandular differentiation in selected patients with low tumor burden and without risk factors. For most HS (*e.g.*, micropapillary, sarcomatoid, plasmacytoid, and nested variant), RC should be considered first-line therapy[11].

Novel treatment options and promising biomarkers

More recently, several novel intravesical treatments and regimens have been discovered and investigated for the treatment of NMIBC[12-14]. Moreover, systemic treatment with check-point inhibition is being tested with or without intravesical instillation therapies[15]. None of these investigations and trials have focused on the antitumor activity in NMIBC with HS. Therefore, these alternative strategies for bladder preservation should only be offered to highly selected patients and ideally within a clinical trial.

Novel biomarkers such as cell-free circulating tumor DNA (ctDNA) in serum or even urine have been discovered[15, 16]. They are thought to reflect the residual tumor more accurately compared to the current standard of care. This approach may be promising in some HS that have been associated with specific genomic alterations. For example, the plasmacytoid variant shows frequent somatic cadherin 1 loss-of-function mutations[17]. Whereas, large nested variant is fibroblast growth factor receptor 3-mutated[18]. Whether ctDNA allows exploitation of these genomic characteristics in specific HS and better reflect residual disease or tumor recurrence remains to be shown. However, more accurate monitoring of the tumor burden and clinical course may allow bladder preservation in such selected situations.

CONCLUSION

The presence of HS is underdiagnosed by TURBT in MIBC, while in NMIBC findings are not consistent. Upfront radical surgery should be offered to these patients whereas bladder preservation may be performed in selected cases or within clinical trials. Predictive models like the European Organization for Research and Treatment of Cancer risk tables should include HS in the future. Novel treatment strategies and biomarkers seem to be promising but require further evaluation before implementation into daily routine.

FOOTNOTES

Author contributions: Giudici N performed the majority of the writing and prepared the tables; Seiler R provided input and supervision in writing the paper; Both authors have read and approved the final manuscript.

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REFERENCES

1 **Netto GJ**, Amin MB, Berney DM, Compérat EM, Gill AJ, Hartmann A, Menon S, Raspollini MR, Rubin MA, Srigley JR, Hoon Tan P, Tickoo SK, Tsuzuki T, Turajlic S, Cree I, Moch H. The 2022 World Health Organization Classification of Tumors of the Urinary System and Male Genital Organs-Part B: Prostate and Urinary Tract Tumors. *Eur Urol* 2022; **82**: 469-482 [PMID: 35965208 DOI: 10.1016/j.eururo.2022.07.002]

2 **Veskimäe E**, Espinos EL, Bruins HM, Yuan Y, Sylvester R, Kamat AM, Shariat SF, Witjes JA, Compérat EM. What Is the Prognostic and Clinical Importance of Urothelial and Nonurothelial Histological Variants of Bladder Cancer in Predicting Oncological Outcomes in Patients with Muscle-invasive and Metastatic Bladder Cancer? A European Association of Urology Muscle Invasive and Metastatic Bladder Cancer Guidelines Panel Systematic Review. *Eur Urol Oncol* 2019; **2**: 625-642 [PMID: 31601522 DOI: 10.1016/j.euo.2019.09.003]

3 **Cai T**, Tiscione D, Verze P, Pomara G, Racioppi M, Nesi G, Barbareschi M, Brausi M, Gacci M, Luciani LG, Liguori G, Gontero P, Campodonico F, Simonato A, Boddi V, Di Stasi SM, Colombo R, Serretta V, Carmignani G, Malossini G, Altieri V, Carini M, Terrone C, Bassi P, Montorsi F, Ficarra V, Selli C, Mirone V, Bartoletti R. Concordance and clinical significance of uncommon variants of bladder urothelial carcinoma in transurethral resection and radical cystectomy specimens. *Urology* 2014; **84**: 1141-1146 [PMID: 25239253 DOI: 10.1016/j.urology.2014.06.032]

4 **Abufaraj M**, Shariat SF, Foerster B, Pozo C, Moschini M, D'Andrea D, Mathieu R, Susani M, Czech AK, Karakiewicz PI, Seebacher V. Accuracy and prognostic value of variant histology and lymphovascular invasion at transurethral resection of bladder. *World J Urol* 2018; **36**: 231-240 [PMID: 29127452 DOI: 10.1007/s00345-017-2116-3]

5 **Kamat AM**, Gee JR, Dinney CP, Grossman HB, Swanson DA, Millikan RE, Detry MA, Robinson TL, Pisters LL. The case for early cystectomy in the treatment of nonmuscle invasive micropapillary bladder carcinoma. *J Urol* 2006; **175**: 881-885 [PMID: 16469571 DOI: 10.1016/S0022-5347(05)00423-4]

6 **Humphrey PA**, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part B: Prostate and Bladder Tumours. *Eur Urol* 2016; **70**: 106-119 [PMID: 26996659 DOI: 10.1016/j.eururo.2016.02.028]

7 **Stroman L**, Nair R, Russell B, Malik N, Desai A, Chandra A, Thurairaja R, Dasgupta P, Khan MS, Malde S. The impact of non-urothelial variant histology on oncological outcomes following radical cystectomy. *BJU Int* 2019; **124**: 418-423 [PMID: 30740862 DOI: 10.1111/bju.14704]

8 **Spaliviero M**, Dalbagni G, Bochner BH, Poon BY, Huang H, Al-Ahmadie HA, Donahue TF, Taylor JM, Meeks JJ, Sjoberg DD, Donat SM, Reuter VE, Herr HW. Clinical outcome of patients with T1 micropapillary urothelial carcinoma of the bladder. *J Urol* 2014; **192**: 702-707 [PMID: 24603101 DOI: 10.1016/j.juro.2014.02.2565]

9 **Willis DL**, Fernandez MI, Dickstein RJ, Parikh S, Shah JB, Pisters LL, Guo CC, Henderson S, Czerniak BA, Grossman HB, Dinney CP, Kamat AM. Clinical outcomes of cT1 micropapillary bladder cancer. *J Urol* 2015; **193**: 1129-1134 [PMID: 25254936 DOI: 10.1016/j.juro.2014.09.092]

10 **Prado K**, Greenberg D, Zhang C, Sun A, Skinner E. PD12-10 management of variant histology in non-muscle invasive bladder cancer. *J Urol* 2020; **203**: e263 [DOI: 10.1097/JU.0000000000000846.010]

11 **Sanguedolce F**, Calò B, Mancini V, Zanelli M, Palicelli A, Zizzo M, Ascani S, Carrieri G, Cormio L. Non-Muscle Invasive Bladder Cancer with Variant Histology: Biological Features and Clinical Implications. *Oncology* 2021; **99**: 345-358 [PMID: 33735905 DOI: 10.1159/000514759]

12 **Boorjian SA**, Alemozaffar M, Konety BR, Shore ND, Gomella LG, Kamat AM, Bivalacqua TJ, Montgomery JS, Lerner SP, Busby JE, Poch M, Crispen PL, Steinberg GD, Schuckman AK, Downs TM, Svatek RS, Mashni J Jr, Lane BR, Guzzo TJ, Bratslavsky G, Karsh LI, Woods ME, Brown G, Canter D, Luchey A, Lotan Y, Krupski T, Inman BA, Williams MB, Cookson MS, Keegan KA, Andriole GL Jr, Sankin AI, Boyd A, O'Donnell MA, Sawutz D, Philipson R, Coll R, Narayan VM, Treasure FP, Yla-Herttuala S, Parker NR, Dinney CPN. Intravesical nadofaragene firadenovec gene therapy for BCG-unresponsive non-muscle-invasive bladder cancer: a single-arm, open-label, repeat-dose clinical trial. *Lancet Oncol* 2021; **22**: 107-117 [PMID: 33253641 DOI: 10.1016/S1470-2045(20)30540-4]

13 **McElree IM**, Steinberg RL, Martin AC, Richards J, Mott SL, Gellhaus PT, Nepple KG, O'Donnell MA, Packiam VT. Sequential Intravesical Gemcitabine and Docetaxel for bacillus Calmette-Guérin-Naïve High-Risk Nonmuscle-Invasive Bladder Cancer. *J Urol* 2022; **208**: 589-599 [PMID: 35892270 DOI: 10.1097/JU.0000000000002740]

14 **Meghani K**, Cooley LF, Choy B, Kocherginsky M, Swaminathan S, Munir SS, Svatek RS, Kuzel T, Meeks JJ. First-in-human Intravesical Delivery of Pembrolizumab Identifies Immune Activation in Bladder Cancer Unresponsive to Bacillus Calmette-Guérin. *Eur Urol* 2022; **82**: 602-610 [PMID: 36008193 DOI: 10.1016/j.eururo.2022.08.004]

15 **Kamat AM**, Shore N, Hahn N, Alanee S, Nishiyama H, Shariat S, Nam K, Kapadia E, Frenkl T, Steinberg G. KEYNOTE-676: Phase III study of BCG and pembrolizumab for persistent/recurrent high-risk NMIBC. *Future Oncol* 2020; **16**: 507-516 [PMID: 32162533 DOI: 10.2217/fon-2019-0817]

16 **Christensen E**, Birkenkamp-Demtröder K, Sethi H, Shchegrova S, Salari R, Nordentoft I, Wu HT, Knudsen M, Lamy P, Lindskrog SV, Taber A, Balcioglu M, Vang S, Assaf Z, Sharma S, Tin AS, Srinivasan R, Hafez D, Reinert T, Navarro S, Olson A, Ram R, Dashner S, Rabinowitz M, Billings P, Sigurjonsson S, Andersen CL, Swenerton R, Aleshin A, Zimmermann B, Agerbæk M, Lin CJ, Jensen JB, Dyrskjøt L. Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma. *J Clin Oncol* 2019; **37**: 1547-1557 [PMID: 31059311 DOI: 10.1200/JCO.18.02052]

17 **Al-Ahmadie HA**, Iyer G, Lee BH, Scott SN, Mehra R, Bagrodia A, Jordan EJ, Gao SP, Ramirez R, Cha EK, Desai NB, Zabor EC, Ostrovnaya I, Gopalan A, Chen YB, Fine SW, Tickoo SK, Gandhi A, Hreiki J, Viale A, Arcila ME, Dalbagni G, Rosenberg JE, Bochner BH, Bajorin DF, Berger MF, Reuter VE, Taylor BS, Solit DB. Frequent somatic CDH1 Loss-of-function mutations in plasmacytoid variant bladder cancer. *Nat Genet* 2016; **48**: 356-358 [PMID: 26901067 DOI: 10.1038/ng.3503]

18 **Weyerer V**, Eckstein M, Compérat E, Juette H, Gaisa NT, Allory Y, Stöhr R, Wullich B, Rouprêt M, Hartmann A, Bertz S. Pure Large Nested Variant of Urothelial Carcinoma (LNUC) Is the Prototype of an FGFR3 Mutated Aggressive Urothelial Carcinoma with Luminal-Papillary Phenotype. *Cancers (Basel)* 2020; **12** [PMID: 32213857 DOI: 10.3390/cancers12030763]

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ORIGINAL ARTICLE

Retrospective Cohort Study

Impact of hyperthermic intraperitoneal chemotherapy on gastric cancer survival: Peritoneal metastasis and cytology perspectives

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Abstract

BACKGROUND

Gastric cancer presenting with peritoneal metastasis is notably associated with diminished survival prospects. The use of cytoreductive surgery in conjunction with hyperthermic intraperitoneal chemotherapy (HIPEC) has been shown to increase survival rates in these patients. Despite these advancements, debates persist regarding the magnitude of survival improvement attributed to this treatment modality. The present investigation examined survival outcomes following HIPEC in individuals diagnosed with gastric cancer and peritoneal metastasis, and it took a comparative analysis of patients exhibiting positive and negative cytological findings.

AIM

To compare the impact of HIPEC on survival in gastric cancer patients with peritoneal metastasis and positive or negative cytology.

METHODS

Between April 2013 and March 2020, 84 patients with advanced gastric cancer treated at our institution were categorized into three cohorts: HIPEC (20 patients with peritoneal metastasis), cytology-positive (23 patients without peritoneal nodules but with positive wash cytology), and cytology-negative (41 patients with advanced gastric cancer, no peritoneal nodules, and negative wash cytology). The HIPEC cohort underwent gastrectomy with HIPEC, while the cytology-positive and cytology-negative groups received gastrectomy alone. The demographic, pathological, and survival data of the groups were compared.

RESULTS
The HIPEC cohort-predominantly younger females-exhibited relatively extended surgical durations and high blood loss. Nevertheless, the complication rates were consistent across all three groups. Median survival in the HIPEC group was 20.00 ± 4.89 months, with 1-year, 2-year, and 3-year overall survival rates of 73.90%, 28.70%, and 9.60%, respectively. These figures paralleled the survival rates of the cytology-positive group (52.20% at 1 year, 28.50% at 2 years, and 19.00% at 3 years). Notably, 47% of patients experienced peritoneal recurrence.

CONCLUSION
HIPEC may offer a modest improvement in short-term survival for patients with gastric cancer and peritoneal metastasis, mirroring the outcomes in cytology-positive patients. However, peritoneal recurrence remained high.

Key Words: Cytoreductive surgery; Gastric cancer; Hyperthermic intraperitoneal chemotherapy; Peritoneal metastasis; Positive cytology

Core Tip: This investigation evaluated the survival outcomes of 84 advanced gastric cancer patients from 2013 to 2020. Among them, the hyperthermic intraperitoneal chemotherapy (HIPEC) cohort, characterized by peritoneal nodules, underwent longer surgeries and experienced greater blood loss; however, the rate of complications did not significantly differ among groups. The HIPEC group's median survival was 20.00 ± 4.89 mo, with 1-year, 2-year, and 3-year survival rates of 73.90%, 28.70%, and 9.60%, respectively. These rates were akin to those of the cytology-positive group. While HIPEC appears to offer a survival benefit, particularly in the short term, the incidence of peritoneal recurrence remains high.

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INTRODUCTION

Although the global incidence and mortality rates of gastric cancer are declining[1], Thailand is experiencing an increase in mortality related to this malignancy[2]. This discrepancy may stem from the disease's advanced stage at the time of diagnosis within the Thai population, with peritoneal carcinomatosis found in the majority of Thai gastric cancer patients [3]. Despite being managed by multidisciplinary teams[4], these patients have historically survived for only 3 to 4 months [5]. Even though systemic chemotherapy offers only marginal benefits, the combination of cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) is recognized as an effective strategy for managing gastric cancer accompanied by carcinomatosis, demonstrating an acceptable complication profile. Rau's *et al* findings[6], alongside additional research, corroborate the survival advantages conferred by CRS and HIPEC, with some patients achieving long-term survival[7-9]. However, the extent of survival improvement with this approach remains variable. Our study endeavored to compare the outcomes of a CRS and HIPEC cohort against those of patients exhibiting positive cytology who were treated solely with systemic chemotherapy.

MATERIALS AND METHODS

Study design

This research was conducted at the Minimally Invasive Unit, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, from April 2013 to March 2020. Patients diagnosed with gastric cancer manifesting either peritoneal carcinomatosis or positive cytology were included. The study population was divided into two primary groups: those who received CRS with HIPEC (referred to as the "HIPEC group") and those who underwent D2 gastrectomy followed by postoperative systemic chemotherapy (termed the "cytology-positive group"). Additionally, the study included a cohort of advanced gastric cancer patients characterized by negative cytology (the "cytology-negative group"). Patients under the age of 18 years were excluded.

Comprehensive demographic data, including age, sex, operative time, duration of hospital stay, blood loss, instances of combined resection, and complications, were collected. The following pathological parameters were meticulously documented: tumor size, histology, anatomical location, margin status, presence of lymphovascular invasion, and number of lymph nodes retrieved. Tumor staging was performed in accordance with the guidelines in the 8th edition of the staging manual of the American Joint Committee on Cancer.

The preoperative evaluation included gastroscopy and abdominal computed tomography scans for all patients. Diagnostic laparoscopy and wash cytology were uniformly conducted to ascertain the extent of disease spread. In the absence of discernible gross peritoneal nodules, patients underwent gastrectomy accompanied by D2 lymph node dissection. For individuals with positive cytology findings, a regimen of postoperative systemic chemotherapy comprising fluoropyrimidines and platinum-based drugs was initiated. When peritoneal nodules were identified during diagnostic laparoscopy, the treatment approach varied by period: CRS with HIPEC was the choice for patients identified before 2017, while those diagnosed from 2017 onward received preoperative chemotherapy.

During diagnostic laparoscopy for CRS with HIPEC, the peritoneal cancer index (PCI) was determined following established protocols[10]. Subsequent to this assessment, gastrectomy with D2 lymph node dissection was performed. In cases where complete (R0) resection was needed, gastrectomy was accompanied by resection of the adjacent involved organs. A comprehensive peritonectomy, involving the removal of the entire abdominal peritoneum, was conducted in every patient to minimize residual disease. After completing the surgical anastomosis, extensive intraperitoneal lavage was carried out using 10 liters of tepid saline, followed by HIPEC at 42 °C for 60 min, utilizing either 100 mg/m^2 cisplatin or 120 mg/m^2 oxaliplatin. The application of CRS combined with HIPEC was contraindicated in patients who presented with distant metastases, para-aortic nodal metastases, or a poor preoperative functional state.

Statistical analyses

We performed the statistical analyses using IBM SPSS Statistics, version 29 (IBM Corp, Armonk, NY, United States). Continuous variables were analyzed by calculating means and standard deviations, whereas categorical variables were expressed as percentages. When the data deviated from a normal distribution, we utilized medians and interquartile ranges. We determined differences in means using either Student's *t* test or the Mann-Whitney *U* test, depending on the parametric nature of the data. Differences among groups were assessed using one-way analysis of variance. The Tukey test was applied for homogeneity of variances, while the Games-Howell test was employed when variances were unequal. Categorical variables across groups were compared using the χ^2 test. Survival durations and their curves were estimated through the Kaplan-Meier method, with intergroup differences evaluated *via* the log-rank test. All *P* values were computed as two-tailed, with the significance threshold set at $P < 0.05$.

RESULTS

Our study included 84 individuals who were diagnosed with gastric cancer. Specifically, the HIPEC cohort included 20 patients who exhibited peritoneal carcinomatosis. The cytology-positive category comprised 23 participants without peritoneal nodules but with affirmative wash cytology results. Conversely, the cytology-negative group consisted of 41 patients with advanced gastric cancer who lacked peritoneal nodules and had negative wash cytology findings. The demographic characteristics are detailed in Table 1. Notably, the mean age within the HIPEC group was significantly younger (45.10 ± 11.34 years) than those in the cytology-positive or cytology-negative cohort. Females predominated in the HIPEC group, constituting 85% of its demographic data. The surgical duration in the HIPEC group averaged 534.60 ± 112.76 min, and blood loss was 1056.50 ± 128.03 mL, with both values notably surpassing those of the other groups. The frequency of combined organ resections, apart from splenectomy, was greater in the HIPEC group, occurring in 90% of patients. This group also experienced a 35% rate of complications, which was higher than the rates of the other two groups, although the differences did not reach statistical significance. The study reported zero mortality.

The HIPEC group exhibited significantly larger tumor sizes than did the cytology-negative group, as detailed in Table 2. Histological analysis revealed that 70% of patients in the HIPEC group presented with poorly differentiated adenocarcinoma or signet ring cell adenocarcinoma. Regarding tumor staging, 50% of these patients were at primary tumor stage IVa, while 30% were at stage IVb. Sixty percent of the patients were classified as having lymph node stage III disease. In addition, 25% of the patients in the HIPEC group exhibited positive resection margins, indicating the presence of tumor cells at the cut edge of the removed tissue. Moreover, 80% of patients demonstrated angiolymphatic invasion, reflecting aggressive tumor behavior. The PCI ranged from 0 to 18, with a median score of 3.0. Complete cytoreduction, indicating no visible residual disease, was achieved in 95% of the patients (19 individuals) with a complete cytoreduction score of zero, while a score of one was recorded in 5% of the patients (1 individual).

All patients underwent follow-up for a median of 88 months (range: 1-179 months). The HIPEC group exhibited a median survival duration of 20.00 ± 4.89 months. The 1-year overall survival rate was 73.90%, declining to 28.70% by the second year and 9.60% by the third year. In comparison, the cytology-positive group had a 1-year survival rate of 52.20%, which decreased slightly to 28.50% by the second year and increased to 19.00% by the third year. The cytology-negative group demonstrated more favorable survival rates of 76.70% at 1 year, 50.10% at 2 years, and 38.90% at 3 years. These survival trends are depicted in Figure 1, which shows that while the survival rate of the HIPEC group aligned with that of the cytology-positive group, it remained substantially lower than that of the cytology-negative group.

Recurrent disease was observed in 80% (16 of 20) of the patients in the HIPEC group. Specifically, peritoneal recurrence was noted in 47% of these patients, with 35% presenting with isolated peritoneal carcinomatosis and 12% showing combined organ and peritoneal metastases. Additionally, 41% of patients experienced distant organ metastasis, and 12% experienced local or lymph node recurrence (Figure 2).

Univariate analysis of prognostic factors revealed a significant association between the PCI score and survival outcomes ($P = 0.041$), underscoring the prognostic relevance of the PCI score in this context.

Table 1 Comparative demographic characteristics of hyperthermic intraperitoneal chemotherapy, cytology-positive, and cytology-negative patient groups

Feature	Cytology-negative group, *n* = 41	Cytology-positive group, *n* = 23	HIPEC group, *n* = 20	*P* value
Age	59.87 ± 10.25	63.17 ± 13.57	45.10 ± 11.34	< 0.0001
Sex				
Male	23 (56.1)	15 (62.5)	3 (15)	0.002
Female	18 (43.9)	8 (34.8)	17 (85)	
Operative time	269.27 ± 98.42	326.43 ± 135.39	534.60 ± 112.76	< 0.0001
Length of stay	14.88 ± 7.76	14.69 ± 6.48	19.05 ± 9.63	0.120
Blood loss	437.56 ± 69.04	616.09 ± 134.64	1056.50 ± 128.03	< 0.0001
Combined resection (other than splenectomy)				
No	37 (90)	18 (78.3)	2 (10)	< 0.0001
Yes	4 (9.8)	5 (21.7)	18 (90)	
Complication				
No	33 (80.5)	17 (73.90)	13 (65.0)	0.419
Yes	8 (19.5)	6 (26.10)	7 (35.0)	

Data are *n* (%). HIPEC: Hyperthermic intraperitoneal chemotherapy.

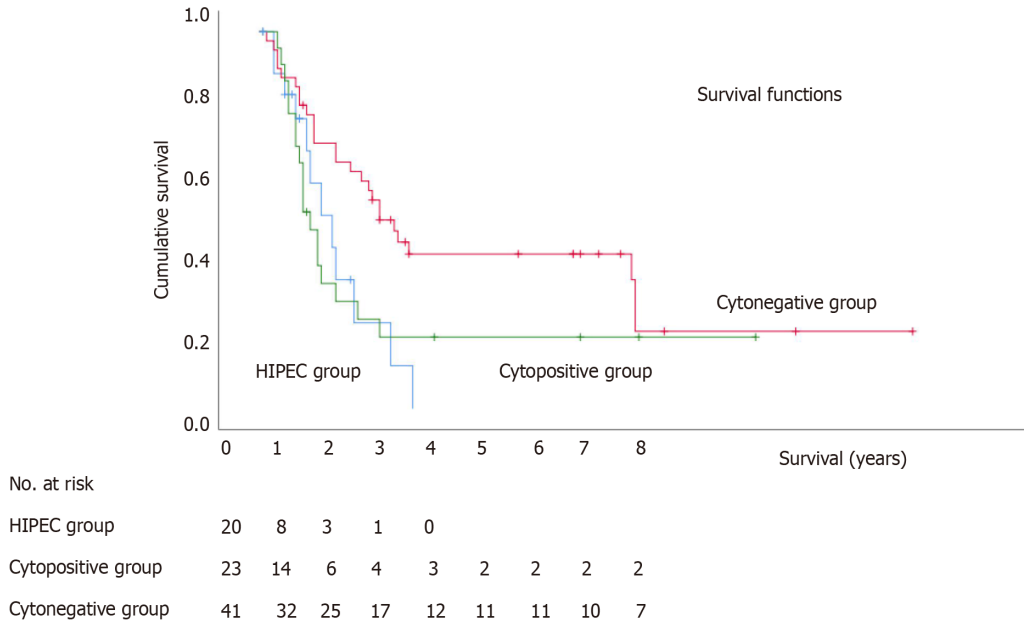

Figure 1 Comparative survival analysis of hyperthermic intraperitoneal chemotherapy *vs* cytology-positive and cytology-negative groups. HIPEC: Hyperthermic intraperitoneal chemotherapy.

DISCUSSION

Our analysis suggested that CRS combined with HIPEC may enhance short-term survival in patients, with 2-year and 3-year survival rates of 28.70% and 9.60%, respectively. The median survival duration for individuals in the HIPEC cohort was 20.00 ± 4.89 months, consistent with the findings of Smith *et al*[11]. Liu's *et al* meta-analysis of 21 randomized controlled trials, encompassing 1674 participants, demonstrated a significantly greater 3-year survival rate in patients receiving HIPEC than in those who did not[12]. Chia's *et al* systematic review also revealed a 5-year survival rate ranging from 6% to 31% for CRS patients within the HIPEC group[13]. Brandl *et al*[14] identified 28 long-term survivors with a median survival exceeding 5 years among 448 patients treated with HIPEC. Despite these encouraging results, our study did not record any 5-year survivors, suggesting the presence of more advanced peritoneal metastases in our patient cohort.

Table 2 Pathological profile comparison among hyperthermic intraperitoneal chemotherapy, cytology-positive, and cytology-negative groups

Feature	Cytology-negative group, *n* = 41	Cytology-positive group, *n* = 23	HIPEC group, *n* = 20	*P* value
Size	6.34 ± 3.40	7.49 ± 3.66	9.13 ± 4.25	0.025
Histology				
Well/moderately differentiated	15 (36.6)	6 (26.1)	6 (30.0)	0.670
Poorly differentiated/Signet ring cell adenocarcinoma	26 (63.4)	17 (73.9)	14 (70.0)	
Location				
Upper	19 (46.3)	8 (34.8)	4 (20.0)	0.548
Middle	6 (14.6)	4 (17.4)	6 (30.0)	
Distal	11 (26.8)	7 (30.4)	6 (30.0)	
Entire	5 (12.2)	4 (17.4)	4 (20.0)	
pT-stage				
Stage I	2 (4.9)	0 (0)	1 (5.0)	0.154
Stage II	6 (14.6)	2 (8.7)	0 (0)	
Stage III	13 (31.7)	3 (13.0)	3 (15)	
Stage IVa	16 (39)	14 (60.9)	10 (50)	
Stage IVb	4 (9.8)	4 (17.4)	6 (30)	
pN-stage				
Stage 0	8 (19.5)	1 (4.3)	2 (10)	0.132
Stage I	8 (19.5)	4 (17.4)	2 (10)	
Stage II	6 (14.6)	2 (8.7)	4 (20)	
Stage IIIa	12 (29.3)	6 (26.1)	2 (10)	
Stage IIIb	7 (17.1)	10 (43.5)	10 (50)	
Margin				
Negative	36 (87.8)	20 (87)	15 (75)	0.401
Positive	5 (12.2)	3 (13)	5 (25)	
Angiolymphatic invasion				
Negative	19 (46.3)	5 (21.7)	4 (20)	0.047
Positive	22 (53.7)	18 (78.3)	16 (80)	
Lymph node retrieval	34.20 ± 15.17	36.74 ± 14.88	39.50 ± 15.74	0.436

Data are *n* (%). HIPEC: Hyperthermic intraperitoneal chemotherapy.

Gastric cancer patients with positive cytology typically have poor prognoses[15]. Individuals with positive cytology generally exhibit superior survival rates to those with visible peritoneal metastases[16]. Mezhir *et al*[17] reported that cytology-positive patients achieved a considerably longer median survival (1.5 years) than patients with macroscopic peritoneal nodules (0.8 years). A study by Jamel *et al*[18] corroborated these findings, demonstrating enhanced survival in cytology-positive patients compared with patients with palpable nodules. In our analysis, HIPEC treatment elevated survival rates for patients with macroscopic nodules to levels comparable to those observed in cytology-positive patients. To the best of our knowledge, this is the first study to present survival data of such a nature. Further research is imperative to discern whether the observed survival benefits stem from HIPEC treatment itself or from concomitant peritonectomy performed during CRS.

The HIPEC group experienced a complication rate of 35%, which, while higher than that of the non-HIPEC group, did not reach statistical significance. This finding aligns with prior research confirming the acceptable safety profile of the HIPEC procedure. A meta-analysis by Patel *et al*[19] encompassing 10 randomized controlled trials concluded that HIPEC does not increase complication rates. Similarly, Marano *et al*[20] reported a complication rate of 29.7% among 91 HIPEC-

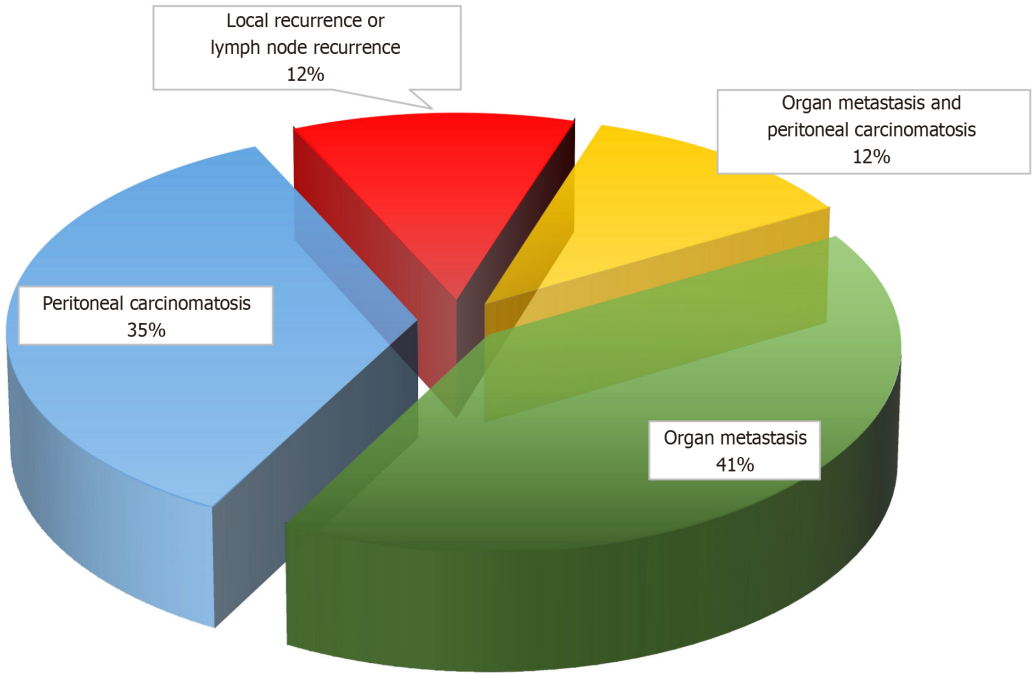

Figure 2 Recurrence patterns post-gastrectomy with and without hyperthermic intraperitoneal chemotherapy adjunct.

treated patients, and Merboth noted a rate of 26.7%[21], further supporting the relative safety of the procedure. Notably, our study recorded no mortality. Comprehensive peritonectomy was performed in all patients, with a high rate of combined organ resection of 90%. These outcomes suggest that CRS with HIPEC can be safely conducted, provided that patients are carefully selected for the procedure.

The application of CRS and HIPEC for treating gastric cancer involves various techniques and remains controversial [22]. Given the prevalent advanced stage of gastric cancer in our region, we opted for total peritonectomy, which, when combined with gastrectomy in patients with peritoneal metastasis, has been shown to be beneficial[23]. Nevertheless, the precise role and scope of peritonectomy within the HIPEC protocol for gastric cancer remain to be explored. Our institution employs an open technique utilizing a cisplatin-based chemotherapy regimen for HIPEC, aligning with findings that underscore the efficacy of cisplatin in such treatments[24]. Although we did not establish a PCI threshold for determining resectability, the median PCI observed in our cohort was 3.0. This value is consistent with recommendations for HIPEC in cases where the PCI is low (< 6)[25], as higher PCIs often correlate with widespread mesenteric nodules that preclude the possibility of achieving complete (R0) resection. Our findings from a multivariate analysis of pathological factors identified the PCI as the only significant predictor of patient survival, thus highlighting its critical role in selecting suitable candidates for HIPEC.

Previous studies have indicated the efficacy of HIPEC in reducing peritoneal recurrence rates[26]. However, our findings show a high overall recurrence rate of 80%, with the peritoneum being the predominant site of recurrence (47%). These results align with the observations by Yu *et al*[27], which noted a decrease in recurrence from 40.3% to 20.9% with HIPEC, yet peritoneal recurrence remained the most common. These outcomes suggest that HIPEC, while beneficial, may not be sufficient as a standalone intervention for controlling peritoneal cancer cell dissemination.

Other modalities aimed at eradicating intraperitoneal cancer cells should be considered. Pressurized intraperitoneal aerosol chemotherapy could produce high intraperitoneal concentrations of chemotherapeutic drugs with low systemic absorption, leading to observed regression of peritoneal metastasis[28]. Meta-analyses have also demonstrated tumor regression, albeit with some heterogeneity across studies[29]. Intraperitoneal chemotherapy, when used in combination with systemic chemotherapy, has shown improved survival rates in patients who underwent surgery after responding to treatment[30]. A combination of these modalities, along with standardization of the HIPEC procedure itself, can help reduce the tumor burden in the peritoneal cavity, thereby lowering the risk of peritoneal recurrence.

CONCLUSION

This study demonstrated that HIPEC can enhance survival rates for patients with gastric cancer and peritoneal metastasis. However, given the advanced stage of the disease at presentation, long-term survival remains elusive for most patients. Although the survival outcomes observed in the HIPEC cohort were comparable to those in the cytology-positive cohort, the difference in the peritoneal recurrence rate continued to be significant.

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FOOTNOTES

Author contributions: Methasate A and Parakonthun T were involved in the conception, design, data handling, manuscript drafting and revision, and final approval; Parakonthun T additionally managed the data and assumed corresponding author responsibilities; Intralawan T, Nampoolsuksan C, and Swangsri J conducted the data analysis, participated in manuscript revision, and provided final approval; All authors reviewed and approved the final version.

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REFERENCES

1 **Shin WS**, Xie F, Chen B, Yu P, Yu J, To KF, Kang W. Updated Epidemiology of Gastric Cancer in Asia: Decreased Incidence but Still a Big Challenge. *Cancers (Basel)* 2023; **15** [PMID: 37174105 DOI: 10.3390/cancers15092639]

2 **Wong MCS**, Huang J, Chan PSF, Choi P, Lao XQ, Chan SM, Teoh A, Liang P. Global Incidence and Mortality of Gastric Cancer, 1980-2018. *JAMA Netw Open* 2021; **4**: e2118457 [PMID: 34309666 DOI: 10.1001/jamanetworkopen.2021.18457]

3 **Methasate A**, Trakarnsanga A, Akaraviputh T, Chinsawangwathanakol V, Lohsiriwat D. Lymph node metastasis in gastric cancer: result of D2 dissection. *J Med Assoc Thai* 2010; **93**: 310-317 [PMID: 20420105]

4 **Nampoolsuksan C**. Short-term Postoperative Outcomes Before and After the Establishment of the Siriraj Upper Gastrointestinal Cancer Center: A Propensity Score Matched Analysis. *Siriraj Med J* 2020; **72**: 321-329 [DOI: 10.33192/Smj.2020.43]

5 **Koizumi W**, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, Toh Y, Nagaie T, Takagi S, Yamamura Y, Yanaoka K, Orita H, Takeuchi M. S-1 plus cisplatin *versus* S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol* 2008; **9**: 215-221 [PMID: 18282805 DOI: 10.1016/S1470-2045(08)70035-4]

6 **Rau B**, Brandl A, Thuss-Patience P, Bergner F, Raue W, Arnold A, Horst D, Pratschke J, Biebl M. The efficacy of treatment options for patients with gastric cancer and peritoneal metastasis. *Gastric Cancer* 2019; **22**: 1226-1237 [PMID: 31065877 DOI: 10.1007/s10120-019-00969-1]

7 **Yu P**, Ye Z, Dai G, Zhang Y, Huang L, Du Y, Cheng X. Neoadjuvant systemic and hyperthermic intraperitoneal chemotherapy combined with cytoreductive surgery for gastric cancer patients with limited peritoneal metastasis: a prospective cohort study. *BMC Cancer* 2020; **20**: 1108 [PMID: 33198674 DOI: 10.1186/s12885-020-07601-x]

8 **Braeuer F**, Fischer I, Brammen L, Pressl G, Fuegger R, Rohregger K, Wundsam H. Outcome in Patients Treated With Cytoreductive Surgery and HIPEC for Gastric Cancer With Peritoneal Carcinomatosis. *Anticancer Res* 2020; **40**: 2151-2156 [PMID: 32234908 DOI: 10.21873/anticanres.14174]

9 **Zhang JF**, Lv L, Zhao S, Zhou Q, Jiang CG. Hyperthermic Intraperitoneal Chemotherapy (HIPEC) Combined with Surgery: A 12-Year Meta-Analysis of this Promising Treatment Strategy for Advanced Gastric Cancer at Different Stages. *Ann Surg Oncol* 2022; **29**: 3170-3186 [PMID: 35175455 DOI: 10.1245/s10434-021-11316-z]

10 **Jacquet P**, Sugarbaker PH. Clinical research methodologies in diagnosis and staging of patients with peritoneal carcinomatosis. *Cancer Treat Res* 1996; **82**: 359-374 [PMID: 8849962 DOI: 10.1007/978-1-4613-1247-5_23]

11 **Smith SD**, Kirkpatrick D, Thomas P, Loggie BW. Current perspectives on hyperthermic intraperitoneal chemotherapy in gastric cancer. *GICTT* 2017; **7**: 19-30 [DOI: 10.2147/GICTT.S139944]

12 **Liu YW**, Du Y, Chen BA. Effect of hyperthermic intraperitoneal chemotherapy for gastric cancer patients: a meta-analysis of the randomized controlled trials. *J Int Med Res* 2019; **47**: 5926-5936 [PMID: 31741406 DOI: 10.1177/0300060519882545]

13 **Chia CS**, Seshadri RA, Kepenekian V, Vaudoyer D, Passot G, Glehen O. Survival outcomes after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy for peritoneal carcinomatosis from gastric cancer: a systematic review. *Pleura Peritoneum* 2016; **1**: 67-77 [PMID: 30911610 DOI: 10.1515/pp-2016-0010]

14 **Brandl A**, Yonemura Y, Glehen O, Sugarbaker P, Rau B. Long term survival in patients with peritoneal metastasised gastric cancer treated with cytoreductive surgery and HIPEC: A multi-institutional cohort from PSOGI. *Eur J Surg Oncol* 2021; **47**: 172-180 [PMID: 33071173 DOI: 10.1016/j.ejso.2020.10.006]

15 **Cieśla S**, Lisiecki R, Ławnicka A, Kudliński B, Ostrowska P, Davì A, Veroux M, Murawa D. Clinical Significance of Peritoneal Fluid Examination for Free Cancer Cells in Patients Qualified for Surgery for Gastric Cancer. *Front Surg* 2021; **8**: 685868 [PMID: 34235174 DOI: 10.3389/fsurg.2021.685868]

16 **Liu X**, Cai H, Sheng W, Wang Y. Long-term results and prognostic factors of gastric cancer patients with microscopic peritoneal carcinomatosis. *PLoS One* 2012; **7**: e37284 [PMID: 22615966 DOI: 10.1371/journal.pone.0037284]

17 **Mezhir JJ**, Shah MA, Jacks LM, Brennan MF, Coit DG, Strong VE. Positive peritoneal cytology in patients with gastric cancer: natural history and outcome of 291 patients. *Ann Surg Oncol* 2010; **17**: 3173-3180 [PMID: 20585870 DOI: 10.1245/s10434-010-1183-0]

18 **Jamel S**, Markar SR, Malietzis G, Acharya A, Athanasiou T, Hanna GB. Prognostic significance of peritoneal lavage cytology in staging gastric cancer: systematic review and meta-analysis. *Gastric Cancer* 2018; **21**: 10-18 [PMID: 28779261 DOI: 10.1007/s10120-017-0749-y]

19 **Patel M**, Arora A, Mukherjee D, Mukherjee S. Effect of hyperthermic intraperitoneal chemotherapy on survival and recurrence rates in advanced gastric cancer: a systematic review and meta-analysis. *Int J Surg* 2023; **109**: 2435-2450 [PMID: 37158149 DOI: 10.1097/JS9.0000000000000457]

20 **Marano L**, Marrelli D, Sammartino P, Biacchi D, Graziosi L, Marino E, Coccolini F, Fugazzola P, Valle M, Federici O, Baratti D, Deraco M, Di Giorgio A, Macrì A, Pasqual EM, Framarini M, Vaira M, Roviello F; Italian Peritoneal Surface Malignancies Oncoteam (S. I.C.O.). Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy for Gastric Cancer with Synchronous Peritoneal Metastases: Multicenter Study of 'Italian Peritoneal Surface Malignancies Oncoteam-S.I.C.O.'. *Ann Surg Oncol* 2021; **28**: 9060-9070 [PMID: 34057569 DOI: 10.1245/s10434-021-10157-0]

21 **Merboth F**, Garcia S, V Renesse J, Distler M, Welsch T, Weitz J, Stange DE. Comparative Analysis of Postoperative Complications after Cytoreductive Surgery and HIPEC in Gastric Cancer. *Oncol Res Treat* 2022; **45**: 45-53 [PMID: 34844244 DOI: 10.1159/000520330]

22 **Gronau F**, Feldbruegge L, Oberwittler F, Gonzalez-Moreno S, Villeneuve L, Eveno C, Glehen O, Kusamura S, Rau B. HIPEC in Peritoneal Metastasis of Gastric Origin: A Systematic Review of Regimens and Techniques. *J Clin Med* 2022; **11** [PMID: 35268546 DOI: 10.3390/jcm11051456]

23 **Li S**, Lan X, Gao H, Wang W, Chen L, Song S, Xue Y. Addition of peritonectomy to gastrectomy can predict good prognosis of gastric adenocarcinoma patients with intraoperatively proven single P1/P2 carcinomatosis. *Tumour Biol* 2017; **39**: 1010428317697567 [PMID: 28618957 DOI: 10.1177/1010428317697567]

24 **Topal B**, Demey K, Topal H, Jaekers J, Van Cutsem E, Vandecaveye V, Sagaert X, Prenen H. Cytoreductive surgery and Hyperthermic intra-operative peritoneal chemotherapy with Cisplatin for gastric peritoneal Carcinomatosis Monocentric phase-2 nonrandomized prospective clinical trial. *BMC Cancer* 2017; **17**: 771 [PMID: 29149865 DOI: 10.1186/s12885-017-3730-6]

25 **Boerner T**, Piso P. Cytoreductive Surgery for Peritoneal Carcinomatosis from Gastric Cancer: Technical Details. *J Clin Med* 2021; **10** [PMID: 34830545 DOI: 10.3390/jcm10225263]

26 **Yap DRY**, Wong JSM, Tan QX, Tan JW, Chia CS, Ong CJ. Effect of HIPEC on Peritoneal Recurrence in Peritoneal Metastasis Treated With Cytoreductive Surgery: A Systematic Review. *Front Oncol* 2021; **11**: 795390 [PMID: 34926311 DOI: 10.3389/fonc.2021.795390]

27 **Yu P**, Huang X, Huang L, Dai G, Xu Q, Fang J, Ye Z, Chai T, Du Y. Hyperthermic intraperitoneal chemotherapy (HIPEC) plus systemic chemotherapy *versus* systemic chemotherapy alone in locally advanced gastric cancer after D2 radical resection: a randomized-controlled study. *J Cancer Res Clin Oncol* 2023; **149**: 11491-11498 [PMID: 37392201 DOI: 10.1007/s00432-023-05019-z]

28 **Solass W**, Kerb R, Mürdter T, Giger-Pabst U, Strumberg D, Tempfer C, Zieren J, Schwab M, Reymond MA. Intraperitoneal chemotherapy of peritoneal carcinomatosis using pressurized aerosol as an alternative to liquid solution: first evidence for efficacy. *Ann Surg Oncol* 2014; **21**: 553-559 [PMID: 24006094 DOI: 10.1245/s10434-013-3213-1]

29 **Di Giorgio A**, Macrì A, Ferracci F, Robella M, Visaloco M, De Manzoni G, Sammartino P, Sommariva A, Biacchi D, Roviello F, Pastorino R, Pires Marafon D, Rotolo S, Casella F, Vaira M. 10 Years of Pressurized Intraperitoneal Aerosol Chemotherapy (PIPAC): A Systematic Review and Meta-Analysis. *Cancers (Basel)* 2023; **15** [PMID: 36831468 DOI: 10.3390/cancers15041125]

30 **Ishigami H**, Yamaguchi H, Yamashita H, Asakage M, Kitayama J. Surgery after intraperitoneal and systemic chemotherapy for gastric cancer with peritoneal metastasis or positive peritoneal cytology findings. *Gastric Cancer* 2017; **20**: 128-134 [PMID: 28028665 DOI: 10.1007/s10120-016-0684-3]

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ORIGINAL ARTICLE

Retrospective Study

Low testing rates and high *BRCA* prevalence: Poly (ADP-ribose) polymerase inhibitor use in Middle East *BRCA*/homologous recombination deficiency-positive cancer patients

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Abstract

BACKGROUND

Poly (ADP-ribose) polymerase inhibitors (PARPis) are approved as first-line therapies for breast cancer gene (*BRCA*)-positive, human epidermal growth factor receptor 2-negative locally advanced or metastatic breast cancer. They are also effective for new and recurrent ovarian cancers that are *BRCA*- or homologous recombination deficiency (HRD)-positive. However, data on these mutations and PARPi use in the Middle East are limited.

AIM

To assess *BRCA*/HRD prevalence and PARPi use in patients in the Middle East with breast/ovarian cancer.

METHODS

This was a single-center retrospective study of 57 of 472 breast cancer patients

tested for *BRCA* mutations, and 25 of 65 ovarian cancer patients tested for HRD. These adult patients participated in at least four visits to the oncology service at our center between August 2021 and May 2023. Data were summarized using descriptive statistics and compared using counts and percentages. Response to treatment was assessed using Response Evaluation Criteria in Solid Tumors criteria.

RESULTS

Among the 472 breast cancer patients, 12.1% underwent *BRCA* testing, and 38.5% of 65 ovarian cancer patients received HRD testing. Pathogenic mutations were found in 25.6% of the tested patients: 26.3% breast cancers had germline *BRCA* (*gBRCA*) mutations and 24.0% ovarian cancers showed HRD. Notably, 40.0% of *gBRCA*-positive breast cancers and 66.0% of HRD-positive ovarian cancers were Middle Eastern and Asian patients, respectively. PARPi treatment was used in 5 (33.3%) *gBRCA*-positive breast cancer patients as first-line therapy (n = 1; 7-months progression-free), for maintenance (n = 2; > 15-months progression-free), or at later stages due to compliance issues (n = 2). Four patients (66.6%) with HRD-positive ovarian cancer received PARPi and all remained progression-free.

CONCLUSION

Lower testing rates but higher *BRCA* mutations in breast cancer were found. Ethnicity reflected United Arab Emirates demographics, with breast cancer in Middle Eastern and ovarian cancer in Asian patients.

Key Words: Homologous recombination repair; *BRCA1*; *BRCA2*; Homologous recombination deficiency; Ovarian cancer; Breast cancer; Poly (ADP-ribose) polymerase inhibitors; Olaparib; DNA double-strand breaks

Core Tip: Following National Comprehensive Cancer Network guidelines, breast cancer patients were tested for *BRCA1/2* mutations, while ovarian cancer patients underwent homologous recombination defect testing. These mutations can be targeted with poly (ADP-ribose) polymerase inhibitor (PARPi) therapy, a form of precision medicine. In this single-center study, we analyzed the proportion of breast and ovarian cancer patients tested for mutations, demographics and disease characteristics of tested patients, and PARPi therapy outcomes. This first-of-its-kind study in the Middle East offers insights into targeted therapy use, contributing to knowledge on PARPis for breast and ovarian cancers.

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INTRODUCTION

Poly (ADP-ribose) polymerase (PARP) is a protein responsible for fixing single-stranded breaks. Proteins translated from other pathways, such as homologous recombination repair (HRR) genes, repair the double-strand breaks (DSBs) in DNA and help to maintain genomic stability[1]. *BRCA1/2* are the most identifiable and actionable genes of the HRR pathway. There is an increased risk of breast (45%-72%) and ovarian (39%-44%) cancers in patients or carriers with *BRCA1* and/or *BRCA2* pathogenic variants compared to the general population[2]. PARP inhibition using drugs called PARP inhibitors (PARPis) leads single-stranded breaks to become DSBs. While normal cells can repair the DSBs because of a normal HRR pathway, cancer cells with these genes mutated cannot repair DSBs, leading to the accumulation of defects and selective cancer cell death.

Breast cancer

The prevalence of *BRCA* mutations ranges widely from 1.8% to 36.9%, depending on whether the tested subjects were selected and the country from which they were reported[3,4]. Nearly 20% of *BRCA* mutated breast cancers are triple-negative breast cancer (TNBC) and have a bad prognosis[5,6]. TNBC with these mutations occur at a younger age, are high grade and highly aggressive, have a worse prognosis, and respond well to platinum-based chemotherapy with or without PARPi[7]. Patients with or carriers of these mutations have higher chances of a second cancer in the opposite breast, ovary, pancreas, and prostate[8]. Olaparib is a PARPi approved for locally advanced or metastatic human epidermal growth factor receptor 2 (HER2)-negative breast cancer[9,10].

Ovarian cancer

The homologous recombination deficiency (HRD) test, which not only tests mutations of HRR pathway genes but also overall deficiency in this pathway, is preferred in ovarian cancer[11]. Ovarian cancers with HRD tend to occur at a young

age, have a higher incidence of high-grade serous ovarian cancer (HGSOC), and respond well to standard platinum-based chemotherapy and PARPi[12-14]. Olaparib is approved as maintenance therapy after primary chemotherapy in germline *BRCA* (*gBRCA*) or somatic *BRCA* mutated patients who achieved complete response (CR) or stable disease (SD). Along with olaparib, niraparib and rucaparib are approved for recurrent ovarian cancer, but the specific indications depend on the *BRCA* or HRD status (SOLO-1, NOVA and ARIEL3) trials[14-16]. Moreover, the field of PARPi use in ovarian cancer is rapidly evolving, and new indications are emerging.

According to 2018 World Health Organization cancer statistics for the United Arab Emirates (UAE), breast cancer was the most common malignancy, accounting for 22.4% of diagnoses[17]. Studies in Gulf countries like UAE, Oman, and Kuwait have reported a 2%-8% prevalence of BRCA1/BRCA2 somatic variants in ovarian cancer[18]. Additionally, the prevalence of *gBRCA* mutations was found to be 10.2% in breast cancer and 30.7% in ovarian cancer in the region[19].

The aim of this study was to uncover the prevalence of *gBRCA* mutations in breast cancer and HRD in ovarian cancer. Beyond mere prevalence, the study compared the characteristics of patients with and without these pathogenic mutations within the tested breast and ovarian cancer groups. Finally, to assess the potential treatment implications of these findings, the study reported the proportion of patients who received PARPi therapy and their response to this targeted treatment. This study not only seeks to illuminate the selected DSB repair gene mutations in the region but also pave the way for understanding targeted treatment strategies.

MATERIALS AND METHODS

Study design

This single-center, retrospective study delves into the genetics of breast and ovarian cancer patients tested from August 2021 to May 2023 (22 months).

Genetic testing

Genetic susceptibility testing adhered to current clinical practice: *BRCA1/2* analysis for breast cancer and full HRD assessment (including gene mutations and functional testing of HRR pathway) for ovarian cancer. We tested for these through ISO 15189-certified labs (Invitae, Genotypos, and Myriad) due to in-house limitations.

Patients

All patients were at least 18-years-old and participated in four or more clinic visits within the study period to ensure sufficient clinical workup for the included cancers.

National Comprehensive Cancer Network criteria

Our analysis focused on the 82 patients who underwent *BRCA* or HRD testing. Resource limitations prevented us from definitively determining testing rates across the entire patient population (including the 455 patients who were not tested). Detailed National Comprehensive Cancer Network criteria were reported only in tested cancer patients (breast cancer: young age, family history of cancer, TNBC, advanced HER2-negative breast cancer and history of first primary cancer; ovarian cancer: all epithelial histological types and recurrent cancers).

Data collection

Prior ethics approval was secured. Data on demographics, diagnoses, mutations, treatment response (Response Evaluation Criteria in Solid Tumors criteria), disease-free interval, progression-free interval, and other clinical details were sourced from electronic medical records and oncologist/radiology reports.

Statistical analyses

We employed descriptive statistics (*e.g.*, central tendency, dispersion, frequency distributions) and compared percentages to identify patterns, trends, and group differences using Datatab software[20]. The sample size calculation was not determined, as the objective of this study was descriptive. Similarly, statistical methods for hypothesis testing were not employed due to the descriptive nature of the analysis.

RESULTS

Proportion of breast and ovarian cancer patients tested

Of the 472 breast cancer patients, 12.1% (n = 57) received *BRCA* testing, and 38.5% (n = 25) of the 65 ovarian cancer patients underwent HRD testing. The distribution of ethnicity differed between the two cancer groups. UAE nationals were the largest ethnic group among breast cancer patients (45.3% or 214), followed by Middle Eastern (25.0%, 118/472), Asian (16.7%, 79/472), and African (10.4%, 49/472). Similarly, UAE nationals were the predominant group for ovarian cancer patients (33.8%, 22/65), followed by Asian (29.2%, 19/65), Middle Eastern (24.6%, 16/65), and African (7.6%, 5/65).

Mutation status and testing criteria

Among the 82 patients who underwent testing, 21 (25.6%) had pathogenic mutations identified. Among the tested breast cancer patients, testing criteria were as follows: 68.4% (39/57 patients) were young (age < 50 years), 15.8% (9/57 patients) had a family history of cancer, 7.0% (4/57 patients) had a history of recurrence or another primary cancer, and 8.8% (5/57 patients) had TNBC. All 25 tested ovarian cancer patients had single or mixed epithelial histology. PARPis were utilized in 42.8% of the patients with pathogenic mutations.

Breast cancer: BRCA mutation analysis and patient characteristics

Among the 57 breast cancer cases tested for *gBRCA* mutations, 15 (26.3%) harbored pathogenic mutations. Of these, 10 (17.7%) involved *gBRCA1* mutations, while the remaining 5 (8.7%) were associated with *gBRCA2* as shown in Figure 1. Four cases had *BRCA2* variants of unknown significance (VUS). The median age at time of diagnosis was 42.1 years in the *BRCA* mutated group *vs* 45.3 years in the non-mutated group. Patients from the Middle East, excluding UAE nationals, were the highest in both groups. UAE national patients were the second highest (33.3%) in the mutated group and the third (19%) in the non-mutated group, as shown in Figure 2. Family history of cancer (53.3% in the mutated group *vs* 38.1% in the non-mutated group) and a history of a first-degree relative having cancer (53.3% in the mutated group *vs* 21.3% in the non-mutated group) were more prevalent in the mutated group. Additionally, Eastern Cooperative Oncology Group performance status (ECOG PS) was distributed differently between the groups, with 86.7% of mutated patients and 95.2% of non-mutated patients having a score of 0, while 13.3% and 4.8% had a score of 1, respectively. The mutated group also showed a higher median grade of breast cancer (3 *vs* 2). Both groups were similar in cancer staging, wherein stage 2 was most common followed by stages 3 and 4. Both groups had invasive ductal carcinoma as the most common histological type (80% in the mutated group *vs* 83.3% in the non-mutated group), followed by invasive lobular carcinoma (6.6% *vs* 9.5%). The distribution of molecular subtypes also differed slightly, namely TNBC (20% *vs* 19%), hormone-positive (HR+)/HER2-negative (40% *vs* 35.7%), and HR+/HER2-positive (20% *vs* 14.3%), as detailed in Table 1.

Notably, the response rates to chemotherapy were similar between the groups, with CR being 40% in the mutated group *vs* 45.2% in the non-mutated group, SD being 40% in the mutated group *vs* 19% in the non-mutated group, partial response (PR) being 0% in the mutated group *vs* 7.2% in the non-mutated group, and progressive disease being 20% in the mutated group *vs* 28.6% in the non-mutated group, as shown in Figure 3.

Within the mutated group (n = 15), 5 patients (33.3%) received PARPi. Of these, 1 patient with advanced disease received PARPi as first-line therapy and progressed after 7 months, the other patient with advanced disease presented to us after progression on ribociclib plus letrazole and could receive PARPi for 5 months only. The other 3 patients received PARPi after adjuvant or neoadjuvant chemotherapy and 2 achieved CR to chemotherapy and were disease-free for more than 15 months on olaparib. Unfortunately, 1 patient progressed on two lines of chemotherapy and could take PARPi for 40 days only. Detailed outcomes are presented in Table 2.

Ovarian cancer: HRD analysis and patient characteristics

Among the 25 ovarian cancer cases, 6 (24.0%) harbored HRD, comprising 2 (8.0%) somatic *BRCA1* and 1 (4.0%) somatic *BRCA2*. The remaining 19 (76.0%) cases were HRD-negative, as shown in Figure 1. The median age at time of diagnosis was slightly higher in the mutated group (56.5 years) compared to the non-mutated group (52.5 years). In terms of ethnicity, Asians formed the majority of the mutated group at 66.6%, followed by UAE nationals and patients from other Middle Eastern countries, both at 16.7% each. The non-mutated group displayed a different distribution, with Asians comprising 36.8%, followed by Middle Easterners at 31.6%, UAE nationals at 21%, and Africans at 10.6%, as detailed in Figure 2. The mutated group reported no such history, whereas 26.3% of the non-mutated group had a positive family history. Both groups were predominantly composed of patients with good PS (ECOG 0: 83.3% *vs* 89.4%). Serous adenocarcinoma was the most prevalent histological type in both groups (83.3% in the mutated and 47.3% in the non-mutated), followed by mixed type (0% and 26.3%, respectively). Notably, the majority in both groups presented with advanced-stage (stages 3 and 4) and high-grade (grade 4) ovarian cancer. Additionally, most patients in the mutated group underwent interval debulking surgery (66.7% and 26.4%, respectively), as detailed in Table 3.

Overall treatment response to chemotherapy was more favorable in the mutated group, with a 50% CR rate compared to 15.7% in the non-mutated group. SD was also achieved by 16.7% of mutated patients *vs* 42.1% of non-mutated patients, while progressive disease rates were lower in the mutated group (16.7%) compared to the non-mutated group (31.6%) as shown in Figure 3. Among the patients with mutated ovarian cancer who received maintenance PARPi, 3 (75%) achieved disease-free status, while the remaining patient (25%) showed no evidence of disease progression. Notably, all patients remained on PARPi at study completion, as shown in Table 2. No deaths were reported in the mutated group, compared to 10.5% (2 patients) in the non-mutated group, as shown in Figure 3.

Recurrence or first primary malignancies

Among the 82 patients who underwent testing, 12 (14.6%) had a history of cancer. Notably, 4 (33.3%) of these patients harbored *gBRCA*- or HRD-positive mutations, while the remaining 8 (66.6%) had no identifiable mutations. Within the *gBRCA* group, 2 (16.7%) had recurrence of breast cancer, and in the HRD-positive group, 1 (16.7%) had recurrence of ovarian cancer. Additionally, 1 HRD-positive ovarian cancer patient had a history of another first primary, thyroid cancer. Among the unmutated group, 4 (50%) had recurrence of breast cancer, 1 (12.5%) had recurrent ovarian cancer, and 3 (37.5%) had different first primary cancer.

Table 1 Demographics and clinical characteristics of germline *BRCA*-positive and *BRCA*-negative breast cancer

gBRCA[1]	Positive	Negative
Total, *n* (%)	15 (26.3)	42 (73.7)
Age in yr at diagnosis, mean ± SD	42.1 ± 11	45.3 ± 8.7
Positive family history, *n* (%)	8 (53.3)	16 (38.1)
First-degree relative, *n* (%)	8 (53.3)	9 (21.3)
Non-first-degree relatives, *n* (%)	4 (26.6)	11 (26.1)
H/o IVF & Rx, *n* (%)	0	0
H/o hormone Rx, *n* (%)	1 (6.6)	4 (9.5)
ECOG PS at diagnosis[2], *n* (%)		
0	13 (86.7)	40 (95.2)
1	2 (12.3)	2 (4.8)
Histology at diagnosis, *n* (%)		
Invasive ductal carcinoma	12 (80)	35 (83.3)
Invasive lobular carcinoma	1 (6.6)	4 (9.5)
Grade at diagnosis, mean ± SD	3 ± 0.6	2 ± 0.7
Stage at diagnosis, *n* (%)		
1	1 (6.6)	0
2	7 (46.6)	21 (50)
3	4 (26.6)	13 (31)
4	3 (20)	8 (19)
Categories[3], *n* (%)		
HER2-positive	4 (26.6)	12 (28.7)
Triple-negative	3 (20)	8 (19.0)
HR-positive and HER2-negative	6 (40)	15 (35.7)
HR-positive and HER2-positive	3 (20)	6 (14.3)

[1]Includes *BRCA1* and *BRCA2*.
[2]Eastern Cooperative Oncology Group Performance Status.
[3]Categories of breast cancer having prognostic significance.
ECOG PS: Eastern Cooperative Oncology Group Performance Status; HER2: Human epidermal growth factor receptor 2; HR: Homologous recombination; IVF: *In vitro* fertilization; Rx: Medical prescription.

DISCUSSION

Our study, the first of its kind in the Middle East, investigated the genetic testing rates in cancer patients, prevalence of *gBRCA* mutations in tested breast cancer patients and HRD in tested ovarian cancer patients. We further compared the demographics, disease characteristics, and response to chemotherapy of these cancers with and without these mutations. Additionally, we analyzed the proportion of patients receiving PARPi therapy and their respective response outcomes. Notably, our cohort comprised 82 tested patients of 537 and 21 (25.6%) harbored pathogenic mutations, exceeding the previously reported rate[21]. Interestingly, 9 (11%) patients carried VUS, falling within the lower range of reported frequencies (0%-44%)[22]. These findings reflect the well-established role of *BRCA* and other HRR genes in these cancers [2].

BRCA/HRD testing rates were 12% (57 of 472 patients) for breast cancer and 38.4% (25 of 65 patients) for ovarian cancer. These rates are modestly lower than the figures of 14.2% and 43% reported elsewhere respectively[23,24]. However, it is important to acknowledge limitations in our data. Due to limited resources, we lacked information on the eligibility criteria for the untested patients (n = 455). This absence makes it challenging to determine the exact proportion of eligible patients who may not have received testing.

Breast cancer

The prevalence of pathogenic *gBRCA* mutations in breast cancer patients tested in our study was 26.3%, more than two

Table 2 Clinical outcomes of poly (ADP-ribose) polymerase inhibitor therapy in *BRCA* gene-positive breast cancer and homologous recombination deficiency-positive ovarian cancer patients

Cancer type	Patient	Stage	Response change to PARPi	Duration of PARPi	PARPi status at end of study
BRCA+ breast cancer, 5/15 (33.3%)	1	II	CR–DF	13 months+	On Rx
	2[1]	II	PR–PR	40 days	Stopped
	3	III	CR–DF	17 months+	On Rx
	4	IV	PR–PR	5 months	Stopped
	5[2]	IV	PR	7 months	Stopped
HRD+ ovarian cancer, 4/6 (66.6%)	1	III	PR–DF	15 months+	On Rx
	2	IV	CR–DF	13 months+	On Rx
	3	IV	SD–PRF	20 months+	On Rx
	4	IV	CR–DF	25 months+	On Rx

[1]Received three cycles of neoadjuvant chemotherapy and then lost to follow-up and received poly (ADP-ribose) polymerase inhibitor in the advanced stage.
[2]Received olaparib as first line. + indicates the status of still receiving poly (ADP-ribose) polymerase inhibitor at the time of data collection/study end.
CR: Complete remission, DF: Disease-free; HRD: Homologous recombination deficiency; PR: Partial response; PRF: Progression-free; SD: Stable disease.

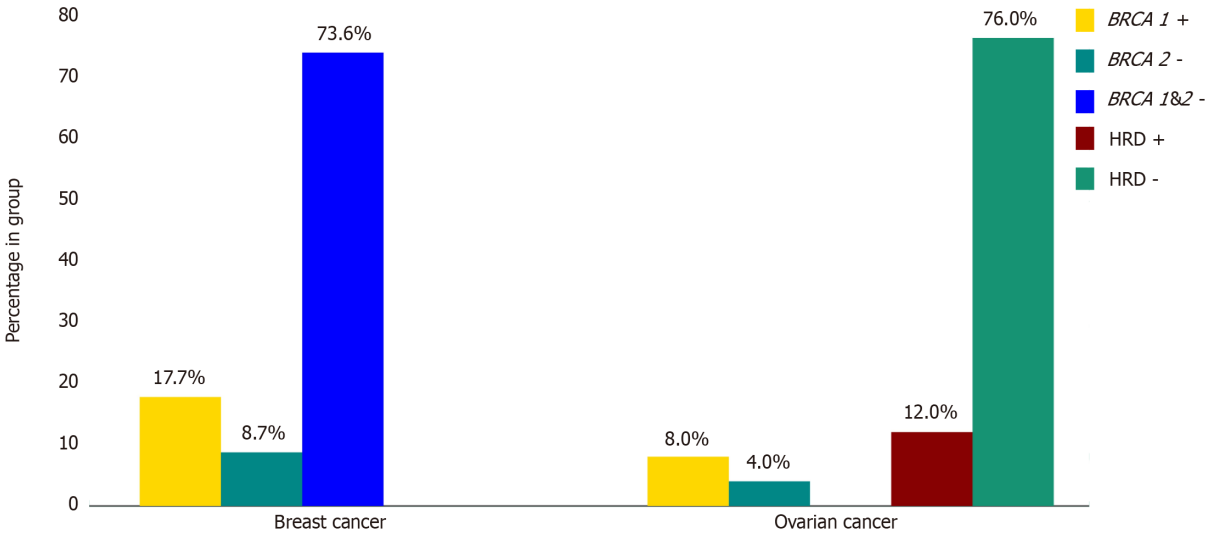

Figure 1 Distribution of breast cancer gene mutations and homologous recombination deficiency in breast and ovarian cancers. Among breast cancer patients, 17.7% (10/57) harbored pathogenic mutations in germline *BRCA1* (*gBRCA1*), while 8.7% (5/57) had mutations in *gBRCA2*. In the ovarian cancer group, 8% (2/25) of patients had *gBRCA1* mutations, 4% (1/25) had *gBRCA2* mutations, and 12% (3/25) had homologous recombination deficiency.

times the 12% reported in a large, multicenter study involving 2733 women[25]. *BRCA* testing is commonly recommended for women diagnosed with breast cancer at a young age, and the lower median age in our cohort aligns with this practice[25]. Moreover the median age of 45.3 years in the non-mutated group aligns with a review confirming the median age of breast cancer in UAE is 48 years[26]. While Figure 2 shows that UAE nationals formed the largest group among tested breast cancer patients, followed by Middle Eastern patients, this is not consistent with the overall demographics of the UAE, as reported elsewhere[27]. This trend is also observed in the overall breast cancer patients within our cohort. Consistent with previous reports of higher histological grade at presentation in *gBRCA*-mutated breast cancers, our study found a significantly higher proportion of high-grade tumors in the mutated group compared to the non-mutated group[28]. The good PS (low ECOG PS) observed in both groups, reflecting their young age, aligns with the reported literature. The proportion of HR-positive cases was slightly lower in both the mutated group (40%) and the non-mutated group (35.7%), which falls within the range observed in a large study (44% HR-positive with 1236 patients)[8, 29]. Overall, the *BRCA* mutated and non-mutated breast cancer groups appeared similar, although the significance of these values could not be calculated due to limitations in statistical analysis.

Table 3 Demographic and clinical characteristics of homologous recombination deficiency-positive and -negative ovarian cancer

Characteristic	HRD-positive	HRD-negative
Total, *n* (%)	6 (24)	19 (76)
Age years at diagnosis, mean ± SD	56.5 ± 11	52.5 ± 13
Positive family history, *n* (%)	0	5 (26.3)
ECOG PS at diagnosis, *n* (%)		
0	5 (83.3)	17 (89.4)
1	1 (16.7)	1 (5.3)
2	0	1 (5.3)
Histology, *n* (%)		
Serous	5 (83.3)	9 (47.3)
Endometrioid/mucinous/clear cell	1 (16.7)	5 (26.3)
Mixed	0	5 (26.3)
Grade of cancer, mean ± SD	4 ± 0	4 ± 0.9
Stage of cancer, *n* (%)		
1	1 (16.7)	2 (10.5)
2	0	0
3	2 (33.3)	11 (58)
4	3 (50)	6 (31.5)
Surgical Rx, *n* (%)	6 (100)	17 (89.5)
Primary	2 (33.3)	12 (63.1)
Interval	4 (66.7)	5 (26.4)

Of the 6 homologous recombination deficiency-positive cases, 3 had somatic *BRCA* gene mutations. ECOG PS: Eastern Cooperative Oncology Group Performance Status; HRD: Homologous recombination deficiency; Rx: Medical prescription.

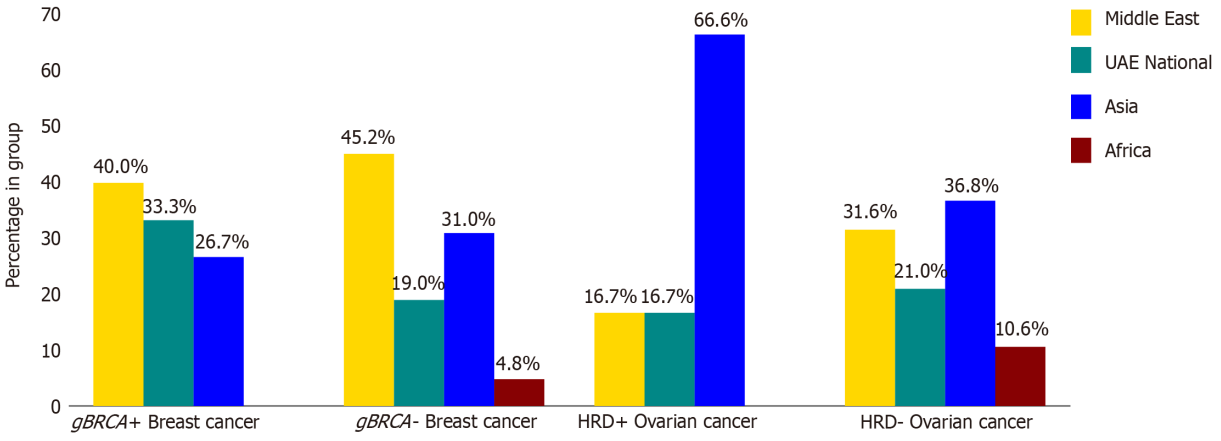

Figure 2 Breast and ovarian cancer distribution based on mutation status and ethnicity. A higher percentage of breast cancer (*n* = 57), both germline *BRCA* (*gBRCA*)-positive and *gBRCA*-negative, was found in Middle Eastern patients excluding United Arab Emirates nationals. Conversely, a higher percentage of ovarian cancer (*n* = 25), both homologous recombination deficiency HRD-positive and HRD-negative, was found in Asian patients.

Response to chemotherapy, 80% of *gBRCA*-mutated patients and 64.2% in the non-mutated group achieved either complete or PR or SD to chemotherapy, as shown in Figure 3.

This negligible difference in outcomes in the mutated group compared to the non-mutated group aligns with findings from the previous reports that suggested no significant difference in response rates[9]. Of the 33% (*n* = 5) of *gBRCA*-positive patients who received PARPi therapy, 4 (80%) had TNBC, while the remaining 1 (20%) was HR+/HER2-negative, as shown in Table 2. Interestingly, the response to PARPi in 3 out of 5 patients was in line with that reported in

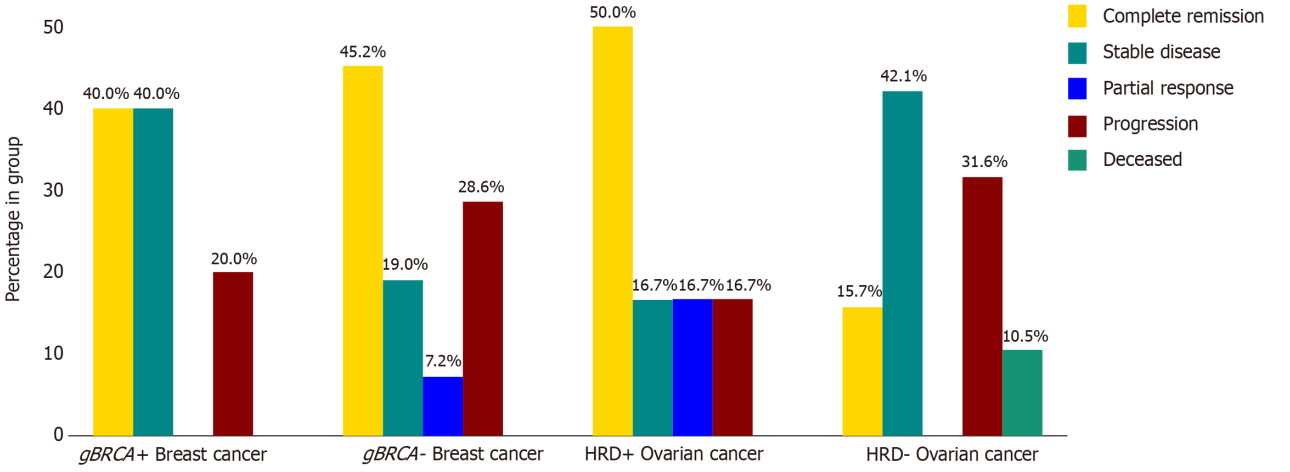

Figure 3 Response status to chemotherapy of breast and ovarian cancers with and without mutations. Homologous recombination-deficient (HRD) ovarian cancer (*n* = 6) had a higher percentage of complete remission (3/6) than cancer not HRD (3/19). While germline *BRCA* gene non-mutated breast cancer (*n* = 42) patients showed a slightly higher complete response rate (19/42), the overall treatment response remained similar in both mutated and non-mutated groups.

the OlympiAD and OlympiA trials. These 3 patients received PARPi/olaparib after neoadjuvant or adjuvant chemotherapy; among them 2 patients remained progression free even after 15 months as in OlympiA trial[30]. Unfortunately, the other patient, who took olaparib for 40 days, presented in advanced stage after she had refused chemotherapy and had been lost to follow up. One patient received PARPi as first line for advanced disease progressed after 7 months similar to the duration that reported in the OlympiAD trial, whereas other advanced stage patients did receive PARPi for 5 months but as second line[9]. Our findings suggest that PARPi therapy is used in approximately one-third of patients with *BRCA*-positive cancer. Interestingly, patients who received PARPi treatment according to established guidelines from major clinical trials seemed to experience better outcomes compared to those who did not receive treatment as per the guidelines.

Ovarian cancer

Our study revealed a 24% prevalence of HRD-positive ovarian cancers, which is in the 20%-25% range observed in similar studies from this region, and studies from North America and China[11,19,31]. The HRD-positive group had a higher median age (56.5 years) compared to the negative group (52.5 years), both of which fall within the 52-60 years range[11]. As shown in Figure 2, Asians were the majority among the tested ovarian cancer patients. This is consistent with the overall demographics of the UAE, where Asians comprise 71% of the population[27,32]. Additionally, Asians were the second-highest ethnicity among all ovarian cancer patients in our cohort, following UAE nationals. Both groups demonstrated similar proportions of patients with good ECOG performance scores, and neither group reported a positive history of family cancer. However, as shown in Table 3, the HRD-positive group displayed a significantly higher prevalence of HGSOC and advanced-stage cancer, reflecting a trend consistent with the reported literature. Most patients had advanced ovarian cancer, and their response to primary treatment was consistent with the expected course of the disease[13,14]. Overall, the HRD-positive and HRD-negative ovarian cancer groups were not similar and were in line with the earlier reports. The significance (*P* values) of these comparative values could not be calculated due to limitations in statistical analysis.

HRD-positive ovarian cancers have been shown to have better overall survival and platinum responsiveness, a trend observed in our cohort where 83.3% of patients with mutations achieved CR or SD, compared to only 57.8% of those without mutations. Notably, the proportion of patients experiencing progressive disease was 50% lower in the HRD-positive group compared to the non-mutated group, as shown in Figure 3[11,33]. We acknowledge that, due to the short duration of study, the majority of newly diagnosed patients may not have yet reached the point of relapse, which is typically 3 years.

Among HRD-positive patients, 66.6% received PARPi therapy as maintenance; among those, 3 patients (75%) remained disease-free and 1 patient (25%) remained progression-free. All of those who received olaparib remained progression-free at the end of study, in line with the trials that led to the approval of PARPi/olaparib alone or with bevacizumab[14,34]. Overall, our findings regarding prevalence, disease characteristics, and treatment response in HRD-positive ovarian cancer align with the existing literature.

Second primary malignancies

Among the 15 *gBRCA*-positive breast cancer patients, 2 (13.3%) had a recurrence of breast cancer, while 2 (33.2%) of the 6 HRD-positive ovarian cancer patients had a recurrence of ovarian cancer. These recurrence rates or history of having first primary cancer in a mutation-positive group fall within the range reported in a large multicenter study[29].

CONCLUSION

Our study identified a higher-than-reported prevalence of *BRCA* mutations (26.3%) in the tested breast cancer patients. HRD positivity rates in ovarian cancer patients were at the upper end of the reported range. Testing rates for *BRCA* in overall breast cancer patients and HRD in ovarian cancer patients, however, remained low. While UAE nationals formed the majority of patients with both cancers in our cohort, the ethnic distribution of tested patients differed. Specifically, Middle Eastern patients dominated the positive *BRCA* mutation and testing group for breast cancer, while Asian patients formed the majority in the positive HRD and testing group for ovarian cancer. This pattern likely reflects the broader demographics of the UAE population, where Asians are the predominant ethnicity.

While most of our findings on disease characteristics (including triple-negative status, histological types, grades, and stages) and treatment responses to chemotherapy and PARPi aligned with published data, the limited sample size restricts definitive conclusions about statistical significance. Larger studies are necessary to confirm our observations and explore potentially unexpected trends, such as the observed ethnic distribution and the high prevalence of *BRCA* positivity in breast cancer patients.

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FOOTNOTES

Author contributions: Syed N contributed to the conceptualization of the study, design of the methodology, formal analysis of the data, and original drafting of the manuscript as well as review and editing of the successive versions; Chintakuntlawar AV contributed to the design of the methodology, formal analysis of the data, review and editing of the successive versions of the manuscript, and supervision of the overall project; Vilasini D provided resources for the study and contributed to the review and editing of the successive versions of the manuscript; Al Salami AM, Al Hasan R, Uttam Chandani K and Chandani AU provided resources for the study; Afrooz I contributed to the original drafting of the manuscript as well as review and editing of the successive versions, provided resources for the study, and performed supervision of the overall project; Chehal A contributed to the conceptualization of the study and design of the methodology, provided resources for the study, and participated in the review and editing of the successive versions and to the supervision of the overall project.

Institutional review board statement: This study was approval by the Ethics Committee of Sheikh Shakhbout Medical City (No. SSMCREC-384).

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REFERENCES

1 **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]

2 **Petrucelli N**, Daly MB, Pal T. BRCA1- and BRCA2-Associated Hereditary Breast and Ovarian Cancer. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024 [PMID: 20301425]

3 **Armstrong N**, Ryder S, Forbes C, Ross J, Quek RG. A systematic review of the international prevalence of BRCA mutation in breast cancer. *Clin Epidemiol* 2019; **11**: 543-561 [PMID: 31372057 DOI: 10.2147/CLEP.S206949]

4 **Creeden JF**, Nanavaty NS, Einloth KR, Gillman CE, Stanbery L, Hamouda DM, Dworkin L, Nemunaitis J. Homologous recombination proficiency in ovarian and breast cancer patients. *BMC Cancer* 2021; **21**: 1154 [PMID: 34711195 DOI: 10.1186/s12885-021-08863-9]

5 **Peshkin BN**, Alabek ML, Isaacs C. BRCA1/2 mutations and triple negative breast cancers. *Breast Dis* 2010; **32**: 25-33 [PMID: 21778580 DOI: 10.3233/BD-2010-0306]

6 **Fu X**, Tan W, Song Q, Pei H, Li J. BRCA1 and Breast Cancer: Molecular Mechanisms and Therapeutic Strategies. *Front Cell Dev Biol* 2022; **10**: 813457 [PMID: 35300412 DOI: 10.3389/fcell.2022.813457]

7 **Wang CJ**, Xu Y, Lin Y, Zhu HJ, Zhou YD, Mao F, Zhang XH, Huang X, Zhong Y, Sun Q, Li CG. Platinum-Based Neoadjuvant Chemotherapy for Breast Cancer With BRCA Mutations: A Meta-Analysis. *Front Oncol* 2020; **10**: 592998 [PMID: 33304851 DOI: 10.3389/fonc.2020.592998]

8 **Azim HA Jr**, Partridge AH. Biology of breast cancer in young women. *Breast Cancer Res* 2014; **16**: 427 [PMID: 25436920 DOI: 10.1186/s13058-014-0427-5]

9 **Robson M**, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, Delaloge S, Li W, Tung N, Armstrong A, Wu W, Goessl C, Runswick S, Conte P. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N Engl J Med* 2017; **377**: 523-533 [PMID: 28578601 DOI: 10.1056/NEJMoa1706450]

10 **Litton JK**, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH, Fehrenbacher L, Yerushalmi R, Mina LA, Martin M, Roché H, Im YH, Quek RGW, Markova D, Tudor IC, Hannah AL, Eiermann W, Blum JL. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. *N Engl J Med* 2018; **379**: 753-763 [PMID: 30110579 DOI: 10.1056/NEJMoa1802905]

11 **Pennington KP**, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, Thornton A, Norquist BM, Casadei S, Nord AS, Agnew KJ, Pritchard CC, Scroggins S, Garcia RL, King MC, Swisher EM. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. *Clin Cancer Res* 2014; **20**: 764-775 [PMID: 24240112 DOI: 10.1158/1078-0432.CCR-13-2287]

12 **Huang XZ**, Jia H, Xiao Q, Li RZ, Wang XS, Yin HY, Zhou X. Efficacy and Prognostic Factors for PARP Inhibitors in Patients With Ovarian Cancer. *Front Oncol* 2020; **10**: 958 [PMID: 32612955 DOI: 10.3389/fonc.2020.00958]

13 **Vergote I**, Tropé CG, Amant F, Kristensen GB, Ehlen T, Johnson N, Verheijen RH, van der Burg ME, Lacave AJ, Panici PB, Kenter GG, Casado A, Mendiola C, Coens C, Verleye L, Stuart GC, Pecorelli S, Reed NS; European Organization for Research and Treatment of Cancer-Gynaecological Cancer Group; NCIC Clinical Trials Group. Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer. *N Engl J Med* 2010; **363**: 943-953 [PMID: 20818904 DOI: 10.1056/NEJMoa0908806]

14 **Moore K**, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, Lisyanskaya A, Floquet A, Leary A, Sonke GS, Gourley C, Banerjee S, Oza A, González-Martín A, Aghajanian C, Bradley W, Mathews C, Liu J, Lowe ES, Bloomfield R, DiSilvestro P. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med* 2018; **379**: 2495-2505 [PMID: 30345884 DOI: 10.1056/NEJMoa1810858]

15 **Mirza MR**, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, Fabbro M, Ledermann JA, Lorusso D, Vergote I, Ben-Baruch NE, Marth C, Mądry R, Christensen RD, Berek JS, Dørum A, Tinker AV, du Bois A, González-Martín A, Follana P, Benigno B, Rosenberg P, Gilbert L, Rimel BJ, Buscema J, Balser JP, Agarwal S, Matulonis UA; ENGOT-OV16/NOVA Investigators. Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer. *N Engl J Med* 2016; **375**: 2154-2164 [PMID: 27717299 DOI: 10.1056/NEJMoa1611310]

16 **Coleman RL**, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, Colombo N, Weberpals JI, Clamp A, Scambia G, Leary A, Holloway RW, Gancedo MA, Fong PC, Goh JC, O'Malley DM, Armstrong DK, Garcia-Donas J, Swisher EM, Floquet A, Konecny GE, McNeish IA, Scott CL, Cameron T, Maloney L, Isaacson J, Goble S, Grace C, Harding TC, Raponi M, Sun J, Lin KK, Giordano H, Ledermann JA; ARIEL3 investigators. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017; **390**: 1949-1961 [PMID: 28916367 DOI: 10.1016/S0140-6736(17)32440-6]

17 **World Health Organization Team**. Cancer United Arab Emirates 2020 country profile. 2020 Jan [cited 22 November 2023]. Available from: https://www.who.int/publications/m/item/cancer-are-2020

18 **Azribi F**, Abdou E, Dawoud E, Ashour M, Kamal A, Al Sayed M, Burney I. Prevalence of BRCA1 and BRCA2 pathogenic sequence variants in ovarian cancer patients in the Gulf region: the PREDICT study. *BMC Cancer* 2021; **21**: 1350 [PMID: 34930165 DOI: 10.1186/s12885-021-09094-8]

19 **Alhuqail AJ**, Alzahrani A, Almubarak H, Al-Qadheeb S, Alghofaili L, Almoghrabi N, Alhussaini H, Park BH, Colak D, Karakas B. High prevalence of deleterious BRCA1 and BRCA2 germline mutations in arab breast and ovarian cancer patients. *Breast Cancer Res Treat* 2018; **168**: 695-702 [PMID: 29297111 DOI: 10.1007/s10549-017-4635-4]

20 **DATAtab Team**. DATAtab: Online Statistics Calculator. DATAtab e.U. Graz, Austria, 2023. [cited 24 November 2023]. Available from: https://datatab.net

21 **Heeke AL**, Pishvaian MJ, Lynce F, Xiu J, Brody JR, Chen WJ, Baker TM, Marshall JL, Isaacs C. Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types. *JCO Precis Oncol* 2018; **2018** [PMID: 30234181 DOI: 10.1200/PO.17.00286]

22 **Shao C**, Wan J, Lam FC, Tang H, Marley AR, Song Y, Miller C, Brown M, Han J, Adeboyeje G. A comprehensive literature review and meta-analysis of the prevalence of pan-cancer BRCA mutations, homologous recombination repair gene mutations, and homologous recombination deficiencies. *Environ Mol Mutagen* 2022; **63**: 308-316 [PMID: 36054589 DOI: 10.1002/em.22505]

23 **Stenehjem DD**, Telford C, Unni SK, Bauer H, Sainski A, Deka R, Schauerhamer MB, Ye X, Tak CR, Ma J, Dalvi TB, Gutierrez L, Kaye JA, Tyczynski JE, Brixner DI, Biskupiak JE. BRCA testing and outcomes in women with breast cancer. *Breast Cancer Res Treat* 2021; **186**: 839-850 [PMID: 33389410 DOI: 10.1007/s10549-020-06038-x]

24 **Neff RT**, Senter L, Salani R. BRCA mutation in ovarian cancer: testing, implications and treatment considerations. *Ther Adv Med Oncol* 2017; **9**: 519-531 [PMID: 28794804 DOI: 10.1177/1758834017714993]

25 **Copson ER**, Maishman TC, Tapper WJ, Cutress RI, Greville-Heygate S, Altman DG, Eccles B, Gerty S, Durcan LT, Jones L, Evans DG, Thompson AM, Pharoah P, Easton DF, Dunning AM, Hanby A, Lakhani S, Eeles R, Gilbert FJ, Hamed H, Hodgson S, Simmonds P, Stanton L, Eccles DM. Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. *Lancet Oncol* 2018; **19**: 169-180 [PMID: 29337092 DOI: 10.1016/S1470-2045(17)30891-4]

26 **Al-Shamsi HO**, Abdelwahed N, Al-Awadhi A, Albashir M, Abyad AM, Rafii S, Afrit M, Al Lababidi B, Abu-Gheida I, Sonawane YP, Nijhawan NA, Haq UU, Dreier N, Joshua TLA, Iqbal F, Yacoub T, Nawaz FA, Abdul Jabbar D, Tirmazy SH, El-Shourbagy DM, Hamza D, Omara M, Al Madhi SAS, Ghazal H, Darr H, Oner M, Vlamaki Z, El Kinge AR, Ramanathan D, Judah M, Almahmeed T, Ahmad M, Jonnada SB, Almansoori N, Razek AA, Al-Hamadi A, Balalaa N, Jamali F, Singarachari RA, Labban A, Das K, Luiten EJT, Abdelgawad T, Al-Khatib F, Alrawi S, Jaafar H. Breast Cancer in the United Arab Emirates. *JCO Glob Oncol* 2023; **9**: e2200247 [PMID: 36608306 DOI: 10.1200/GO.22.00247]

27 **World Population Review Team**. Dubai-population 2023. [cited 25 December 2023]. Available from: https://worldpopulationreview.com/world-cities/dubai-population

28 **Yoshida R**. Hereditary breast and ovarian cancer (HBOC): review of its molecular characteristics, screening, treatment, and prognosis. *Breast Cancer* 2021; **28**: 1167-1180 [PMID: 32862296 DOI: 10.1007/s12282-020-01148-2]

29 **Lambertini M**, Ceppi M, Hamy AS, Caron O, Poorvu PD, Carrasco E, Grinshpun A, Punie K, Rousset-Jablonski C, Ferrari A, Paluch-Shimon S, Toss A, Senechal C, Puglisi F, Pogoda K, Pérez-Fidalgo JA, De Marchis L, Ponzone R, Livraghi L, Estevez-Diz MDP, Villarreal-Garza C, Dieci MV, Clatot F, Duhoux FP, Graffeo R, Teixeira L, Córdoba O, Sonnenblick A, Ferreira AR, Partridge AH, Di Meglio A, Saule C, Peccatori FA, Bruzzone M, t'Kint de Roodenbeke MD, Ameye L, Balmaña J, Del Mastro L, Azim HA Jr. Clinical behavior and outcomes of breast cancer in young women with germline BRCA pathogenic variants. *NPJ Breast Cancer* 2021; **7**: 16 [PMID: 33579978 DOI: 10.1038/s41523-021-00224-w]

30 **Tutt ANJ**, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P, Gelber RD, de Azambuja E, Fielding A, Balmaña J, Domchek SM, Gelmon KA, Hollingsworth SJ, Korde LA, Linderholm B, Bandos H, Senkus E, Suga JM, Shao Z, Pippas AW, Nowecki Z, Huzarski T, Ganz PA, Lucas PC, Baker N, Loibl S, McConnell R, Piccart M, Schmutzler R, Steger GG, Costantino JP, Arahmani A, Wolmark N, McFadden E, Karantza V, Lakhani SR, Yothers G, Campbell C, Geyer CE Jr; OlympiA Clinical Trial Steering Committee and Investigators. Adjuvant Olaparib for Patients with BRCA1- or BRCA2-Mutated Breast Cancer. *N Engl J Med* 2021; **384**: 2394-2405 [PMID: 34081848 DOI: 10.1056/NEJMoa2105215]

31 **Feng Z**, Shao D, Cai Y, Bi R, Ju X, Chen D, Song C, Chen X, Li J, An N, Li Y, Zhou Q, Xiu Z, Zhu S, Wu X, Wen H. Homologous recombination deficiency status predicts response to platinum-based chemotherapy in Chinese patients with high-grade serous ovarian carcinoma. *J Ovarian Res* 2023; **16**: 53 [PMID: 36922847 DOI: 10.1186/s13048-023-01129-x]

32 **Zhang Y**, Luo G, Li M, Guo P, Xiao Y, Ji H, Hao Y. Global patterns and trends in ovarian cancer incidence: age, period and birth cohort analysis. *BMC Cancer* 2019; **19**: 984 [PMID: 31640608 DOI: 10.1186/s12885-019-6139-6]

33 **Alsop K**, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, Dobrovic A, Birrer MJ, Webb PM, Stewart C, Friedlander M, Fox S, Bowtell D, Mitchell G. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *J Clin Oncol* 2012; **30**: 2654-2663 [PMID: 22711857 DOI: 10.1200/JCO.2011.39.8545]

34 **Ray-Coquard I**, Pautier P, Pignata S, Pérol D, González-Martín A, Berger R, Fujiwara K, Vergote I, Colombo N, Mäenpää J, Selle F, Sehouli J, Lorusso D, Guerra Alía EM, Reinthaller A, Nagao S, Lefeuvre-Plesse C, Canzler U, Scambia G, Lortholary A, Marmé F, Combe P, de Gregorio N, Rodrigues M, Buderath P, Dubot C, Burges A, You B, Pujade-Lauraine E, Harter P; PAOLA-1 Investigators. Olaparib plus Bevacizumab as First-Line Maintenance in Ovarian Cancer. *N Engl J Med* 2019; **381**: 2416-2428 [PMID: 31851799 DOI: 10.1056/NEJMoa1911361]

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ORIGINAL ARTICLE

Retrospective Study

Programmed cell death 1 inhibitor sintilimab plus concurrent chemoradiotherapy for locally advanced pancreatic adenocarcinoma

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Abstract

BACKGROUND

Pancreatic adenocarcinoma, a malignancy that arises in the cells of the pancreas, is a devastating disease with unclear etiology and often poor prognosis. Locally advanced pancreatic cancer, a stage where the tumor has grown significantly but has not yet spread to distant organs, presents unique challenges in treatment. This article aims to discuss the current strategies, challenges, and future directions in the management of locally advanced pancreatic adenocarcinoma (LAPC).

AIM

To investigate the feasibility and efficacy of programmed cell death 1 (PD-1) inhibitor sintilimab plus concurrent chemoradiotherapy for LAPC.

METHODS

Eligible patients had LAPC, an Eastern cooperative oncology group performance status of 0 or 1, adequate organ and marrow functions, and no prior anticancer therapy. In the observation group, participants received intravenous sintilimab 200 mg once every 3 wk, and received concurrent chemoradiotherapy (concurrent conventional fractionated radiotherapy with doses planning target volume 50.4 Gy and gross tumor volume 60 Gy in 28 fractions and oral S-1 40 mg/m^2 twice daily on days 1-14 of a 21-d cycle and intravenous gemcitabine 1000 mg/m^2 on days 1 and 8 of a 21-d cycle for eight cycles until disease progression, death, or unacceptable toxicity). In the control group, participants only received concurrent chemoradiotherapy. From April 2020 to November 2021, 64 participants were finally enrolled with 34 in the observation group and 30 in the control group.

RESULTS
Thirty-four patients completed the scheduled course of chemoradiotherapy, while 32 (94.1%) received sintilimab plus concurrent chemoradiotherapy with 2 patients discontinuing sintilimab in the observation group. Thirty patients completed the scheduled course of chemoradiotherapy in the control group. Based on the Response Evaluation Criteria in Solid Tumors guidelines, the analysis of the observation group revealed that a partial response was observed in 11 patients (32.4%), stable disease was evident in 19 patients (55.9%), and 4 patients (11.8%) experienced progressive disease; a partial response was observed in 6 (20.0%) patients, stable disease in 18 (60%), and progressive disease in 6 (20%) in the control group. The major toxic effects were leukopenia and nausea. The incidence of severe adverse events (AEs) (grade 3 or 4) was 26.5% (9/34) in the observation group and 23.3% (7/30) in the control group. There were no treatment-related deaths. The observation group demonstrated a significantly longer median overall survival (22.1 mo compared to 15.8 mo) ($P < 0.05$) and progression-free survival (12.2 mo *vs* 10.1 mo) ($P < 0.05$) in comparison to the control group. The occurrence of severe AEs did not exhibit a statistically significant difference between the observation group and the control group ($P > 0.05$).

CONCLUSION
Sintilimab plus concurrent chemoradiotherapy was effective and safe for LAPC patients, and warrants further investigation.

Key Words: Immunotherapy; Concurrent chemoradiotherapy; Locally advanced pancreatic adenocarcinoma; Programmed cell death 1; Sintilimab

Core Tip: The article presents an insightful exploration of a study of the combination of sintilimab with S-1 and gemcitabine concurrent radiotherapy for locally advanced pancreatic cancer (LAPC). The observation group had significantly longer median progression-free survival and overall survival than the control group. The occurrence of severe adverse events did not exhibit a statistically significant difference between the observation group and the control group, with a *P* value greater than 0.05. It is considered a promising, effective, and well-tolerated treatment for LAPC.

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INTRODUCTION

The prognosis of patients with locally advanced pancreatic cancer (LAPC) remains extremely poor and, in most historical studies, the median survival duration typically falls within a range of 8 to 12 mo. However, the 5-year overall survival (OS) rate remains relatively low, hovering at approximately 9%[1].

Currently, the treatment of LAPC is multidisciplinary, often combining surgical resection, chemotherapy, and radiation therapy. Surgical resection, known as pancreatoduodenectomy or Whipple procedure, is the preferred approach for patients with resectable tumors. However, most patients present with locally advanced disease that is not amenable to surgical resection due to tumor invasion of surrounding structures or metastasis[2].

In such cases, chemotherapy plays a crucial role. The most commonly used chemotherapeutic agents include gemcitabine, fluorouracil, and platinum-based drugs. These agents are administered either as monotherapy or in combination to achieve synergistic antitumor effects. Chemotherapy is typically administered before surgery to shrink the tumor and increase the chances of successful resection (neoadjuvant therapy), or after surgery to eliminate residual cancer cells (adjuvant therapy).

Radiation therapy, either alone or combined with chemotherapy, is also used to treat LAPC. This procedure involves the utilization of high-energy radiation to effectively eliminate cancerous cells and reduce the size of the tumor. Advanced techniques such as stereotactic body radiation therapy and intensity-modulated radiation therapy (IMRT) allow for more precise delivery of radiation to the tumor while minimizing damage to surrounding healthy tissue.

Despite these advancements, locally advanced pancreatic adenocarcinoma remains a challenging disease to treat. The prognosis for patients with this condition is often poor, with limited survival rates. This is partly due to the aggressive nature of the cancer and its resistance to traditional treatment modalities.

Moreover, the complex anatomy of the pancreas and its close proximity to vital structures make surgical resection challenging. Even with resection, the risk of recurrence and metastasis remains high. Additionally, the side effects of chemotherapy and radiation therapy can be significant, further compromising the quality of life for patients.

To address these challenges, researchers are exploring novel treatment strategies. A promising avenue lies in the advancement of targeted therapies, which are designed to specifically target and eliminate cancer cells while minimizing collateral damage to healthy cells. These therapies, including immunotherapy and gene-based therapies, are in various stages of clinical development and show promise in improving outcomes for patients with LAPC[3-5].

Treatment options specifically for patients with LAPC are scarce and chemotherapy or radiotherapy alone delivers limited efficacy. Twenty percent of patients undergoing initial chemoradiation therapy unexpectedly exhibited immediate metastases following treatment[6,7], and in some cases, they even suffer from higher toxicity levels compared to those who solely receive chemotherapy. Recent Phase 2/3 studies have demonstrated a notable increase in median survival rates through the utilization of programmed cell death 1 (PD-1) inhibitors, providing promising results in the field of cancer treatment. PD-1 inhibitor sintilimab, a human IgG4 monoclonal antibody, has shown efficacy in liver cancer, non-small cell lung cancer, classical Hodgkin's lymphoma, rectal cancer, and cervical cancer[8-12].

Immunotherapy and radiotherapy are promising therapeutic options for LAPC, and they have the potential to enhance the effects of chemotherapy when used in combination. Therefore, in this retrospective study, we aimed to comprehensively assess and compare the efficacy and safety profile of the PD-1 inhibitor, sintilimab, combined with S-1 plus gemcitabine concurrent chemoradiotherapy *vs* S-1 plus gemcitabine concurrent chemoradiotherapy in the treatment of LAPC.

MATERIALS AND METHODS

Patient eligibility

We enrolled patients aged 18-80 years whose estimated life expectancy was 12 wk. The patients had been confirmed histologically or cytologically to have unresectable LAPC. The inclusion criteria were as follows: Eastern cooperative oncology group performance status of 0 or 1; no earlier treatment for pancreatic cancer; no evidence of distant metastasis; adequate hematological function; adequate hepatic and renal function; adequate oral intake; and written informed consent. Exclusion criteria were as follows: active infection; watery diarrhea; active gastroduodenal ulcers; pleural effusion or ascites; complications such as history of drug hypersensitivity, active concomitant malignancy, heart disease or renal disease; mental disorders; pregnant and lactating women; and women of childbearing age unless using effective contraception.

For pretreatment staging, thoracic and abdominal computed tomography (CT) was needed to exclude the presence of distant metastasis and to assess local extension of the tumor. Tumor unresectability criteria included tumor encasement of the superior mesenteric artery, bilateral portal vein, common hepatic artery, or celiac trunk. Before treatment, all patients with obstructive jaundice underwent percutaneous transhepatic or an endoscopic retrograde biliary drainage.

Characteristics of patients

From April 2020 to November 2021, a total of 64 patients participated in the study conducted at the First Affiliated Hospital of Yangtze University, located in Jingzhou, China. The comprehensive characteristics of these patients have been comprehensively outlined in Table 1 for a clear and detailed understanding. Sixty-four participants were finally enrolled with 34 in the observation group and 30 in the control group. There was no significant difference in any baseline characteristics between these two groups (Table 1). All patients were thoroughly briefed on the pros and cons of both treatment options, including potential outcomes, morbidity associated with treatment, as well as financial implications. Consequently, the ultimate decision regarding their treatment was primarily made by each patient.

Treatment schedule

In the observation group, participants received intravenous sintilimab 200 mg once every 3 wk, and concurrent chemoradiotherapy [concurrent conventional fractionated radiotherapy with doses planning target volume (PTV) 50.4 Gy and gross tumor volume (GTV) 60 Gy in 28 fractions and oral S-1 40 mg/m^2 twice daily on days 1-14 of a 21-d cycle, and intravenous gemcitabine 1000 mg/m^2 on days 1 and 8 of a 21-d cycle for eight cycles until disease progression, death, or unacceptable toxicity]. In the control group, participants received only concurrent chemoradiotherapy (concurrent conventional fractionated radiotherapy with doses PTV 50.4 Gy and GTV 60 Gy in 28 fractions and oral S-1 40 mg/m^2 twice daily on days 1-14 of a 21-d cycle and intravenous gemcitabine 1000 mg/m^2 on days 1 and 8 of a 21-d cycle for eight cycles until disease progression, death, or unacceptable toxicity).

IMRT was precisely administered through three-dimensional (3D) treatment planning, utilizing 15 MV photons for optimal precision. The overall dosage consisted of 50.4 Gy targeted to the PTV and 60 Gy delivered to the GTV, distributed across 28 fractions over approximately 5.5 wk. This approach ensured a controlled and effective administration of radiation therapy. The GTV was delineated as the region of solid, macroscopic tumor tissue that exhibited contrast enhancement on CT and magnetic resonance imaging (MRI), and/or positron emission tomography. The clinical target volume (CTV) was defined as encompassing the GTV with an additional margin of at least 5 mm, considering any potential areas of microscopic tumor spread as well as the involved regional lymph nodes. The CTV, inclusive of a 5-mm lateral margin to compensate for potential inaccuracies, and a 10-mm craniocaudal margin to account for daily set-up errors and respiratory organ motion, was collectively designated as the PTV. Not more than 30% of the total volume received ≥ 18 Gy in both kidneys. If only one kidney was functional, not more than 10% of the total volume received ≥ 18 Gy, and the liver mean dose was limited to ≤ 30 Gy. The stomach received a maximum dose of ≤ 55 Gy, and not more than 30% of the volume received 45-55 Gy. The dosage delivered to the spinal cord was consistently kept below 45 Gy, ensuring safety and precision throughout the treatment process.

Table 1 Patient characteristics

Feature	Observation group	Control group
Age in yr		
Median	57	55
Range	18-70	49-80
Sex		
Male	20	16
Female	14	14
Performance status		
0	29	28
1	5	2
Stage		
Stage A	21	17
Stage B	13	13

Evaluation

All incoming patients were comprehensively included in both response and toxicity assessments. Throughout the chemotherapy process, thorough physical examinations, biochemistry tests, and complete blood cell counts were meticulously evaluated on both the first and eighth days of each treatment cycle. In accordance with the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0, an objective assessment of tumor response was conducted every 4 to 6 wk utilizing CT or MRI. Additionally, the levels of carcinoembryonic antigen and carbohydrate antigen 19-9 were monitored at the same frequency to track any potential changes. To confirm the objective response, the patient status was evaluated at an interval of no less than 4 wk, with complete response (CR), partial response (PR), and stable disease being the criteria used for assessment. The duration of the response was determined by measuring the time interval between the initial documentation of a clinical response (either CR or PR) and the subsequent documentation of tumor progression. This period provides a quantitative assessment of the treatment's effectiveness in maintaining a favorable outcome before disease progression occurs. Adverse events (AEs) were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Objective responses and AEs were confirmed by an external review committee. The progression-free survival (PFS) was determined by calculating the duration from the initiation of treatment to the occurrence of either documented disease progression or death from any cause. The date of treatment initiation to the censored date of follow-up or death was calculated as OS.

RESULTS

Efficacy

Thirty-four patients completed the scheduled course of chemoradiotherapy, while 32 (94.1%) received sintilimab plus concurrent chemoradiotherapy, with 2 patients discontinuing sintilimab in the observation group. Thirty patients completed the scheduled course of chemoradiotherapy in the control group. The objective response rate (ORR), as determined by the RECIST 1.0 criteria, stood at 32.4% in the observation group, significantly higher than the 20.0% observed in the control group. Similarly, when assessing the disease control rate (DCR) utilizing the RECIST 1.0 criteria, the observation group demonstrated a significant achievement of 88.2%, representing a noteworthy enhancement in comparison to the 80.0% observed in the control group. The observation group underwent a median follow-up duration of 16.3 mo, varying between 12.2 and 26.5 mo. In contrast, the control group exhibited a median follow-up period of 17.4 mo, spanning from 15.1 to 25.6 mo. The median OS for the observation group was 22.1 mo, with a 95%CI extending from 16.3 to 27.6 mo. In contrast, the control group demonstrated a median OS of 15.8 mo, accompanied by a 95%CI varying from 5.5 to 18.3 mo. This comparison clearly highlights the disparities in survival outcomes between the two groups. This difference was statistically significant ($P < 0.05$; Table 2). The median PFS in the observation group was 12.2 mo (95%CI: 5.5-18.3 mo), whereas in the control group, it was 10.1 mo (95%CI: 5.8-14.2 mo) ($P < 0.05$; Table 2). Detailed univariable and multivariable analyses revealed that the sole independent prognostic factor significantly influencing both OS [hazard ratio (HR) = 0.484; 95%CI: 0.245-0.948; $P < 0.05$] and PFS (HR = 0.579; 95%CI: 0.334-0.991; $P < 0.05$; Table 2) was the allocation of treatment. During the follow-up phase, it was observed that there was no notable variation in the occurrence of treatment failure or the need for post-protocol intervention among the two groups.

Adverse events

Table 3 presents a comprehensive overview of Grade 1-4 AEs. Notably, no unexpected toxicities were observed

Table 2 Summary of tumor response and survival outcomes according to Response Evaluation Criteria in Solid Tumors 1.0 criteria

Outcomes	Observation group, *n* = 34	Control group, *n* = 30	*P* value
Best tumor response			
Complete response	0 (0)	0 (0)	
Partial response	11 (32.4)	6 (20.0)	
Stable disease	19 (55.9)	18 (60.0)	
Progressive disease	4 (11.8)	6 (20)	
Objective response rate	11 (32.4)	6 (20)	
Disease control rate	30 (88.2)	24 (80)	
Median OS in month	22.1 ± 2.6 (16.3-27.6)	17.8 ± 2.3 (12.9-22.8)	< 0.05
Median PFS in month	12.2 ± 3.1 (5.5-18.3)	10.1 ± 2.2 (5.8-14.2)	< 0.05

[1]Data are *n* (%) or mean ± standard deviation (95% confidence interval).
OS: Overall survival; PFS: Progression-free survival.

Table 3 Toxicity

Symptom	Control group, No. of patients				Observation group, No. of patients			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Leukopenia	11	7	3	1	12	8	4	1
Fatigue	15	6	2	0	16	9	2	0
Anemia	14	5	1	0	15	6	1	0
Nausea	18	8	0	0	20	10	1	0
Anorexia	8	5	0	0	11	7	0	0

throughout the study period, indicating a favorable safety profile. Furthermore, there were no fatalities attributed to the treatment administered, underscoring its tolerability. The occurrence of severe AEs is also detailed in the table, providing crucial insights into the potential risks associated with the treatment. Grade 3 or 4 was 26.5% (9/34) in the observation group and 23.3% (7/30) in the control group. In the observation group, the most frequent (≥ 10% incidence) Grade 3 AEs were leukopenia (*n* = 5; 14.7%), fatigue (*n* = 2; 5.9%), and anemia (*n* = 1; 2.9%). In the control group, the most frequent AEs were leukopenia (*n* = 4; 13.3%), fatigue (*n* = 2; 6.7%), and anemia (*n* = 1; 3.3%).

DISCUSSION

LAPC has a poor prognosis and is one of the most lethal cancers globally[1]. Optimizing patient selection is imperative to strike a balance between disease control, toxicity management, and the maintenance of a high quality of life, because many patients with LAPC are not curable through multidisciplinary treatment[13]. Research has demonstrated that the use of 3D conformal radiotherapy, IMRT, or stereotactic body radiotherapy, combined with concurrent chemotherapy, effectively controls local disease progression. This combined approach has been shown to not only prevent the development of metastatic disease but also significantly enhance survival rates when compared to chemotherapy alone [14-18]. However, the effect is limited, and many patients with LAPC who received upfront chemoradiotherapy experienced metastases soon after they completed therapy.

Immunotherapy, which harnesses the power of the immune system to attack cancer cells, is a particularly exciting area of research. Clinical trials are underway to evaluate the efficacy of immunotherapy agents, such as immune checkpoint inhibitors, in combination with chemotherapy and/or radiation therapy for the treatment of LAPC.

Gene-based therapies, such as CRISPR-Cas9 gene editing and oncogenic virus-based therapies, are also being explored as potential treatment options. These therapies aim to correct genetic mutations that drive cancer growth or activate the immune system to target cancer cells more effectively.

Moreover, the development of personalized medicine approaches based on tumor genomics and proteomics is expected to improve treatment outcomes. By understanding the unique genetic and molecular characteristics of each patient's tumor, doctors can tailor treatment plans that are more likely to be effective and less likely to cause adverse side effects.

LAPC remains a significant challenge in oncology. However, with ongoing research and the development of novel treatment strategies, we are hopeful that the prognosis for these patients will improve in the future. A multifaceted approach, combining surgical resection, chemotherapy, radiation therapy, and novel therapeutic strategies, is likely to be the key to overcoming this devastating disease[19-23].

In recent years, there has been intensive research on checkpoint inhibitor immunotherapy for LAPC[24-27]. Anti-PD-1 immunotherapy holds the potential to effectively collaborate with radiotherapy, leveraging immunogenic cell death to enhance T-cell priming and reversing the immunosuppressive microenvironment, thereby fostering a synergistic therapeutic effect[28,29]. Chen *et al*[30] demonstrated encouraging results in their study: the combination of nab-paclitaxel and gemcitabine, along with the PD-1 inhibitor camrelizumab and radiotherapy, exhibited both efficacy and safety in treating patients with LAPC. This integrated approach led to a notable extension of the median OS to 22.3 mo, which was significantly higher than the 18.6 mo observed in the control group ($P < 0.05$). This demonstrates the effectiveness of our comprehensive strategy in prolonging survival rates, and similarly improved the median PFS to 12.0 mo, *vs* 10.5 mo in the comparator arm ($P < 0.05$). It has been reported that, in combination with chemotherapy, this exhibits a synergistic effect, leading to a reduction in tumor burden by mitigating chemotherapy resistance and modifying the microenvironment[31-34]. Therefore, there is a compelling rationale for combining these three therapies to effectively improve both local and systemic tumor control. However, it is noteworthy that there is a significant lack of clinical data specifically addressing this aspect.

Accordingly, we conducted a retrospective study aimed at comprehensively evaluating and contrasting the therapeutic efficacy and safety profile of S-1 plus gemcitabine chemoradiotherapy administered alongside anti-PD-1 immunotherapy (sintilimab) with the standard treatment of S-1 plus gemcitabine chemoradiotherapy alone in patients suffering from LAPC. Based on the RECIST 1.0 criteria, the ORR was calculated to be 32.4% in the observation group, whereas it was lower, at 20.0%, in the control group. While the DCR achieved in the observation group, utilizing the RECIST 1.0 criteria, stood at an impressive 88.2%, it was slightly lower in the control group, registering a rate of 80.0%. The median OS for patients in the observation group was 22.1 mo, significantly longer than the 15.8 mo observed in the control group ($P < 0.05$). Median PFS was 12.2 mo in the observation group and 10.1 mo in the control group ($P < 0.05$). Univariate and multivariate analyses revealed that the sole independent prognostic factor for both OS and PFS was the allocation of treatment. Notably, during the follow-up period, no statistically significant disparities were observed in terms of the patterns of treatment failure or post-protocol interventions among the two groups. No unexpected toxicity was detected, and there were no fatalities attributed to the treatment. The occurrence of severe AEs did not exhibit a statistically significant difference between the two groups, with rates of 26.5% and 23.3%, respectively. In the observation group, the most prevalent Grade ≥ 3 AEs occurring at a frequency of ≥ 10% were leukopenia (accounting for 14.7% of cases), fatigue (5.9%), and anemia (2.9%). When compared to the control group, these rates were comparable, with leukopenia occurring in 13.3% of cases, fatigue in 6.7%, and anemia in 3.3%.

CONCLUSION

In conclusion, our study demonstrates for the first time that the combination of S-1 and gemcitabine chemoradiotherapy, coupled with anti-PD-1 immunotherapy (sintilimab), exhibits both efficacy and safety in patients with LAPC. This finding holds promise for future exploration and investigation.

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FOOTNOTES

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Institutional review board statement: The present study was approved by the Ethics Committee of The First Affiliated Hospital of Yangtze University (No. KY202429) and was performed according to the Declaration of Helsinki.

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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 **De Luca R**, Gianotti L, Pedrazzoli P, Brunetti O, Rizzo A, Sandini M, Paiella S, Pecorelli N, Pugliese L, Pietrabissa A, Zerbi A, Salvia R, Boggi U, Casirati A, Falconi M, Caccialanza R. Immunonutrition and prehabilitation in pancreatic cancer surgery: A new concept in the era of ERAS® and neoadjuvant treatment. *Eur J Surg Oncol* 2023; **49**: 542-549 [PMID: 36577556 DOI: 10.1016/j.ejso.2022.12.006]
3 **Di Federico A**, Tateo V, Parisi C, Formica F, Carloni R, Frega G, Rizzo A, Ricci D, Di Marco M, Palloni A, Brandi G. Hacking Pancreatic Cancer: Present and Future of Personalized Medicine. *Pharmaceuticals (Basel)* 2021; **14** [PMID: 34358103 DOI: 10.3390/ph14070677]
4 **Di Federico A**, Mosca M, Pagani R, Carloni R, Frega G, De Giglio A, Rizzo A, Ricci D, Tavolari S, Di Marco M, Palloni A, Brandi G. Immunotherapy in Pancreatic Cancer: Why Do We Keep Failing? A Focus on Tumor Immune Microenvironment, Predictive Biomarkers and Treatment Outcomes. *Cancers (Basel)* 2022; **14** [PMID: 35626033 DOI: 10.3390/cancers14102429]
5 **Rizzo A**, Mollica V, Tateo V, Tassinari E, Marchetti A, Rosellini M, De Luca R, Santoni M, Massari F. Hypertransaminasemia in cancer patients receiving immunotherapy and immune-based combinations: the MOUSEION-05 study. *Cancer Immunol Immunother* 2023; **72**: 1381-1394 [PMID: 36695827 DOI: 10.1007/s00262-023-03366-x]
6 **Sudo K**, Yamaguchi T, Ishihara T, Nakamura K, Hara T, Denda T, Tawada K, Imagumbai T, Araki H, Sakai M, Hatano K, Kawakami H, Uno T, Ito H, Yokosuka O. Phase II study of oral S-1 and concurrent radiotherapy in patients with unresectable locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2011; **80**: 119-125 [PMID: 20605363 DOI: 10.1016/j.ijrobp.2010.01.027]
7 **Tempero MA**, Arnoletti JP, Behrman SW, Ben-Josef E, Benson AB 3rd, Casper ES, Cohen SJ, Czito B, Ellenhorn JD, Hawkins WG, Herman J, Hoffman JP, Ko A, Komanduri S, Koong A, Ma WW, Malafa MP, Merchant NB, Mulvihill SJ, Muscarella P 2nd, Nakakura EK, Obando J, Pitman MB, Sasson AR, Tally A, Thayer SP, Whiting S, Wolff RA, Wolpin BM, Freedman-Cass DA, Shead DA; National Comprehensive Cancer Networks. Pancreatic Adenocarcinoma, version 2.2012: featured updates to the NCCN Guidelines. *J Natl Compr Canc Netw* 2012; **10**: 703-713 [PMID: 22679115 DOI: 10.6004/jnccn.2012.0073]
8 **Ren Z**, Xu J, Bai Y, Xu A, Cang S, Du C, Li Q, Lu Y, Chen Y, Guo Y, Chen Z, Liu B, Jia W, Wu J, Wang J, Shao G, Zhang B, Shan Y, Meng Z, Wu J, Gu S, Yang W, Liu C, Shi X, Gao Z, Yin T, Cui J, Huang M, Xing B, Mao Y, Teng G, Qin Y, Wang J, Xia F, Yin G, Yang Y, Chen M, Wang Y, Zhou H, Fan J; ORIENT-32 study group. Sintilimab plus a bevacizumab biosimilar (IBI305) versus sorafenib in unresectable hepatocellular carcinoma (ORIENT-32): a randomised, open-label, phase 2-3 study. *Lancet Oncol* 2021; **22**: 977-990 [PMID: 34143971 DOI: 10.1016/S1470-2045(21)00252-7]
9 **Lu S**, Wu L, Jian H, Chen Y, Wang Q, Fang J, Wang Z, Hu Y, Sun M, Han L, Miao L, Ding C, Cui J, Li B, Pan Y, Li X, Ye F, Liu A, Wang K, Cang S, Zhou H, Sun X, Ferry D, Lin Y, Wang S, Zhang W, Zhang C. Sintilimab plus bevacizumab biosimilar IBI305 and chemotherapy for patients with EGFR-mutated non-squamous non-small-cell lung cancer who progressed on EGFR tyrosine-kinase inhibitor therapy (ORIENT-31): first interim results from a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol* 2022; **23**: 1167-1179 [PMID: 35908558 DOI: 10.1016/S1470-2045(22)00382-5]
10 **Shi Y**, Su H, Song Y, Jiang W, Sun X, Qian W, Zhang W, Gao Y, Jin Z, Zhou J, Jin C, Zou L, Qiu L, Li W, Yang J, Hou M, Zeng S, Zhang Q, Hu J, Zhou H, Xiong Y, Liu P. Safety and activity of sintilimab in patients with relapsed or refractory classical Hodgkin lymphoma (ORIENT-1): a multicentre, single-arm, phase 2 trial. *Lancet Haematol* 2019; **6**: e12-e19 [PMID: 30612710 DOI: 10.1016/S2352-3026(18)30192-3]
11 **Chen G**, Jin Y, Guan WL, Zhang RX, Xiao WW, Cai PQ, Liu M, Lin JZ, Wang FL, Li C, Quan TT, Xi SY, Zhang HZ, Pan ZZ, Wang F, Xu RH. Neoadjuvant PD-1 blockade with sintilimab in mismatch-repair deficient, locally advanced rectal cancer: an open-label, single-centre phase 2 study. *Lancet Gastroenterol Hepatol* 2023; **8**: 422-431 [PMID: 36870360 DOI: 10.1016/S2468-1253(22)00439-3]
12 **Xu Q**, Wang J, Sun Y, Lin Y, Liu J, Zhuo Y, Huang Z, Huang S, Chen Y, Chen L, Ke M, Li L, Li Z, Pan J, Song Y, Liu R, Chen C. Efficacy and Safety of Sintilimab Plus Anlotinib for PD-L1-Positive Recurrent or Metastatic Cervical Cancer: A Multicenter, Single-Arm, Prospective Phase II Trial. *J Clin Oncol* 2022; **40**: 1795-1805 [PMID: 35192397 DOI: 10.1200/JCO.21.02091]
13 **Wolfgang CL**, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, Hruban RH. Recent progress in pancreatic cancer. *CA Cancer J Clin* 2013; **63**: 318-348 [PMID: 23856911 DOI: 10.3322/caac.21190]
14 **Zhu X**, Liu W, Cao Y, Feng Z, Zhao X, Jiang L, Ye Y, Zhang H. Immune profiling of pancreatic cancer for radiotherapy with immunotherapy and targeted therapy: Biomarker analysis of a randomized phase 2 trial. *Radiother Oncol* 2024; **190**: 109941 [PMID: 37820884 DOI: 10.1016/j.radonc.2023.109941]
15 **Reddy AV**, Hill CS, Sehgal S, Zheng L, He J, Laheru DA, Jesus-Acosta A, Herman JM, Meyer J, Narang AK. Post-radiation neutrophil-to-lymphocyte ratio is a prognostic marker in patients with localized pancreatic adenocarcinoma treated with anti-PD-1 antibody and stereotactic body radiation therapy. *Radiat Oncol J* 2022; **40**: 111-119 [PMID: 35796114 DOI: 10.3857/roj.2021.01060]
16 **Hughson AL**, Hannon G, Salama NA, Vrooman TG, Stockwell CA, Mills BN, Garrett-Larsen J, Qiu H, Katerji R, Benoodt L, Johnston CJ, Murphy JD, Kruger E, Ye J, Gavras NW, Keeley DC, Qin SS, Lesch ML, Muhitch JB, Love TMT, Calvi LM, Lord EM, Luheshi N, Elyes J,

Linehan DC, Gerber SA. Local Delivery of SBRT and IL12 by mRNA Technology Overcomes Immunosuppressive Barriers to Eliminate Pancreatic Cancer. *bioRxiv* 2023 [PMID: 37961513 DOI: 10.1101/2023.10.30.564833]

17 **Benkhaled S**, Peters C, Jullian N, Arsenijevic T, Navez J, Van Gestel D, Moretti L, Van Laethem JL, Bouchart C. Combination, Modulation and Interplay of Modern Radiotherapy with the Tumor Microenvironment and Targeted Therapies in Pancreatic Cancer: Which Candidates to Boost Radiotherapy? *Cancers (Basel)* 2023; **15** [PMID: 36765726 DOI: 10.3390/cancers15030768]

18 **Zhu X**, Cao Y, Liu W, Ju X, Zhao X, Jiang L, Ye Y, Jin G, Zhang H. Stereotactic body radiotherapy plus pembrolizumab and trametinib versus stereotactic body radiotherapy plus gemcitabine for locally recurrent pancreatic cancer after surgical resection: an open-label, randomised, controlled, phase 2 trial. *Lancet Oncol* 2022; **23**: e105-e115 [PMID: 35240087 DOI: 10.1016/S1470-2045(22)00066-3]

19 **Song D**, Yang X, Guo X, Sun H. Safety and efficacy analysis of PD-1 inhibitors in combination with chemotherapy for advanced pancreatic cancer. *Immunotherapy* 2022; **14**: 1307-1313 [PMID: 36341552 DOI: 10.2217/imt-2022-0196]

20 **Du J**, Zhu L, Sha H, Zou Z, Shen J, Kong W, Zhao L, Gu Q, Yu L, Qiu Y, Liu B. Therapeutic effect and safety of individualized chemotherapy combined with sequential immunotherapy based on BRCA1 mRNA expression level in unresectable pancreatic cancer. *Front Oncol* 2022; **12**: 1015232 [PMID: 36387089 DOI: 10.3389/fonc.2022.1015232]

21 **Huang Y**, Yan X, Ren T, Yi F, Li Q, Zhang C. The safety and efficacy of chemotherapy combined with immunotherapy for pancreatic cancer: A meta-analysis. *Medicine (Baltimore)* 2021; **100**: e26673 [PMID: 34398033 DOI: 10.1097/MD.0000000000026673]

22 **Fu Q**, Chen Y, Huang D, Guo C, Zhang X, Xiao W, Xue X, Zhang Q, Li X, Gao S, Que R, Shen Y, Wu J, Zhang M, Bai X, Liang T. Sintilimab Plus Modified FOLFIRINOX in Metastatic or Recurrent Pancreatic Cancer: The Randomized Phase II CISPD3 Trial. *Ann Surg Oncol* 2023; **30**: 5071-5080 [PMID: 37052821 DOI: 10.1245/s10434-023-13383-w]

23 **Liu Q**, Zhao G, Zhang X, Jiang N, Zhao Z, Wang Y, Xu S, Zhu L, Lau WY, Dai G, Liu R. Nab-paclitaxel plus S-1 with or without PD-1 inhibitor in pancreatic ductal adenocarcinoma with only hepatic metastases: a retrospective cohort study. *Langenbecks Arch Surg* 2022; **407**: 633-643 [PMID: 34518900 DOI: 10.1007/s00423-021-02321-7]

24 **Katz MHG**, Petroni GR, Bauer T, Reilley MJ, Wolpin BM, Stucky CC, Bekaii-Saab TS, Elias R, Merchant N, Dias Costa A, Lenehan P, Cardot-Ruffino V, Rodig S, Pfaff K, Dougan SK, Nowak JA, Varadhachary GR, Slingluff CL, Rahma O. Multicenter randomized controlled trial of neoadjuvant chemoradiotherapy alone or in combination with pembrolizumab in patients with resectable or borderline resectable pancreatic adenocarcinoma. *J Immunother Cancer* 2023; **11** [PMID: 38040420 DOI: 10.1136/jitc-2023-007586]

25 **Zhou B**, Zhang SR, Chen G, Chen P. Developments and challenges in neoadjuvant therapy for locally advanced pancreatic cancer. *World J Gastroenterol* 2023; **29**: 5094-5103 [PMID: 37744290 DOI: 10.3748/wjg.v29.i35.5094]

26 **Li Y**, Xiang S, Pan W, Wang J, Zhan H, Liu S. Targeting tumor immunosuppressive microenvironment for pancreatic cancer immunotherapy: Current research and future perspective. *Front Oncol* 2023; **13**: 1166860 [PMID: 37064113 DOI: 10.3389/fonc.2023.1166860]

27 **Chick RC**, Gunderson AJ, Rahman S, Cloyd JM. Neoadjuvant Immunotherapy for Localized Pancreatic Cancer: Challenges and Early Results. *Cancers (Basel)* 2023; **15** [PMID: 37568782 DOI: 10.3390/cancers15153967]

28 **Zhu X**, Liu W, Cao Y, Ju X, Zhao X, Jiang L, Ye Y, Zhang H. Effect of stereotactic body radiotherapy dose escalation plus pembrolizumab and trametinib versus stereotactic body radiotherapy dose escalation plus gemcitabine for locally recurrent pancreatic cancer after surgical resection on survival outcomes: A secondary analysis of an open-label, randomised, controlled, phase 2 trial. *EClinicalMedicine* 2023; **55**: 101764 [PMID: 36471691 DOI: 10.1016/j.eclinm.2022.101764]

29 **Chen IM**, Donia M, Chamberlain CA, Jensen AWP, Draghi A, Theile S, Madsen K, Hasselby JP, Toxværd A, Høgdall E, Lorentzen T, Wilken EE, Geertsen P, Svane IM, Johansen JS, Nielsen D. Phase 2 study of ipilimumab, nivolumab, and tocilizumab combined with stereotactic body radiotherapy in patients with refractory pancreatic cancer (TRIPLE-R). *Eur J Cancer* 2023; **180**: 125-133 [PMID: 36592507 DOI: 10.1016/j.ejca.2022.11.035]

30 **Chen S**, Li J, Dong A, Liu Z, Zhu M, Jin M, Wei G, Wu S, Wang Y, Chen Y, Peng Z. Nab-paclitaxel and gemcitabine plus camrelizumab and radiotherapy versus nab-paclitaxel and gemcitabine alone for locally advanced pancreatic adenocarcinoma: a prospective cohort study. *J Hematol Oncol* 2023; **16**: 26 [PMID: 36941671 DOI: 10.1186/s13045-023-01422-8]

31 **Samanta K**, Setua S, Kumari S, Jaggi M, Yallapu MM, Chauhan SC. Gemcitabine Combination Nano Therapies for Pancreatic Cancer. *Pharmaceutics* 2019; **11** [PMID: 31689930 DOI: 10.3390/pharmaceutics11110574]

32 **Saung MT**, Zheng L. Adding combination immunotherapy consisting of cancer vaccine, anti-PD-1 and anti-CSF1R antibodies to gemcitabine improves anti-tumor efficacy in murine model of pancreatic ductal adenocarcinoma. *Ann Pancreat Cancer* 2019; **2** [PMID: 32405624 DOI: 10.21037/apc.2019.11.01]

33 **Zhang F**, Wang Y, Yang F, Zhang Y, Jiang M, Zhang X. The Efficacy and Safety of PD-1 Inhibitors Combined with Nab-Paclitaxel Plus Gemcitabine versus Nab-Paclitaxel Plus Gemcitabine in the First-Line Treatment of Advanced Pancreatic Cancer: A Retrospective Monocentric Study. *Cancer Manag Res* 2022; **14**: 535-546 [PMID: 35173487 DOI: 10.2147/CMAR.S349442]

34 **Kamath SD**, Kalyan A, Kircher S, Nimeiri H, Fought AJ, Benson A 3rd, Mulcahy M. Ipilimumab and Gemcitabine for Advanced Pancreatic Cancer: A Phase Ib Study. *Oncologist* 2020; **25**: e808-e815 [PMID: 31740568 DOI: 10.1634/theoncologist.2019-0473]

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ORIGINAL ARTICLE

Clinical and Translational Research

Bibliometric analysis of phosphoglycerate kinase 1 expression in breast cancer and its distinct upregulation in triple-negative breast cancer

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Abstract

BACKGROUND

Phosphoglycerate kinase 1 (PGK1) has been identified as a possible biomarker for breast cancer (BC) and may play a role in the development and advancement of triple-negative BC (TNBC).

AIM

To explore the PGK1 and BC research status and PGK1 expression and mechanism differences among TNBC, non-TNBC, and normal breast tissue.

METHODS

PGK1 and BC related literature was downloaded from Web of Science Core Collection Core Collection. Publication counts, key-word frequency, cooperation networks, and theme trends were analyzed. Normal breast, TNBC, and non-TNBC mRNA data were gathered, and differentially expressed genes obtained. Area under the summary receiver operating characteristic curves, sensitivity and specificity of PGK1 expression were determined. Kaplan Meier revealed PGK1's prognostic implication. PGK1 co-expressed genes were explored, and Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and Disease Ontology applied. Protein-protein interaction networks were constructed. Hub genes identified.

RESULTS

PGK1 and BC related publications have surged since 2020, with China leading the way. The most frequent keyword was "Expression". Collaborative networks were found among co-citations, countries, institutions, and authors. PGK1 expression

and BC progression were research hotspots, and PGK1 expression and BC survival were research frontiers. In 16 TNBC *vs* non-cancerous breast and 15 TNBC *vs* non-TNBC datasets, PGK1 mRNA levels were higher in 1159 TNBC than 1205 non-cancerous breast cases [standardized mean differences (SMD): 0.85, 95% confidence interval (95%CI): 0.54-1.16, I^2 = 86%, P < 0.001]. PGK1 expression was higher in 1520 TNBC than 7072 non-TNBC cases (SMD: 0.25, 95%CI: 0.03-0.47, I^2 = 91%, P = 0.02). Recurrence free survival was lower in PGK1-high-expression than PGK1-low-expression group (hazard ratio: 1.282, P = 0.023). PGK1 co-expressed genes were concentrated in ATP metabolic process, HIF-1 signaling, and glycolysis/gluconeogenesis pathways.

CONCLUSION

PGK1 expression is a research hotspot and frontier direction in the BC field. PGK1 may play a strong role in promoting cancer in TNBC by mediating metabolism and HIF-1 signaling pathways.

Key Words: Phosphoglycerate kinase 1; Breast cancer; Triple-negative breast cancer; Bibliometric analysis; Computational pathology

Core Tip: The expression of phosphoglycerate kinase 1 (PGK1) in breast cancer (BC) was shown to be both a research hotspot and frontier of BC research. PGK1 mRNA levels were significantly higher in triple-negative BC (TNBC) than in non-cancerous breast tissue. Within the population with BC, PGK1 mRNA expression levels were distinctly upregulated in TNBC compared with non-TNBC. Recurrence-free survival was markedly lower in the PGK1-high-expression than the PGK1-low-expression group. PGK1 has a significant role in promoting TNBC through metabolism and HIF-1 signaling pathways.

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INTRODUCTION

Breast cancer (BC) has become the most frequent malignancy to affect females. Based on the 2024 statistics from the American Cancer Society, BC accounted for 32.0% of the estimated new cases of female cancer, and the incidence of female BC has been said to exhibit a 0.6% annual growth rate[1]. Obtaining the best prognosis for BC patients is mainly dependent on early BC detection and intervention[2-8]. Thus, it is necessary that we explore more accurate BC biomarkers to improve methods for early, accurate identification and treatment to reduce mortality from BC[9-18].

BC is classified according to the presence or absence of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2). BC that does not express these receptors is classified as triple-negative BC (TNBC)[19-21], and this subtype accounts for approximately 12%-20% of all BC cases[22]. Compared with non-TNBC, TNBC has its own clinical characteristics: It is more common in younger women (< 50 years old), and it has higher degree of invasiveness, higher recurrence rate, shorter recurrence time, lower survival rate, and more *BRCA1/2* gene mutations[23-27]. TNBC patients do not benefit from endocrine therapy, unlike ER/PR-positive cases, or from targeted therapy, as used for HER-2-positive cases[23-29]. The mechanisms of TNBC occurrence and development have been investigated by some researchers. Studies have found gene mutations, DNA repair defects, and non-coding RNA to be involved in the carcinogenic, proliferative, and invasive processes of TNBC. The Notch, Wnt/β-catenin, and Hedgehog signaling pathways have also been reported to be important contributors to incidences of TNBC[30-32]. Nevertheless, there are different opinions on the key mechanisms of TNBC[33,34], and many molecular events believed to be related to TNBC need more scientific attention.

Phosphoglycerate kinase 1 (PGK1) is a core enzymes of the glycolysis pathway[35], being involved in a reversible reaction that produces ATP during glycolysis[36], and plays a pivotal part in balancing energy production, redox, and biosynthesis[37]. The phenomenon of PGK1 overexpression has been observed in a variety of malignant tumors, *e.g.*, hepatocellular carcinoma[38], non-small cell lung cancer[39], and pancreatic ductal adenocarcinoma[40]. The presence of higher PGK1 levels in BC tissue had been linked to a worse prognosis, and this trend is mirrored in TNBC[35,41-46]. This suggests that PGK1 could serve as a promising biomarker for BC and may play a role in the initiation and progression of TNBC.

In this research, we concentrated on employing bibliometric analysis to gain insights into the current status and future directions of PGK1 and BC studies, with a focus on comparing the expression levels and underlying mechanisms of PGK1 in BC *vs* normal breast tissue, as well as in TNBC *vs* non-TNBC, through computational pathology and various methods of high-throughput data mining.

MATERIALS AND METHODS

PGK1 and BC bibliometric analysis

Bibliometric analysis data sources: The Web of Science Core Collection (WOS) was used to search the scientific literature related to PGK1 and BC with the following search formula: ("PGK1" OR "phosphoglycerate kinase 1" OR "HEL-S-68p" OR "MIG10" OR "PGKA") AND ("breast" OR "mammary" OR "nipple") AND ("cancer" OR "tumor" OR "carcinoma" OR "neoplasm" OR "neoplasia" OR "malignancy" OR "malignant"). WOS was searched until September 3, 2022. The inclusion and exclusion criteria were as follows: (1) Including BC and PGK1 related studies, and excluding studies unrelated to PGK1; (2) Including English literature in WOS, and excluding literature in other languages (such as German, Spanish, *etc.*); (3) Including original research and review articles, and excluding comments, letters, and conference abstracts; and (4) Excluding retracted articles. The total number of papers irrelevant to BC and PGK1 was 39, as shown in Figure 1.

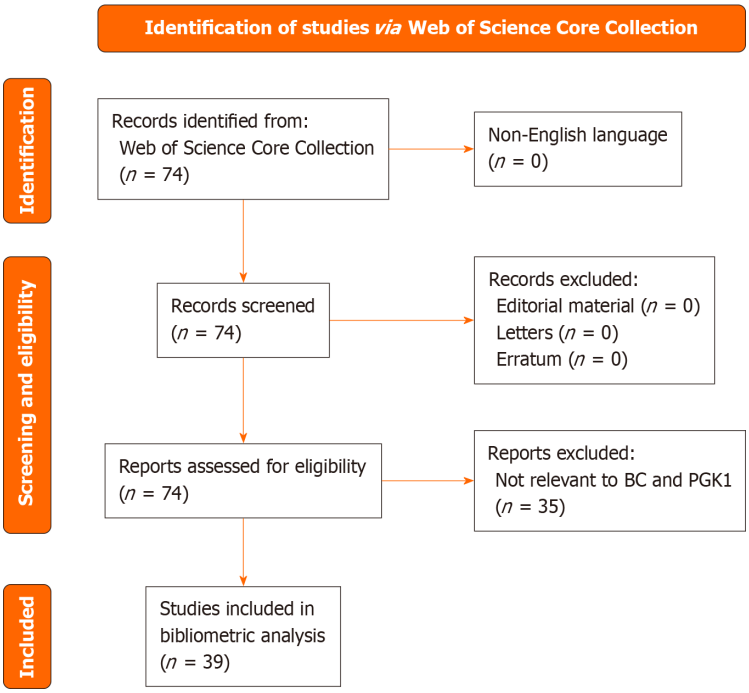


Figure 1 PRISMA flow diagram of the data screening procedure used in the current study. BC: Breast cancer; PGK1: Phosphoglycerate kinase 1.

Data processing methods: The Bibliometrix program in R language was employed to retrieve document data, draw the annual scientific publication curve and geographic distribution map of the corresponding authors; and making a word cloud, thematic plot, and trending topics. VOSviewer (version 1.6.18) was used to analyze the key word co-occurrence plot of the included BC- and PGK1-related literature. Using the leading Eigen clustering algorithm, cooperative networks of co-cited references, cooperative countries, university institutions, and authors were constructed.

Computational pathology associated with PGK1 in TNBC and non-TNBC

Transcriptome data for PGK1 mRNA levels in TNBC: The mRNA expression data for PGK1 in TNBC tissue were downloaded from the GEO, TCGA/GTEx, Metabric, ArrayExpress, and SRA databases. The first search term used was "breast cancer." The pathological parameters of ER/PR/HER-2-negative patients were collected, including pathological diagnosis, molecular subtypes, and relevant follow-up data such as overall survival (OS), distant-metastasis-free survival (DMFS), and relapse-free survival (RFS). The search term was then changed to "triple-negative breast cancer" to collect relevant datasets relating to TNBC. The pathological parameters of each patient were also collected. The inclusion principles for transcriptome data of TNBC were as follows: (1) Primary TNBC tissue; and (2) Sample size ≥ 6. The following data sets were removed: (1) Animal and cell line data; (2) Relapse and metastatic TNBC; and (3) Duplicated samples. The molecular diagnosis of TNBC was provided by Pam50 molecular typing of the data sets. Data sets that provided ER/PR/HER-2 status without molecular typing were included if all were negative, otherwise they were excluded. A PRISMA flow diagram of data set filtering is presented in Figure 2.

Data integration: Expression data from data sets obtained using the same platform were combined after removing batch effects using the limma-voom and sva packages. Global BC data sets were used to pinpoint upregulated genes in TNBC tissue (*i.e.*, TNBC tissue *vs* non-cancerous breast tissue, and TNBC *vs* non-TN BC tissue). The criteria were [standardized mean differences (SMD)] > 0 and $P < 0.05$.

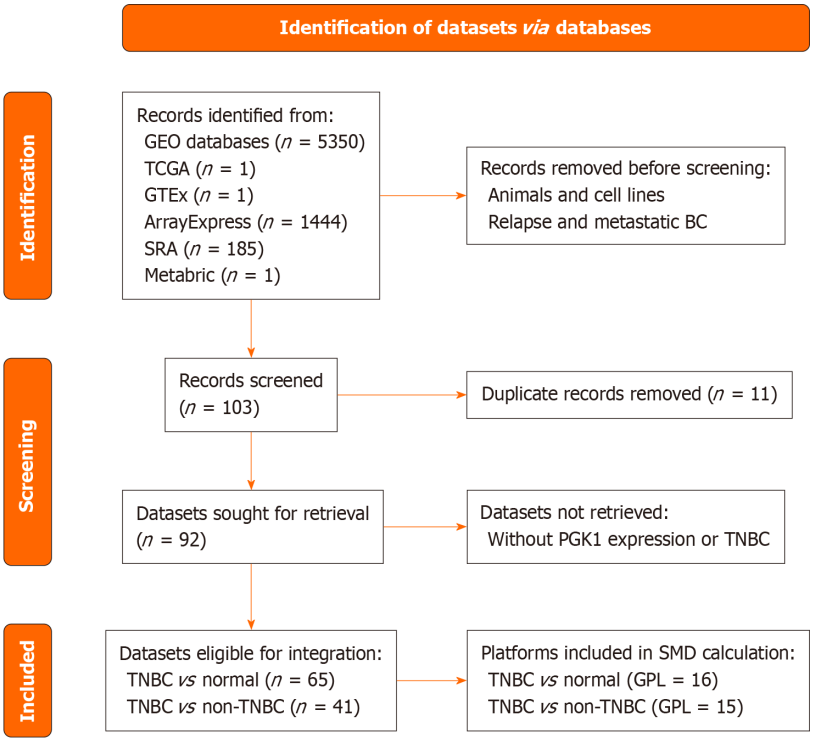


Figure 2 PRISMA flow diagram of the screening procedure used for triple-negative breast cancer data sets including phosphoglycerate kinase 1 expression. BC: Breast cancer; PGK1: Phosphoglycerate kinase 1; TNBC: Triple-negative breast cancer; SMD: Standardized mean differences.

Protein levels of PGK1 in The Human Protein Atlas: The Human Protein Atlas (THPA) (https://www.proteinatlas.org/) is one of the largest human protein maps in the world and includes immunohistochemical results from tissue chips. This research initially examined variations in the location and intensity of PGK1 protein expression between normal breast cells and BC cells with the assistance of the THPA database.

Prognostic analysis of PGK1 in TNBC patients: TNBC patients were grouped using median PGK1 expression. TNBC samples were assigned to a PGK1-overexpression group and PGK1-underexpression group, according to the ideal cutoff point, using survminer v0.4.9 and ggplot2 v3.3.6 packages. Kaplan-Meier analysis was used to filter for candidate prognostic factors in TNBC patients. The hazard ratios (HRs) for PGK1 expression in the different TNBC cohorts were integrated using the meta v4.18.2 package.

Prospective mechanisms of PGK1's effect in TNBC: Genes co-expressed with PGK1 were identified in TNBC tissue samples. The criteria for the co-expressed genes were as follows: (1) Pearson $r \geq 0.3$; and (2) $P < 0.05$. The intersections of overexpressed genes in TNBC *vs* non-TNBC and genes co-expressed with PGK1 were taken for further signaling pathway exploration. Analyses of Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Disease Ontology (DO) were accomplished with Clusterprofiler v4.4.2 to investigate the potential function of PGK1 in TNBC. STRING v11.5 was used to construct protein-protein interaction (PPI) networks. Cytoscape v3.9.0 was used to identify hub genes.

Statistical analysis

A Wilcoxon test was applied to evaluate the differential expression of PGK1 in the BC subgroups (*i.e.*, BC *vs* non-cancerous breast tissue, TNBC *vs* non-cancerous breast tissue, and TNBC *vs* non-TNBC). Violin plots and receiver operating characteristic curves (ROCs) were drawn. Statistical analysis results with $P < 0.05$ were regarded as being significant.

RESULTS

PGK1 and BC bibliometric analysis results

Analysis of the published literature: Although the number of studies on PGK1 in BC was small, the annual number of PGK1 and BC publications has shown an upward trend since 2005 (Figure 3A). The numbers of articles published in 2020 and 2021 (8 and 9, respectively) were significantly increased from previous years, indicating that the interest of researchers in the link between PGK1 and BC had gradually increased.

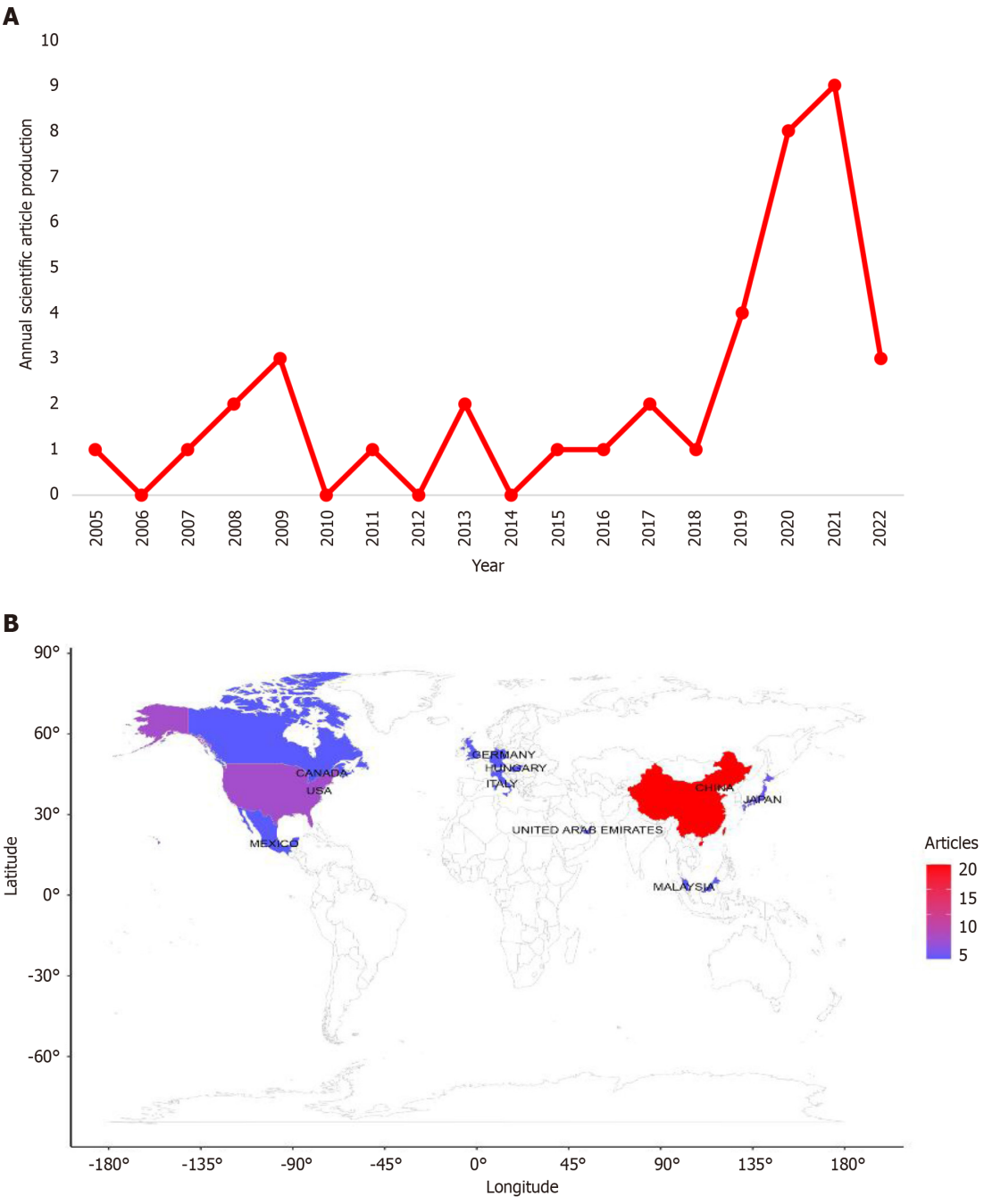


Figure 3 Global scientific research on phosphoglycerate kinase 1 and breast cancer. A: Annual scientific production. Abscissa is the year, and ordinate is number of articles; B: Geographic distribution of corresponding authors. The more articles, the redder the area on the map. Horizontal and vertical coordinates represent longitude and latitude, respectively.

Furthermore, national and regional analysis of the literature suggested that the authors of PGK1-related BC studies were from 15 countries, with 20 articles from China, 5 from the United States, and 2 from Japan, as shown in Figure 3B. A total of 33 journals have published literature on this topic. The four journals with the highest number of articles appear in Table 1.

Key word analysis: The key words with the highest frequency, as defined by the author and added to WOS, appear in Table 2. Figure 4A shows a word cloud, and Figure 4B shows a key word co-occurrence plot. The term "expression" emerged as the most prevalent key word and served as a pivotal hub in the key word co-occurrence relationship, underscoring its significance in studies linking PGK1 and BC. Other key words with a high-frequency of co-occurrence relationships included "progression", "gene", and "survival", showing these have been the focus of BC research. Key words closely related to PGK1 co-occurrence included "survival", "progression", "invasion", and "glycolysis".

Analysis of cooperation network: To complete the cooperative network analysis, first, a co-citation network (Figure 5A) was visualized. Three co-citation clusters were formed with Grandjean G, Ahmad SS, and Warburg O as the core authors, among which Sun S had the highest number of co-citations and was at the center of the co-citation network. Second, data

Table 1 Journals with the most published articles

Journal	Number
Journal of Proteome Research	3
Scientific Reports	3
Frontiers in Cell and Developmental Biology	2
Journal of Translational Medicine	2

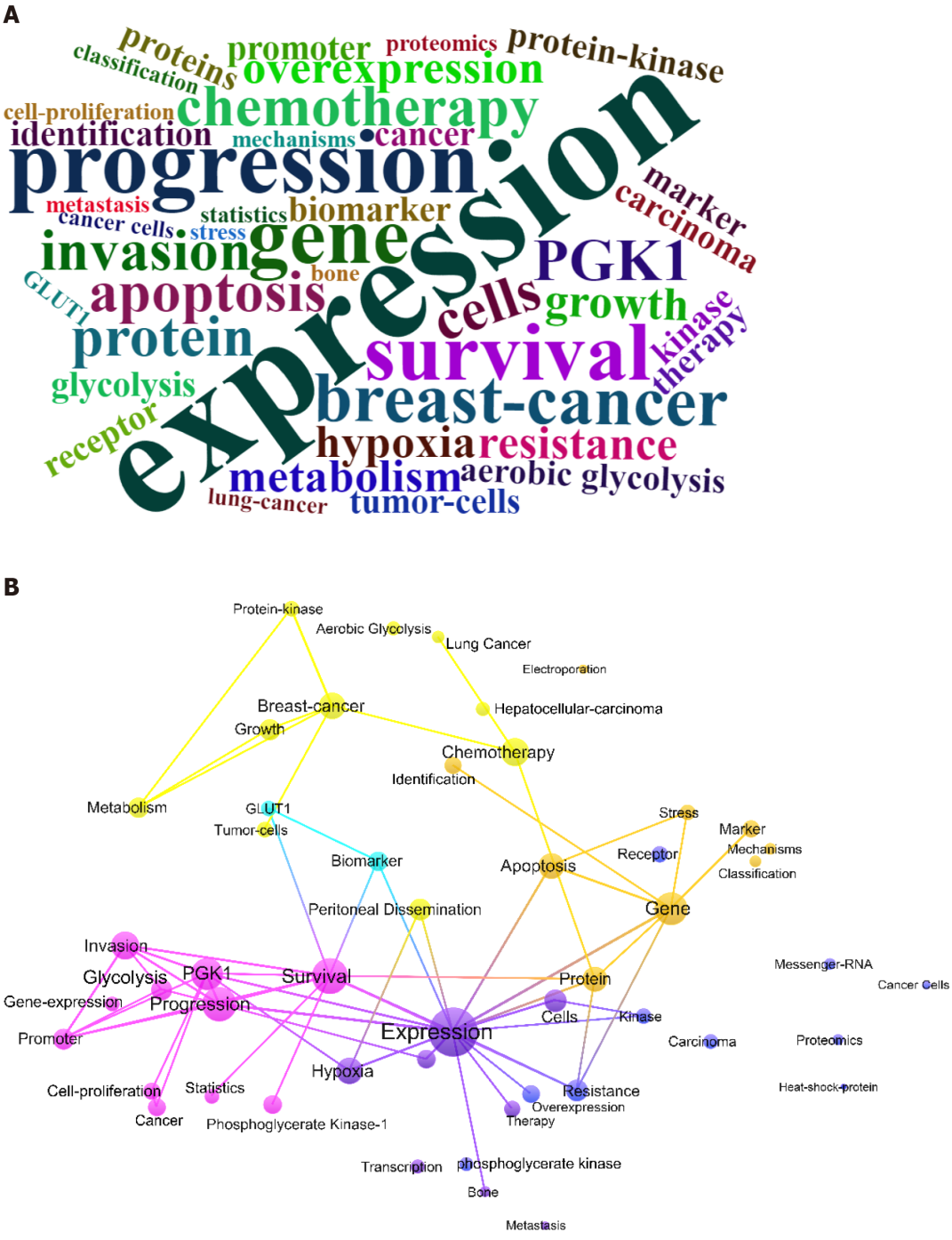


Figure 4 Key words. A: Word cloud of key words. Word frequency was counted according to the Web of Science key words for each document. The word cloud plot is randomly colored. A larger font size indicates a higher frequency; B: Key word co-occurrence plot for breast cancer and phosphoglycerate kinase 1 research. Web of Science key words were clustered using VOSviewer algorithm. Different colors represent different clusters. Font size positively correlates with total co-occurrent numbers. Edges indicate co-occurrence relationships.

Table 2 Key words with the highest frequency

Key word	Frequency
Expression	13
Progression	8
Gene	7
Survival	7
Breast cancer	6

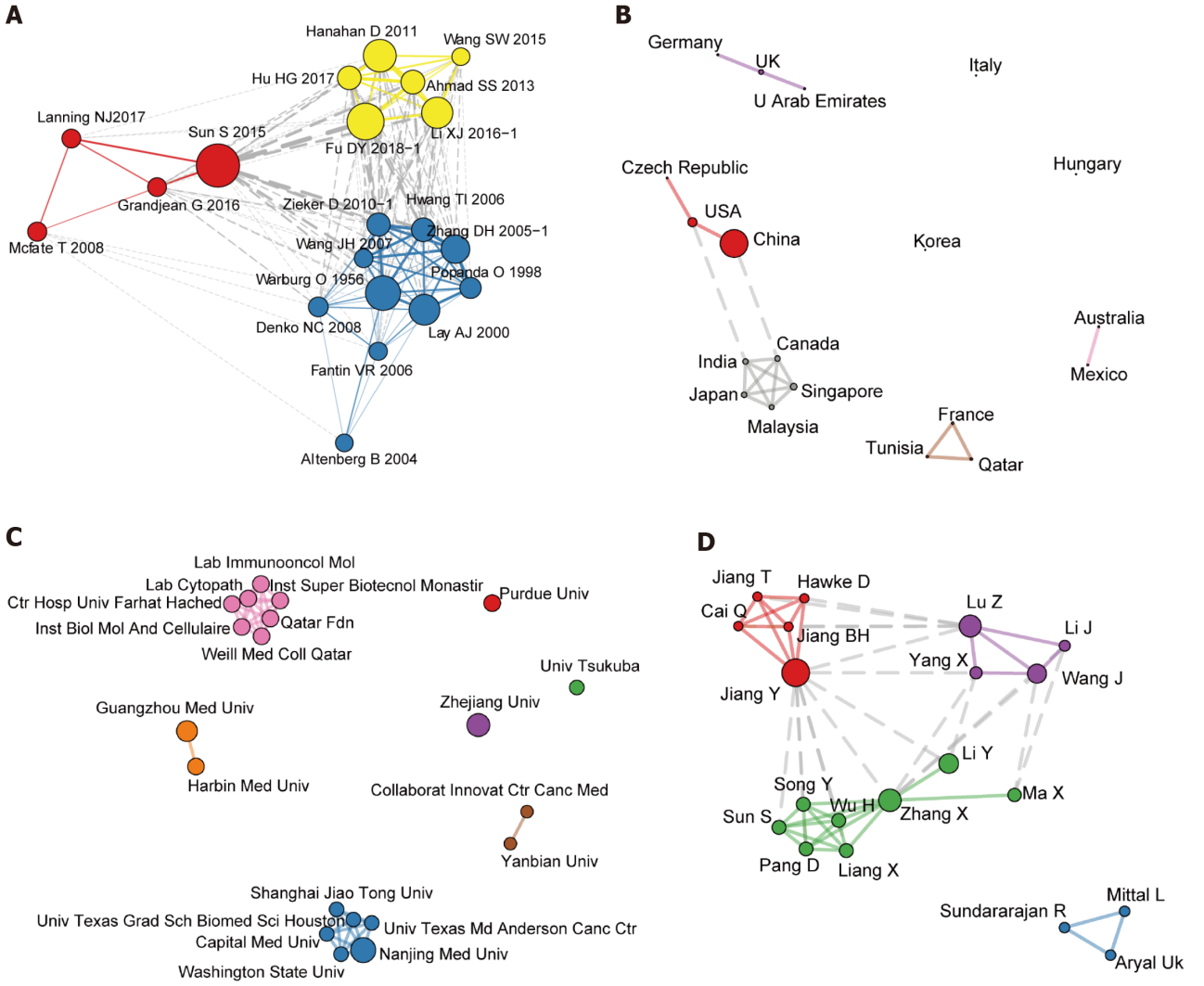


Figure 5 Cooperation network analysis for research on breast cancer and phosphoglycerate kinase 1. A: Co-citation network; B: Collaborations among countries; C: University collaborations; D: Author collaborations. The co-cited documents and cooperating countries, universities, and authors were clustered using a "leading Eigen" algorithm. Different colors represent different clusters. Node size is positively correlated with the degree of connectivity of each node in the network. Edges indicate a co-citation or collaborative relationship.

on collaborations between countries were calculated, as shown in Figure 5B. The country that published the most papers was China, while working collaboratively with the United States. A network of cooperation was formed by Canada, India, Japan, Malaysia, and Singapore. France, Tunisia, and Qatar were also involved in cooperations.

Regarding university collaborations (Figure 5C), a number of Chinese and American academic institutions, including Shanghai Jiao Tong University, Capital Medical University, Washington State University, Nanjing Medical University, and the University of Texas MD Anderson Cancer Center, had established tight-knit collaborative networks. Another partnership cluster consisted of Laboratory Immunooncol Molecular, Institut Supérieur de Biotechnologie de Monastir, Qatar Foundation, *etc*. However, as a whole, university research institutions did not form close cooperative relationships.

Author collaborations also led to the formation of different cooperation clusters (Figure 5D). Jiang Y had the largest node area, indicating that he had the highest degree of connectivity in the author cooperation network.

Citation analysis: The most relevant authors and the H-indexes of authors are shown in Tables 3 and 4, respectively. The authors with the highest number of publications and H-indexes were Jiang Y and Zhang X. In the most cited documents (Table 5), Zhang *et al*[44] showed that PGK1 was overexpressed in tissues and cell lines with receptor tyrosine kinase ErbB2 (HER-2/NEU)-positivity, particularly HER-2/NEU1-positive BC in which PGK1 exhibited even higher levels of expression. Its expression was related to the state of the HER-2/NEU signal pathway. Shashni *et al*[47] believed that the generation of ATP in the cytoplasm and mitochondria decreased with the downregulation of PGK1, which initiated cell apoptosis and inhibited tumor metabolism. Qian *et al*[48] reported that PGK1 was overexpressed in an invasive ductal carcinoma of the breast. The most cited sources can provide research directions for scientific and technological efforts in the BC- and PGK1-related fields (Table 6)[48].

Table 3 Most relevant authors in breast cancer and phosphoglycerate kinase 1 literature

Authors	Number of articles	Articles fractionalized
Zhang X	5	0.84
Jiang Y	5	0.47
Wang J	4	0.73
Li Y	4	0.59
Aryal UK	3	0.62
Mittal L	3	0.62
Sundararajan R	3	0.62
Lu Z	3	0.28
Liang Y	2	0.50
Ye T	2	0.50

Table 4 Top authors by H-index in breast cancer and phosphoglycerate kinase 1 literature

Authors	H-index	G-index	M-index	Total citations	Number of publications	Publication year
Jiang Y	3	4	0.375	256	4	2015
Zhang X	3	4	0.375	79	4	2015
Wang J	3	4	0.250	35	4	2011
Aryal UK	3	3	0.750	42	3	2019
Mittal L	3	3	0.750	42	3	2019
Sundararajan R	3	3	0.750	42	3	2019
Li Y	2	3	0.500	53	3	2019
Camarillo IG	2	2	0.667	27	2	2020
Liang Y	2	2	0.667	15	2	2020
Ye T	2	2	0.667	15	2	2020

Thematic analysis: In the thematic plot, the horizontal axis shows a higher degree of importance from left to right, and the vertical axis displays a better degree of development from bottom to top. PGK1 and BC research topics that have attracted the most attention are "expression", "gene", "apoptosis", "survival", "invasion", and "protein" as shown in Figure 6A.

The trends among topics demonstrated once again that the key words with the highest attention since 2005 have been "expression", "progress", "survival", and "gene", *etc*. According to this trend, new hot topics in PGK1 and BC in the last two years have been "survival", "protein", "invasion", and "biomarkers", *etc*. (Figure 6B).

Computational pathology results relating to PGK1 expression in TNBC and non-TNBC

mRNA expression levels of PGK1 in TNBC tissue: In a preliminary investigation, 31 high-throughput data sets from 7543 BC tissue samples were compared with 1685 non-cancerous breast tissue samples, and our analysis revealed the

Table 5 Most cited documents in breast cancer and phosphoglycerate kinase 1 literature

Ref.	Journal	DOI	Local citations	Global citations
Zhang *et al*[44], 2005	*Mol Cell Proteomics*	10.1074/mcp.M400221-MCP200	9	244
Shashni *et al*[47], 2013	*J Drug Target*	10.3109/1061186X.2012.736998	4	33
Qian *et al*[48], 2017	*Mol Cell*	10.1016/j.molcel.2017.01.027	3	133
Kabbage *et al*[53], 2008	*J Biomed Biotechnol*	10.1155/2008/564127	2	25
Fu *et al*[35], 2018	*Life Sci*	10.1016/j.lfs.2020.117863	2	11

Table 6 Most cited sources of breast cancer and phosphoglycerate kinase 1 research

Sources	Number of articles
Cancer Research	96
Journal of Biological Chemistry	65
Proceedings of the National Academy of Sciences of the United States of America	38
Clinical Cancer Research	36
Cell	35

definitive overexpression of PGK1 in BC, with an SMD of 0.76 and area under the curve (AUC) of 0.8525 for the summary ROC (sROC) (Figures 7 and 8). Tables 7 and 8 showed the included data sets for TNBC *vs* non-cancerous breast tissue, and TNBC *vs* non-TNBC tissue, respectively. Upon analyzing 16 data sets comprising 1159 cases of TNBC and 1205 cases of non-cancerous breast tissue, a clear upregulation of PGK1 mRNA expression in TNBC was observed compared to non-cancerous tissue (Figure 9A), with an SMD of 0.85 and 95% confidence interval (95%CI) of 0.54-1.16, as shown in Figure 10A. More importantly, we compared PGK1 expression across 1520 cases of TNBC and 7072 cases of non-TNBC for the first time and found that TNBC exhibited higher PGK1 mRNA expression than non-TNBC tissue in 9 out of the 15 datasets analyzed (Figure 9B). As Figure 10B shows, the comparative difference in expression was obvious (SMD: 0.25, 95%CI: 0.03-0.47).

Table 7 Included data sets for triple-negative breast cancer tissue *vs* non-cancerous breast tissue

ID	Series			
GPL1390	GSE10885-GPL1390, GSE10886, GSE10893-GPL1390		GSE1992-GPL1390, GSE2607-GPL1390, GSE6128	
GPL13607	GSE59246		GSE70951-GPL13607	
GPL17077	GSE57297		GSE80754	
GPL17586	GSE115144	GSE73540	GSE76250	GSE134359
GPL570	GSE20711	GSE50567	GSE22544	GSE146558
	GSE45827	GSE61304	GSE25407	GSE103865
	GSE65194	GSE42568	GSE26910	GSE153796
	GSE29431	GSE5764	GSE54002	GSE135565
	GSE7904	GSE10780	GSE71053	GSE7307
	GSE31448	GSE10810	GSE147472	GSE3744
	GSE29044	GSE21422	GSE140494	
GPL6244	GSE36295	GSE61724		GSE118432
	GSE86374	GSE37751		GSE81838
GPL6848	GSE26304		GSE18672	
GPL8269	GSE22384-GPL8269		GSE41119	
GPL887	GSE10885-GPL887		GSE24124	

	GSE2607-GPL887		GSE9309	
GPL96	GSE15852	GSE5364	GSE158309	GSE6883-GPL96
GSE29174				
GSE41970-GPL16299				
GSE50428				
GSE64790				
GSE92252				
TCGA_TNBC				

TNBC: Triple-negative breast cancer.

Table 8 Included data sets for triple-negative breast cancer tissue vs non- triple-negative breast cancer tissue

ID	Series				
GPL1390	GSE10885-GPL1390				
GPL13607	GSE59246				
GPL17077	GSE57297				
GPL17586	GSE115144				
GPL570	GSE20711	GSE29044	GSE10810	GSE54002	GSE103865
	GSE45827	GSE50567	GSE21422	GSE71053	GSE153796
	GSE65194	GSE61304	GSE22544	GSE147472	GSE135565
	GSE29431	GSE42568	GSE25407	GSE140494	GSE7307
	GSE7904	GSE5764	GSE26910	GSE146558	GSE3744
	GSE31448	GSE10780			
GPL6244	GSE36295				
GPL6848	GSE26304				
GPL8269	GSE22384-GPL8269				
GPL887	GSE10885-GPL887				
GPL96	GSE15852				
GSE148425					
GSE29174					
GSE50428					
METABRIC_mRNA_Zscores					
TCGA TNBC-nonTNBC					

TNBC: Triple-negative breast cancer.

The overexpression of PGK1 mRNA had an excellent ability to distinguish TNBC from non-cancerous breast tissue (AUC of sROC = 0.8578, sensitivity = 0.7637, and specificity = 0.8240, Figure 11A and Figure 10C). In Figure 12, the 13 ROC charts of TNBC *vs* non-cancerous breast tissue indicated that six data sets yielded an AUC of more than 0.7. Figure 11B showed that PGK1's ability to discern TNBC from non-TNBC tissue was moderate (AUC of sROC = 0.688, sensitivity = 0.7571, and specificity = 0.4754; Figure 10D, Figures 13-15).

Protein expression level of PGK1 in BC tissue: The THPA database showed that, in glandular epithelial cells of non-cancerous breast tissue and tumor cells, PGK1 was expressed in nuclear and cytoplasmic membranes. BC tumor cells showed diffuse but strong expression of PGK1 (Figure 16). However, owing to the restricted number of cases available in the public database, it was not feasible to conduct statistical analyses. In the future, we will increase the number of cases included and continue to verify the clinical significance of PGK1 protein expression.

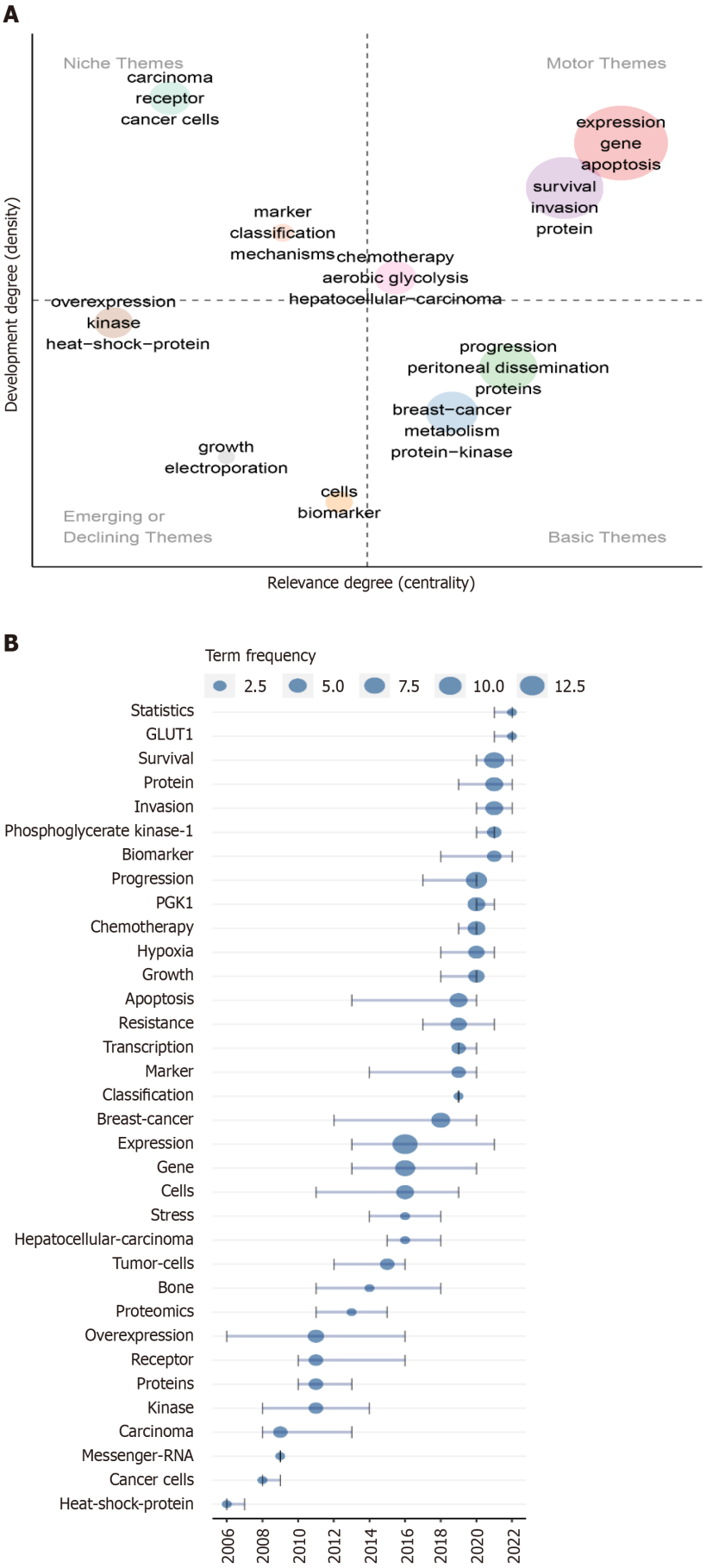


Figure 6 Thematic plot of research on breast cancer and phosphoglycerate kinase 1 and trends in topics related to breast cancer and

phosphoglycerate kinase 1 research. A: Using Web of Science key words, various research themes were detected *via* co-occurrence analysis and walktrap clustering analysis. Different colors represent different themes. Density represents the connection strength of basic knowledge units within a single topic. A larger topic density value indicates greater topic maturity. Centrality represents the strength of the connection between a topic and other topics. A higher centrality value of a topic suggests a strong interconnection with other topics, placing the topic at the heart of the research landscape. According to the density and centripetal degree values, the rectangular coordinate system was divided into four quadrants. The first quadrant contains core themes of high maturity; the second quadrant has isolated themes of high maturity, and the third quadrant contains new themes or themes that are about to disappear. The fourth quadrant encompasses basic themes of low maturity that may become research hotspots or future research trends; B: Topic trends were detected using co-word analysis. For each topic, the quartiles of publication year were calculated. The horizontal axis represents the first and third quantile years. Each node refers to the median publication year of each term. The larger the node, the more frequent the term.

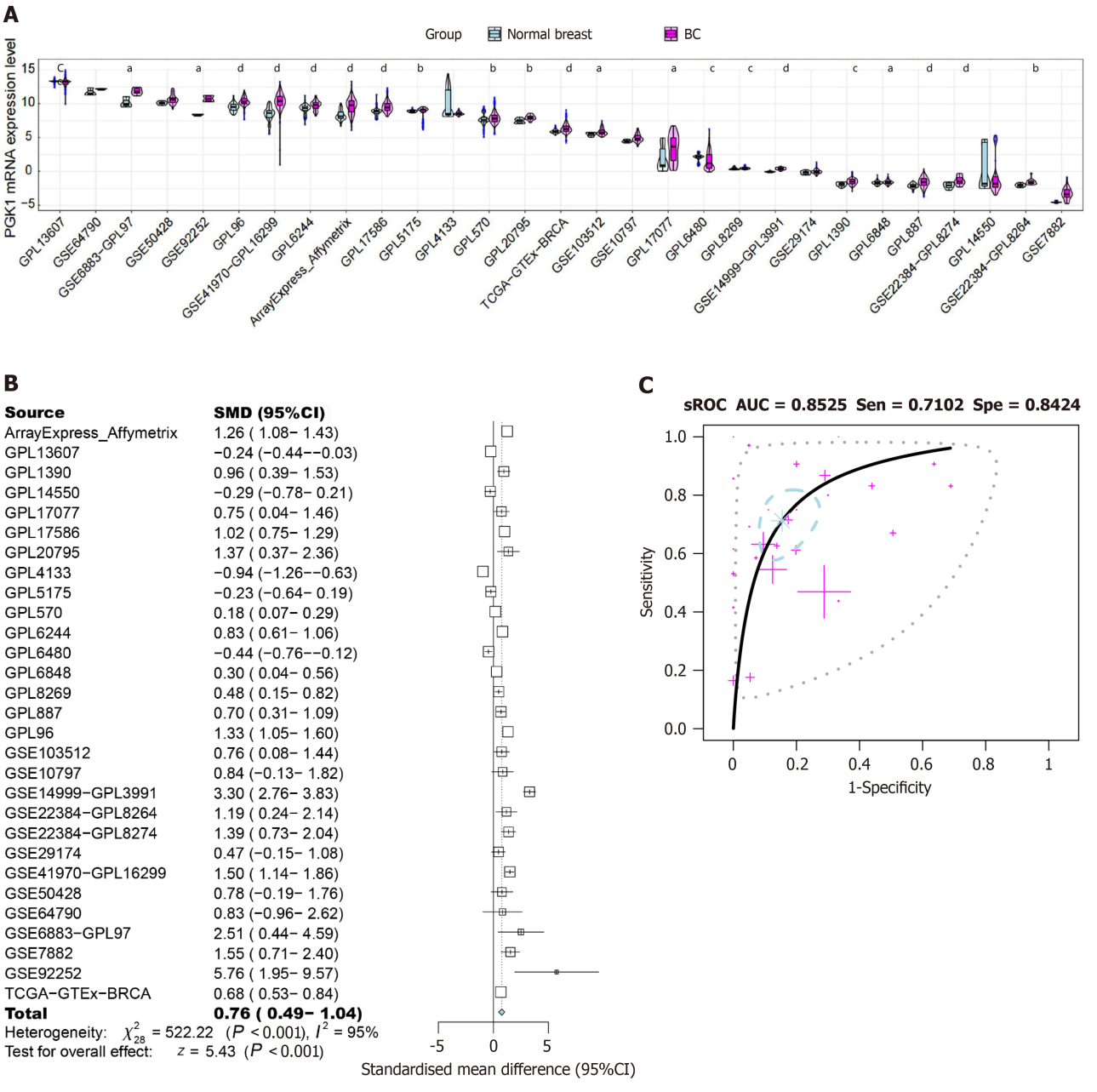


Figure 7 Expression levels of phosphoglycerate kinase 1 in breast cancer tissue *vs* normal breast tissue. A: mRNA expression level; B: Standardized mean differences; C: Summary receiver operating characteristic curves. [a]P < 0.05; [b]P < 0.01; [c]P < 0.001; [d]P < 0.0001. BC: Breast cancer; sROC: Summary receiver operating characteristic curve; AUC: Area under the curve; Sen: Sensitivity; Spe: Specificity; SMD: Standardized mean difference; 95%CI: 95% confidence interval.

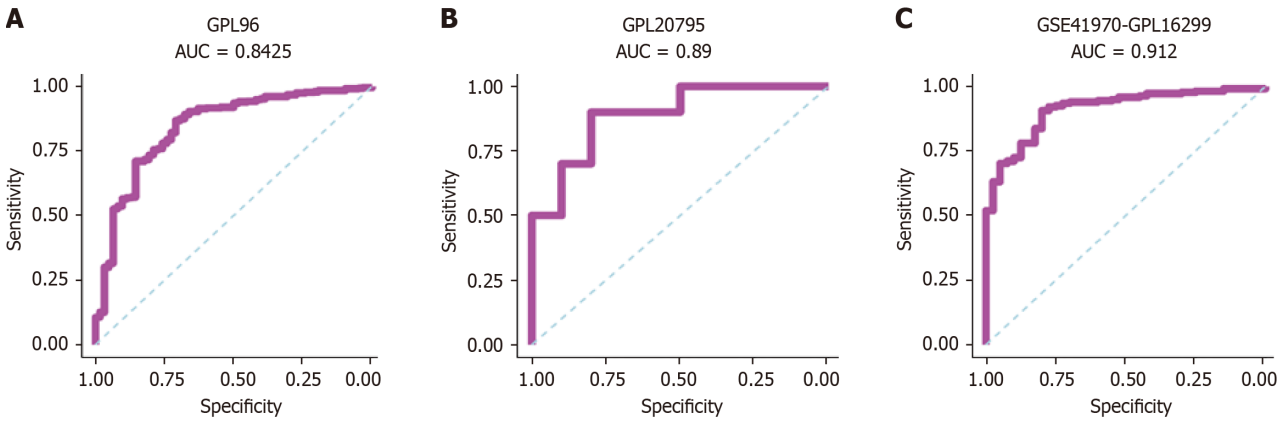


Figure 8 Receiver operating characteristic curves. A-C: Receiver operating characteristic curves of phosphoglycerate kinase 1 expression in breast cancer tissue *vs* normal breast tissue. AUC: Area under the curve.

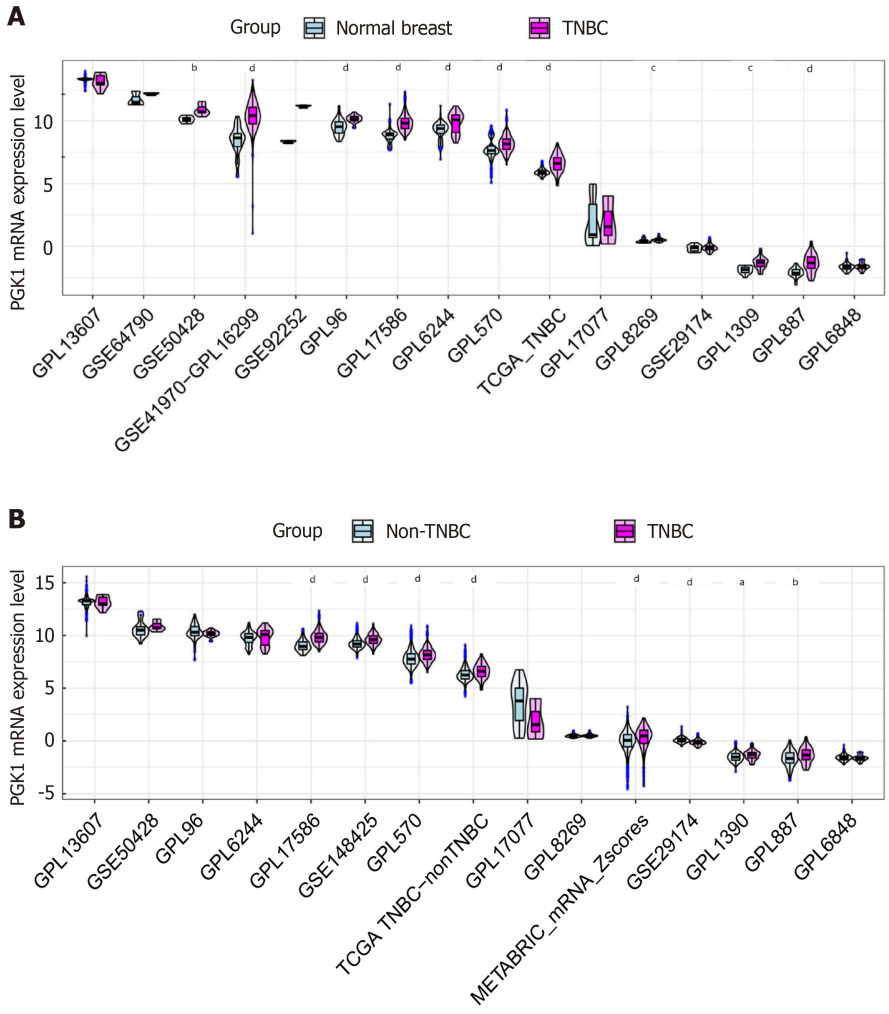
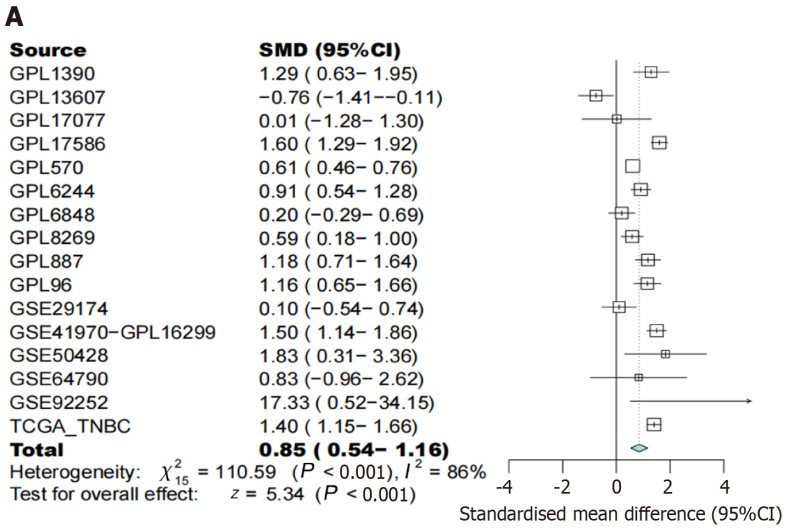
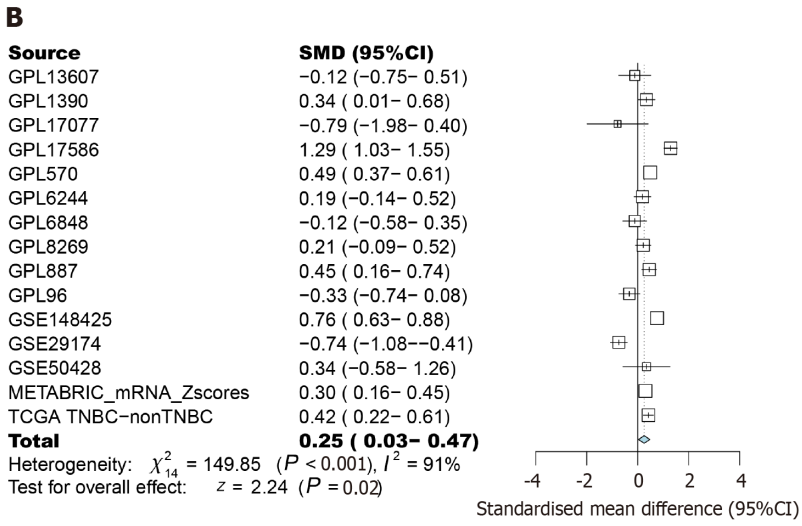


Figure 9 mRNA expression level of phosphoglycerate kinase 1 in triple-negative breast cancer. A: Triple-negative breast cancer (TNBC) tissue *vs* normal breast tissue; B: TNBC tissue *vs* non-TNBC tissue. TNBC: Triple-negative breast cancer. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; $^{d}P < 0.0001$.

Prognostic value of PGK1 in TNBC patients: Compared with the PGK1-low-expression group, the PGK1-high-expression group had a substantially shorter RFS time (HR: 1.282, $P = 0.023$). Similar trends were observed for OS and DMFS, indicating that higher expression of PGK1 is detrimental to the prognosis of TNBC, as shown in Figure 17.

Potential functional mechanisms of PGK1 in TNBC: GO enrichment analysis demonstrated that genes co-expressed with PGK1 participate in biological processes that include "generation of precursor metabolites and energy", "ATP metabolic process", and "ATP metabolic process" (Figure 18A). Simultaneously, the co-expressed genes were notably





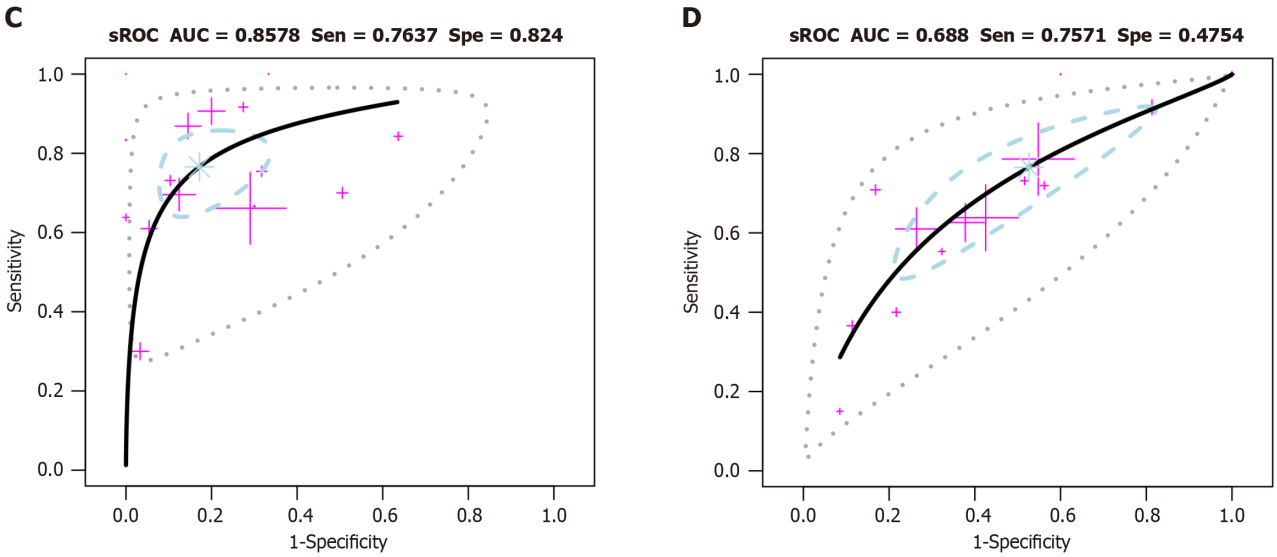


Figure 10 Comprehensive expression levels of phosphoglycerate kinase 1 in triple-negative breast cancer. A and C: Triple-negative breast cancer (TNBC) tissue *vs* normal breast tissue; B and D: TNBC tissue *vs* non-TNBC tissue. SMD: Standardized mean difference; 95%CI: 95% confidence interval; sROC: Summary receiver operating characteristic curve; AUC: Area under the curve; Sen: Sensitivity; Spe: Specificity.

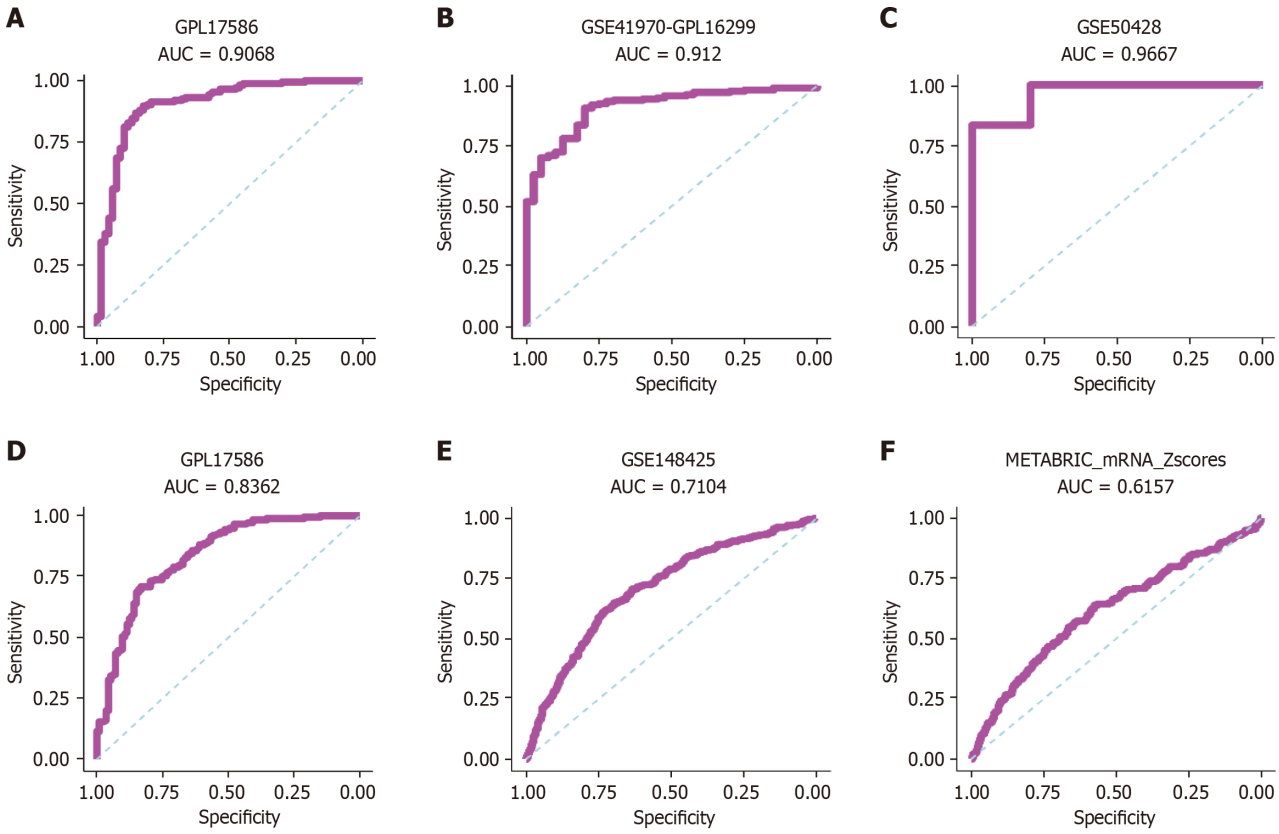


Figure 11 Receiver operating characteristic curves for phosphoglycerate kinase 1 expression in triple-negative breast cancer. A: Triple-negative breast cancer (TNBC) tissue *vs* normal breast tissue; B: TNBC tissue *vs* non-TNBC tissue. AUC: Area under the curve.

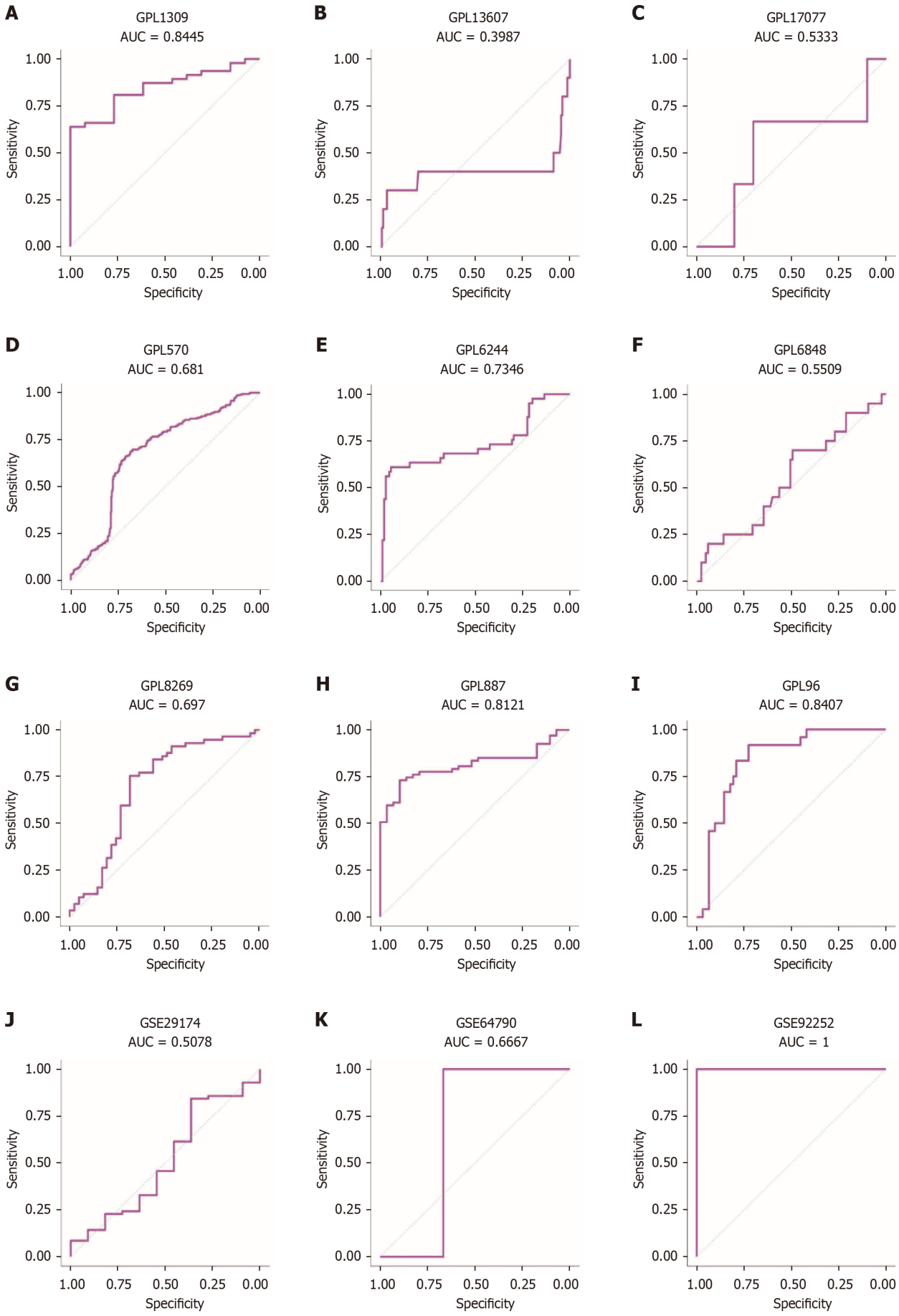
enriched in the following KEGG pathways: "Carbon metabolism", "Proteasome", "HIF-1 signaling pathway", "Central carbon metabolism in cancer", "Cell cycle", "Glycolysis/Gluconeogenesis", and "Pentose phosphate pathway", shown in Figure 18B. DO enrichment analysis showed that BC was the disease most correlated with PGK1 (Figure 18C). The major pathways relevant to the genes co-expressed with PGK1 suggest that PGK1 is closely related to metabolism and HIF-1 signaling.

The PPI network revealed 10 co-expressed genes closely related to PGK1, and TPI1 was a key gene among these 10 genes (Figure 19). The intersections of highly expressed genes between TNBC *vs* non-TNBC and genes co-expressed with PGK1 in TNBC (occurrence frequency ≥ 4) were shown in Figure 20.

DISCUSSION

There are few research documents on PGK1 and TNBC. Therefore, in this study, we concentrated on exploring the scientific knowledge gathered on BC and PGK1 in the literature in WOS with bibliometric methods to reveal the foci of recent research and suggest future research directions.

This investigation found that the total volume of PGK1 and BC related literature had been relatively small since 2005, but the number of annual publications had increased significantly from 2020, reflecting a growing interest among researchers in this area. More relevant articles came out of China than any other country. The Chinese researchers Jiang Y and Zhang X ranked first and second in terms of the number of publications and H-index. China had been an international leader in PGK1 research in BC. Different aggregations were formed among co-citations, countries, universities, and authors in the cooperation network. Although several close regional cooperative relationships had formed, some countries and institutions had not joined a cooperation cluster, and cooperation among cluster groups was also less interconnection. A lack of comprehensive collaboration might hinder communication and progress across the entire research domain. Key word analysis demonstrated that researchers were most concerned about the expression of PGK1 in BC and its link with BC progression. In the top three most cited papers, Zhang *et al*[44] and Qian *et al*[48] reported PGK1 overexpression separately in HER-2/NEU1-positive BC and invasive breast ductal carcinoma, and Shashni *et al*[47] believed that ATP generation and cancer metabolism could be affected by PGK1 downregulation. The journals with the most articles were the Journal of Proteome Research and Scientific Reports, and Cancer Research was the most cited journal. Overall, hot topics in PGK1 and BC were "expression" and "progression". BC survival, invasion, and PGK1 proteins were several new areas of attention in this field that have emerged in the past two years.


A
GPL1309
AUC = 0.8445
B
GPL13607
AUC = 0.3987
C
GPL17077
AUC = 0.5333
D
GPL570
AUC = 0.681
E
GPL6244
AUC = 0.7346
F
GPL6848
AUC = 0.5509
G
GPL8269
AUC = 0.697
H
GPL887
AUC = 0.8121
I
GPL96
AUC = 0.8407
J
GSE29174
AUC = 0.5078
K
GSE64790
AUC = 0.6667
L
GSE92252
AUC = 1
Sensitivity
Specificity
1.00
0.75
0.50
0.25
0.00

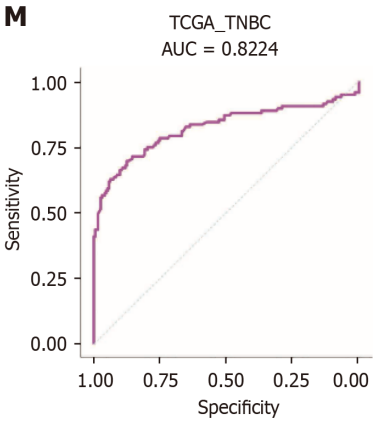


Figure 12 Receiver operating characteristic curves. A-M: Receiver operating characteristic curves for phosphoglycerate kinase 1 expression in triple-negative breast cancer *vs* normal breast tissue. AUC: Area under the curve.

This research gathered 31 high-throughput datasets on BC tissue (7543 cases) and non-cancerous breast tissue (1685 cases) and confirmed that PGK1 exhibited a markedly higher expression state in BC (SMD: 0.76; AUC of sROC = 0.8525), which was consistent with the results reported in previous items of literature. Li *et al*[42] compared 112 non-cancerous tissues and 1096 BC tissues and found that BC had stronger PGK1 expression than non-cancerous breast tissue. Similarly, Shao *et al*[49] found a rise in PGK1 mRNA levels in 145 BC tissues, which that contrasted with those of 69 non-cancerous tissues, and PGK1 mRNA was inversely correlated with the OS of BC. He *et al*[50] also compared 1066 BC and 112 non-cancerous samples and reached a conclusion in line with the above reports. Moreover, the expression level of PGK1 protein was shown to be increased in BC tissues compared with non-cancerous tissues.

The expression of PGK1 also depends on the type of BC[51]. Our study, for the first time, revealed that PGK1 expression was increased in TNBC and surpassed the levels found in both normal breast tissue and non-TNBC. Furthermore, genes co-expressed with PGK1 had been shown to be enriched in "generation of precursor metabolites and energy", "ATP metabolic process", and "response to hypoxia". In KEGG pathways analysis, they were enriched in the pathways of "proteasome", "HIF-1 signaling", and "glycolysis/gluconeogenesis".

It was discovered that the mRNA expression levels of 1159 TNBC tissue cases were noticeably higher than those of 1205 non-cancerous breast tissue cases in 16 high-throughput data sets, with an SMD of 0.85 and integrated AUC of 0.8578. This indicated that the increase in PGK1 mRNA might influence the occurrence of BC, including TNBC.

More crucially, this study also found that PGK1 might play different roles in promoting TNBC and non-TNBC. When compared with non-TNBC, TNBC samples showed much higher expression of PGK1, with the SMD of 15 data sets (including 1520 cases of TNBC and 7072 cases of non-TNBC) being 0.25, and the integrated AUC being 0.688. Previously, PGK1 was reported to be associated with the status of HER-2/ER. PGK1 expression in HER-2-positive patients (37 out of 88 cases) and ER-positive patients (70 out of 167 cases) was upregulated compared with that in the HER-2-negative group (74 out of 254 cases) and ER-negative group (66 out of 234 cases)[43]. One study also mentioned that, compared with the HER-2-positive group (19 cases), the TNBC group's (15 cases) mRNA and protein levels of PGK1 were down-regulated [52]. These findings suggested that PGK1 expression was down-regulated in BC with ER/HER-2 negative status and TNBC. Through an analysis of large data sets combining global gene chip and RNA-seq data from multi-center studies (1520 cases of TNBC and 7072 cases of non-TNBC), it was finally concluded that, though PGK1 in TNBC had both high and low expression, PGK1 showed an overall trend of increased expression in TNBC.

The prognostic analysis showed that increased PGK1 expression was closely associated with a shorter RFS in TNBC cases, and a similar pattern was observed for OS, indicating that high PGK1 expression was detrimental to the survival prognosis of TNBC patients. Therefore, we concluded that PGK1 was overexpressed in TNBC compared to normal breast tissue and non-TNBC, and that its upregulation correlated with more adverse prognoses in patients with TNBC, implying a potential role for PGK1 in tumor progression.

In this study, GO and KEGG enrichment analyses demonstrated that genes co-expressed with PGK1 were related to glycolysis, hypoxia, and HIF-1 pathways, which was reflected in previous findings in the literature. PGK1 had been shown to not only be a crucial glycolytic enzyme in ATP generation during glycolysis but also to act as a protein kinase to suppress pyruvate metabolism in the mitochondria and enhance the glycolysis of tumor cells[53].

Obviously, the metabolism of BC cells, including TNBC cells, undergoes changes. Under aerobic conditions, normal cells obtain energy by oxidative phosphorylation (OXPHOS) in the mitochondria. In hypoxia, cells acquire energy *via* the metabolic pathway of glycolysis rather than mitochondrial processes that consume oxygen[54]. Despite the availability of sufficient oxygen, malignant tumor cells continue to fulfill their metabolic demands through the glycolytic mode rather than OXPHOS, and this phenomenon is referred to aerobic glycolysis or the Warburg effect[55,56]. The generation of tumor stromal blood vessels lags behind growth rate of tumor cells, and oxygen delivery is unable to keep up with the rapid growth and proliferation of tumor cells, putting tumor cells in a continuous hypoxic microenvironment, with interrupted mitochondrial OXPHOS and a shift in energy metabolism to aerobic glycolysis[57]. Tumor cells that utilize glycolysis would then have a survival advantage in a rapidly proliferating hypoxic environments[58]. In studies, TNBC tissue displays higher glucose uptake and lactic acid (glycolytic product) secretion, and more dependence on glycolysis

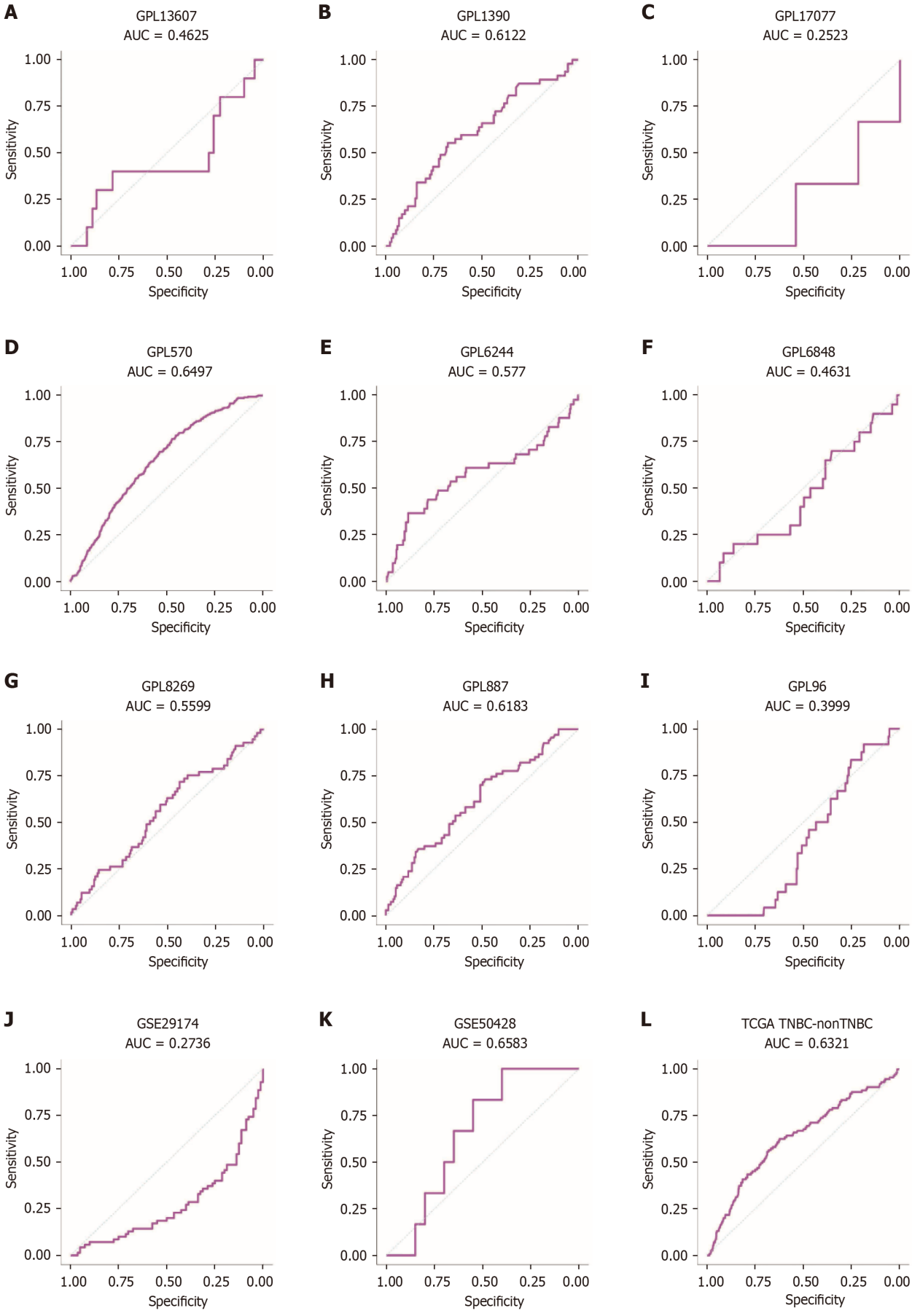


Figure 13 Receiver operating characteristic curves. A-L: Receiver operating characteristic curves for phosphoglycerate kinase 1 expression in triple-negative breast cancer *vs* non-triple-negative breast cancers. AUC: Area under the curve.

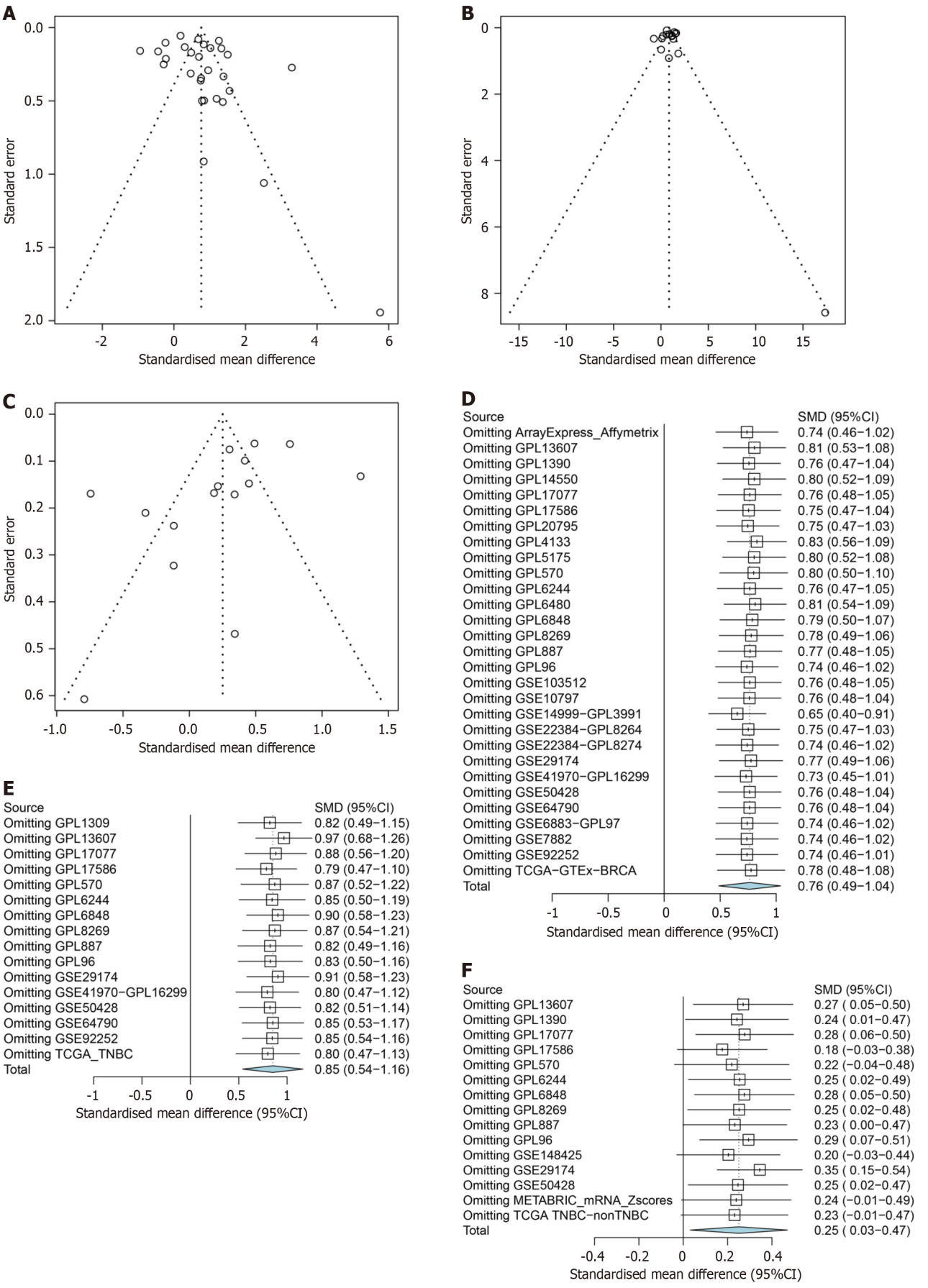
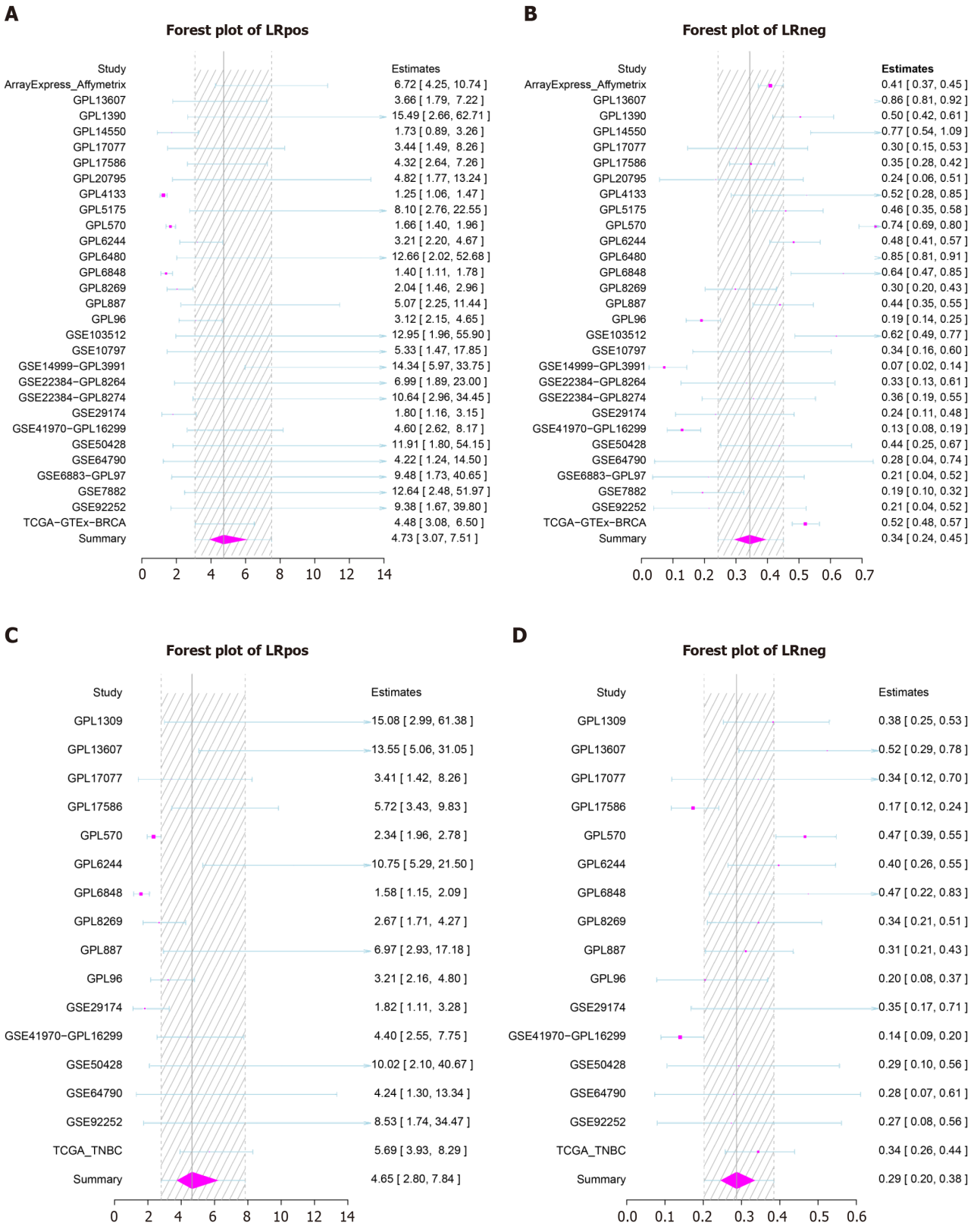


Figure 14 Funnel plots and forest plots for phosphoglycerate kinase 1 expression in breast cancer. A-C: Publication bias detection based on breast cancer (BC) *vs* normal breast tissue, triple-negative BC (TNBC) *vs* normal breast tissue, and TNBC *vs* non-TNBC tissue; D-F: Sensitivity analysis based on BC *vs* normal breast tissue, TNBC *vs* normal breast tissue, and TNBC *vs* non-TNBC tissue.


A
Forest plot of LRpos
Study
Estimates
ArrayExpress_Affymetrix 6.72 [4.25, 10.74]
GPL13607 3.66 [1.79, 7.22]
GPL1390 15.49 [2.66, 62.71]
GPL14550 1.73 [0.89, 3.26]
GPL17077 3.44 [1.49, 8.26]
GPL17586 4.32 [2.64, 7.26]
GPL20795 4.82 [1.77, 13.24]
GPL4133 1.25 [1.06, 1.47]
GPL5175 8.10 [2.76, 22.55]
GPL570 1.66 [1.40, 1.96]
GPL6244 3.21 [2.20, 4.67]
GPL6480 12.66 [2.02, 52.68]
GPL6848 1.40 [1.11, 1.78]
GPL8269 2.04 [1.46, 2.96]
GPL887 5.07 [2.25, 11.44]
GPL96 3.12 [2.15, 4.65]
GSE103512 12.95 [1.96, 55.90]
GSE10797 5.33 [1.47, 17.85]
GSE14999−GPL3991 14.34 [5.97, 33.75]
GSE22384−GPL8264 6.99 [1.89, 23.00]
GSE22384−GPL8274 10.64 [2.96, 34.45]
GSE29174 1.80 [1.16, 3.15]
GSE41970−GPL16299 4.60 [2.62, 8.17]
GSE50428 11.91 [1.80, 54.15]
GSE64790 4.22 [1.24, 14.50]
GSE6883−GPL97 9.48 [1.73, 40.65]
GSE7882 12.64 [2.48, 51.97]
GSE92252 9.38 [1.67, 39.80]
TCGA−GTEx−BRCA 4.48 [3.08, 6.50]
Summary 4.73 [3.07, 7.51]
0 2 4 6 8 10 12 14
B
Forest plot of LRneg
Study
Estimates
ArrayExpress_Affymetrix 0.41 [0.37, 0.45]
GPL13607 0.86 [0.81, 0.92]
GPL1390 0.50 [0.42, 0.61]
GPL14550 0.77 [0.54, 1.09]
GPL17077 0.30 [0.15, 0.53]
GPL17586 0.35 [0.28, 0.42]
GPL20795 0.24 [0.06, 0.51]
GPL4133 0.52 [0.28, 0.85]
GPL5175 0.46 [0.35, 0.58]
GPL570 0.74 [0.69, 0.80]
GPL6244 0.48 [0.41, 0.57]
GPL6480 0.85 [0.81, 0.91]
GPL6848 0.64 [0.47, 0.85]
GPL8269 0.30 [0.20, 0.43]
GPL887 0.44 [0.35, 0.55]
GPL96 0.19 [0.14, 0.25]
GSE103512 0.62 [0.49, 0.77]
GSE10797 0.34 [0.16, 0.60]
GSE14999−GPL3991 0.07 [0.02, 0.14]
GSE22384−GPL8264 0.33 [0.13, 0.61]
GSE22384−GPL8274 0.36 [0.19, 0.55]
GSE29174 0.24 [0.11, 0.48]
GSE41970−GPL16299 0.13 [0.08, 0.19]
GSE50428 0.44 [0.25, 0.67]
GSE64790 0.28 [0.04, 0.74]
GSE6883−GPL97 0.21 [0.04, 0.52]
GSE7882 0.19 [0.10, 0.32]
GSE92252 0.21 [0.04, 0.52]
TCGA−GTEx−BRCA 0.52 [0.48, 0.57]
Summary 0.34 [0.24, 0.45]
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
C
Forest plot of LRpos
Study
Estimates
GPL1309 15.08 [2.99, 61.38]
GPL13607 13.55 [5.06, 31.05]
GPL17077 3.41 [1.42, 8.26]
GPL17586 5.72 [3.43, 9.83]
GPL570 2.34 [1.96, 2.78]
GPL6244 10.75 [5.29, 21.50]
GPL6848 1.58 [1.15, 2.09]
GPL8269 2.67 [1.71, 4.27]
GPL887 6.97 [2.93, 17.18]
GPL96 3.21 [2.16, 4.80]
GSE29174 1.82 [1.11, 3.28]
GSE41970−GPL16299 4.40 [2.55, 7.75]
GSE50428 10.02 [2.10, 40.67]
GSE64790 4.24 [1.30, 13.34]
GSE92252 8.53 [1.74, 34.47]
TCGA_TNBC 5.69 [3.93, 8.29]
Summary 4.65 [2.80, 7.84]
0 2 4 6 8 10 12 14
D
Forest plot of LRneg
Study
Estimates
GPL1309 0.38 [0.25, 0.53]
GPL13607 0.52 [0.29, 0.78]
GPL17077 0.34 [0.12, 0.70]
GPL17586 0.17 [0.12, 0.24]
GPL570 0.47 [0.39, 0.55]
GPL6244 0.40 [0.26, 0.55]
GPL6848 0.47 [0.22, 0.83]
GPL8269 0.34 [0.21, 0.51]
GPL887 0.31 [0.21, 0.43]
GPL96 0.20 [0.08, 0.37]
GSE29174 0.35 [0.17, 0.71]
GSE41970−GPL16299 0.14 [0.09, 0.20]
GSE50428 0.29 [0.10, 0.56]
GSE64790 0.28 [0.07, 0.61]
GSE92252 0.27 [0.08, 0.56]
TCGA_TNBC 0.34 [0.26, 0.44]
Summary 0.29 [0.20, 0.38]
0.0 0.1 0.2 0.3 0.4 0.5 0.6

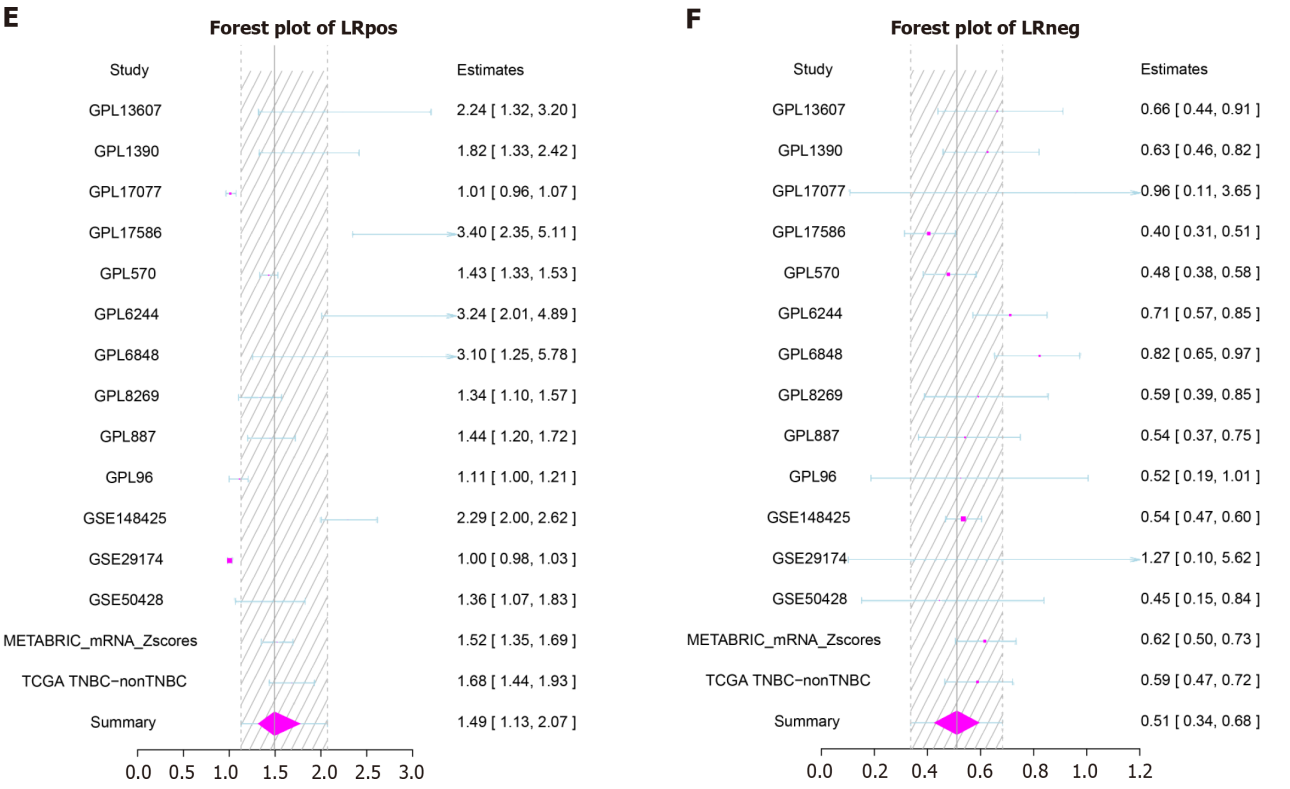


Figure 15 Forest plots of positive likelihood ratio (LRpos) and negative likelihood ratio (LRneg) for phosphoglycerate kinase 1 expression based on different groups. A and B: Breast cancer (BC) *vs* normal breast tissue; C and D: Triple-negative BC (TNBC) *vs* normal breast tissue; E and F: TNBC *vs* non-TNBC tissue.

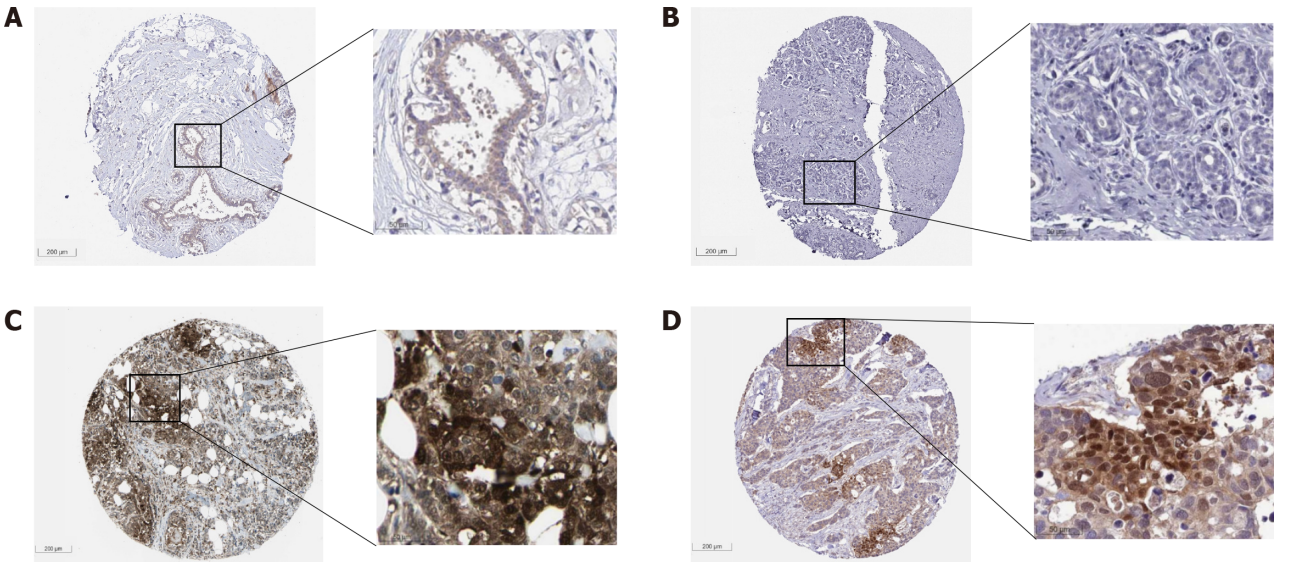


Figure 16 Immunohistochemistry staining from the Human Protein Atlas showing low or no expression of phosphoglycerate kinase 1 in normal breast tissue cells, and high expression in breast cancer cells. A: HPA045385, female, age 45, breast (T-04000), normal tissue, NOS (M-00100), Patient ID: 3544 (low or no expression). (1) Adipocytes. Staining: Not detected; Intensity: Weak; Quantity: < 25%; Location: cytoplasmic/membranous; (2) Glandular cells. Staining: Low; Intensity: Weak; Quantity: > 75%; Location: cytoplasmic/membranous; and (3) Myoepithelial cells. Staining: Low; Intensity: Weak; Quantity: > 75%; Location: Cytoplasmic/membranous; B: HPA073644, female, age 43, breast (T-04000), skin (T-01000), normal tissue, NOS (M-00100), Patient ID: 2104 (low or no expression). Adipocytes, glandular cells, and myoepithelial cells. Staining: Not detected; Intensity: Negative; Quantity: None; C: CAB010065, female, age 59, breast (T-04000), lobular carcinoma (M-85203), Patient ID: 2805 (high expression). (1) Tumor cells. Staining: High; Intensity: Strong; Quantity: 75%-25%; Location: Cytoplasmic/membranous nuclear; D: HPA045385, female, age 83, breast (T-04000), duct carcinoma (M-85003), Patient ID: 2160 (high expression). (1) Tumor cells. Staining: High. Intensity: Strong. Quantity: 75%-25%. Location: Cytoplasmic/membranous nuclear. These immunohistochemical protein expression figures were from the Human Protein Atlas (THPA) database [71].

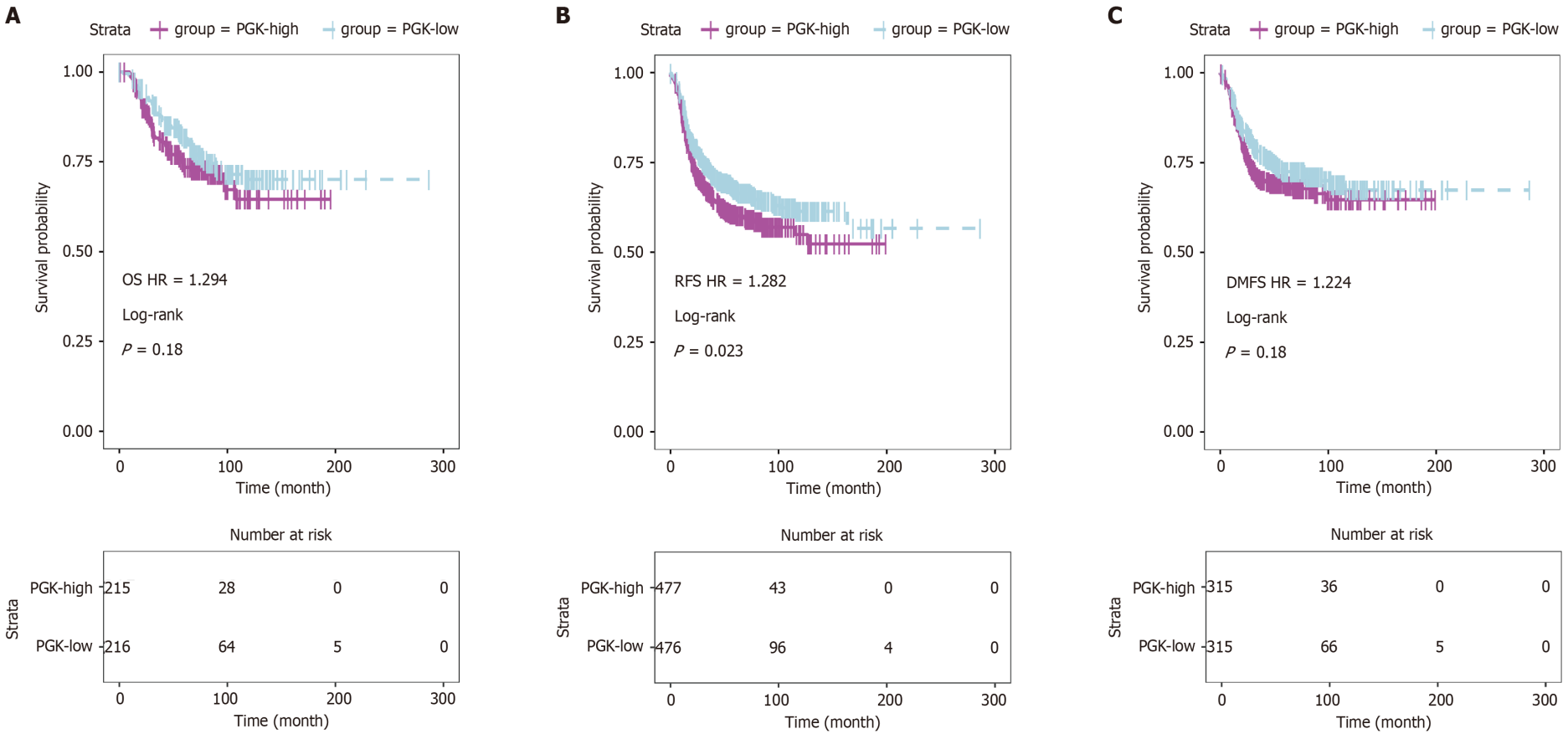


Figure 17 Kaplan-Meier curves for association between phosphoglycerate kinase 1 expression of triple-negative breast cancer patients. A: Overall survival; B: Recurrence-free survival; C: Distant metastasis free survival. OS: Overall survival; RFS: Recurrence-free survival; DMFS: Distant metastasis free survival; HR: Hazard ratio.

than other BC subtypes[59]. PGK1, as the initial rate-limiting enzyme of the cellular glycolytic pathway to ATP synthesis, directly supplies energy for cellular activities, while the other reaction product, 3-PG, is oxidized by one-carbon metabolism. This is probably the reason why PGK1 is more significant than other glycolysis enzymes in contributing to the Warburg effect in tumors[56,60,61]. Several glycolytic enzyme genes, including *PGK1*, were reported to be upregulated by HIF-1 induction under a hypoxic microenvironment, providing a molecular basis for the Warburg effect in tumor cells[57,62]. In this way, processes, including cell growth, metastasis, drug resistance, and immune evasion, are regulated *via* the Warburg effect. The high levels of PGK1 in cancerous tissues could potentiate the Warburg effect, thereby influencing the progression and prognosis of both BC and TNBC.

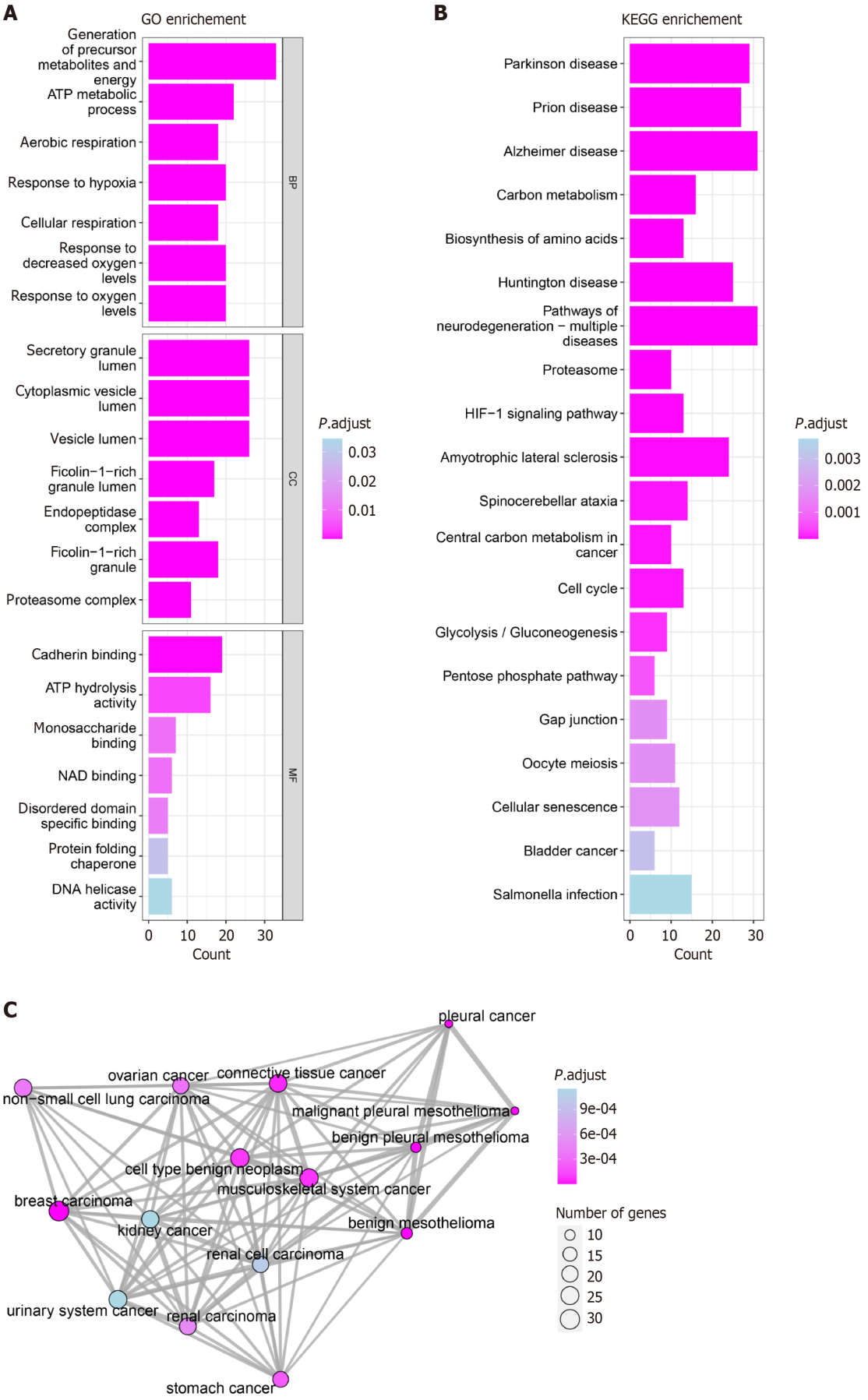


Figure 18 Pathway and disease enrichment analysis annotation. A: Gene Ontology enrichment; B: Kyoto Encyclopedia of Genes and Genomes enrichment. Columnar length represents the number of gene enrichments; the longer the column, the more gene enrichment. Color indicates the size of the *P* value;

the darker the color, the smaller the *P* value; C: Disease Ontology enrichment. The area of each circle represents the number of genes; the larger the circle, the more genes are involved. The color of each circle represents the *P* value; the darker the color, the smaller the *P* value. GO: Gene Ontology enrichment; KEGG: Kyoto Encyclopedia of Genes and Genomes.

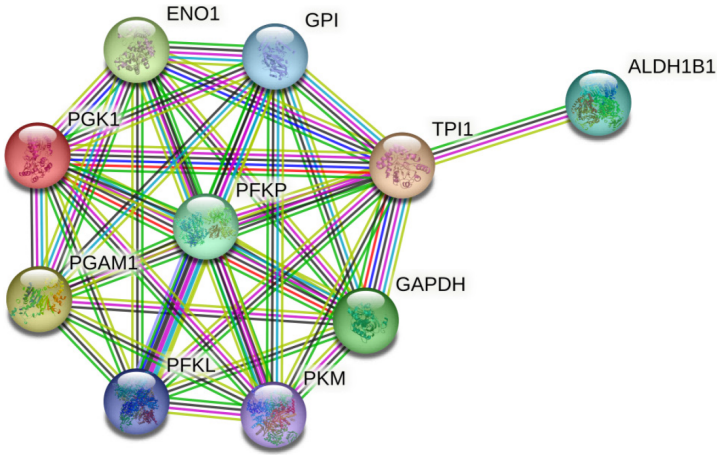


Figure 19 Protein-protein interaction network analysis of phosphoglycerate kinase 1 related genes.

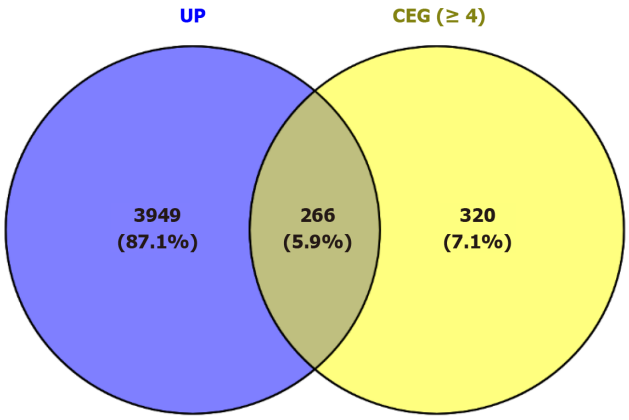


Figure 20 Intersection of triple-negative breast cancer *vs* non-triple-negative breast cancer gene overexpression and positively phosphoglycerate kinase 1 correlated gene co-expression in triple-negative breast cancer (occurrence ≥ 4). UP: Upregulated gene; CEG: Co-expressed gene.

The HIF-1/PGK1 pathway is one of the most prominent pathways in cancer cells[63]. PGK1 has been identified as a target gene of HIF-1[64] and is capable of establishing a positive-feedback loop with HIF-1[45]. HIF-1 can detect hypoxia within the tumor microenvironment, increase the expression of glucose transporters, activate genes involved in cancer glycolysis metabolism, and drive glucose toward glycolysis in cancerous cells, the latter of which is a pivotal regulatory step in the metabolic shift of tumor cells from oxidative phosphorylation to glycolysis[65-67]. Under hypoxia stress, protein receptor activation, or carcinogenic gene mutation expression, about 12% of cytoplasmic PGK1 is translocated by the accumulated HIF-1 to mitochondria, where PGK1 contributes to protein kinase activity. Pyruvate is diverted from the mitochondria into the cytoplasm to produce lactic acid, inhibiting the mitochondrial tricarboxylic acid cycle and enhancing glycolysis to support the energy and metabolite requirements of cancerous cells[64,68]. Additionally, HIF-1α expression is correlated with an ER-negative status[69]. The expression of HIF-1 was shown to be upregulated in TNBC and associated with a poorer prognosis, and it also affected the level of PGK1[66]. Regarding TNBC treatments, Sun *et al* [70] found that PGK1 expression inhibition increased the sensitivity of TNBC to paclitaxel treatment. Therefore, as PGK1 is a latent biomarker for different therapy options for TNBC, inhibiting its expression might represent a new therapeutic strategy.

CONCLUSION

Research into PGK1, a potential biomarker in BC, has made great advancements recently. PGK1 expression levels in

TNBC are subject to regulation by upstream HIF-1 and are evidently upregulated compared to those in non-TNBC and non-cancerous breast tissue. PGK1 has been shown to enhance glycolysis in TNBC. Therefore, we believe that PGK1 provides a valuable marker for predicting the disease progression of TNBC. How to inhibit the occurrence and progress of TNBC through PGK1 needs further in-depth studies. There were some limitations to our study. Our bibliometric analysis was accomplished with a single data base, and specific BC histological classifications were not assigned to the cases in the non-TNBC group.

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FOOTNOTES

Author contributions: He RQ, Huang ZG, Chen G, and Zou W designed the paper; Chen JY and Li JD performed literature and dataset screening, conducted bibliometric statistics, and carried out all computational analyses, including mRNA and protein expression, prognosis and signaling pathways; Chen JY, Li JD, Huang ZG, and Zou W constructed the figures and tables; Chen JY and Li JD wrote the draft; He RQ, Huang ZG, Chen G, and Zou W corrected the draft; and all authors have read and approved the final manuscript.

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REFERENCES

1 **Siegel RL**, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin* 2024; **74**: 12-49 [PMID: 38230766 DOI: 10.3322/caac.21820]
2 **Wang X**, Wang C, Guan J, Chen B, Xu L, Chen C. Progress of Breast Cancer basic research in China. *Int J Biol Sci* 2021; **17**: 2069-2079 [PMID: 34131406 DOI: 10.7150/ijbs.60631]
3 **Brahimetaj R**, Willekens I, Massart A, Forsyth R, Cornelis J, Mey J, Jansen B. Improved automated early detection of breast cancer based on high resolution 3D micro-CT microcalcification images. *BMC Cancer* 2022; **22**: 162 [PMID: 35148703 DOI: 10.1186/s12885-021-09133-4]
4 **Schliemann D**, Hoe WMK, Mohan D, Allotey P, Reidpath DD, Tan MM, Taib NAM, Donnelly M, Su TT. Challenges and opportunities for breast cancer early detection among rural dwelling women in Segamat District, Malaysia: A qualitative study. *PLoS One* 2022; **17**: e0267308 [PMID: 35594267 DOI: 10.1371/journal.pone.0267308]
5 **Barbirou M**, Miller AA, Gafni E, Mezlini A, Zidi A, Boley N, Tonellato PJ. Evaluation of cfDNA as an early detection assay for dense tissue breast cancer. *Sci Rep* 2022; **12**: 8458 [PMID: 35589867 DOI: 10.1038/s41598-022-12457-1]
6 **Jones MA**, Islam W, Faiz R, Chen X, Zheng B. Applying artificial intelligence technology to assist with breast cancer diagnosis and prognosis prediction. *Front Oncol* 2022; **12**: 980793 [PMID: 36119479 DOI: 10.3389/fonc.2022.980793]
7 **Din NMU**, Dar RA, Rasool M, Assad A. Breast cancer detection using deep learning: Datasets, methods, and challenges ahead. *Comput Biol Med* 2022; **149**: 106073 [PMID: 36103745 DOI: 10.1016/j.compbiomed.2022.106073]
8 **Wu M**, Yuan K, Lyu S, Li Y. Screening potential immune signatures for early-stage basal-like/triple-negative breast cancer. *World J Surg Oncol* 2022; **20**: 214 [PMID: 35751103 DOI: 10.1186/s12957-022-02683-2]
9 **Sun L**, Qi M, Cui X, Song Q. The Clinical Application of Combined Ultrasound, Mammography, and Tumor Markers in Screening Breast Cancer among High-Risk Women. *Comput Math Methods Med* 2022; **2022**: 4074628 [PMID: 35872933 DOI: 10.1155/2022/4074628]
10 **Zhang DX**, Dang XTT, Vu LT, Lim CMH, Yeo EYM, Lam BWS, Leong SM, Omar N, Putti TC, Yeh YC, Ma V, Luo JY, Cho WC, Chen G, Lee VKM, Grimson A, Le MTN. αvβ1 integrin is enriched in extracellular vesicles of metastatic breast cancer cells: A mechanism mediated by galectin-3. *J Extracell Vesicles* 2022; **11**: e12234 [PMID: 35923105 DOI: 10.1002/jev2.12234]
11 **Ebeid SA**, Abd El Moneim NA, El-Benhawy SA, Ramadan R, Ismail SE. Znhit1 and HIF-2α are correlated with cancer stem cell markers in breast cancer patients. *Sci Rep* 2022; **12**: 13918 [PMID: 35978075 DOI: 10.1038/s41598-022-18133-8]

12 **Cedano-Prieto DM**, Bergez-Hernandez F, Leal-Leon EA, Garcia-Magallanes N, Luque-Ortega F, Picos-Cardenas V, Guerrero-Arambula E, Gutierrez-Zepeda B, Romo-Martinez E, Arambula-Meraz E. Altered Expression of Survivin Variants S-2B and S-WT in Breast Cancer Is Related to Adipokine Expression. *J Oncol* 2022; **2022**: 7398444 [PMID: 35342410 DOI: 10.1155/2022/7398444]

13 **Peng W**, Li JD, Zeng JJ, Zou XP, Tang D, Tang W, Rong MH, Li Y, Dai WB, Tang ZQ, Feng ZB, Chen G. Clinical value and potential mechanisms of COL8A1 upregulation in breast cancer: a comprehensive analysis. *Cancer Cell Int* 2020; **20**: 392 [PMID: 32818022 DOI: 10.1186/s12935-020-01465-8]

14 **Li JD**, Chen G, Wu M, Huang Y, Tang W. Downregulation of CDC14B in 5218 breast cancer patients: A novel prognosticator for triple-negative breast cancer. *Math Biosci Eng* 2020; **17**: 8152-8181 [PMID: 33378938 DOI: 10.3934/mbe.2020414]

15 **Cloud AS**, Vargheese AM, Gunewardena S, Shimak RM, Ganeshkumar S, Kumaraswamy E, Jensen RA, Chennathukuzhi VM. Loss of REST in breast cancer promotes tumor progression through estrogen sensitization, MMP24 and CEMIP overexpression. *BMC Cancer* 2022; **22**: 180 [PMID: 35177031 DOI: 10.1186/s12885-022-09280-2]

16 **Tang W**, Li GS, Li JD, Pan WY, Shi Q, Xiong DD, Mo CH, Zeng JJ, Chen G, Feng ZB, Huang SN, Rong MH. The role of upregulated miR-375 expression in breast cancer: An *in vitro* and in silico study. *Pathol Res Pract* 2020; **216**: 152754 [PMID: 31787478 DOI: 10.1016/j.prp.2019.152754]

17 **Thomas CE**, Dahl L, Byström S, Chen Y, Uhlén M, Mälarstig A, Czene K, Hall P, Schwenk JM, Gabrielson M. Circulating proteins reveal prior use of menopausal hormonal therapy and increased risk of breast cancer. *Transl Oncol* 2022; **17**: 101339 [PMID: 35033985 DOI: 10.1016/j.tranon.2022.101339]

18 **Lee YJ**, Kim Y, Choi BB, Kim JR, Ko HM, Suh KH, Lee JS. The blood level of thioredoxin 1 as a supporting biomarker in the detection of breast cancer. *BMC Cancer* 2022; **22**: 12 [PMID: 34979986 DOI: 10.1186/s12885-021-09055-1]

19 **Lu B**, Natarajan E, Balaji Raghavendran HR, Markandan UD. Molecular Classification, Treatment, and Genetic Biomarkers in Triple-Negative Breast Cancer: A Review. *Technol Cancer Res Treat* 2023; **22**: 15330338221145246 [PMID: 36601658 DOI: 10.1177/15330338221145246]

20 **Yang Y**, Zhang L, Tian W, Li Y, Qin Q, Mao Y, Liu X, Hong J, Hu L, Zeng Q, Zhang Q, Zhao H. Prognosis prediction and risk factors for triple-negative breast cancer patients with brain metastasis: A population-based study. *Cancer Med* 2023; **12**: 7951-7961 [PMID: 36629093 DOI: 10.1002/cam4.5575]

21 **Deng H**, Wang L, Wang N, Zhang K, Zhao Y, Qiu P, Qi X, Zhang D, Xu F, Liu J. Neoadjuvant checkpoint blockade in combination with Chemotherapy in patients with tripe-negative breast cancer: exploratory analysis of real-world, multicenter data. *BMC Cancer* 2023; **23**: 29 [PMID: 36611131 DOI: 10.1186/s12885-023-10515-z]

22 **Wang Q**, Xu M, Sun Y, Chen J, Chen C, Qian C, Chen Y, Cao L, Xu Q, Du X, Yang W. Gene Expression Profiling for Diagnosis of Triple-Negative Breast Cancer: A Multicenter, Retrospective Cohort Study. *Front Oncol* 2019; **9**: 354 [PMID: 31134153 DOI: 10.3389/fonc.2019.00354]

23 **Lin C**, Cui J, Peng Z, Qian K, Wu R, Cheng Y, Yin W. Efficacy of platinum-based and non-platinum-based drugs on triple-negative breast cancer: meta-analysis. *Eur J Med Res* 2022; **27**: 201 [PMID: 36242046 DOI: 10.1186/s40001-022-00839-0]

24 **Li Y**, Zhang H, Merkher Y, Chen L, Liu N, Leonov S, Chen Y. Recent advances in therapeutic strategies for triple-negative breast cancer. *J Hematol Oncol* 2022; **15**: 121 [PMID: 36038913 DOI: 10.1186/s13045-022-01341-0]

25 **Bakherad H**, Ghasemi F, Hosseindokht M, Zare H. Nanobodies; new molecular instruments with special specifications for targeting, diagnosis and treatment of triple-negative breast cancer. *Cancer Cell Int* 2022; **22**: 245 [PMID: 35933373 DOI: 10.1186/s12935-022-02665-0]

26 **Yin J**, Zhu C, Wang G, Gu J. Treatment for Triple-Negative Breast Cancer: An Umbrella Review of Meta-Analyses. *Int J Gen Med* 2022; **15**: 5901-5914 [PMID: 35795302 DOI: 10.2147/IJGM.S370351]

27 **Sun H**, Xu J, Dai S, Ma Y, Sun T. Breast cancer brain metastasis: Current evidence and future directions. *Cancer Med* 2023; **12**: 1007-1024 [PMID: 35822637 DOI: 10.1002/cam4.5021]

28 **Swain SM**, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions. *Nat Rev Drug Discov* 2023; **22**: 101-126 [PMID: 36344672 DOI: 10.1038/s41573-022-00579-0]

29 **Yang R**, Li Y, Wang H, Qin T, Yin X, Ma X. Therapeutic progress and challenges for triple negative breast cancer: targeted therapy and immunotherapy. *Mol Biomed* 2022; **3**: 8 [PMID: 35243562 DOI: 10.1186/s43556-022-00071-6]

30 **Chai Y**, Chen Y, Zhang D, Wei Y, Li Z, Li Q, Xu B. Homologous Recombination Deficiency (HRD) and BRCA 1/2 Gene Mutation for Predicting the Effect of Platinum-Based Neoadjuvant Chemotherapy of Early-Stage Triple-Negative Breast Cancer (TNBC): A Systematic Review and Meta-Analysis. *J Pers Med* 2022; **12** [PMID: 35207810 DOI: 10.3390/jpm12020323]

31 **Alghazali MW**, Al-Hetty HRAK, Ali ZMM, Saleh MM, Suleiman AA, Jalil AT. Non-coding RNAs, another side of immune regulation during triple-negative breast cancer. *Pathol Res Pract* 2022; **239**: 154132 [PMID: 36183439 DOI: 10.1016/j.prp.2022.154132]

32 **Hua Z**, White J, Zhou J. Cancer stem cells in TNBC. *Semin Cancer Biol* 2022; **82**: 26-34 [PMID: 34147641 DOI: 10.1016/j.semcancer.2021.06.015]

33 **Su YH**, Wu YZ, Ann DK, Chen JL, Kuo CY. Obesity promotes radioresistance through SERPINE1-mediated aggressiveness and DNA repair of triple-negative breast cancer. *Cell Death Dis* 2023; **14**: 53 [PMID: 36681663 DOI: 10.1038/s41419-023-05576-8]

34 **See SHC**, Smith SH, Finkelman BS, LaBoy C, Novo JE, Siziopikou KP, Blanco LZ Jr. The role of PRAME and NY-ESO-1 as potential therapeutic and prognostic biomarkers in triple-negative breast carcinomas. *Pathol Res Pract* 2023; **241**: 154299 [PMID: 36603407 DOI: 10.1016/j.prp.2022.154299]

35 **Fu D**, He C, Wei J, Zhang Z, Luo Y, Tan H, Ren C. PGK1 is a Potential Survival Biomarker and Invasion Promoter by Regulating the HIF-1α-Mediated Epithelial-Mesenchymal Transition Process in Breast Cancer. *Cell Physiol Biochem* 2018; **51**: 2434-2444 [PMID: 30537744 DOI: 10.1159/000495900]

36 **Chu Z**, Huo N, Zhu X, Liu H, Cong R, Ma L, Kang X, Xue C, Li J, Li Q, You H, Zhang Q, Xu X. FOXO3A-induced LINC00926 suppresses breast tumor growth and metastasis through inhibition of PGK1-mediated Warburg effect. *Mol Ther* 2021; **29**: 2737-2753 [PMID: 33940159 DOI: 10.1016/j.ymthe.2021.04.036]

37 **Nie H**, Ju H, Fan J, Shi X, Cheng Y, Cang X, Zheng Z, Duan X, Yi W. O-GlcNAcylation of PGK1 coordinates glycolysis and TCA cycle to promote tumor growth. *Nat Commun* 2020; **11**: 36 [PMID: 31911580 DOI: 10.1038/s41467-019-13601-8]

38 **Yi J**, Luo X, Huang W, Yang W, Qi Y, He J, Xie H. PGK1 is a potential biomarker for early diagnosis and prognosis of hepatocellular carcinoma. *Oncol Lett* 2024; **27**: 109 [PMID: 38304170 DOI: 10.3892/ol.2024.14242]

39 **Tian T**, Leng Y, Tang B, Dong X, Ren Q, Liang J, Liu T, Liu Y, Feng W, Liu S, Zhou Y, Zhao H, Shen L. The oncogenic role and regulatory mechanism of PGK1 in human non-small cell lung cancer. *Biol Direct* 2024; **19**: 1 [PMID: 38163864 DOI: 10.1186/s13062-023-00448-9]

40 **Hwang TL**, Liang Y, Chien KY, Yu JS. Overexpression and elevated serum levels of phosphoglycerate kinase 1 in pancreatic ductal

adenocarcinoma. *Proteomics* 2006; **6**: 2259-2272 [PMID: 16493704 DOI: 10.1002/pmic.200500345]

41 **Pei S**, Zhang P, Yang L, Kang Y, Chen H, Zhao S, Dai Y, Zheng M, Xia Y, Xie H. Exploring the role of sphingolipid-related genes in clinical outcomes of breast cancer. *Front Immunol* 2023; **14**: 1116839 [PMID: 36860848 DOI: 10.3389/fimmu.2023.1116839]

42 **Li W**, Xu M, Li Y, Huang Z, Zhou J, Zhao Q, Le K, Dong F, Wan C, Yi P. Comprehensive analysis of the association between tumor glycolysis and immune/inflammation function in breast cancer. *J Transl Med* 2020; **18**: 92 [PMID: 32070368 DOI: 10.1186/s12967-020-02267-2]

43 **Sun S**, Liang X, Zhang X, Liu T, Shi Q, Song Y, Jiang Y, Wu H, Jiang Y, Lu X, Pang D. Phosphoglycerate kinase-1 is a predictor of poor survival and a novel prognostic biomarker of chemoresistance to paclitaxel treatment in breast cancer. *Br J Cancer* 2015; **112**: 1332-1339 [PMID: 25867275 DOI: 10.1038/bjc.2015.114]

44 **Zhang D**, Tai LK, Wong LL, Chiu LL, Sethi SK, Koay ES. Proteomic study reveals that proteins involved in metabolic and detoxification pathways are highly expressed in HER-2/neu-positive breast cancer. *Mol Cell Proteomics* 2005; **4**: 1686-1696 [PMID: 16048908 DOI: 10.1074/mcp.M400221-MCP200]

45 **Yang H**, Geng YH, Wang P, Zhou YT, Yang H, Huo YF, Zhang HQ, Li Y, He HY, Tian XX, Fang WG. Extracellular ATP promotes breast cancer invasion and epithelial-mesenchymal transition *via* hypoxia-inducible factor 2α signaling. *Cancer Sci* 2019; **110**: 2456-2470 [PMID: 31148343 DOI: 10.1111/cas.14086]

46 **Vishnubalaji R**, Alajez NM. Single-Cell Transcriptome Analysis Revealed Heterogeneity and Identified Novel Therapeutic Targets for Breast Cancer Subtypes. *Cells* 2023; **12** [PMID: 37190091 DOI: 10.3390/cells12081182]

47 **Shashni B**, Sakharkar KR, Nagasaki Y, Sakharkar MK. Glycolytic enzymes PGK1 and PKM2 as novel transcriptional targets of PPARγ in breast cancer pathophysiology. *J Drug Target* 2013; **21**: 161-174 [PMID: 23130662 DOI: 10.3109/1061186X.2012.736998]

48 **Qian X**, Li X, Lu Z. Protein kinase activity of the glycolytic enzyme PGK1 regulates autophagy to promote tumorigenesis. *Autophagy* 2017; **13**: 1246-1247 [PMID: 28486006 DOI: 10.1080/15548627.2017.1313945]

49 **Shao F**, Yang X, Wang W, Wang J, Guo W, Feng X, Shi S, Xue Q, Gao S, Gao Y, Lu Z, He J. Associations of PGK1 promoter hypomethylation and PGK1-mediated PDHK1 phosphorylation with cancer stage and prognosis: a TCGA pan-cancer analysis. *Cancer Commun (Lond)* 2019; **39**: 54 [PMID: 31578148 DOI: 10.1186/s40880-019-0401-9]

50 **He M**, Hu C, Deng J, Ji H, Tian W. Identification of a novel glycolysis-related signature to predict the prognosis of patients with breast cancer. *World J Surg Oncol* 2021; **19**: 294 [PMID: 34600547 DOI: 10.1186/s12957-021-02409-w]

51 **Li Y**, Wang S, Zhang X, Yang R, Wei X, Yan R, Jiang Y, Shen W. Expression Characteristics and Significant Prognostic Values of PGK1 in Breast Cancer. *Front Mol Biosci* 2021; **8**: 695420 [PMID: 34291087 DOI: 10.3389/fmolb.2021.695420]

52 **Schulz DM**, Böllner C, Thomas G, Atkinson M, Esposito I, Höfler H, Aubele M. Identification of differentially expressed proteins in triple-negative breast carcinomas using DIGE and mass spectrometry. *J Proteome Res* 2009; **8**: 3430-3438 [PMID: 19485423 DOI: 10.1021/pr900071h]

53 **Kabbage M**, Chahed K, Hamrita B, Guillier CL, Trimeche M, Remadi S, Hoebeke J, Chouchane L. Protein alterations in infiltrating ductal carcinomas of the breast as detected by nonequilibrium pH gradient electrophoresis and mass spectrometry. *J Biomed Biotechnol* 2008; **2008**: 564127 [PMID: 18401453 DOI: 10.1155/2008/564127]

54 **Mei Y**, Zhao L, Jiang M, Yang F, Zhang X, Jia Y, Zhou N. Characterization of glucose metabolism in breast cancer to guide clinical therapy. *Front Surg* 2022; **9**: 973410 [PMID: 36277284 DOI: 10.3389/fsurg.2022.973410]

55 **Tran Q**, Lee H, Park J, Kim SH, Park J. Targeting Cancer Metabolism - Revisiting the Warburg Effects. *Toxicol Res* 2016; **32**: 177-193 [PMID: 27437085 DOI: 10.5487/TR.2016.32.3.177]

56 **Fukushi A**, Kim HD, Chang YC, Kim CH. Revisited Metabolic Control and Reprogramming Cancers by Means of the Warburg Effect in Tumor Cells. *Int J Mol Sci* 2022; **23** [PMID: 36077431 DOI: 10.3390/ijms231710037]

57 **Liu S**, Li Y, Yuan M, Song Q, Liu M. Correlation between the Warburg effect and progression of triple-negative breast cancer. *Front Oncol* 2022; **12**: 1060495 [PMID: 36776368 DOI: 10.3389/fonc.2022.1060495]

58 **Mittal L**, Aryal UK, Camarillo IG, Ferreira RM, Sundararajan R. Author Correction: Quantitative proteomic analysis of enhanced cellular effects of electrochemotherapy with Cisplatin in triple-negative breast cancer cells. *Sci Rep* 2019; **9**: 19124 [PMID: 31819154 DOI: 10.1038/s41598-019-55880-7]

59 **Wang Z**, Jiang Q, Dong C. Metabolic reprogramming in triple-negative breast cancer. *Cancer Biol Med* 2020; **17**: 44-59 [PMID: 32296576 DOI: 10.20892/j.issn.2095-3941.2019.0210]

60 **Chen Y**, Cen L, Guo R, Huang S, Chen D. Roles and mechanisms of phosphoglycerate kinase 1 in cancer. *Bull Cancer* 2022; **109**: 1298-1307 [PMID: 36096942 DOI: 10.1016/j.bulcan.2022.07.004]

61 **He Y**, Wang X, Lu W, Zhang D, Huang L, Luo Y, Xiong L, Li H, Zhang P, Li Q, Liang S. PGK1 contributes to tumorigenesis and sorafenib resistance of renal clear cell carcinoma *via* activating CXCR4/ERK signaling pathway and accelerating glycolysis. *Cell Death Dis* 2022; **13**: 118 [PMID: 35121728 DOI: 10.1038/s41419-022-04576-4]

62 **Yuan Y**, Li H, Pu W, Chen L, Guo D, Jiang H, He B, Qin S, Wang K, Li N, Feng J, Wen J, Cheng S, Zhang Y, Yang W, Ye D, Lu Z, Huang C, Mei J, Zhang HF, Gao P, Jiang P, Su S, Sun B, Zhao SM. Cancer metabolism and tumor microenvironment: fostering each other? *Sci China Life Sci* 2022; **65**: 236-279 [PMID: 34846643 DOI: 10.1007/s11427-021-1999-2]

63 **Duncan L**, Shay C, Teng Y. PGK1 : An Essential Player in Modulating Tumor Metabolism. *Methods Mol Biol* 2022; **2343**: 57-70 [PMID: 34473315 DOI: 10.1007/978-1-0716-1558-4_4]

64 **Li X**, Jiang Y, Meisenhelder J, Yang W, Hawke DH, Zheng Y, Xia Y, Aldape K, He J, Hunter T, Wang L, Lu Z. Mitochondria-Translocated PGK1 Functions as a Protein Kinase to Coordinate Glycolysis and the TCA Cycle in Tumorigenesis. *Mol Cell* 2016; **61**: 705-719 [PMID: 26942675 DOI: 10.1016/j.molcel.2016.02.009]

65 **Xu M**, Liu X, Zhou X, Qin Y, Yang L, Wen S, Qiu Y, Chen S, Tang R, Guo Y, Liu M, Sun Y. Hypoxia-induced circSTT3A enhances serine synthesis and promotes H3K4me3 modification to facilitate breast cancer stem cell formation. *Pharmacol Res* 2023; **197**: 106964 [PMID: 37865128 DOI: 10.1016/j.phrs.2023.106964]

66 **Williams SD**, Smith TM, Stewart LV, Sakwe AM. Hypoxia-Inducible Expression of Annexin A6 Enhances the Resistance of Triple-Negative Breast Cancer Cells to EGFR and AR Antagonists. *Cells* 2022; **11** [PMID: 36230969 DOI: 10.3390/cells11193007]

67 **Jin MS**, Lee H, Park IA, Chung YR, Im SA, Lee KH, Moon HG, Han W, Kim K, Kim TY, Noh DY, Ryu HS. Overexpression of HIF1α and CAXI predicts poor outcome in early-stage triple negative breast cancer. *Virchows Arch* 2016; **469**: 183-190 [PMID: 27184798 DOI: 10.1007/s00428-016-1953-6]

68 **Li X**, Zheng Y, Lu Z. PGK1 is a new member of the protein kinome. *Cell Cycle* 2016; **15**: 1803-1804 [PMID: 27105392 DOI:

10.1080/15384101.2016.1179037]

69 **Yan M**, Rayoo M, Takano EA; KConFab Investigators, Fox SB. BRCA1 tumours correlate with a HIF-1alpha phenotype and have a poor prognosis through modulation of hydroxylase enzyme profile expression. *Br J Cancer* 2009; **101**: 1168-1174 [PMID: 19724277 DOI: 10.1038/sj.bjc.6605287]

70 **Sun S**, Wu H, Wu X, You Z, Jiang Y, Liang X, Chen Z, Zhang Y, Wei W, Jiang Y, Chen Y, Song Y, Pang D. Silencing of PGK1 Promotes Sensitivity to Paclitaxel Treatment by Upregulating XAF1-Mediated Apoptosis in Triple-Negative Breast Cancer. *Front Oncol* 2021; **11**: 535230 [PMID: 33747900 DOI: 10.3389/fonc.2021.535230]

71 **Uhlen M**, Zhang C, Lee S, Sjöstedt E, Fagerberg L, Bidkhori G, Benfeitas R, Arif M, Liu Z, Edfors F, Sanli K, von Feilitzen K, Oksvold P, Lundberg E, Hober S, Nilsson P, Mattsson J, Schwenk JM, Brunnström H, Glimelius B, Sjöblom T, Edqvist PH, Djureinovic D, Micke P, Lindskog C, Mardinoglu A, Ponten F. A pathology atlas of the human cancer transcriptome. *Science* 2017; **357** [PMID: 28818916 DOI: 10.1126/science.aan2507]

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ORIGINAL ARTICLE

Basic Study

Parthenolide enhances the metronomic chemotherapy effect of cyclophosphamide in lung cancer by inhibiting the NF-kB signaling pathway

Zheng Cai, Lang Gao, Kai Hu, Qi-Ming Wang

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Abstract

BACKGROUND

Parthenolide (PTL), a sesquiterpene lactone derived from the medicinal herb Chrysanthemum parthenium, exhibits various biological effects by targeting NF-kB, STAT3, and other pathways. It has emerged as a promising adjunct therapy for multiple malignancies.

AIM

To evaluate the *in vitro* and *in vivo* effect of PTL on cyclophosphamide (CTX) metronomic chemotherapy.

METHODS

The cytotoxicity of PTL and CTX on Lewis lung cancer cells (LLC cells) was assessed by measuring cell activity and apoptosis. The anti-tumor efficiency was evaluated using a tumor xenograft mice model, and the survival of mice and tumor volume were monitored. Additionally, the collected tumor tissues were analyzed for tumor microenvironment indicators and inflammatory factors.

RESULTS

In vitro, PTL demonstrated a synergistic effect with CTX in inhibiting the growth of LLC cells and promoting apoptosis. *In vivo*, metronomic chemotherapy combined with PTL and CTX improved the survival rate of tumor-bearing mice and reduced tumor growth rate. Furthermore, metronomic chemotherapy combined with PTL and CTX reduced NF-κB activation and improved the tumor immune microenvironment by decreasing tumor angiogenesis, reducing Transforming

growth factor β, and α-SMA positive cells.

CONCLUSION
PTL is an efficient compound that enhances the metronomic chemotherapy effects of CTX both *in vitro* and *in vivo*, suggesting its potential as a supplementary therapeutic strategy in metronomic chemotherapy to improve the chemotherapy effects.

Key Words: Lung cancer; Parthenolide; Cyclophosphamide; Rhythmic chemotherapy; NF-κB pathway

Core Tip: Our present study found that as a specific inhibitor of NF-kB, Parthenolide can promote the efficiency of cyclophosphamide in lung cancer *via* inhibiting NF-kB signaling *in vitro* and *in vivo*. Our present study will help scientists and clinicians to draw up novel rhythmic chemotherapy strategies.

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INTRODUCTION

Toxic effects and chemotherapy resistance are the main obstacles to traditional chemotherapy clinically. To avoid these problems caused by traditional chemotherapy regimens, a new type of administration method called "metronomic chemotherapy" has emerged[1]. This approach involves frequent administration of conventional chemotherapeutics at very low doses. The term “metronomic chemotherapy” was mentioned preclinically as early as 2000[2,3]. Apart from its advantages including minimizing side effects and reducing opportunities for acquired drug resistance, it is proposed that metronomic chemotherapy targets activated endothelial cells in tumors, modulates hosts’ immune system, and affects tumor progenitor cells and neighboring stromal cells[4]. The most studied drugs for metronomic chemotherapy include cyclophosphamide (CTX), methotrexate, capecitabine, and vinorelbine[5]. CTX is a prodrug that undergoes conversion *in vivo* by hepatic cytochrome P450 enzymes to active forms of metabolites, such as phosphoramide mustard. These active forms undergo covalent cross-linking with DNA, leading to DNA strand breaks and cross-links, which impede the process of DNA replication and transcription[6-8]. However, CTX involves a wide range of cytotoxicity and can cause several serious side effects such as myelosuppression, immunosuppression, alopecia, and urinary toxicity. Because metronomic chemotherapy has a good weakening effect on toxic effects and chemotherapy resistance, CTX is the most widely used metronomic chemotherapy agent preclinically and clinically[9,10]. CTX has been used in combination with other chemotherapy agent, such as vincristine, lomustine, and etoposide, possibly by regulating the host immune system to enhance the efficacy of lung cancer therapy[11,12]. However, uncertainty about treatment efficacy, challenges with compliance, and patient applicability limit the use of CTX-related metronomic chemotherapy. Therefore, there is an urgent need to find more effective drugs for lung cancer treatment in combination with CTX.

Natural products have long been used as the primary arsenal for anti-cancer drug development[13]. Novel natural products act on tumors solely or in combination with traditional chemotherapy drugs. Feverfew (*Tanacetum parthenium L.*) is a traditional medicinal plant that has been used for centuries to treat fevers, migraine headaches, rheumatoid arthritis, and various other inflammation-related diseases[14]. In addition, feverfew extracts have demonstrated the ability to inhibit the proliferation of tumor cells in laboratory studies[15]. This plant contains a large number of natural products, its active compounds include sesquiterpene lactones, like parthenolide (PTL)[14]. PTL was isolated from feverfew extract half a century ago and has been reported to exhibit tumor cell growth inhibition effects *in vitro*[16,17]. In 2001, it was discovered that PTL directly targets Ikappa B kinase, providing a clear explanation for its anti-inflammatory mechanism of action[18]. NF-kB signaling is also involved in tumor progression[19], its activation suppresses the apoptotic potential of chemotherapeutic agents and contributes to drug resistance. It also reported that NF-kB inhibitors could sensitize metronomic chemotherapy[20].

Several chemicals have been reported to sensitize the efficacy of metronomic chemotherapy. However, whether PTL can sensitize CTX metronomic chemotherapy has never been investigated. Considering the reported characteristics of PTL, especially its strong effect on NF-kB inhibition, we hypothesized that PTL can enhance the efficacy of CTX. In the present study, we tested the biological effect of PTL on CTX both *in vitro* and *in vivo* and observed that PTL strongly enhanced the efficacy of CTX, partially by NF-kB inhibition.

MATERIALS AND METHODS

Cell line and mice

Mouse Lewis lung carcinoma (LLC) cell line was purchased from Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI-1640 medium containing 10% FBS at 37 °C in a humidified 5% CO_2 incubator as previously described[21]. For *in vitro* cell assays, LLC Cells were transfected with CYP2B6 constant express plasmid, and the expression of CYP2B6 was quantified by Q-PCR and Western blot. The cell medium was changed every day and the cells were used in their logarithmic growth phase for all experiments.

Male wild-type C57BL/6J mice were used for all experiments. Mice weighed 20-22 g were purchased from Kunming Medical University [Grade SPF II, SCXK(Yunnan)K2020-0004] and fed a standard laboratory diet with water ad libitum and were kept under constant environmental conditions in the Biological Center Lab of Kunming Medical University. All the experimental procedures were approved by the ethics committee of Kunming Medical University and performed following the institutional animal care guidelines and the ARRIVE guidelines.

Cell proliferation assay

LLC cells transfected with CYP2B6 were exposed to a culture medium with different concentrations of CTX and/or PTL. Cell viability was assessed by an Enhanced CCK8 Cell Proliferation Assay kit (Elabscience, Wuhan, China) according to the manufacturer's instructions. About 2 × 10^3 cells in 200 μL of cell culture medium were seeded into 96-well plates. After 24 hours of attachment, different concentrations of CTX and/or PTL were added to the culture medium. The cell viability was assayed every 24 hours.

PI/annexin double staining

Apoptosis was assayed by PI/annexin V double staining using a cell apoptosis assay kit (Elabscience, Wuhan, China) according to the manufacturer's instructions. A total of 5 × 10^4 cells were collected and resuspended in 195 μL Annexin V-FITC binding buffer and then stained with 5 μL Annexin V-FITC and 10 μL PI, then incubated at room temperature in the dark for 20 minutes. The apoptotic cells were monitored by flow cytometry assay using a flow cytometer (BD Bioscience).

Western blot assay

Protein expression levels were quantified by Western blot. In brief, total proteins were extracted using RIPA Lysis (Beyotime Biotechnology, Shanghai, China) according to the protocols provided by the manufacturer. Then, proteins were isolated by 10% SDS-PAGE and electro-transferred on PVDF membranes (Millipore, United States). Subsequently, the membranes containing proteins were seriatim incubated with the primary and secondary antibodies for the indicated time. Antibodies used in the experiment including CYP2B6 antibody (A1463, ABclonal, Wuhan, China) anti-NF-kB P65 (ab16502, Abcam, Cambridge, MA, United States), Anti-NF-kB p65 (phospho S536) (ab76302, Abcam), anti-β-Actin (E-AB-20034, Elabscience) and Goat Anti-Rabbit IgG (H + L) (peroxidase/HRP conjugated) (E-AB-1003, Elabscience). The protein bolts were developed by the Immobilon Western HRP Substrate (Millipore) and pictured with a chemiluminescence imager (Tanon, China), then quantified with Image J software (NIH, Bethesda, MA, United States).

NF-κB p65 transcription factor activity assay

Transcription factor activity for NF-kB was carried out with a commercial NF-κB p65 transcription factor activity assay kit (RayBiotech Inc., United States) which coded plate with NF-κB binding DNA sequence that specifically captured the active NF-κB p65 contained in whole cell lysate or nuclear extracts. The active NF-κB p65 was further quantified by NF-κB p65 primary antibody and HRP-conjugated secondary antibody. After a short incubation with CTX and PTL, the nuclear protein was extracted from 1 × 10^7 LLC cells using a Nuclear/Cytoplasmic Protein Extraction Kit (SINP001, Viagene Biotech Inc., United States). The Nuclear protein lysis was loaded onto the NF-κB p65 transcription factor activity detecting plate provided by the manufacturer, and the signal was detected according to the manufacturer's instructions.

Subcutaneous tumor inoculation

LLC cells (1 × 10^6 cells/mouse) re-suspended in serum-free DMEM were injected under the skin of C57BL/6 mice in the back or neck area. Fourteen days after tumor cell injection, the tumor-bearing mice were euthanatized by cervical dislocation, the tumor was harvested, segmented, and digested with collagenase IV (Sigma), about 1 × 10^8 cells were harvested after digestion, and 2 × 10^6 cells/mouse were subcutaneously injected in the back of 40 mice. Randomly distributed these mice into four groups, *i.e.*, control, CTX, PTL, and CTX + PTL group, each including 10 mice. After one week of the tumor cell implantation, the daily CTX and/or PTL administrated was performed by intragastric infusion and lasted for about 3 weeks. The survival of mice and the tumor size (measured with a vernier caliper) were recorded. When the tumor's long diameter reached 2 cm, the mouse was killed, and the tumor was isolated from the skin and weighed after exsanguination. The tumor mass was kept in a formalin solution and embedded in paraffin for Immunohistochemistry (IHC). Both the natural death and euthanasia of mice were recorded as death events in the Kaplan-Meier assay.

Immunohistochemistry assay

Immunohistochemistry assay was carried out as previously described[22]. Briefly, the paraffin section of tumor tissues from mice was dewaxed and rehydrated. Antigen retrieval was first carried out. Then tissue sections were quenched in 0.3% hydrogen peroxide and blocked using 5% goat serum. The slides were incubated with the primary antibody at 4 °C

overnight and then probed with HRP-conjugated secondary antibody at room temperature for 1 hour. Afterward, the slides were stained using diaminobenzidine (DAB). The presentation of dark brown was considered positive. Antibodies used in this section were as followed: Ant-CD31 (ab124432, Abcam), anti-F4/80 (A1256, ABclonal), Anti-alpha smooth muscle Actin [1A4] (ab7817, Abcam), Anti-Mannose Receptor/CD206 (ab64693, Abcam), Anti-NF-kB p65 (phospho S536) (ab76302; Abcam), Anti-transforming growth factor β (TGF β1) antibody (ab215715, Abcam), tumor necrosis factor α (TNF-α) antibody (A11534, ABclonal), IL-6 antibody (A0286, ABclonal), Goat Anti-Rabbit IgG (H + L) (peroxidase/HRP conjugated) (E-AB-1003, Elabscience) and Goat Anti-mouse IgG (H + L) (peroxidase/HRP conjugated) (E-AB-1001, Elabscience).

Statistical analysis

Survival data were analyzed using the Kaplan-Meier method, and survival curves were compared using the log-rank test in univariate analysis. A one-way ANOVA test was performed to evaluate the difference between groups. Two-group comparisons were performed using the Student *t*-test.

RESULTS

Overexpression of CTX metabolic enzyme P450 in LLCs

CTX is a prodrug that requires metabolic activation by cytochrome P450 enzymes in the liver to convert it into its active forms. There are currently 57 known human cytochrome P450 genes, which exhibit significant inter-individual genetic variation. CTX hydroxylation can be catalyzed by various P450 (CYP) enzymes, including CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, and CYP2J2[23-30]. Among them, CYP2B6 and CYP2C19 are the enzymes with the highest bioactivation activity for CTX[31]. Several studies have shown that overexpression of P450, especially CYP2B6, in tumor cells can enhance the sensitivity of tumor cells to CTX[32-35].

To evaluate the synergistic effect of PTL in combination with CTX *in vitro*, we developed a lung cancer cell line with constitutive expression of cytochrome P450 enzymes to enable efficient metabolism of CTX. We confirmed the overexpression of CYP2B6 in the P450-overexpressing (P540-OE) LLC cells at both the mRNA and protein levels (Figure 1A and B). While the wild-type LLC cells, human lung cancer cells (A549, H838, Hcc827, H1299, H1975, H1734, and XWCL-05), and normal bronchial epithelial cells (Beas-2B), expressed a very low level of P450 protein (Figure 1B and Supplementary Figure 1). It is worth noting that the expression of exogenous genes may impact cell growth. Therefore, to evaluate the inhibitory effect of CTX on P540-OE LLC cells, it is essential to account for any potential influence exerted by the exogenous expression of CYP2B6 on cell growth. The results of the CCK8 cell activity assay showed that P450 overexpression or vector transfection had no significant effect on cell growth during the 4-day growth process (Figure 1C). Therefore, in subsequent experiments, P540-OE LLC cells were used to evaluate the antitumor activity of PTL and CTX treatment regimens.

PTL enhanced cytotoxicity of CTX on P450 overexpressed LLC cells

To assess whether overexpression of cytochrome P450 sensitizes LLC cells to CTX, we used the CCK8 assay to evaluate cell proliferation activity. P450 overexpression significantly increased the sensitivity of LLC cells to CTX (Figure 2A and B). As the treatment time and concentration increased, there was a remarkable decrease in the proliferation activity of LLC P450-OE cells compared to the control group (Figure 2A and B). These findings indicate that P450 overexpression enhances the cytotoxic effect of CTX. By comparing the percentage of inhibition, we observed that the highest inhibitory effect was achieved at 48 hours of treatment (Figure 2C). Therefore, for subsequent experiments, we selected the 48-hour treatment as the optimal time for collecting cell samples.

To assess the cytotoxic effects of CTX and PTL on LLC cells, this study established a range of concentration gradients for individual treatments of CTX and PTL on P450-OE LLC cells. PTL inhibited the proliferation of P450-OE LLC cells in a dose-dependent manner within the concentration range of 2.5 uM to 20 uM (Figure 2D and E). Similarly, CTX also exhibited a dose-dependent inhibition of P450-OE LLC cell proliferation within a concentration range of 2.5 ug/mL to 20 ug/mL (Figure 2D and E). To determine the synergistic potential of combining these two drugs, we calculated the combination index (CI). Inhibition of cell proliferation was more pronounced when CTX and PTL were used in combination (Figure 2F). Specifically, when the inhibitory effect ranged from 0.2 to 1.0, the CI remained less than 1, and it gradually decreased as the inhibitory effect increased (Figure 2F). These findings collectively demonstrate that the combination of CTX and PTL produces a synergistic effect on cell toxicity.

To further elucidate the cytotoxic effects of the combined treatment of CTX and PTL, we examined the changes in apoptosis levels in P450-OE LLC cells when exposed to CTX and PTL individually as well as in combination. When administered alone, CTX at a concentration of 5 ug/mL or PTL at 5 uM significantly promoted apoptosis in P450-OE LLC cells. Specifically, the apoptosis ratio was 22.88% ± 1.67% for CTX at 5 ug/mL and 16.1% ± 1.62% for PTL at 5 uM (Figure 2G). Strikingly, the proportion of induced apoptosis dramatically increased to 48.57% ± 1.86% when the two drugs were combined (Figure 2G). Importantly, the overall level of apoptosis observed in the combined treatment group surpassed that observed with either drug alone. These findings underscore the enhanced efficacy of the combination therapy, suggesting a promising approach for targeted tumor cytotoxicity.

Further, this study evaluated the effects of 5 μg/mL CTX and 5 μM PTL individually as well as in combination on the viability of hepatic (LO2), alveolar (RLE-6TN), and renal (HK2) cells. As indicated in Figure 2H, 5 μg/mL CTX was significantly cytotoxic to LO2 and HK2, and 5 μM PTL enhanced the cell activity of LO2 and HK2 after 48 hours of treatment. Moreover, the combination of CTX and PTL alleviated the cytotoxicity of CTX on LO2 and HK2. Notably, CTX

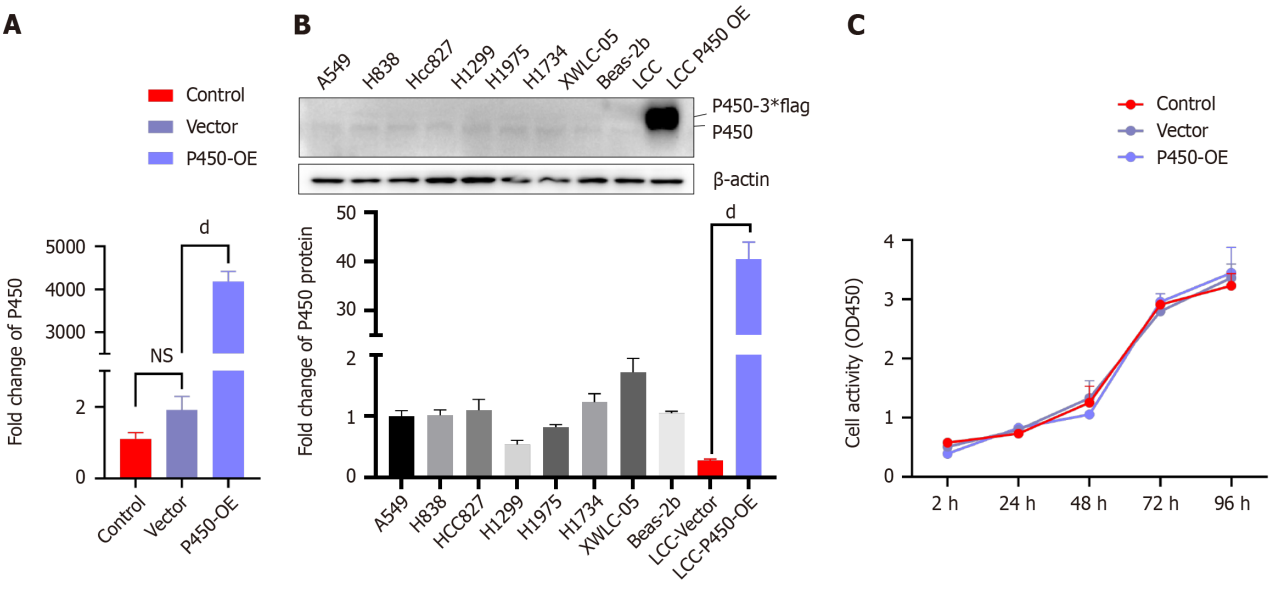

Figure 1 P450 expression detection in different cell lines and the effect of its overexpression on Lewis lung cancer cells cell activity. A: Q-PCR detection of P450 mRNA expression in Lewis lung cancer cells (LLC) cells transfected with P450 overexpression plasmid (P450-OE); B: Western blot detection of P450 protein in different cell lines. Full undamaged Gels and Blots images are shown in Supplementary Figure 1; C: CCK8 assay of LLC cells transfected with P450-OE plasmid or the vector plasmid. $n = 3$, $^{d}P < 0.0001$; NS: Not significant; LCC: Lewis lung cancer cells.

and PTL had no significant effect on the cell activity of RLE-6TN. This suggests that CTX is toxic to normal hepatic and renal cells and that its toxicity can be mitigated by combination therapy with PTL.

PTL improved the survival of tumor-bearing mice in combination with low-dose CTX

In a study conducted by Dynes *et al*[36], it was demonstrated that metronomic chemotherapy using a dosage of 20 mg/kg/day CTX in mice was effective. Therefore, for this study, metronomic chemotherapy was administered to mice with a CTX dose of 20 mg/kg/day. The reported anti-tumor dose of PTL ranged from 4 to 40 mg/kg[37-39]. Generally, doses of 4-12 mg/kg were administered intravenously or peritoneally, while a dose of 40 mg/kg/day was given *via* intragastric administration as per the former report[38]. For this study, the reference dosage of 40 mg/kg/day was employed for intragastric administration of PTL.

The animal experiment procedure is displayed in Figure 3A. A significant difference in survival among the four groups of mice is indicated in Figure 3B. Survival curve analysis revealed significant differences between the PTL and Control group, as well as between the CTX and Control group (Figure 3C and D). Notably, the PTL + CTX combination group exhibited the longest survival time, with 40% of tumor-bearing mice living extended to the end of the experiment (Figure 3B and E). Additionally, a statistical difference in survival was observed between the CTX treatment group and the CTX + PTL combined administration group (Figure 3E), indicating an enhanced tumor inhibition effect of the combination of PTL and CTX.

The tumor growth in the control group exhibited rapid progression, with a mean tumor diameter of 2 cm reached as early as day 19. While there were no significant differences in the tumor growth curves between the CTX-only, PTL-only, and CTX + PTL combined chemotherapy groups (Figure 3F), a noteworthy observation was the significant difference in tumor growth rates when normalized by growth time between the control group and the CTX + PTL combined groups (Figure 3G). Although the tumor growth rate in the PTL and CTX treatment group was lower than that in the control group, statistical analysis showed no significant difference, suggesting that the effect of PTL and CTX combined chemotherapy had a more favorable impact compared to their individual use.

Effects of PTL combined with CTX on tissue morphology, angiogenesis, and immune microenvironment of transplanted tumors

To further evaluate the impact of PTL combined with CTX on tumor tissue pathology, we conducted Hematoxylin and eosin staining on the collected tumor tissues. Compared to the control group, the CTX group displayed a significant amount of cell detachments and shrinkage within the tumor, accompanied by the presence of necrotic areas. In the PTL group, there were loose connections between tumor cells, along with a considerable amount of cell detachments and fragmented cell nuclei, indicating apoptotic tumor cell death. Notably, the PTL combined with the CTX group exhibited extensive necrotic regions within the tumor, along with disrupted tumor morphology and visible congestion, suggesting a more severe collapse of the tumor tissue structure (Figure 4A). A combination of PTL and CTX significantly enhanced CTX anti-angiogenesis effects, reduced the vascular density to nearly 50% percent (Figure 4B), and demonstrated a superior effect on tumor toxicity and angiogenesis inhibition compared to single CTX.

To assess the inhibitory effects of PTL combined with CTX on tumor graft growth and changes in the tumor immune microenvironment, we conducted IHC staining for macrophage marker genes CD206 and F4/80 in the tumor tissues.

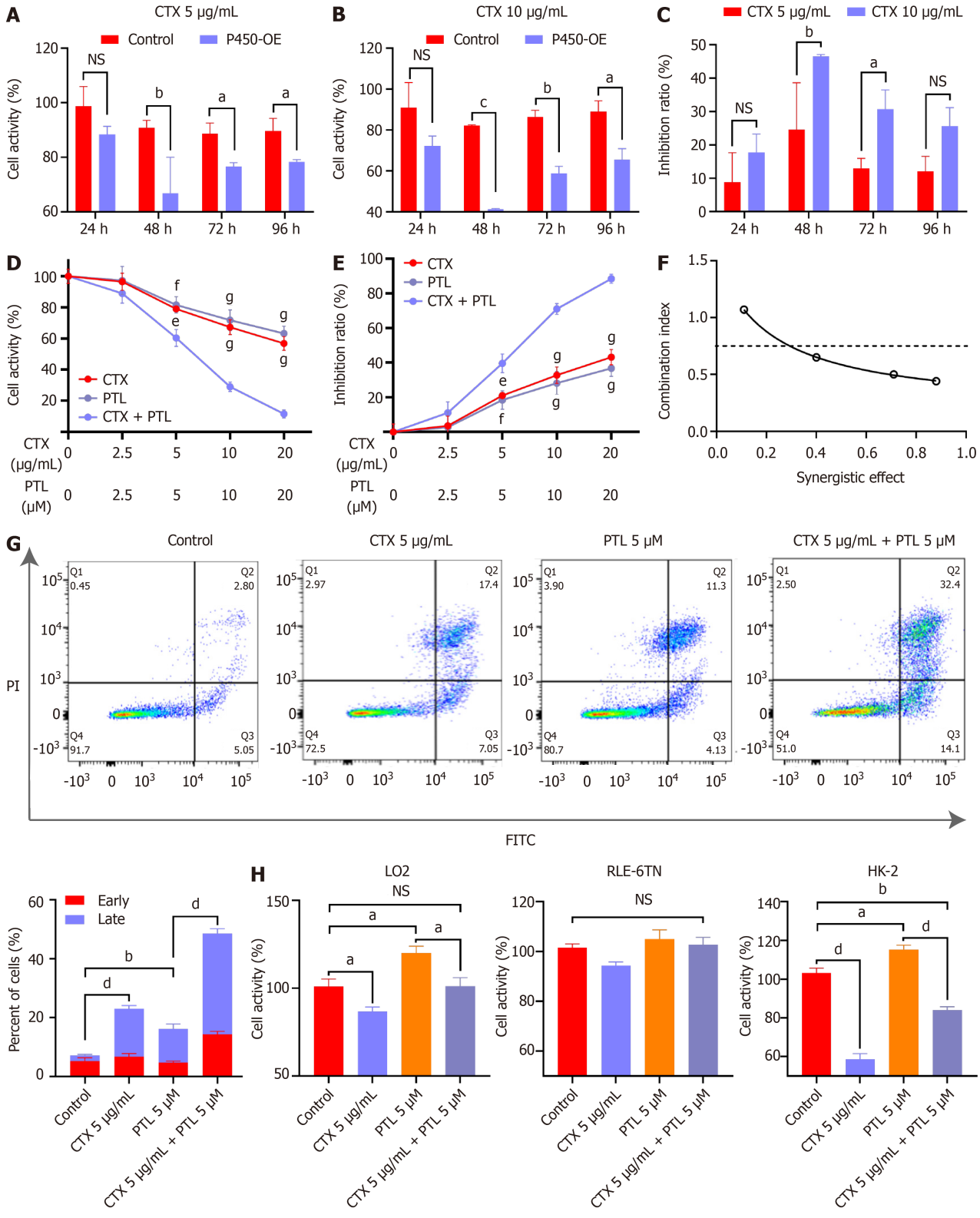

Figure 2 Parthenolide enhanced cytotoxicity of cyclophosphamide on P450 overexpressed Lewis lung cancer cells. A and B: P450 overexpression plasmid (P450-OE) and Control groups were treated with 5 ug/mL (A) or 10 ug/mL (B) cyclophosphamide (CTX), and its inhibitory effect on cell proliferation was evaluated using CCK8 assay; C: Comparison of proliferation inhibition percentage of P450-OE Lewis lung cancer cells (LLCs) treated with 5 and 10 ug/mL CTX; D: Cells were treated with different concentrations of CTX, Parthenolide (PTL), or a combination of the two drugs for 48 hours, and cell viability was detected by CCK-8 assay; E: Inhibition curve of different groups; F: The Combination Index was calculated using CompuSyn software; G: The apoptosis percentage of P450-OE LLC cells was evaluated after 48 hours of exposure to 5 μg/mL CTX and 5 μM PTL individually as well as in combination; H: CCK-8 evaluated the viability of LO2, RLE-6TN and HK-2 cells after treatment with 5 μg/mL CTX and 5 μM PTL individually as well as in combination for 48 hours. $n = 3$, $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; $^{d}P < 0.0001$. Compared with CTX + PTL group, $^{e}P < 0.01$; $^{f}P < 0.001$; $^{g}P < 0.0001$; NS: Not significant. PTL: Parthenolide; CTX: Cyclophosphamide.

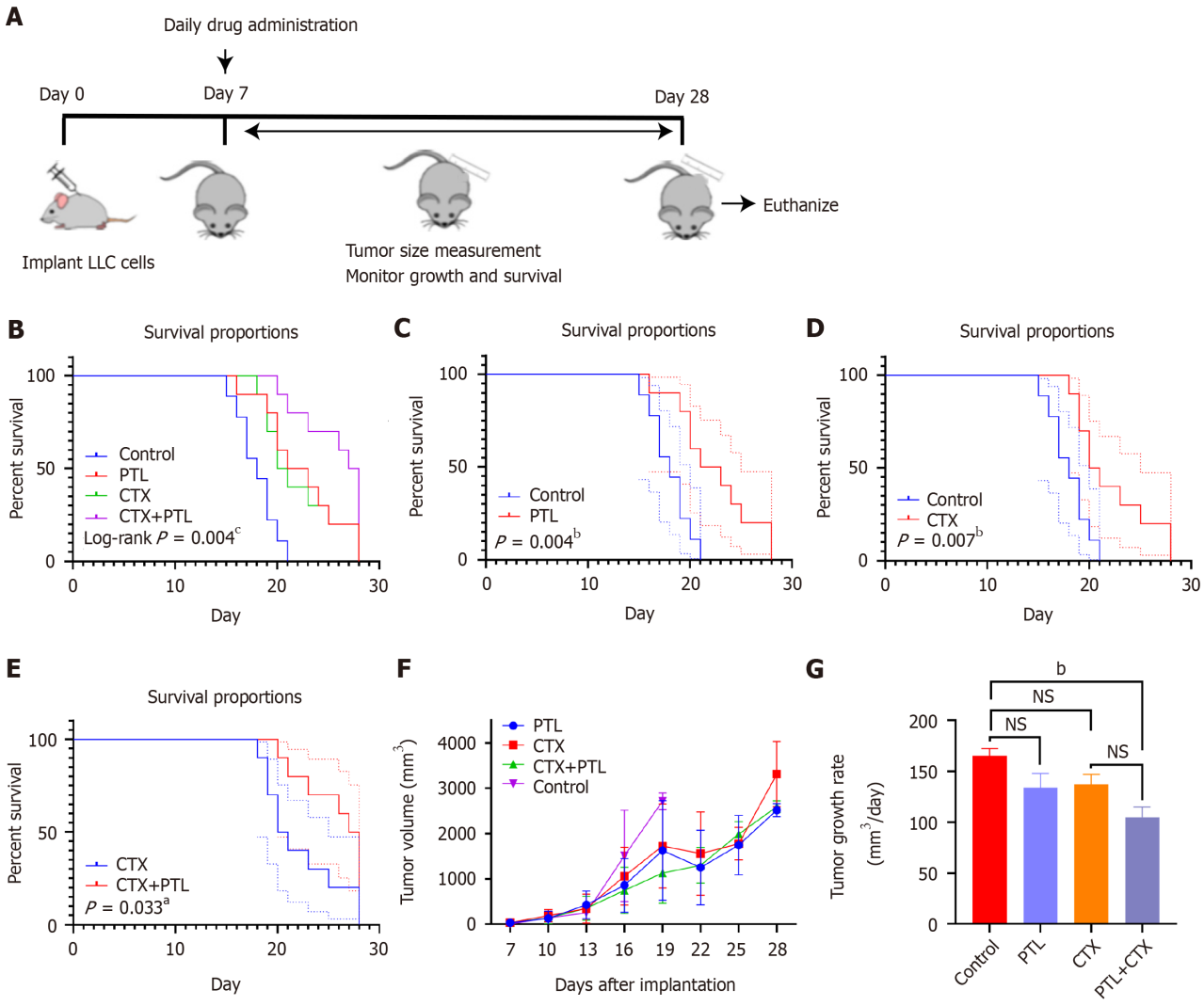

Figure 3 Survival analysis of tumor-bearing mice. A: Animal experiment process; B: Survival curve and log-rank analysis of 4 groups of tumor-bearing mice; C: Survival comparison between control and parthenolide (PTL) groups; D: Survival comparison between control and cyclophosphamide (CTX) chemotherapy groups; E: Comparison between CTX and PTL + CTX combined groups; F: Tumor growth curve of different groups; G: Comparison of tumor growth rate. n = 10, $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. NS: Not significant; PTL: Parthenolide; CTX: Cyclophosphamide.

Additionally, we analyzed immune regulatory factors TGF-β, pro-inflammatory factors IL-6 and TNF-a, and invasive marker gene α-SMA expression in tumor tissues. The proportion of F4/80-positive cells significantly increased after PTL or CTX treatment. However, when the two drugs were combined and administrated, the proportion of the F4/80-positive cell population did not further increase (Figure 4C). The proportion of CD206-positive cells showed no significant differences among tumors of the four groups (Figure 4C). Hence, the increase of F4/80 positive macrophages might be due to the import of proinflammatory M1 cells, whereas PTL combined CTX administration did not enhance or decrease macrophage recruitment. Similar expression of the inflammatory factors (IL-6 and TNF-α) among the control group, the CTX treatment group, and the PTL treatment group. However, in the PTL combined with the CTX group, there was a slight decrease in the expression levels of IL-6 and TNF-α; however, this decrease was not statistically significant (Figure 5A). There was no significant change in the expression level of TGF-β in the tumor tissues of the CTX group when compared to the control group. In contrast, PTL treatment led to a significant down-regulation in the expression level of TGF-β (Figure 5A). Furthermore, the combination of PTL and CTX exerted a substantial inhibitory effect on the expression level of TGF-β in the tumor microenvironment (Figure 5A). CTX alone had no significant effect on the expression level of α-SMA (Figure 5B). PTL treatment alone significantly downregulated the expression level of α-SMA, and PTL combined with CTX also significantly inhibited the expression level of α-SMA (Figure 5B). Thus, the expression level of α-SMA is primarily influenced by PTL treatment.

In conclusion, these results indicated that PTL promotes CTX efficiency not only by acting on tumor cells but also by influencing the tumor microenvironment. PTL could enhance the angiogenesis inhibition effect of CTX. It also inhibited TGF-β and a-SMA expression in tumor tissue. Since the involvement of TGF-β and a-SMA in tissue reparation and tumor metastasis, the inhibition of them might contribute to the tumor sensitivity to CTX.

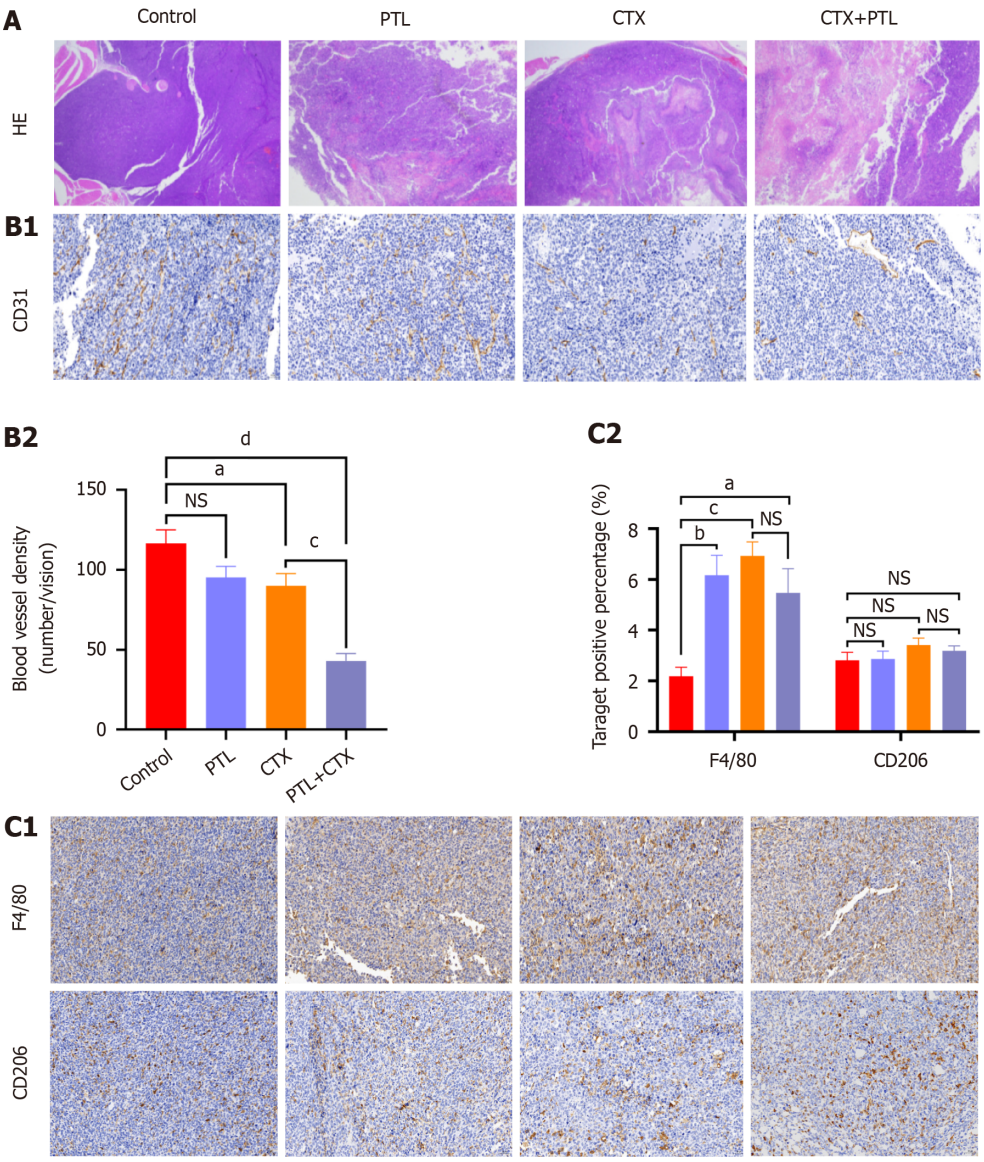

Figure 4 Effects of parthenolide combined with cyclophosphamide on tissue morphology and macrophage infiltration of transplanted tumors. A: Hematoxylin and eosin staining; B: CD31 staining and statistical results of tumor vascular density; C: F4/80 and CD206 staining in tumor tissues as well as comparison of F4/80 and CD206 positive cell proportion. Magnification: 200 ×. n = 10; $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; $^{d}P < 0.0001$; NS: Not significant. Con: Control; PTL: Parthenolide; CTX: Cyclophosphamide; HE: Hematoxylin and eosin staining.

PTL alleviated CTX-induced NF-kB activation

Many literatures have reported that the inhibition of PTL on NF-κB is the basis of its anti-tumor activity, and the activation of NF-κB leads to its transfer into the nucleus. CTX alone had no significant effect on the overall expression level of NF-κB in transplanted tumors, but could slightly increase (without significance) the proportion of NF-κB staining in the nucleus (Figure 6A). Compared to the CTX group, PTL combined with CTX significantly inhibited the expression level of NF-κB in transplanted tumor tissues and the transfer of NF-κB to the nucleus (Figure 6A), suggesting that PTL combined with CTX could inhibit the activation of NF-κB induced by CTX chemotherapy. To further verify the inhibitory effect of PTL on NF-κB in CTX, P450 OE LLC cells treated with CTX or PTL + CTX were collected and the expression of NF-κB was detected by Western blot. CTX did not induce the up-regulation of NF-κB p65 expression but could induce the phosphorylation of NF-κB p65 (Figure 6B). The up-regulation of p-NF-κB p65 induced by CTX could be down-regulated when PTL is combined with CTX (Figure 6B and Supplementary Figure 2). The results of the transcription factor activity assay further confirmed that PTL combined with CTX can inhibit CTX-induced NF-κB p65 transcription activity (Figure 6C). Therefore, PTL combined with CTX may have a synergistic anticancer effect by inhibiting CTX-induced NF-κB activation. Overall, these results demonstrated that parthenolide, as an NF-kB inhibitor, alleviated CTX-induced NF-kB activation, and thus may improve the sensitivity of LLC cells to CTX.

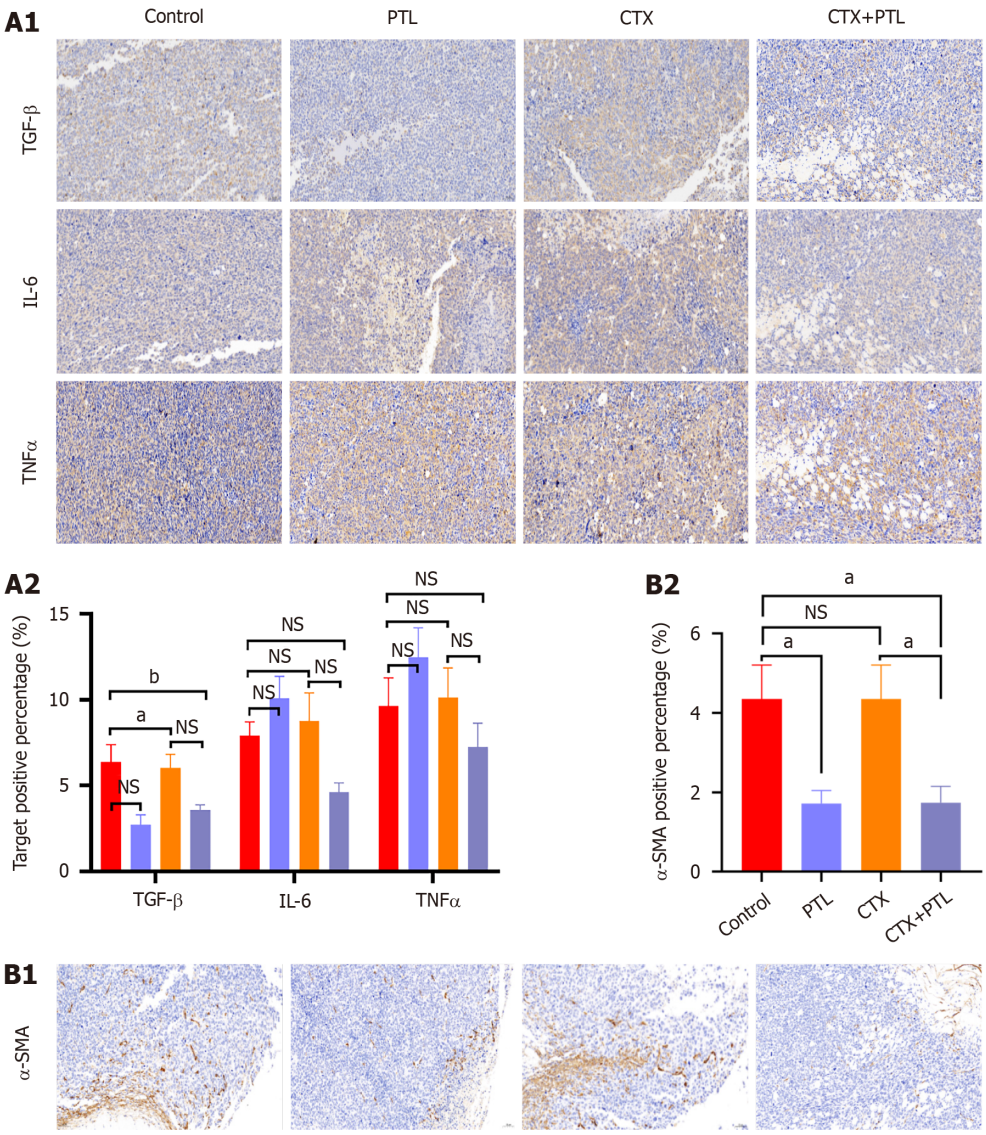

Figure 5 Influence of parthenolide combined with cyclophosphamide on the immune microenvironment in the transplanted tumor. A: Immunohistochemistry (IHC) staining of transforming growth factor β (TGF-β), IL-6, and tumor necrosis factor α (TNF-a), and the percentage of TGF-β, IL-6, and TNF-a positive cells in different treatment groups were compared; B: A-SMA IHC staining in tumor tissues. Magnification: 200 ×. n = 10; $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; $^{d}P < 0.0001$; NS: Not significant. PTL: Parthenolide; CTX: Cyclophosphamide.

DISCUSSION

There are a variety of ways for cancer therapy, including surgery, radiation therapy, chemotherapy, immunotherapy, and targeted drug therapy. And most of them relied on novel chemicals or combinations of chemicals. Conventional therapies including surgery, radiation therapy, and chemotherapy for cancer treatment have posed many challenges, including toxicity, and multidrug resistance. Newly developed therapies, such as immunotherapy and targeted drug therapy have other challenges such as high economic expenses[40]. Complementary alternative medicine by employing phyto-chemicals received increased attention because of their capability to modulate a myriad of molecular mechanisms with a less toxic effect. Phytochemicals can favorably inhibit several signaling pathways involved in cancer development and progression. Some combinations of phytochemicals promote cell death, inhibit cell proliferation and invasion, sensitize tumor cells, or boost the immune system, thus making them striking alternatives in cancer therapy. Some chemicals have also been proven to promote the efficiency of CTX, such as dexamethasone and celecoxib[41]. However, the role of natural products including phytochemicals in metronomic chemotherapy has not been widely investigated. Our present study proposed a novel combination of PTL and low-dose CTX to enhance the efficiency of metronomic chemotherapy. The present study demonstrated that the combination of CTX (5 μg/mL) and PLT (5 μM) enhanced the toxicity of CTX on LLC cells overexpressing P450 *in vitro*. In addition, CTX exhibited toxicity in normal hepatic and renal cells. This is consistent with previous reports[42,43]. Surprisingly, PLT alleviated CTX-induced activity inhibition of normal hepatic and renal cells. This suggests that PLT can effectively control the toxic and side effects of CTX on normal cells.

Though metronomic chemotherapy showed many advantages including minimizing side effects and reducing opportunities for acquired drug resistance, many signal pathways have still been activated to promote tumor cell survival, such as NF-kB. NF-kB has long been considered a double sword in cell death and cell survival[44]. Targeting

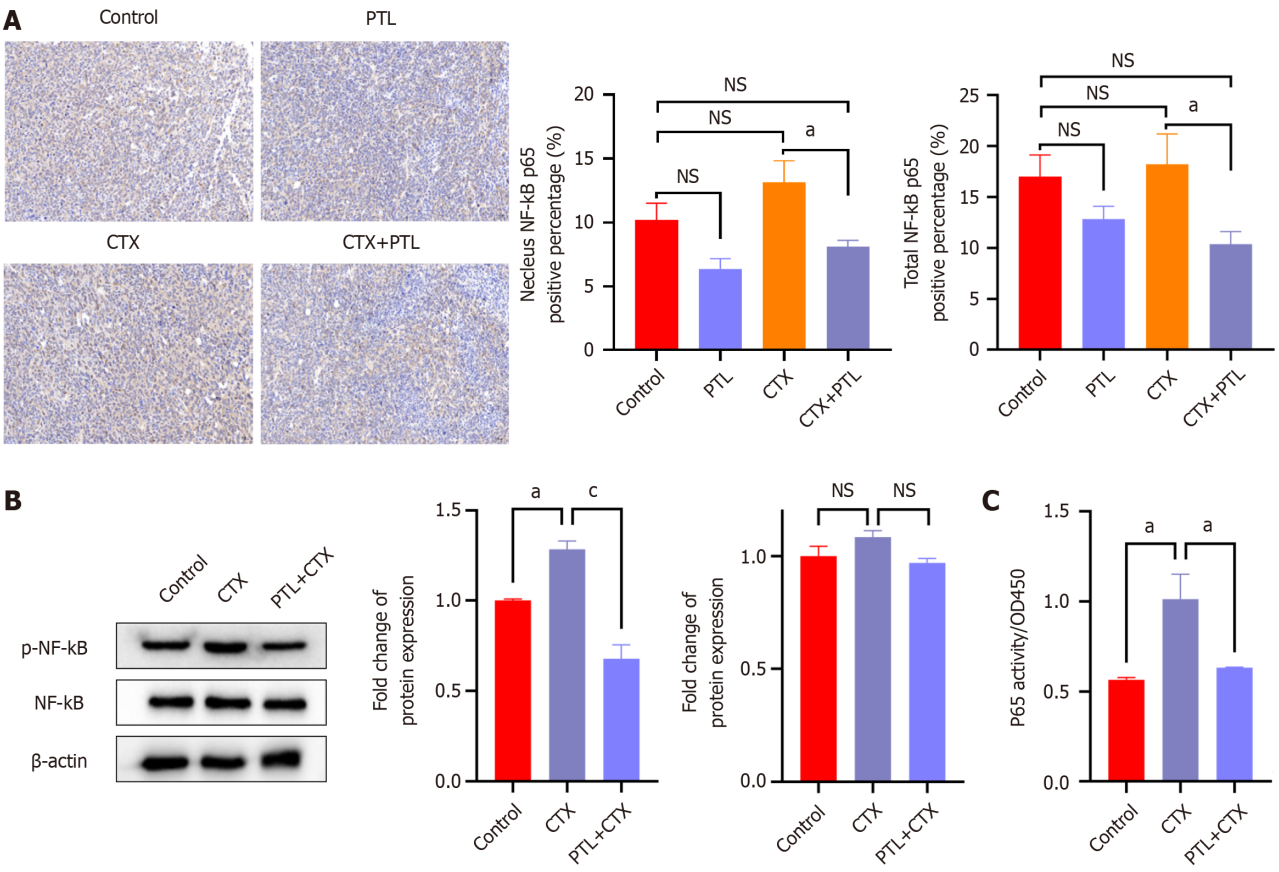

Figure 6 Parthenolide alleviated cyclophosphamide-induced NF-kB activation. A: NF-κB immunohistochemistry staining as well as comparison of the percentage of total and nuclei NF-κB positive cells in transplanted tumors of mice in different treatment groups. Magnification: 200 ×; B: P-NF-κB and NF-κB protein immunoblotting as well as gray analysis. Full undamaged Gels and Blots images are shown in Supplementary Figure 2; C: Analysis of NF-κB P65 transcriptional activity. n = 10, $^{a}P < 0.05$; $^{c}P < 0.001$; NS: Not significant. PTL: Parthenolide; CTX: Cyclophosphamide.

nuclear factor-kappa B can be used to overcome resistance to chemotherapy[45]. However, whether NF-kB inhibition boosts CTX efficiency has never been investigated. Our present study showed that parthenolide, which is a well-accepted NF-kB inhibitor, significantly increased the efficiency of CTX to kill tumor cells either *in vitro* or *in vivo*. The results showed in our present study will help scientists and clinicians draw up novel metronomic chemotherapy strategies.

The schedule of standard therapy with CTX was a 21-day cycle of either 100 or 150 mg/kg CTX, administered intraperitoneally once every other day over 6 days. And 10-40 mg/kg daily are considered low dose CTX[46]. Our present study showed that even at low doses, CTX can activate NF-kB (shown in Figure 6). While PTL significantly attenuated NF-kB activity induced by CTX. These results reminded us that NF-kB inhibition in CTX may be useful clinically to promote the therapy efficiency. This is also consistent with previous studies that CTX can activate NF-kB in other cells and tissues[47], and inhibition of NF-kB activation by CTX was involved in the reduction of side effects.

Angiogenesis is critical for solid tumor formation[48], and vascular formation is even considered the hallmark of solid tumors[49]. NF-kB activation has been reported to be critical in tumor angiogenesis[50]. Though the efficacy of CTX can be significantly increased when administered in combination with anti-angiogenic drugs[51], whether NF-kB inhibition can inhibit angiogenesis in CTX has never been investigated. Our present study showed that NF-kB inhibition in CTX significantly reduced the CD31 positive staining in the tumors, indicating reduced angiogenesis.

In many solid tumor types, tumor-associated macrophages are important components of the tumor microenvironment. And macrophage infiltration is strongly associated with poor survival in solid tumor patients[52]. Here we demonstrated that CD206 positive M2-like tumor-associated macrophages were not reduced by either CTX or PTL administration, but both CTX and PTL increased the total F4/80 positive cells, this indicated the infiltration of CD206 negative macrophages, possibly M1 macrophages. Thus, CTX or PTL administration might boost the immune system. However, we did not observe the synergistic effect of the two drugs in macrophage recruitment. Proinflammatory factors IL-6 and TNF-a also have no significant difference among groups. Overall, these results indicate that PTL combined with CTX did not synergistically enhance the inflammatory reaction.

TGF-β expression in tumors enables cancer cells to undergo epithelial-to-mesenchymal transition and correlates with chemoresistance[53]. TGF-β expression in tumors hinders the release of cancer cell antigens, subverts dendritic cell function, reduces T cell trafficking and infiltration to tumors, antagonizes recognition of cancer cells by T cells, and hinders the killing of cancer cells[54]. TGF-β expression was directly or indirectly related to the treatment activity. High levels of TGF-β are associated with therapeutic resistance[55]. Elevated levels of TGF-β are associated with desmoplasia, which results from abnormal signaling of the TGF-β pathway and the remodeling of the microenvironment[54].

Desmoplasia refers to the dense extracellular matrix (ECM) composed of fibrous collagen, hyaluronic acid, fibrin, proteoglycan, and tendinous protein C[56], which can compress the blood vessels within the tumor, resulting in hypoperfusion, thus obstructing the delivery of therapeutic drugs, and renders the tumor hypoxic and drug resistant[54]. Here, our results indicated that NF-κB inhibition with PTL reduces TGF-β expression, which might render cancer cells sensitive to CTX.

α-SMA is another ECM and tissue-remolding-associated gene in cancer progression. Tumor cells that express α-SMA are predicted to be the cells that have an invasive nature and tend to metastasize[17]. Here we demonstrated that NF-kB inhibition with PTL reduced the proportion of α-SMA positive cells. It might be another mechanism of PTL's synergistic effect on CTX.

CONCLUSION

Thus, our present study found that NF-kB inhibition in CTX is not only involved in the balance of cell survival and cell death of tumor cells but also involved in the tumor microenvironment by reducing angiogenesis, macrophage infiltration, and inhibiting metastasis. In conclusion, our present study for the first time finds that as a specific inhibitor of NF-kB, PTL can promote the efficiency of CTX in lung cancer *via* inhibiting NF-kB signaling *in vitro* and *in vivo*. By directly promoting the cytotoxicity of CTX or improving the tumor microenvironment. Our present study will help scientists and clinicians to draw up novel metronomic chemotherapy strategies.

FOOTNOTES

Author contributions: Cai Z and Wang QM conceived and designed the experiments; Cai Z, Gao L, Hu K, and Wang QM performed the experiments; Cai Z and Gao L analyzed and interpreted the data; Hu K, and Wang QM contributed reagents/materials/analysis tools; Cai Z wrote original draft; Gao L, Hu K, and Wang QM reviewed and edited draft; All authors have read and agreed to the published version of the manuscript.

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REFERENCES

1 **Maiti R**. Metronomic chemotherapy. *J Pharmacol Pharmacother* 2014; **5**: 186-192 [PMID: 25210398 DOI: 10.4103/0976-500X.136098]
2 **Hanahan D**, Bergers G, Bergsland E. Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. *J Clin Invest* 2000; **105**: 1045-1047 [PMID: 10772648 DOI: 10.1172/JCI9872]
3 **Bocci G**, Kerbel RS. Pharmacokinetics of metronomic chemotherapy: a neglected but crucial aspect. *Nat Rev Clin Oncol* 2016; **13**: 659-673 [PMID: 27184418 DOI: 10.1038/nrclinonc.2016.64]
4 **Simsek C**, Esin E, Yalcin S. Metronomic Chemotherapy: A Systematic Review of the Literature and Clinical Experience. *J Oncol* 2019; **2019**:

5483791 [PMID: 31015835 DOI: 10.1155/2019/5483791]
5 **Cazzaniga ME**, Cordani N, Capici S, Cogliati V, Riva F, Cerrito MG. Metronomic Chemotherapy. *Cancers (Basel)* 2021; **13** [PMID: 34066606 DOI: 10.3390/cancers13092236]
6 **Emadi A**, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: golden anniversary. *Nat Rev Clin Oncol* 2009; **6**: 638-647 [PMID: 19786984 DOI: 10.1038/nrclinonc.2009.146]
7 **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]
8 **Fleming RA**. An overview of cyclophosphamide and ifosfamide pharmacology. *Pharmacotherapy* 1997; **17**: 146S-154S [PMID: 9322882]
9 **Mainetti LE**, Rico MJ, Fernández-Zenobi MV, Perroud HA, Roggero EA, Rozados VR, Scharovsky OG. Therapeutic efficacy of metronomic chemotherapy with cyclophosphamide and doxorubicin on murine mammary adenocarcinomas. *Ann Oncol* 2013; **24**: 2310-2316 [PMID: 23666914 DOI: 10.1093/annonc/mdt164]
10 **Stölting S**, Klink T, Bela C, Engels C, Wagner T. Metronomic scheduling of trofosfamide chemotherapy in human NSCLC xenografts highly increases therapeutic efficacy compared to conventional scheduling by inhibition of angiogenesis. *Int J Clin Pharmacol Ther* 2004; **42**: 652-653 [PMID: 15598036 DOI: 10.5414/CPP42652]
11 **Lebeau B**, Chouaïd C, Baud M, Masanès MJ, Febvre M. Oral second- and third-line lomustine-etoposide-cyclophosphamide chemotherapy for small cell lung cancer. *Lung Cancer* 2010; **67**: 188-193 [PMID: 19394109 DOI: 10.1016/j.lungcan.2009.03.024]
12 **Holoye PY**, Samuels ML. Cyclophosphamide, vincristine and sequential split-course radiotherapy in the treatment of small cell lung cancer. *Chest* 1975; **67**: 675-679 [PMID: 165045 DOI: 10.1378/chest.67.6.675]
13 **Demain AL**, Vaishnav P. Natural products for cancer chemotherapy. *Microb Biotechnol* 2011; **4**: 687-699 [PMID: 21375717 DOI: 10.1111/j.1751-7915.2010.00221.x]
14 **Pareek A**, Suthar M, Rathore GS, Bansal V. Feverfew (Tanacetum parthenium L.): A systematic review. *Pharmacogn Rev* 2011; **5**: 103-110 [PMID: 22096324 DOI: 10.4103/0973-7847.79105]
15 **Wu C**, Chen F, Rushing JW, Wang X, Kim HJ, Huang G, Haley-Zitlin V, He G. Antiproliferative activities of parthenolide and golden feverfew extract against three human cancer cell lines. *J Med Food* 2006; **9**: 55-61 [PMID: 16579729 DOI: 10.1089/jmf.2006.9.55]
16 **Wiedhopf RM**, Young M, Bianchi E, Cole JR. Tumor inhibitory agent from Magnolia grandiflora (Magnoliaceae). I. Parthenolide. *J Pharm Sci* 1973; **62**: 345 [PMID: 4686424 DOI: 10.1002/jps.2600620244]
17 **Anggorowati N**, Ratna Kurniasari Ch, Damayanti K, Cahyanti T, Widodo I, Ghozali A, Romi MM, Sari DC, Arfian N. Histochemical and Immunohistochemical Study of α-SMA, Collagen, and PCNA in Epithelial Ovarian Neoplasm. *Asian Pac J Cancer Prev* 2017; **18**: 667-671 [PMID: 28440973 DOI: 10.22034/APJCP.2017.18.3.667]
18 **Kwok BH**, Koh B, Ndubuisi MI, Elofsson M, Crews CM. The anti-inflammatory natural product parthenolide from the medicinal herb Feverfew directly binds to and inhibits IkappaB kinase. *Chem Biol* 2001; **8**: 759-766 [PMID: 11514225 DOI: 10.1016/S1074-5521(01)00049-7]
19 **Taniguchi K**, Karin M. NF-κB, inflammation, immunity and cancer: coming of age. *Nat Rev Immunol* 2018; **18**: 309-324 [PMID: 29379212 DOI: 10.1038/nri.2017.142]
20 **Nakanishi C**, Toi M. Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. *Nat Rev Cancer* 2005; **5**: 297-309 [PMID: 15803156 DOI: 10.1038/nrc1588]
21 **Zhou L**, Wu F, Jin W, Yan B, Chen X, He Y, Yang W, Du W, Zhang Q, Guo Y, Yuan Q, Dong X, Yu W, Zhang J, Xiao L, Tong P, Shan L, Efferth T. Theabrownin Inhibits Cell Cycle Progression and Tumor Growth of Lung Carcinoma through c-myc-Related Mechanism. *Front Pharmacol* 2017; **8**: 75 [PMID: 28289384 DOI: 10.3389/fphar.2017.00075]
22 **Li M**, Wang Q, Zhang X, Yan N, Li X. Exosomal miR-126 blocks the development of non-small cell lung cancer through the inhibition of ITGA6. *Cancer Cell Int* 2020; **20**: 574 [PMID: 33317527 DOI: 10.1186/s12935-020-01653-6]
23 **Chang TK**, Weber GF, Crespi CL, Waxman DJ. Differential activation of cyclophosphamide and ifosphamide by cytochromes P-450 2B and 3A in human liver microsomes. *Cancer Res* 1993; **53**: 5629-5637 [PMID: 8242617]
24 **Xie HJ**, Yasar U, Lundgren S, Griskevicius L, Terelius Y, Hassan M, Rane A. Role of polymorphic human CYP2B6 in cyclophosphamide bioactivation. *Pharmacogenomics J* 2003; **3**: 53-61 [PMID: 12629583 DOI: 10.1038/sj.tpj.6500157]
25 **Roy P**, Yu LJ, Crespi CL, Waxman DJ. Development of a substrate-activity based approach to identify the major human liver P-450 catalysts of cyclophosphamide and ifosfamide activation based on cDNA-expressed activities and liver microsomal P-450 profiles. *Drug Metab Dispos* 1999; **27**: 655-666 [PMID: 10348794]
26 **Chang TK**, Yu L, Goldstein JA, Waxman DJ. Identification of the polymorphically expressed CYP2C19 and the wild-type CYP2C9-ILE359 allele as low-Km catalysts of cyclophosphamide and ifosfamide activation. *Pharmacogenetics* 1997; **7**: 211-221 [PMID: 9241661 DOI: 10.1097/00008571-199706000-00006]
27 **Griskevicius L**, Yasar U, Sandberg M, Hidestrand M, Eliasson E, Tybring G, Hassan M, Dahl ML. Bioactivation of cyclophosphamide: the role of polymorphic CYP2C enzymes. *Eur J Clin Pharmacol* 2003; **59**: 103-109 [PMID: 12684728 DOI: 10.1007/s00228-003-0590-6]
28 **El-Serafi I**, Fares M, Abedi-Valugerdi M, Afsharian P, Moshfegh A, Terelius Y, Potácová Z, Hassan M. Cytochrome P450 2J2, a new key enzyme in cyclophosphamide bioactivation and a potential biomarker for hematological malignancies. *Pharmacogenomics J* 2015; **15**: 405-413 [PMID: 25601761 DOI: 10.1038/tpj.2014.82]
29 **Raccor BS**, Claessens AJ, Dinh JC, Park JR, Hawkins DS, Thomas SS, Makar KW, McCune JS, Totah RA. Potential contribution of cytochrome P450 2B6 to hepatic 4-hydroxycyclophosphamide formation in vitro and *in vivo*. *Drug Metab Dispos* 2012; **40**: 54-63 [PMID: 21976622 DOI: 10.1124/dmd.111.039347]
30 **Helsby NA**, Hui CY, Goldthorpe MA, Coller JK, Soh MC, Gow PJ, De Zoysa JZ, Tingle MD. The combined impact of CYP2C19 and CYP2B6 pharmacogenetics on cyclophosphamide bioactivation. *Br J Clin Pharmacol* 2010; **70**: 844-853 [PMID: 21175440 DOI: 10.1111/j.1365-2125.2010.03789.x]
31 **Helsby NA**, Yong M, van Kan M, de Zoysa JR, Burns KE. The importance of both CYP2C19 and CYP2B6 germline variations in cyclophosphamide pharmacokinetics and clinical outcomes. *Br J Clin Pharmacol* 2019; **85**: 1925-1934 [PMID: 31218720 DOI: 10.1111/bcp.14031]
32 **Freund RRA**, Gobrecht P, Rao Z, Gerstmeier J, Schlosser R, Görls H, Werz O, Fischer D, Arndt HD. Stereoselective total synthesis of parthenolides indicates target selectivity for tubulin carboxypeptidase activity. *Chem Sci* 2019; **10**: 7358-7364 [PMID: 31489157 DOI: 10.1039/c9sc01473j]

33 **Chen L**, Waxman DJ, Chen D, Kufe DW. Sensitization of human breast cancer cells to cyclophosphamide and ifosfamide by transfer of a liver cytochrome P450 gene. *Cancer Res* 1996; **56**: 1331-1340 [PMID: 8640822]

34 **Schwartz PS**, Waxman DJ. Cyclophosphamide induces caspase 9-dependent apoptosis in 9L tumor cells. *Mol Pharmacol* 2001; **60**: 1268-1279 [PMID: 11723234 DOI: 10.1124/mol.60.6.1268]

35 **Doloff JC**, Waxman DJ. Adenoviral vectors for prodrug activation-based gene therapy for cancer. *Anticancer Agents Med Chem* 2014; **14**: 115-126 [PMID: 23869779 DOI: 10.2174/18715206113139990309]

36 **Dynes J**, Osz K, Hooper A, Petrik J. Low-dose metronomic delivery of cyclophosphamide is less detrimental to granulosa cell viability, ovarian function, and fertility than maximum tolerated dose delivery in the mouse. *Biol Reprod* 2017; **97**: 449-465 [PMID: 29024988 DOI: 10.1093/biolre/iox110]

37 **Jin X**, Zhou J, Zhang Z, Lv H. The combined administration of parthenolide and ginsenoside CK in long circulation liposomes with targeted tLyp-1 ligand induce mitochondria-mediated lung cancer apoptosis. *Artif Cells Nanomed Biotechnol* 2018; **46**: S931-S942 [PMID: 30307334 DOI: 10.1080/21691401.2018.1518913]

38 **Sweeney CJ**, Mehrotra S, Sadaria MR, Kumar S, Shortle NH, Roman Y, Sheridan C, Campbell RA, Murry DJ, Badve S, Nakshatri H. The sesquiterpene lactone parthenolide in combination with docetaxel reduces metastasis and improves survival in a xenograft model of breast cancer. *Mol Cancer Ther* 2005; **4**: 1004-1012 [PMID: 15956258 DOI: 10.1158/1535-7163.MCT-05-0030]

39 **Li X**, Yang H, Ke J, Liu B, Lv X, Li X, Zhang Y. Smad4 re-expression increases the sensitivity to parthenolide in colorectal cancer. *Oncol Rep* 2017; **38**: 2317-2324 [PMID: 28902368 DOI: 10.3892/or.2017.5929]

40 **Rizeq B**, Gupta I, Ilesanmi J, AlSafran M, Rahman MM, Ouhtit A. The Power of Phytochemicals Combination in Cancer Chemoprevention. *J Cancer* 2020; **11**: 4521-4533 [PMID: 32489469 DOI: 10.7150/jca.34374]

41 **Jeong Y**, Lee JL. Efficacy of metronomic oral cyclophosphamide with low dose dexamethasone and celecoxib in metastatic castration-resistant prostate cancer. *Asia Pac J Clin Oncol* 2017; **13**: 204-211 [PMID: 27521286 DOI: 10.1111/ajco.12583]

42 **Haubitz M**, Bohnenstengel F, Brunkhorst R, Schwab M, Hofmann U, Busse D. Cyclophosphamide pharmacokinetics and dose requirements in patients with renal insufficiency. *Kidney Int* 2002; **61**: 1495-1501 [PMID: 11918757 DOI: 10.1046/j.1523-1755.2002.00279.x]

43 **Jali AM**, Alam MF, Hanbashi A, Mawkili W, Abdlasaed BM, Alshahrani S, Qahl AM, Alrashah ASS, Shahi HA. Sesamin's Therapeutic Actions on Cyclophosphamide-Induced Hepatotoxicity, Molecular Mechanisms, and Histopathological Characteristics. *Biomedicines* 2023; **11** [PMID: 38137459 DOI: 10.3390/biomedicines11123238]

44 **Aggarwal BB**, Sung B. NF-κB in cancer: a matter of life and death. *Cancer Discov* 2011; **1**: 469-471 [PMID: 22586649 DOI: 10.1158/2159-8290.CD-11-0260]

45 **Godwin P**, Baird AM, Heavey S, Barr MP, O'Byrne KJ, Gately K. Targeting nuclear factor-kappa B to overcome resistance to chemotherapy. *Front Oncol* 2013; **3**: 120 [PMID: 23720710 DOI: 10.3389/fonc.2013.00120]

46 **Man S**, Bocci G, Francia G, Green SK, Jothy S, Hanahan D, Bohlen P, Hicklin DJ, Bergers G, Kerbel RS. Antitumor effects in mice of low-dose (metronomic) cyclophosphamide administered continuously through the drinking water. *Cancer Res* 2002; **62**: 2731-2735 [PMID: 12019144]

47 **El-Sheikh AA**, Morsy MA, Okasha AM. Inhibition of NF-κB/TNF-α pathway may be involved in the protective effect of resveratrol against cyclophosphamide-induced multi-organ toxicity. *Immunopharmacol Immunotoxicol* 2017; **39**: 180-187 [PMID: 28463035 DOI: 10.1080/08923973.2017.1318913]

48 **Makrilia N**, Lappa T, Xyla V, Nikolaidis I, Syrigos K. The role of angiogenesis in solid tumours: an overview. *Eur J Intern Med* 2009; **20**: 663-671 [PMID: 19818284 DOI: 10.1016/j.ejim.2009.07.009]

49 **Majidpoor J**, Mortezaee K. Angiogenesis as a hallmark of solid tumors - clinical perspectives. *Cell Oncol (Dordr)* 2021; **44**: 715-737 [PMID: 33835425 DOI: 10.1007/s13402-021-00602-3]

50 **Tabruyn SP**, Griffioen AW. NF-kappa B: a new player in angiostatic therapy. *Angiogenesis* 2008; **11**: 101-106 [PMID: 18283548 DOI: 10.1007/s10456-008-9094-4]

51 **Kerbel RS**, Kamen BA. The anti-angiogenic basis of metronomic chemotherapy. *Nat Rev Cancer* 2004; **4**: 423-436 [PMID: 15170445 DOI: 10.1038/nrc1369]

52 **Chen Y**, Song Y, Du W, Gong L, Chang H, Zou Z. Tumor-associated macrophages: an accomplice in solid tumor progression. *J Biomed Sci* 2019; **26**: 78 [PMID: 31629410 DOI: 10.1186/s12929-019-0568-z]

53 **Mousset A**, Lecorgne E, Bourget I, Lopez P, Jenovai K, Cherfils-Vicini J, Dominici C, Rios G, Girard-Riboulleau C, Liu B, Spector DL, Ehmsen S, Renault S, Hego C, Mechta-Grigoriou F, Bidard FC, Terp MG, Egeblad M, Gaggioli C, Albrengues J. Neutrophil extracellular traps formed during chemotherapy confer treatment resistance *via* TGF-β activation. *Cancer Cell* 2023; **41**: 757-775.e10 [PMID: 37037615 DOI: 10.1016/j.ccell.2023.03.008]

54 **Larson C**, Oronsky B, Carter CA, Oronsky A, Knox SJ, Sher D, Reid TR. TGF-beta: a master immune regulator. *Expert Opin Ther Targets* 2020; **24**: 427-438 [PMID: 32228232 DOI: 10.1080/14728222.2020.1744568]

55 **Faivre SJ**, Santoro A, Gane E, Kelley RK, Hourmand IO, Assenat E, Gueorguieva I, Cleverly A, Desaiah D, Lahn MMF, Raymond E, Benhadji KA, Giannelli G. A phase 2 study of galunisertib, a novel transforming growth factor-beta (TGF-β) receptor I kinase inhibitor, in patients with advanced hepatocellular carcinoma (HCC) and low serum alpha fetoprotein (AFP). *JCO* 2016; **34**: 4070-4070 [DOI: 10.1200/JCO.2016.34.15_suppl.4070]

56 **Gkretsi V**, Stylianou A, Papageorgis P, Polydorou C, Stylianopoulos T. Remodeling Components of the Tumor Microenvironment to Enhance Cancer Therapy. *Front Oncol* 2015; **5**: 214 [PMID: 26528429 DOI: 10.3389/fonc.2015.00214]

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SYSTEMATIC REVIEWS

Investigating the therapeutic efficacy of psilocybin in advanced cancer patients: A comprehensive review and meta-analysis

Husam Bader, Husam Farraj, Joud Maghnam, Yazan Abu Omar

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Abstract

BACKGROUND

Psilocybin, a naturally occurring psychedelic compound found in certain species of mushrooms, is known for its effects on anxiety and depression. It has recently gained increasing interest for its potential therapeutic effects, particularly in patients with advanced cancer. This systematic review and meta-analysis aim to evaluate the effects of psilocybin on adult patients with advanced cancer.

AIM

To investigate the therapeutic effect of psilocybin in patients with advanced cancer.

METHODS

A comprehensive search of electronic databases was conducted in PubMed, Cochrane Central Register of Controlled Trials, and Google Scholar for articles published up to February 2023. The reference lists of the included studies were also searched to retrieve possible additional studies.

RESULTS

A total of 7 studies met the inclusion criteria for the systematic review, comprising 132 participants. The results revealed significant improvements in quality of life, pain control, and anxiety relief following psilocybin-assisted therapy, specifically results on anxiety relief. Pooled effect sizes indicated statistically significant reductions in symptoms of anxiety at both 4 to 4.5 months [35.15 (95%CI: 32.28-38.01)] and 6 to 6.5 months [33.06 (95%CI: 28.73-37.40)]. Post-administration compared to baseline assessments ($P < 0.05$). Additionally, patients reported sustained improvements in psychological well-being and existential distress fo-

llowing psilocybin therapy.

CONCLUSION
The findings provided compelling evidence for the potential benefits of psilocybin-assisted therapy in improving quality of life, pain control, and anxiety relief in patients with advanced cancer.

Key Words: Quality of life; Advanced cancer; Psilocybin; Systemic review; Meta analysis

Core Tip: Psilocybin-assisted therapy shows promising results in improving quality of life, pain control, and anxiety relief for patients with advanced cancer. This systematic review and meta-analysis of 7 studies involving 132 participants demonstrated significant reductions in anxiety symptoms and sustained improvements in psychological well-being following psilocybin therapy. These findings highlight the potential therapeutic benefits of psilocybin in palliative care settings.

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INTRODUCTION

In recent years, there has been an increasing interest in exploring alternative therapeutic approaches to address the complex physical, psychological, and existential distress experienced by patients with advanced cancers (Mok *et al*[1], 2010; Henoch and Danielson[2], 2009). Among these approaches, the use of psychedelics, particularly psilocybin, has gained considerable attention for its potential to alleviate symptoms such as depression, anxiety, and existential distress, while also facilitating profound spiritual experiences and promoting existential well-being (Schimmers *et al*[3], 2022). Advanced cancer presents a myriad of challenges for patients and healthcare providers alike. The progression of the disease is often accompanied by a spectrum of physical symptoms, including pain, nausea, fatigue, and shortness of breath, which can significantly impact patients' quality of life and functional status (Paice and Ferrell[4], 2011; Gupta *et al* [5], 2007). Moreover, the psychological burden of living with advanced cancer is substantial, with many patients experiencing heightened levels of anxiety, depression, and existential distress as they confront the uncertainties of their prognosis and the existential implications of their illness (Vehling and Kissane[6], 2018 and Greer *et al*[7], 2020).

Conventional treatment approaches for advanced cancer typically involve a combination of modalities such as radiation therapy, chemotherapy, surgery, and palliative care interventions. While these treatments aim to control disease progression, alleviate symptoms, and improve overall well-being, they often fall short in addressing the multidimensional needs of patients. Despite advancements in medical technology and therapeutic strategies, many individuals with advanced cancer continue to experience unmet physical, psychosocial, and spiritual needs throughout their illness (Holland[8], 2002). Although conventional treatments can effectively target cancer burden and manage certain symptoms, they are frequently limited in their ability to address the holistic needs of patients. For example, while chemotherapy may shrink tumors and alleviate pain, it can also cause debilitating side effects such as nausea, vomiting, and neuropathy, further compromising patients' quality of life (Anand *et al*[9], 2022; Carey and Burish[10], 1988). Similarly, while palliative care focuses on improving symptom management and enhancing quality of life, it may not adequately address the existential distress and spiritual suffering that often accompany advanced cancer (Boston *et al*[11], 2011).

In recent years, however, there has been a remarkable shift in attitudes toward psychedelics, fueled in part by a re-evaluation of their therapeutic potential and a burgeoning body of scientific evidence supporting their safety and efficacy (Sessa[12], 2014). Advances in neuroimaging technology and psychopharmacology have shed new light on the mechanisms of action underlying psychedelic-induced alterations in consciousness, revealing their profound effects on brain function, cognition, and emotion regulation (Moujaes *et al*[13], 2023). Central to this resurgence has been the concept of psychedelic-assisted therapy, which combines the administration of a psychedelic substance with psychotherapeutic support to facilitate profound psychological insights, emotional processing, and therapeutic breakthroughs. Emerging research suggests that psychedelic-assisted therapy holds promise for a wide range of mental health conditions, including treatment-resistant depression, post-traumatic stress disorder (PTSD), and illicit substance use disorders (Reiff *et al*[14], 2020; Schenberg[15], 2018).

In the field of oncology, the potential utility of psychedelics therapy is particularly compelling, given the profound psychological and existential distress experienced by patients with advanced cancer. By facilitating transformative experiences, enhancing existential coping mechanisms, and promoting spiritual well-being, psychedelic therapy has the potential to complement existing cancer treatments and improve the overall quality of life for patients facing life-threatening illnesses. Psilocybin, a psychedelic chemical that's naturally found in certain species of mushrooms, has emerged as a focal point of interest in the context of advanced cancer care due to its unique pharmacological properties

and demonstrated therapeutic potential (Grob *et al*[16], 2011; Griffiths *et al*[17], 2016). Unlike traditional pharmacotherapies, which primarily target symptoms through direct modulation of neurotransmitter systems, psilocybin acts as a serotonin receptor agonist, particularly at the 5-HT2A receptor, leading to profound alterations in consciousness, perception, and self-awareness (Rahbarnia *et al*[18], 2023 and Carter *et al*[19], 2005).

Research into the effects of psilocybin has shown therapeutic potential for a different range of psychiatric and existential conditions, including depression, anxiety, addiction, and existential distress. In the context of advanced cancer care, where patients grapple with the existential realities of mortality, identity, and meaning, psilocybin therapy offers a novel approach to addressing the multidimensional needs of this population. Existing systematic reviews and meta-analyses have mainly focused on the broader application of psychedelics in psychiatric disorders, such as depression and PTSD, rather than specifically examining the effects of psilocybin in patients with advanced cancer This emphasis gains significance given the progressive strides in cancer therapy and the emergence of novel medications, which have not been paralleled by commensurate investigations into interventions aimed at enhancing the quality of life for individuals grappling with advanced cancer. As a result, there is a true need for a thorough synthesis of the available evidence to clarify the effects of psilocybin on psychological outcomes, existential well-being, and quality of life in this vulnerable patient population. The purpose of this systematic review and meta-analysis is to address this research gap by critically evaluating the existing literature on the use of psilocybin.

MATERIALS AND METHODS

Research question

What is the impact of psilocybin therapy on psychological distress, existential concerns, and quality of life in adult patients with advanced cancer?

Methods

This systematic review and meta-analysis are reported following guidelines outlined in Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (Page *et al*[20], 2021).

Information sources and study selection

A comprehensive systematic search of the literature was carried out, encompassing articles released until February 1st, 2023. Primary databases including PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), and Google Scholar were systematically explored using specific topic-related keywords to construct search queries. Following this, identified studies underwent a rigorous selection process.

Primary search

In formulating the search strategy for PubMed, a blend of free keyword searches and controlled MeSH terms was utilized to ensure comprehensive coverage. Keyword searches were tailored to scrutinize the entirety of the text, augmenting the sensitivity of the search strategy. For CENTRAL, slight modifications were made to adapt the PubMed strategy accordingly. Table 1 illustrates the assortment of keywords employed for each search, delineating the approach for comprehensive literature retrieval.

Secondary search

In addition to searching the specified databases, a direct inquiry was conducted using the Google Scholar database. To ensure the retrieval of the most relevant outcomes within the initial pages, the exploration integrated precise terms associated with the topic "psilocybin in patients with advanced cancer", encompassing a range of keywords including Psilocybin, Psilocybine, Hallucinogens, Serotonin agonists, Mushroom poisoning, Neoplasms, Cancer, Oncology, Neoplasm metastasis, Neoplasm staging, Neoplasm recurrence (local), Disease progression, Palliative care, Terminal care, Hospice care, Terminal illness, Quality of life, Symptom assessment, Symptom management and Psychological adaptation to identify any relevant articles.

Eligibility criteria

The research question for this study was developed from the Population Intervention Comparison Outcomes Study Design (PICOS) framework. The PICOS criteria for eligible studies were defined as follows:

Population (P): Adults diagnosed with advanced cancer.

Intervention (I): Administration of psilocybin or psilocybin-containing substances.

Comparison (C): Not applicable (as this review primarily focuses on single-arm studies)

Outcomes (O): Reporting outcomes related to quality of life, pain control, or anxiety relief.

Study design (S): Including randomized controlled trials, quasi-experimental studies, observational studies, and case series published in English-language peer-reviewed journals.

Inclusion criteria

All studies had to meet the following pre-defined inclusion criteria: (1) Original studies; (2) Studies involving adult patients diagnosed with advanced cancer; (3) Interventions that involve the administration of psilocybin or psilocybin-containing substances; (4) Studies reporting outcomes related to quality of life, pain control, or anxiety relief; (5) Ran-

Table 1 Search strings

Database	Search field	Search string
PubMed	Title, abstract	(psilocybin OR psilocybine OR hallucinogens OR serotonin agonists OR mushroom poisoning) AND (neoplasms OR cancer OR oncology OR neoplasm metastasis OR neoplasm staging OR neoplasm recurrence, local OR disease progression OR palliative care OR terminal care OR hospice care OR terminal illness OR quality of life)
CENTRAL	All fields	(psilocybin OR magic mushrooms) AND (cancer OR oncology) AND (advanced OR metastatic)

CENTRAL: Cochrane Central Register of Controlled Trials.

domized controlled trials, quasi-experimental studies, observational studies, and case series; (6) Studies published in peer-reviewed journals; (7) Studies published in the English language; and (8) Studies conducted on human subjects.

Exclusion criteria

Studies that satisfied the following criteria were excluded: (1) Studies involving pediatric patients or patients without advanced cancer; (2) Studies without clear methodology or outcome measures; (3) Studies not published in peer-reviewed journals (*e.g.*, conference abstracts, posters); (4) Animal studies or *in vitro* studies; or (5) Duplicate publications or multiple reports from the same study (only the most comprehensive report will be included).

Data extraction

We utilized a systematic methodology to gather information from the studies included in our analysis. In this study, the data extraction process was conducted by two independent reviewers (Husam B and Farraj H) utilizing a predefined form specifically designed for this purpose. Any discrepancies or inconsistencies between the reviewers were addressed through constructive dialogue, with careful reference to the predetermined criteria outlined for the study. In instances where disagreements persisted, resolution was facilitated by third-party arbitration, overseen by reviewer Abu Omar Y. This approach ensured strict adherence to established guidelines and served to mitigate potential subjective biases inherent in the analysis process. The extracted data included the following categories: (1) Author name; (2) Publication year; (3) Study design; (4) Sample size; (5) Mean age (SD); (6) Cancer diagnosis; (7) Intervention details; and (8) Findings.

Handling data from studies with multiple reports

In instances where studies were accompanied by multiple reports, we diligently examined all accessible publications and chose the most pertinent one for integration into our analysis. If several reports were incorporated for a singular study, we took measures to avoid data redundancy and worked to amalgamate information across these reports. This approach was adopted to uphold the integrity and thoroughness of our analysis.

Quality appraisal

The Cochrane Handbook's Risk of Bias assessment tool will be employed to evaluate randomized controlled trials (Higgins *et al*[21], 2011). Each study will undergo scrutiny and categorization into "high risk", "low risk", or "unclear" across various domains, including random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other potential biases.

Statistical analysis

In our meta-analysis, we employed a single proportion rate to assess the efficacy of anxiety relief measured by The State-Trait Anxiety Inventory (STAI) TRAIT scale. For categorical factors, proportions along with their corresponding 95%CIs were calculated, while mean or median values were determined for continuous data whenever possible. Pooled means and proportions were then computed utilizing random-effects models, accounting for the homogeneity or heterogeneity of the included studies. Statistical heterogeneity was evaluated using I^2 statistics and Cochran Q test values, with an I^2 value exceeding 50% indicative of high statistical heterogeneity ($I^2 > 50\%$ and $P < 0.05$). A forest plot was generated to assess the potential presence of publication bias. All statistical analyses were performed using R software (version 4.3.2).

Our meta-analysis primarily focused on assessing the impact of psilocybin on anxiety levels. It is important to note that while several studies examined various effects of psilocybin on patients with advanced cancers beyond anxiety, the heterogeneity in symptom reporting and study structures precluded achieving consistent homogeneity necessary for conducting a meta-analysis across all these symptoms. For instance, while some studies reported on "existential well-being", others focused on "quality of life". Although these concepts may exhibit proximity or overlap in definition, deeming them interchangeable for mathematical meta-analysis could introduce ambiguity. Consequently, the investigators opted for a more stringent approach in their quantitative analysis to maintain methodological rigor.

RESULTS

Search results

The primary search yielded a total of 6291 articles across the databases: 14 from CENTRAL, 6077 from PubMed, and 200 from Google Scholar. Following the removal of 453 duplicate articles, 5810 articles were excluded during the title and abstract screening phase based on the eligibility criteria. Subsequently, the remaining 23 articles underwent full-text review, resulting in the exclusion of 16 articles due to incomplete fulfilment of the inclusion criteria. Ultimately, 7 studies were included in the systematic review, while 3 studies were incorporated into the meta-analysis. The rationales for exclusion are delineated in the PRISMA flowchart depicted in Figure 1.

Results of quality appraisal

Four of the randomized clinical trials (RCTs) were deemed to have low-risk overall quality, while one trial raised some concerns in this regard. This assessment is depicted in Figure 2.

Results of data extraction

Characteristics of included studies: As shown in Table 2, this paper includes findings from a total of 7 studies, comprising 5 RCTs, 1 observational study, and 1 qualitative interview study. Across these studies, the sample sizes varied, with RCTs ranging from 11 to 51 participants, while the observational study involved a single participant. In terms of gender distribution, the percentage of male participants ranged from 0% in the observational study to 54% in the qualitative interview study. The mean ages of participants ranged from 50 to 60.3 years across the studies. The cancer diagnoses encompassed a wide spectrum, including breast, reproductive, lymphoma, colon, ovarian, peritoneal, salivary gland, multiple myeloma, and other types of cancer. Intervention details varied among the studies, with treatments including psilocybin administration at different dosages, niacin as a placebo, and qualitative interviews exploring participants' experiences with psilocybin therapy.

Results of included studies: In a study conducted by Agin-Liebes *et al*[22], a randomized controlled trial explored the long-term effects within a subset of participants who had completed the initial trial. Out of the 16 participants still living, all were approached for follow-up, with 15 agreeing to participate. The follow-up assessments were conducted on average at 3.2- and 4.5 years post-psilocybin administration. The findings revealed sustained reductions in symptoms of anxiety, depression, despair, and death anxiety at both follow-up points. Furthermore, a significant majority of participants attributed positive life changes to their experience with psilocybin-assisted therapy, rating it as a profound, personally meaningful, and spiritually insightful experience. The authors concluded that psilocybin-assisted psychotherapy shows promise in providing long-term relief from psychiatric distress related to cancer. A study conducted by Griffiths *et al*[17] explored the potential of psilocybin in reducing depression and anxiety among cancer patients. The authors found that administering high doses of psilocybin led to significant reductions in reported levels of depressed mood and anxiety. Additionally, participants reported improvements in their quality of life, sense of life meaning, and optimism, while experiencing a decrease in death anxiety. These positive effects were maintained at the 6-month follow-up, with approximately 80% of participants still showing clinically significant decreases in depression and anxiety. Participants attributed their improved attitudes towards self, mood, life, personal relationships, and spirituality to their experiences with high-dose psilocybin, with over 80% reporting moderate to substantial increases in overall well-being and life satisfaction.

Another investigation conducted by Grob *et al*[16] focused on examining the safety and effectiveness of psilocybin in individuals with advanced-stage cancer and reactive anxiety. The researchers noted a positive trend toward enhanced mood and reduced anxiety. No clinically significant adverse events were reported in association with psilocybin administration. Analysis of the State-Trait Anxiety Inventory trait anxiety subscale indicated a significant decrease in anxiety levels at 1- and 3 months post-treatment. The Beck Depression Inventory also highlighted a mood improvement that became significant at the 6-month mark. Furthermore, the profile of mood states identified an enhancement in mood following psilocybin treatment, although it did not reach statistical significance. According to a case report by Patchett-Marble *et al*[23], similar to findings in clinical trials involving psilocybin, a single session of psilocybin induced a mystical encounter for the patient. This encounter was later described by the patient as the most profoundly meaningful experience of her life. As a result, there were immediate, significant, and enduring enhancements observed in her distress levels and overall quality of life. In an RCT conducted by Ross *et al*[24] in 2021, the efficacy of a single dose of psilocybin, combined with psychotherapy, in generating immediate and lasting ant-suicidal effects among advanced cancer patients was examined. The authors found that exploratory analyses corroborated the hypothesis, indicating that psilocybin-assisted psychotherapy could potentially serve as a valuable intervention against suicidality following a cancer diagnosis. This conclusion was drawn based on the therapy's observed positive influence on feelings of hopelessness and demoralization, with particular emphasis on its effects on meaning-making.

A study conducted by Ross *et al*[25] found that psilocybin elicited immediate, considerable, and lasting improvements in anxiety and depression, while also decreasing cancer-related demoralization and hopelessness. Additionally, it enhanced spiritual well-being and elevated overall quality of life. Follow-up examinations at the 6.5-month mark revealed persistent anxiolytic and antidepressant effects, with approximately 60%-80% of participants maintaining clinically significant reductions in depression or anxiety. Moreover, sustained enhancements were observed in existential distress and quality of life, accompanied by a positive shift in attitudes toward death. The therapeutic impact of psilocybin on anxiety and depression was noted to be mediated by the psilocybin-induced mystical experience. The authors concluded that when combined with psychotherapy, a single moderate dose of psilocybin yielded rapid, robust, and enduring

Table 2 Study descriptor table

Ref.	Publication year	Study design	Sample size	Mean age (SD)	Cancer diagnosis	Intervention details	Findings
Agin-Liebes *et al*[22]	2020	Randomized controlled trial	15, 40% Male	53 (15.5)	Various cancer types (breast, Reproductive, Lymphoma, and other types), stage I to IV	Psilocybin (0.3 mg/kg) on the first medication session followed by niacin (250 mg) on the second session (*i.e.* psilocybin-first group), or niacin (250 mg) on the first medication session followed by psilocybin (0.3 mg/kg) on the second session (*i.e.* niacin-first group)	Reductions in anxiety, depression, hopelessness, demoralization, and death anxiety were sustained at the first and second follow-ups. Participants overwhelmingly attributed positive life changes to the psilocybin-assisted therapy experience and rated it among the most personally meaningful and spiritually significant experiences of their lives
Griffiths *et al*[17]	2016	Randomized controlled trial	51, 51% Male	56.3	All 51 participants had a potentially life-threatening cancer diagnosis, with 65% having recurrent or metastatic disease. Types of cancer included breast (13 participants), upper aerodigestive (7), gastrointestinal (4), genitourinary (18), hematologic malignancies (8), and other (1)	The low-dose-1st group received the low dose (1 or 3 mg/70 kg) of psilocybin on the first session and the high dose on the second session, whereas the high-dose-1st group (22 or 30 mg/70 kg) received the high dose on the first session and the low dose on the second session	High-dose psilocybin produced large decreases in clinician- and self-rated measures of depressed mood and anxiety, along with increases in quality of life, life meaning, and optimism, and decreases in death anxiety. At 6-month follow-up, these changes were sustained, with about 80% of participants continuing to show clinically significant decreases in depressed mood and anxiety. Participants attributed improvements in attitudes about life/self, mood, relationships, and spirituality to the high-dose experience, with > 80% endorsing moderately or greater increased well-being/life satisfaction
Grob *et al* [16]	2011	Randomized controlled trial	12, 8% Male	Subjects ages ranged from 36 to 58 years	Primary cancers included breast cancer in 4 subjects, colon cancer in 3, ovarian cancer in 2, peritoneal cancer in 1, salivary gland cancer in 1, and multiple myeloma in 1. All subjects were in the advanced stages of their illness.	Each subject acted as his or her control and was provided 2 experimental treatment sessions spaced several weeks apart. They were informed that they would receive active psilocybin (0.2 mg/kg) on one occasion and the placebo, niacin (250 mg), on the other occasion. Psilocybin and placebo were administered in clear 00 capsules with corn starch and swallowed with 100 mL of water. A niacin placebo was chosen because it often induces a mild physiological reaction (*e.g.*, flushing) without altering the psychological state. The order in which subjects received the 2 different treatments was randomized and known only by the research pharmacist. Treatment team personnel remained at the bedside with the subject for the entire 6-hour session	During treatment sessions, safe physiological and psychological reactions were observed. No significant adverse events related to psilocybin were reported. The State-Trait Anxiety Inventory showed a notable decrease in anxiety levels at 1 and 3 months post-treatment. Improvement in mood, as measured by the Beck Depression Inventory, became significant by the 6-month mark. Additionally, the Profile of Mood States indicated an enhancement in mood following psilocybin treatment, although this improvement did not quite reach statistical significance
Patchett-Marble *et al*[23]	2022	Observational study	1, 0% Male	54	Advanced lung cancer and substantial existential and psychological distress	The patient consumed 5 g of dried psilocybin mushrooms as a tea and was directed to go inward as she laid down with eye shades on and headphones playing gentle, guiding music. A quantity of 5 g was selected to approximate the dose of psilocybin used in clinical trials, based on reports of psilocybin concentrations in the Psilocybe cubensis mushrooms that she was to consume	In line with psilocybin administration in clinical studies, the single psilocybin session prompted a mystical encounter for the patient, which she later regarded as the most profoundly meaningful experience of her life. This encounter resulted in immediate, significant, and lasting enhancements in her well-being and overall quality of life
Ross *et al* [24]	2021	Randomized controlled trial	11, 36.4% Male	60.3 (7.1)	Patients were diagnosed with cancer at various sites, including breast, reproductive, lymphoma/leukemia, colon, and others, across stages I through IV	A controlled trial was designed to assess the efficacy of a single, moderate-to-high dose of oral psilocybin per session (0.3 mg/kg) *vs* a single-dose session of an orally administered active control (niacin 250 mg)	In individuals exhibiting elevated SI at baseline, PAP demonstrated significant reductions in suicidal ideation as early as 8 hours post-administration, persisting for 6.5 months thereafter. Additionally, PAP led to substantial decreases in Loss of Meaning from baseline, evident 2 weeks post-

							treatment and maintaining significance at 6.5 months, as well as at the 3.2 and 4.5-year follow-ups. Exploratory analyses support the hypothesis that PAP could serve as an effective intervention against suicidality following a cancer diagnosis, attributed to its positive effects on hopelessness, demoralization, and particularly its impact on meaning-making
Ross *et al* [25]	2016	Randomized controlled trial	29, 38% Male	56.28 (12.93)	Nearly two-thirds of participants (62%) had advanced cancers (stages III or IV). The types of cancer included: Breast or reproductive (59%); gastrointestinal (17%); hematologic (14%); and other (10%)	Psilocybin (0.3 mg/kg) first then niacin (250 mg) second, or niacin (250 mg) first then psilocybin (0.3 mg/kg) second	Psilocybin elicited immediate, significant, and enduring enhancements in anxiety and depression levels, alongside reductions in cancer-related demoralization and hopelessness. It also resulted in improved spiritual well-being and heightened quality of life. Follow-up assessments at 6.5 months revealed persistent anxiolytic and antidepressant effects, with approximately 60-80% of participants maintaining clinically significant reductions in depression or anxiety. Furthermore, sustained improvements were noted in existential distress and overall quality of life, along with a positive shift in attitudes towards death. It was observed that the therapeutic impact of psilocybin on anxiety and depression was mediated by the psilocybin-induced mystical experience
Swift *et al* [26]	2017	Qualitative interview study	13, 54% male	50 (15.77)	The distribution of cancer stages among participants is as follows: 31% were diagnosed with Stage I cancer, 15% with Stage II, 31% with Stage III, 15% with Stage IV, and 8% with other stages. Regarding the site of cancer, the breakdown is as follows: 23% of cases were in the breast, 15% in lymphoma, 31% in other locations, and 31% in the ovarian region	Participants were randomized to receive either: (1) Psilocybin (0.3 mg/kg) first and niacin (250 mg) second; or (2) niacin (250 mg) first and psilocybin (0.3 mg/kg) second	Participants recounted the intense and emotionally challenging impact of the psilocybin session, resulting in a profound acceptance of mortality, recognition of cancer's role in life, and a detachment from the emotional burden of the disease. Many participants interpreted their experiences through a spiritual or religious lens, finding that psilocybin therapy aided in reestablishing a deep connection with life, reclaiming a sense of presence, and fostering increased resilience against potential cancer relapse

SI: Suicidal ideation; PAP: Psilocybin-assisted psychotherapy.

anxiolytic and antidepressant effects in patients experiencing psychological distress related to cancer. A study by Swift *et al*[26] examined the effectiveness of psilocybin-assisted psychotherapy for cancer patients, revealing significant reductions in anxiety and depression, alongside improvements in attitudes toward disease progression and death, quality of life, and spirituality. The authors reported that their findings provided evidence supporting the efficacy of psilocybin-assisted psychotherapy, a treatment approach distinguished by its ability to swiftly and significantly alleviate anxiety and depression, while also fostering profound and enduring experiences that offer new perspectives for individuals grappling with the existential challenges posed by cancer. Consequently, the authors deduced that the psilocybin-assisted psychotherapy paradigm holds promise as a complementary approach to delivering medical and psychological care for individuals facing cancer diagnoses, particularly those grappling with profound psychological and existential distress.

Meta-analysis

The Figure 3 illustrates the changes in anxiety levels, as measured by the STAI scale, at two-time points following the administration of psilocybin to patients with advanced cancer. At the initial assessment conducted 4 to 5 months after psilocybin administration, the pooled mean anxiety level was 35.15 (95%CI: 32.28-38.01). Subsequent evaluation at 6 to 6.5

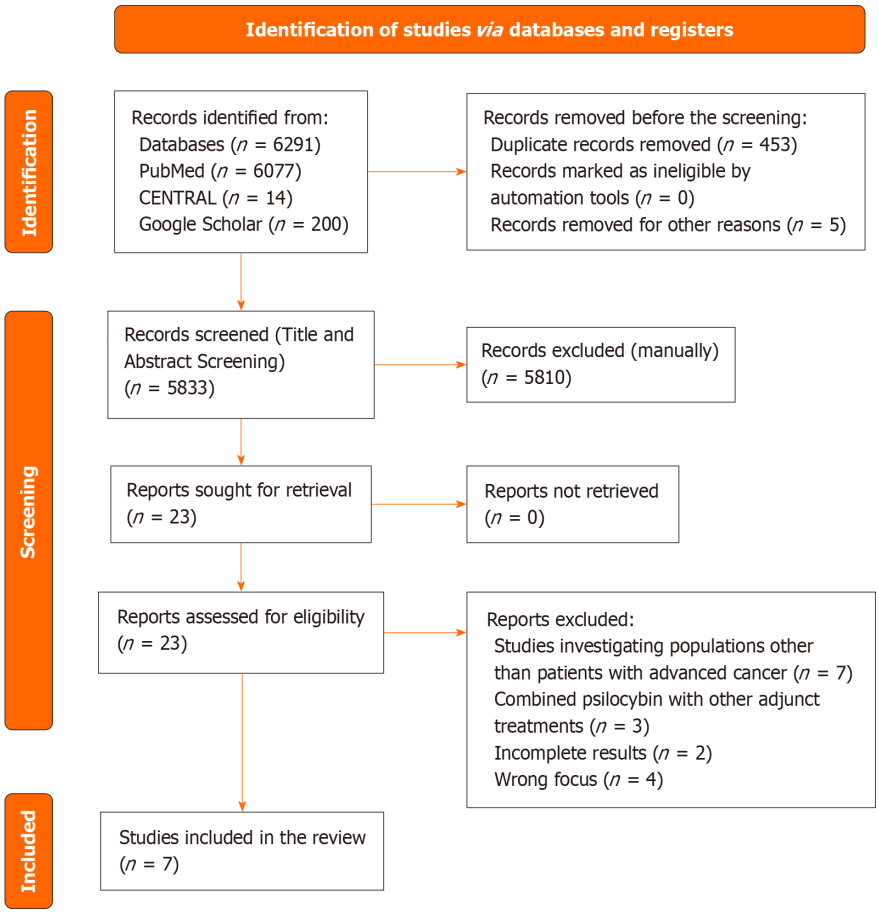

Figure 1 The preferred reporting items for systematic review and meta-analysis flowchart showing the study selection process. CENTRAL: Cochrane Central Register of Controlled Trials.

months post-administration revealed a decrease in the pooled mean anxiety level to 33.06 (95%CI: 28.73-37.40). The observed decrease in anxiety levels suggested a potential therapeutic effect of psilocybin in mitigating anxiety among patients with advanced cancer over time. The non-overlapping confidence intervals between the two-time points indicated a statistically significant difference in anxiety levels.

DISCUSSION

Psilocybin, a naturally occurring psychedelic compound found in certain species of mushrooms, has gained increasing attention in recent years for its potential therapeutic effects, particularly in the context of palliative care for patients with advanced cancer. Considering this growing interest, the present systematic review and meta-analysis sought to investigate the effects of psilocybin on the quality of life, pain control, and anxiety relief in adults with advanced cancer, addressing the pressing need for novel interventions to alleviate the psychological distress associated with this terminal illness.

The findings from the included studies provided compelling evidence for the therapeutic potential of psilocybin-assisted therapy in this patient population. For instance, the study by Agin-Liebes *et al*[22] revealed sustained reductions in symptoms of anxiety, depression, hopelessness, demoralization, and death anxiety among participants, with many attributing profound positive life changes to their experiences with psilocybin. Similarly, Griffiths *et al*[17] demonstrated significant improvements in depression, anxiety, and quality of life following high-dose psilocybin administration, with these effects persisting at the 6-month follow-up. Additionally, Grob *et al*[16] and Swift *et al*[26] reported notable reductions in anxiety and depression, alongside improvements in existential well-being and spirituality, further supporting the therapeutic potential of psilocybin-assisted therapy in enhancing the psychological and existential well-being of patients with advanced cancer.

The findings of this review are largely consistent with existing literature on the therapeutic effects of psilocybin in patients with advanced cancer. Several previous studies have reported similar outcomes, demonstrating significant reductions in symptoms of anxiety, depression, and existential distress following psilocybin-assisted therapy. For example, the results of Agin-Liebes *et al*[22] in 2020 align with those of previous research by Lewis *et al*[27] and Malone *et al*[28] in 2018, which also documented sustained improvements in psychological well-being and quality of life among cancer patients treated with psilocybin. Similarly, the study by Agrawal *et al*[29] reported positive trends toward en-

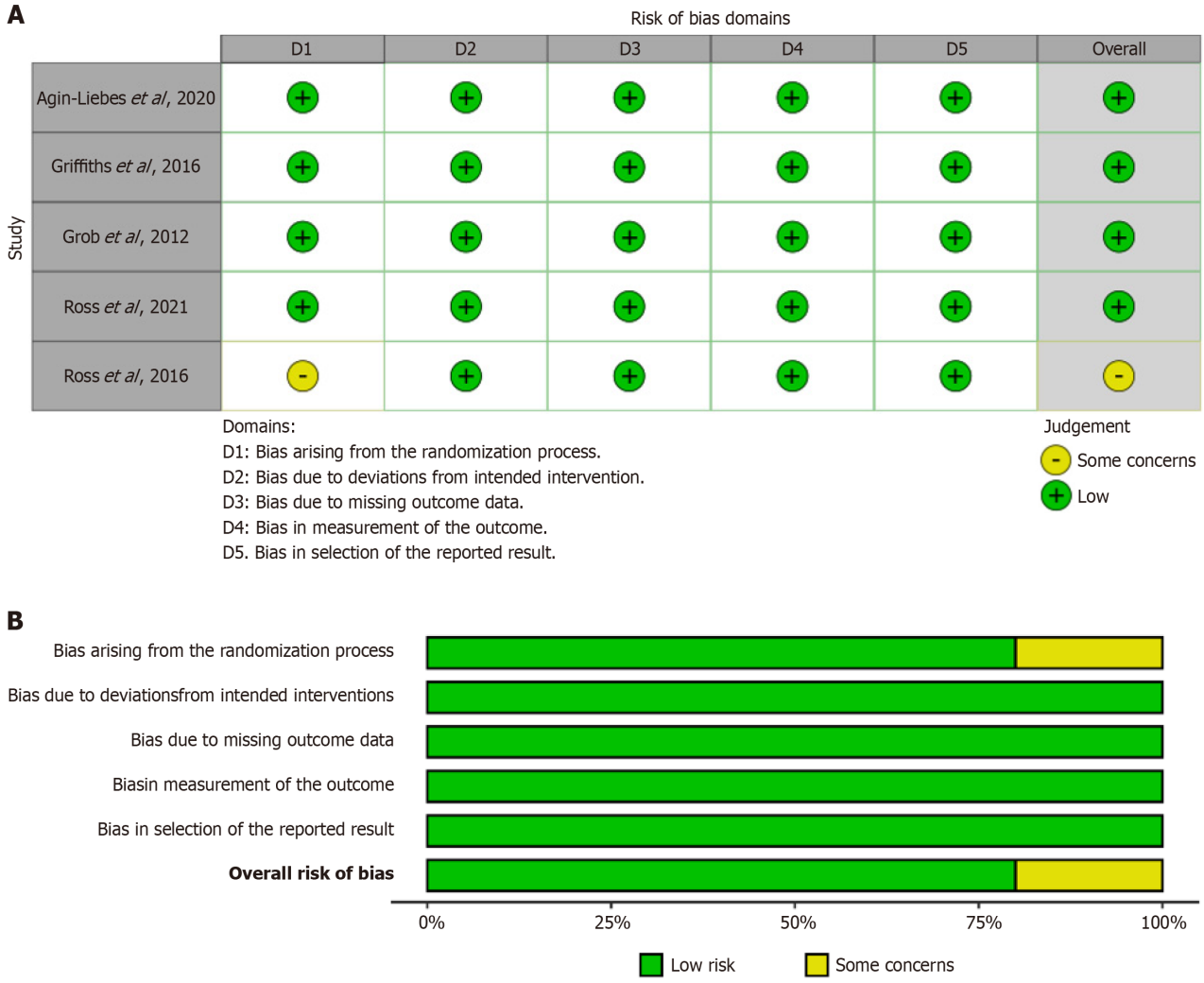

Figure 2 Traffic light plot and Summary plot. A: Traffic light plot; B: Summary plot.

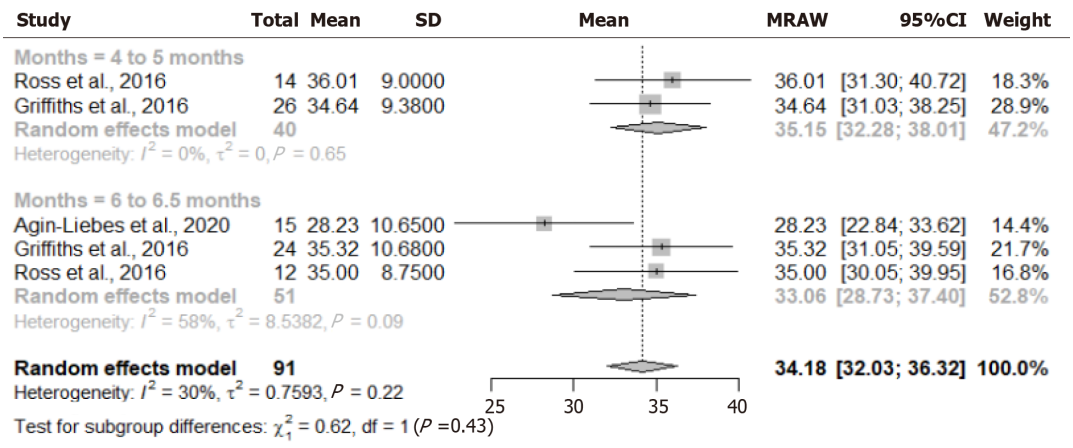

Figure 3 Changes in anxiety levels over time following psilocybin administration in patients with advanced cancer.

hanced mood and reduced anxiety following psilocybin administration, further corroborating the therapeutic potential of this intervention. However, while the overall findings of this review are consistent with previous research, there are some discrepancies worth noting. For instance, while Agin-Liebes *et al*[22] found sustained reductions in symptoms of anxiety and depression at long-term follow-up, other studies, such as that by Ross *et al*[25], reported slightly lower rates of sustained response over time. These differences may be attributed to variations in study design, participant characteristics, or intervention protocols across studies.

One potential explanation for variations in treatment response could be differences in the dosage or administration of psilocybin. For example, Griffiths *et al*[17] administered high doses of psilocybin, whereas Grob *et al*[16] used a moderate dose, and Ross *et al*[25] employed a single moderate dose combined with psychotherapy. These variations in dosing and administration may have influenced the magnitude and duration of therapeutic effects observed in each study. Additionally, differences in patient populations, such as variations in cancer stage, treatment history, or psychological comorbidities, may also contribute to variability in treatment outcomes. For instance, patients with more advanced disease or greater psychological distress may exhibit different response patterns compared to those with earlier-stage disease or milder symptoms.

Overall, while there may be some discrepancies in the literature, the consensus among studies suggests that psilocybin-assisted therapy holds promise as a valuable intervention for improving the psychological well-being and quality of life of patients with advanced cancer. Future research efforts should address these discrepancies through well-designed, controlled trials that systematically investigate the optimal dosing, administration, and patient selection criteria for psilocybin-assisted therapy in this population.

Reflecting on the strengths and limitations of the studies included in this review is essential for interpreting the findings and understanding their implications. One of the strengths of the studies included in this review is their use of RCT designs, which provide a rigorous methodological framework for evaluating the efficacy and safety of psilocybin therapy. Additionally, many of the studies employed standardized outcome measures, allowing for comparability across studies and enhancing the validity of the findings. However, sample size limitations were a common issue across the studies, with small sample sizes in some cases limiting the generalizability of the findings. This is particularly relevant given the variability in patient populations and treatment protocols across studies, which may influence the magnitude and durability of treatment effects.

CONCLUSION

This systematic review and meta-analysis provided a comprehensive synthesis of the evidence regarding the effects of psilocybin-assisted therapy on quality of life, pain control, and anxiety relief in patients with advanced cancer. The findings of this review highlight the potential of psilocybin as a novel therapeutic intervention for addressing the complex psychological and existential needs of patients facing terminal illness. The meta-analysis results revealed significant reductions in symptoms of anxiety among patients receiving psilocybin-assisted therapy. Specifically, analysis of anxiety levels measured by the STAI scale demonstrated a statistically significant decrease in anxiety levels at 6 to 6.5 months post-administration compared to baseline assessments. This finding suggests a potential therapeutic effect of psilocybin in mitigating anxiety among patients with advanced cancer over time, further supporting the utility of psilocybin-assisted therapy as a holistic approach to palliative care. Moreover, the findings from individual studies included in this review consistently demonstrated improvements in quality of life, pain control, and psychological well-being following psilocybin administration. Patients reported sustained reductions in symptoms of anxiety, depression, and existential distress, along with enhancements in spiritual well-being and overall quality of life. These outcomes underscore the potential of psilocybin-assisted therapy to provide holistic support for patients grappling with the profound challenges of terminal illness. Furthermore, the potential implications of psilocybin therapy extend beyond clinical practice to research and policy. Continued research efforts are needed to further elucidate the mechanisms of action underlying the therapeutic effects of psilocybin, optimize treatment protocols, and address remaining questions regarding long-term efficacy and safety. Policymakers and regulatory agencies play a crucial role in facilitating research and ensuring ethical and legal frameworks for the clinical use of psilocybin in patients with advanced cancer.

FOOTNOTES

Author contributions: Bader H and Farraj H performed data extraction and data analysis; Maghnam J assisted in manuscript writing; Abu Omar Y designed the study and assisted in manuscript writing.

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REFERENCES

1 **Mok E**, Lau KP, Lam WM, Chan LN, Ng JS, Chan KS. Healthcare professionals' perceptions of existential distress in patients with advanced cancer. *J Adv Nurs* 2010; **66**: 1510-1522 [PMID: 20492015 DOI: 10.1111/j.1365-2648.2010.05330.x]
2 **Henoch I**, Danielson E. Existential concerns among patients with cancer and interventions to meet them: an integrative literature review. *Psychooncology* 2009; **18**: 225-236 [PMID: 18792088 DOI: 10.1002/pon.1424]
3 **Schimmers N**, Breeksema JJ, Smith-Apeldoorn SY, Veraart J, van den Brink W, Schoevers RA. Psychedelics for the treatment of depression, anxiety, and existential distress in patients with a terminal illness: a systematic review. *Psychopharmacology (Berl)* 2022; **239**: 15-33 [PMID: 34812901 DOI: 10.1007/s00213-021-06027-y]
4 **Paice JA**, Ferrell B. The management of cancer pain. *CA Cancer J Clin* 2011; **61**: 157-182 [PMID: 21543825 DOI: 10.3322/caac.20112]
5 **Gupta D**, Lis CG, Grutsch JF. The relationship between cancer-related fatigue and patient satisfaction with quality of life in cancer. *J Pain Symptom Manage* 2007; **34**: 40-47 [PMID: 17532179 DOI: 10.1016/j.jpainsymman.2006.10.012]
6 **Vehling S**, Kissane DW. Existential distress in cancer: Alleviating suffering from fundamental loss and change. *Psychooncology* 2018; **27**: 2525-2530 [PMID: 30307088 DOI: 10.1002/pon.4872]
7 **Greer JA**, Applebaum AJ, Jacobsen JC, Temel JS, Jackson VA. Understanding and Addressing the Role of Coping in Palliative Care for Patients With Advanced Cancer. *J Clin Oncol* 2020; **38**: 915-925 [PMID: 32023161 DOI: 10.1200/JCO.19.00013]
8 **Holland JC**. History of psycho-oncology: overcoming attitudinal and conceptual barriers. *Psychosom Med* 2002; **64**: 206-221 [PMID: 11914437 DOI: 10.1097/00006842-200203000-00004]
9 **Anand U**, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, De Falco V, Upadhyay A, Kandimalla R, Chaudhary A, Dhanjal JK, Dewanjee S, Vallamkondu J, Pérez de la Lastra JM. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes Dis* 2023; **10**: 1367-1401 [PMID: 37397557 DOI: 10.1016/j.gendis.2022.02.007]
10 **Carey MP**, Burish TG. Etiology and treatment of the psychological side effects associated with cancer chemotherapy: a critical review and discussion. *Psychol Bull* 1988; **104**: 307-325 [PMID: 3062654 DOI: 10.1037/0033-2909.104.3.307]
11 **Boston P**, Bruce A, Schreiber R. Existential suffering in the palliative care setting: an integrated literature review. *J Pain Symptom Manage* 2011; **41**: 604-618 [PMID: 21145202 DOI: 10.1016/j.jpainsymman.2010.05.010]
12 **Sessa B**. Why psychiatry needs psychedelics and psychedelics need psychiatry. *J Psychoactive Drugs* 2014; **46**: 57-62 [PMID: 24830186 DOI: 10.1080/02791072.2014.877322]
13 **Moujaes F**, Preller KH, Ji JL, Murray JD, Berkovitch L, Vollenweider FX, Anticevic A. Toward Mapping Neurobehavioral Heterogeneity of Psychedelic Neurobiology in Humans. *Biol Psychiatry* 2023; **93**: 1061-1070 [PMID: 36715317 DOI: 10.1016/j.biopsych.2022.10.021]
14 **Reiff CM**, Richman EE, Nemeroff CB, Carpenter LL, Widge AS, Rodriguez CI, Kalin NH, McDonald WM; the Work Group on Biomarkers and Novel Treatments, a Division of the American Psychiatric Association Council of Research. Psychedelics and Psychedelic-Assisted Psychotherapy. *Am J Psychiatry* 2020; **177**: 391-410 [PMID: 32098487 DOI: 10.1176/appi.ajp.2019.19010035]
15 **Schenberg EE**. Psychedelic-Assisted Psychotherapy: A Paradigm Shift in Psychiatric Research and Development. *Front Pharmacol* 2018; **9**: 733 [PMID: 30026698 DOI: 10.3389/fphar.2018.00733]
16 **Grob CS**, Danforth AL, Chopra GS, Hagerty M, McKay CR, Halberstadt AL, Greer GR. Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. *Arch Gen Psychiatry* 2011; **68**: 71-78 [PMID: 20819978 DOI: 10.1001/archgenpsychiatry.2010.116]
17 **Griffiths RR**, Johnson MW, Carducci MA, Umbricht A, Richards WA, Richards BD, Cosimano MP, Klinedinst MA. Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *J Psychopharmacol* 2016; **30**: 1181-1197 [PMID: 27909165 DOI: 10.1177/0269881116675513]
18 **Rahbarnia A**, Li Z, Fletcher PJ. Effects of psilocybin, the 5-HT(2A) receptor agonist TCB-2, and the 5-HT(2A) receptor antagonist M100907 on visual attention in male mice in the continuous performance test. *Psychopharmacology (Berl)* 2023 [PMID: 37855864 DOI: 10.1007/s00213-023-06474-9]
19 **Carter OL**, Burr DC, Pettigrew JD, Wallis GM, Hasler F, Vollenweider FX. Using psilocybin to investigate the relationship between attention, working memory, and the serotonin 1A and 2A receptors. *J Cogn Neurosci* 2005; **17**: 1497-1508 [PMID: 16269092 DOI: 10.1162/089892905774597191]
20 **Page MJ**, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Syst Rev* 2021; **10**: 89 [PMID: 33781348 DOI: 10.1186/s13643-021-01626-4]
21 **Higgins JP**, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; **343**: d5928 [PMID: 22008217 DOI: 10.1136/bmj.d5928]
22 **Agin-Liebes GI**, Malone T, Yalch MM, Mennenga SE, Ponté KL, Guss J, Bossis AP, Grigsby J, Fischer S, Ross S. Long-term follow-up of psilocybin-assisted psychotherapy for psychiatric and existential distress in patients with life-threatening cancer. *J Psychopharmacol* 2020; **34**: 155-166 [PMID: 31916890 DOI: 10.1177/0269881119897615]
23 **Patchett-Marble R**, O'Sullivan S, Tadwalkar S, Hapke E. Psilocybin mushrooms for psychological and existential distress: Treatment for a patient with palliative lung cancer. *Can Fam Physician* 2022; **68**: 823-827 [PMID: 36376043 DOI: 10.46747/cfp.6811823]
24 **Ross S**, Agin-Liebes G, Lo S, Zeifman RJ, Ghazal L, Benville J, Franco Corso S, Bjerre Real C, Guss J, Bossis A, Mennenga SE. Acute and Sustained Reductions in Loss of Meaning and Suicidal Ideation Following Psilocybin-Assisted Psychotherapy for Psychiatric and Existential Distress in Life-Threatening Cancer. *ACS Pharmacol Transl Sci* 2021; **4**: 553-562 [PMID: 33860185 DOI: 10.1021/acsptsci.1c00020]
25 **Ross S**, Bossis A, Guss J, Agin-Liebes G, Malone T, Cohen B, Mennenga SE, Belser A, Kalliontzi K, Babb J, Su Z, Corby P, Schmidt BL. Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a

randomized controlled trial. *J Psychopharmacol* 2016; **30**: 1165-1180 [PMID: 27909164 DOI: 10.1177/0269881116675512]

26 **Swift TC**, Belser AB, Agin-liebes G, Devenot N, Terrana S, Friedman HL, Guss J, Bossis AP, Ross S. Cancer at the Dinner Table: Experiences of Psilocybin-Assisted Psychotherapy for the Treatment of Cancer-Related Distress. *J Humanisti Psychol* 2017; **57**: 488-519 [DOI: 10.1177/0022167817715966]

27 **Lewis BR**, Garland EL, Byrne K, Durns T, Hendrick J, Beck A, Thielking P. HOPE: A Pilot Study of Psilocybin Enhanced Group Psychotherapy in Patients With Cancer. *J Pain Symptom Manage* 2023; **66**: 258-269 [PMID: 37302533 DOI: 10.1016/j.jpainsymman.2023.06.006]

28 **Malone TC**, Mennenga SE, Guss J, Podrebarac SK, Owens LT, Bossis AP, Belser AB, Agin-Liebes G, Bogenschutz MP, Ross S. Individual Experiences in Four Cancer Patients Following Psilocybin-Assisted Psychotherapy. *Front Pharmacol* 2018; **9**: 256 [PMID: 29666578 DOI: 10.3389/fphar.2018.00256]

29 **Agrawal M**, Richards W, Beaussant Y, Shnayder S, Ameli R, Roddy K, Stevens N, Richards B, Schor N, Honstein H, Jenkins B, Bates M, Thambi P. Psilocybin-assisted group therapy in patients with cancer diagnosed with a major depressive disorder. *Cancer* 2024; **130**: 1137-1146 [PMID: 38105655 DOI: 10.1002/cncr.35010]

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META-ANALYSIS

Predictive value of tumor-infiltrating lymphocytes for neoadjuvant therapy response in triple-negative breast cancer: A systematic review and meta-analysis

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Abstract

BACKGROUND

The association between tumor-infiltrating lymphocyte (TIL) levels and the response to neoadjuvant therapy (NAT) in patients with triple-negative breast cancer (TNBC) remains unclear.

AIM

To investigate the predictive potential of TIL levels for the response to NAT in TNBC patients.

METHODS

A systematic search of the National Center for Biotechnology Information PubMed database was performed to collect relevant published literature prior to August 31, 2023. The correlation between TIL levels and the NAT pathologic complete response (pCR) in TNBC patients was assessed using a systematic review and meta-analysis. Subgroup analysis, sensitivity analysis, and publication bias analysis were also conducted.

RESULTS

A total of 32 studies were included in this meta-analysis. The overall meta-analysis results indicated that the pCR rate after NAT treatment in TNBC patients in the high TIL subgroup was significantly greater than that in patients in the low TIL subgroup (48.0% *vs* 27.7%) (risk ratio 2.01; 95% confidence interval 1.77-2.29; $P < 0.001$, $I^2 = 56\%$). Subgroup analysis revealed that the between-study heterogeneity originated from differences in study design, TIL level cutoffs, and study

populations. Publication bias could have existed in the included studies. The meta-analysis based on different NAT protocols revealed that all TNBC patients with high levels of TILs had a greater rate of pCR after NAT treatment in all protocols (all $P \leq 0.01$), and there was no significant between-protocol difference in the statistics among the different NAT protocols ($P = 0.29$). Additionally, sensitivity analysis demonstrated that the overall results of the meta-analysis remained consistent when the included studies were individually excluded.

CONCLUSION

TILs can serve as a predictor of the response to NAT treatment in TNBC patients. TNBC patients with high levels of TILs exhibit a greater NAT pCR rate than those with low levels of TILs, and this predictive capability is consistent across different NAT regimens.

Key Words: Breast cancer; Tumor-infiltrating lymphocyte; Neoadjuvant therapy; Treatment response; Systematic review; Meta-analysis

Core Tip: The immune response status may have a significant impact on the effectiveness of chemotherapy. Tumor-infiltrating lymphocytes (TILs) can directly or indirectly participate in specific immune responses against tumor cells. However, the association between TIL levels and the response to neoadjuvant therapy (NAT) in patients with triple-negative breast cancer (TNBC) remains unclear. This systematic review and meta-analysis first investigated the relationship between TIL status and the response to NAT in TNBC patients. This systematic review and meta-analysis will provide clinical physicians with systematic evidence on the role of TILs to predict the response of TNBC patients to NAT.

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INTRODUCTION

Global Cancer Statistics 2020 reported that in 2020, breast cancer (BC) was becoming the most common malignant tumor globally[1]. Triple-negative BC (TNBC) is characterized by extremely aggressive biological behavior and has a high recurrence rate and poor survival[2,3]. Extensive investigations on early diagnosis, precision treatment, and prognostic prediction have been conducted to improve TNBC patient survival[4-6]. Neoadjuvant therapy (NAT) can effectively decrease the clinical stage of TNBC, and patients who attain pathologic complete response (pCR) following NAT have significantly prolonged event-free survival (EFS) and overall survival compared with those having residual infiltrative carcinoma. Consequently, NAT has been widely recommended as the preferred preoperative standard treatment modality for TNBC patients with lymph node involvement and/or stage ≥ T1c disease[7,8].

The immune response status may have a significant impact on the effectiveness of chemotherapy[9,10]. Research findings indicate that in early-stage TNBC patients, the NAT protocol combining the immune checkpoint inhibitor pembrolizumab, which enhances the functionality of activated T cells, with conventional chemotherapy drugs has been correlated with increased rates of pCR and prolonged EFS[11,12]. Tumor-infiltrating lymphocytes (TILs) can directly or indirectly participate in specific immune responses against tumor cells, and their aggregation, interaction, and costimulation are essential for successful antitumor immune responses[13,14]. High levels of TILs within the tumor or the stroma are associated with a more favorable response to NAT in early-stage and locally advanced TNBC patients[15-19]. However, this result was not substantiated in a study that conducted a meta-analysis of individual patient data from a phase II study of TNBC NATs involving five different platinum-based regimens[20]. Therefore, further investigations are warranted to explore the correlation between TIL levels and therapeutic response in TNBC NATs.

Previously, a systematic review and meta-analysis on the correlation between TIL levels in different molecular subtypes of BC and NAT response showed that high levels of TILs are associated with pCR in a TNBC subgroup analysis including four studies[21]. Over the past decade, many clinical trials have further investigated the effectiveness of different NAT regimens for TNBC and employed TIL levels to predict treatment response and long-term prognosis. Consequently, this study was designed to analyze the ability of TILs in TNBC patients to predict the response to NAT through a more comprehensive systematic review and meta-analysis, with the objective of obtaining more current and robust research evidence. Additionally, this study examined the predictive importance of TIL levels for the therapeutic efficacy of different NAT regimens in TNBC patients.

MATERIALS AND METHODS

The present meta-analysis adhered to the reporting suggestions provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[22].

Literature search and inclusion criteria

The literature search was conducted on the National Center for Biotechnology Information PubMed (MEDLINE) database to identify pertinent articles published prior to August 31, 2023. The search strategy involved utilizing a combination of the following MeSH terms, title/abstract keywords, or full-text search terms: "breast cancer, or breast carcinoma" , "triple-negative, or TNBC" , "neoadjuvant therapy, or neoadjuvant" , and "tumor-infiltrating lymphocytes, T lymphocytes, or TILs" . Additionally, a manual search of the literature and reference tracing were performed to identify any additional relevant studies.

The studies eligible for this meta-analysis met the following criteria: (1) Pathological and immunohistochemical-based molecular subtyping confirming the diagnosis of TNBC; (2) Reported TIL levels by hematoxylin and eosin staining evaluation according to the standardized method presented by the International TILs Working Group in 2014 or other explicit assays; (3) Reported the number or rate of pCR events in TNBC patients based on different TIL levels; and (4) Were published in either English or Chinese.

Three researchers (Sun HK, Jiang WL, and Zhang SL) independently evaluated the titles and abstracts of the candidate studies, excluding those not pertinent to the topic. Subsequently, both researchers thoroughly examined the full texts to determine their eligibility for inclusion. In cases where uncertainty arose or disagreements occurred regarding inclusion, the researchers resorted to the study designer (Liu JB) for a review and discussion to achieve consensus. Furthermore, if multiple publications involved the same study population, priority was given to the publication with a larger sample size or the most recent study for eligibility in the meta-analysis.

Data extraction and quality assessment

Three researchers (Sun HK, Jiang WL, and Zhang SL) independently collected the relevant information and data for each study that met the inclusion criteria using a predesigned table. These included details such as the first author, geographical location of the study population, publication year, study design, recruitment year, TNM staging, NAT regimen, number of high/low TILs, cutoff values and methodology used, treatment response endpoints and pCR criteria as well as the number and ratio of pCR events. Next, the quality of the cohort studies included was independently assessed by two researchers (Sun HK and Jiang WL) using the Newcastle-Ottawa Scale (NOS)[23].

Statistical analysis

The meta-analysis was conducted in RevMan 5.4 software. The total cases of patients and the cases of patients who achieved pCR were recorded separately for the high TIL level group and low TIL level group in each study and input into RevMan software. The relative risk ratio (RR) and the associated 95% confidence interval (CI) were calculated per the following formula: The pCR rate in the high TIL level group divided by the pCR rate in the low TIL level group. RR > 1 and $P < 0.05$ indicated a greater pCR rate in the high TIL level subgroup than in the low TIL level subgroup.

In the meta-analysis, between-study heterogeneity was assessed using the I^2 statistic (ranging from 0% to 100%). If an I^2 value less than 50% or a P value greater than 0.05 indicated the absence or low between-study heterogeneity, a fixed-effects model was used for meta-analysis; otherwise, a random-effects model (REM) was used. Additionally, subgroup analysis was conducted to explore the source of between-study heterogeneity when significant heterogeneity was observed, and sensitivity analysis was performed to evaluate the influence of individual studies on the overall meta-analysis results. Publication bias was investigated using a funnel plot and Egger's test. If funnel plot is asymmetric or a P value is less than 0.05 according to Egger's test, publication bias was considered present[24]. Duval and Tweedie trim-and-fill method was used for testing and adjusting for publication bias in meta-analysis[25]. All the statistical tests were two-tailed, and $P < 0.05$ was considered indicative of statistical significance.

RESULTS

Study selection

A preliminary literature search identified 269 articles, and after reviewing the titles and abstracts, we selected 158 articles for full-text reading. Subsequently, 125 articles were excluded because of the eligibility criteria. Finally, 32 eligible studies comprising 5406 TNBC patients were included in this systematic review and meta-analysis. The NOS quality scores of the eligible studies ranged from 6 to 9, with a median score of 8 (Figure 1).

Characteristics of the included studies

Table 1 displays the characteristics of all the studies included in the analysis. Among the 32 included studies, 16 studies provided descriptions of TNBC before NAT based on T staging, including 4051 cases in T1/T2, 48 cases in T2/T3, 341 cases in T2-T4, and 1007 cases in T3/T4; fifteen (15) studies described N staging of pre-NAT TNBC, including 2937 cases in N0 and 2,704 cases in N1-N3; additionally, 11 studies described the clinical TNM staging of pre-NAT TNBC, including 91 cases in stage I, 923 cases in stage II, and 762 cases in stage III; and five studies did not report T or N stage or clinical TNM staging. Among the 27 included studies, TIL levels were assessed per the standardized method proposed by the

Table 1 Characteristics of the impact of tumor-infiltrating lymphocytes on the response to neoadjuvant therapy in triple-negative breast cancer patients included in the meta-analysis

Ref.	Data collection	Recruitment period	Sample size	Age in yr, median/mean (range)	TNM stage	Neoadjuvant regimen	Number of high/middle TILs as %, cut-off, and method	End point and pCR standard	Number of overall pCR as %	pCR rates as high TILs *vs* low TILs	OR or RR
Cerbelli *et al*[36], Germany	Retrospective consecutive cohort	2011.6-2017.6	61	50 (28-74)	T1: 8; T2: 46; T3: 3; T4: 4; N0: 32; N1-N3: 29	AC×4 (Q3W) →T×12 (QW)	49 (17/32) (80.3), (50%) 10%, HE	pCR, ypT0	23 (37.7)	18 (36.7) *vs* 5 (41.7)	OR: [U] 0.41 (0.17-0.95), 0.037; [M] 2.39 (0.96-5.96), 0.062
Galvez *et al*[17], Peru	Retrospective cohort	2003.1-2014.12	435	49 (24-84)	II: 72, III: 363;	AC×4 (Q3W) →T×12 (QW)	181 (41.6), 50%, HE	pCR, ypT0	46 (11.0)	26 (14.4) *vs* 20 (7.9)	NR
Abdelrahman *et al*[39], Egypt	Prospective cohort	2017.1-2019.5	50	45 (22-72)	T1: 20; T2: 30; N0: 18; N1-N3: 32	AC→T	14 (28.0), 50%, HE	pCR, ypT0	20 (40.0)	10 (71.4) *vs* 10 (27.8)	NR
Jung *et al*[53], Korea	Retrospective cohort	2009.1-2014.12	143	NR	T1-T2: 91; T3: 52; N0: 64; N1-N3: 79	AC→T	74 (51.7), 30%, HE	pCR, ypT0	66 (46.2)	43 (58.1) *vs* 23 (33.3)	OR: [U] 2.774 (1.404-5.481), 0.003; [M] 3.484 (1.407-8.627), 0.007
Russo *et al*[47], Venezuela	Retrospective cohort	2008-2013	41	NR	II: 80, III: 107;	AC→T	14 (34.1), 30%, HE	pCR, ypT0	15 (36.6)	11 (78.6) *vs* 4 (14.8)	OR: [U] 8.85 (3.62-21.66), 0.001
Vicent *et al*[48], Spain	Retrospective cohort	1998-2015	164	49 (29-81)	II: 63, III: 37	AC×4 (Q3W) →T×12 (QW)	58 (35.4), 40%, HE	pCR, ypT0/is, ypN0	61 (37.2)	51 (88.0) *vs* 10 (9.0)	NR
Ochi *et al*[32], Japan	Retrospective consecutive cohort	2001-2009	80	52 (27-75)	NR	AC→T	55 (19/36) (68.8), (50%) 10%, HE	pCR, ypT0	25 (31.3)	24 (43.6) *vs* 1 (4.0)	NR
Bockstal *et al*[49], Belgium	Retrospective consecutive cohort	2015.1-2020.3	35	55.8 ± 13.3	NR	AC→T	10 (28.6), 40%, HE	pCR, ypT0	13 (37.1)	8 (80.0) *vs* 5 (20.0)	NR
Rangan *et al*[43], India	NR	NR	75	NR	T1-T3: 49; T4: 26; N0: 36; N1-N3: 39	NR	57 (76.0), 50%, HE	pCR, ypT0	27 (36.0)	25 (43.9) *vs* 2 (11.1)	OR: [U] 6.25 (1.312-29.763), 0.025
Pang *et al*[18], ChiNR	Retrospective cohort	2010.1-2018.12	310	NR	T1-2: 298; T3-4: 97	AC→T	177 (85/92) (57.1), (20%) 10%, HE	pCR, ypT0	88 (28.4)	53 (31.1) *vs* 33 (34.5)	NR
Zhang *et al*[52], America	Retrospective cohort	2005-2016	58	46 (24-64)	T1: 7; T2-T4: 51; N0: 30; N1-N3: 28	AC×4 (Q3W) →T×12 (QW)	17 (29.3), 60%, HE	pCR, ypT0	26 (44.8)	12 (70.6) *vs* 14 (34.1)	NR
Zhao *et al*[50], ChiNR	Retrospective cohort	2017-2018	126	50.1 ± 11.2	T1: 78; T2-T3: 48; N0: 74; N1-N3: 52	AC→T	42 (33.3), 40%, HE	pCR, ypT0	76 (60.3)	38 (90.5) *vs* 38 (45.2)	NR
Cerbelli *et al*[40],	Retrospective	2011.1-2016.12	54	50 (28-75)	T1: 7; T2-T4:	AC×4 (Q3W) →T×12	22 (40.7), 50%, HE	pCR, ypT0/is,	19 (35.2)	11 (50.0) *vs* 8	OR: [U] 1.61 (0.40-6.52),

Italy	consecutive cohort				47; N0: 24; N1-N3: 30	(QW)		N0		(25.0)	0.025
Rao *et al*[30], ChiNR	Retrospective consecutive cohort	2009.7-2014.6	52	46.9 (23-67)	II: 34, III: 16;	TAC	21 (40.4), CD8: ≥ 0.15, HE	pCR, ypT0 DFS OS	14 (26.9)	CD8: 10 (47.6) *vs* 4 (12.9)	CD8 OR: [U] 6.14 (1.6-23.8), 0.010
Lusho *et al*[28], Japan	Retrospective consecutive cohort	2008-2019	120	56 (28-86)	NR	TAC	18 (15.0), 30%, HE	pCR, ypT0/Tis ypN0	34 (28.3)	10 (55.6) *vs* 24 (23.5)	NR
Hida *et al*[37], Japan	Retrospective cohort	2007-2014	48	56 (22-79)	T1: 93; T2: 59; T3: 2; N0: 98; N1-N3: 56	AC×4 (Q3W) →T×12 (QW)	31 (11/20) (64.6), (50%) 10%, HE	pCR, ypT0/is, ypN0	21 (43.8)	18 (58.0) *vs* 3 (17.6)	NR
Hida *et al*[27], Japan	Retrospective consecutive cohort	2007-2014	80	NR	N0: 56; N1-N3: 24	TAC	23 (28.8), 50%, HE	pCR, ypT0/is, N0	28 (35.0)	12 (52.2) *vs* 16 (28.1)	NR
Kolberg *et al*[51], Germany	Retrospective cohort	NR	311	NR	NR	AC→T	59 (19.0), 60%, HE	pCR, ypT0	110 (35.4)	35 (59.3) *vs* 75 (29.8)	OR: [U] 3.44 (1.92-6.18), 0.001
Foldi *et al*[38], America	II RCT	2015.12-2018.11	54	NR	I: 12, II: 33, III: 14;	T→ddAC- Durvalumab (3 and 10 mg/kg)	26 (16/10) (48.1), (30%) 10%, HE	pCR, ypT0/Tis ypN0	23 (42.6)	15 (57.7) *vs* 8 (28.6)	NR
Abuhadra *et al* [16], America	Prospective cohort	2015.10-2019.11	318	52.5 (24-77)	I: 38, II: 210, III: 70;	ddAC→T+ (Atezolizumab/ Panitumumab/ Bevacizumab)	106 (33.3), 20%, HE	pCR, ypT0	130 (40.9)	68 (64.2) *vs* 62 (29.2)	NR
Denkert *et al* [33], Germany	RCT IPD pooled analysis	2010.1-2016.12	906	NR	NR	T+ Bevacizumab	646 (273/373) (71.3), (60%) 10%, HE	pCR, ypT0	333 (36.8)	253 (39.2) *vs* 80 (30.8)	NR
Yuan *et al*[34], America	II RCT	2012.1-2018.8	63	52 (28-79)	II: 55, III: 12;	TCb	28 (6/22) (45.9), (60%) 10%, HE	pCR, ypT0	30 (47.6)	17 (60.7) *vs* 13 (39.3)	Medium *vs* low[1]: OR: [U] 2.23 (0.74- 6.69), 0.16; high *vs* low[1]: OR: [U] 3.06 (0.49-9.30), 0.23
Sharma *et al*[46], America	II RCT	2015.7-2018.5	100	51 (29–70)	T1: 19; T2: 70; T3-T4: 11; N0: 70; N1-N3: 30	Arm-A: CbP + AC; Arm-B: CbD	39 (43.3), 20%, HE	pCR ypT0/is, ypN0	51 (56.7)	26 (66.7) *vs* 25 (49.0)	OR: [U] 2.08 (0.88-4.93), 0.096
Pons *et al*[45], Spain	NR	2016-2022	67	NR	T1-T2: 59; T3: 10; N0: 43; N1-N3: 26	TCb + ddAC	24 (35.8), 20%, HE	pCR, ypT0/is, ypN0	36 (53.7)	14 (58.3) *vs* 22 (51.2)	NR
Abuhadra *et al* [15], America	NR	2015.10-2020.10	408	51 (23–77)	I: 41, II: 284, III: 83	AC→TCb	143 (35.0), 20%, HE	pCR, ypT0/is, N0	166 (40.7)	85 (59.4) *vs* 81 (30.6)	NR
Asano *et al*[31], Japan	Retrospective cohort	2007-2013	61	NR	T1: 24; T2-T4: 153; N0: 41; N1-N3: 136	FEC→T	48 (78.7), 10%, HE	pCR, ypT0	28 (45.9)	26 (54.2) *vs* 2 (15.4)	NR
Ono *et al*[54],	NR	1999-2007	92	52 (23-76)	II: 23, III: 36;	AC→T	67 (72.8)[1], high: (3-5),	pCR, ypT0	29 (31.5)	25 (37.3) *vs* 4	NR

Japan						CEF	HE			(16.0)	
Wang *et al*[35], America	NR	2007-2014	72	NR	T1: 5; T2: 48; T3: 15; T4: 5; N0: 38; N1-N3: 34	NR	53 (1/52) (73.6), (50%) 10%, HE	pCR, ypT0	38 (52.8)	35 (66.0) *vs* 3 (15.8)	NR
Dong *et al*[29], ChiNR	Retrospective cohort	2010.1-2014.12	170	NR	T1-2: 110; T3-4: 60	TAC	122 (74/48) (71.8), (20%) 10%, HE	pCR, ypT0 DFS OS	48 (28.2)	38 (31.1) *vs* 10 (24.8)	NR
Würfel *et al* [44], Germany	NR	2015.5-2017.4	146	NR	T1: 59; T2-T4: 90	NR	24 (16.4), 50%, HE	pCR ypT0 ypN0	56 (38.4)	16 (66.7) *vs* 40 (32.8)	NR
Hamy *et al*[42], France	NR	2015.1-2017.3	717	NR	T1-T2: 529; T3: 189; N0: 282; N1-N3: 435	NR	81 (11.3), 50%, HE	pCR, ypT0	202 (28.2)	48 (59.2) *vs* 154 (24.2)	OR: [U] 5.02 (4.27-5.77), 0.001
Cerbelli *et al*[41], Italy	Retrospective consecutive cohort	NR	59	49 (28-74)	II: 36, III: 24	NR	17 (28.8), 50%, HE	pCR, ypT0	22 (37.3)	13 (76.5) *vs* 9 (21.4)	NR

[1]High: Tumor-infiltrating lymphocyte proportion > 10% (2 points) combined with mild (1 point) or marked (2 points) intensity.

DFS: Disease-free survival; HE: Hematoxylin eosin staining; IPD: Individual patient data; M: Multivariate analysis; NR: Not reported; OR: Odds ratio; OS: Overall survival; pCR: Pathological complete response; RCT: Randomized controlled trial; RR: Risk ratio; TIL: Tumor-infiltrating lymphocyte; U: Univariate analysis.

International TILs Working Group in 2014[26], while five studies[27-30] did not report the specific method used for TIL assessment. The cutoff value for TIL level most commonly reported was 10% (n = 10)[18,29,31-38], followed by 50% (n = 8)[17,27,39-44], 20% (n = 4)[15,16,45,46], 30% (n = 3)[17,28,47], 40% (n = 3)[48-50], and 60% (n = 2)[51,52].

Association between preoperative TIL levels and therapeutic efficacy of NAT in TNBC patients

Overall meta-analysis: A meta-analysis of 32 studies revealed that the patients with high TIL levels had a high proportion of pCR events (46.7%, 1004/2092) than patients with low TIL levels (26.4%, 900/3254) with a significant difference (P < 0.001, REM, I^2 = 56%) (Figure 2). Sensitivity analysis using leave-one-out approach indicated that the meta-analytical statistics were not changed by any single study: Excluding the study with the largest effect size[32], the calculated RR was 1.99 (95%CI: 1.75-2.26, REM, I^2 = 55%).

Publication bias analysis: An asymmetric funnel plot and Egger's test P value (P = 0.001) less than 0.05 suggested potential publication bias in the included studies of overall meta-analysis. Additionally, the trim-and-fill method was further employed for assessing and adjusting for publication bias, the analytical result showed that nine missing studies were interpolated during the analysis to account for potential bias. It was observed that there was no significant asymmetry in the trimmed funnel plot and still significant overall meta-analytical effect size after adjusting for publication bias, suggesting that there was limited or insignificant publication bias (Figure 3).

Subgroup analysis: Due to significant heterogeneity among the included studies in the overall meta-analysis, subgroup analysis was conducted based on important variables, including study design, TIL cutoff value, sample size, and geographical region, to explore the sources of between-study heterogeneity. The analytical results indicated that the statistical effect sizes of all subgroup analyses were consistent with the overall meta-analysis results, and there were no

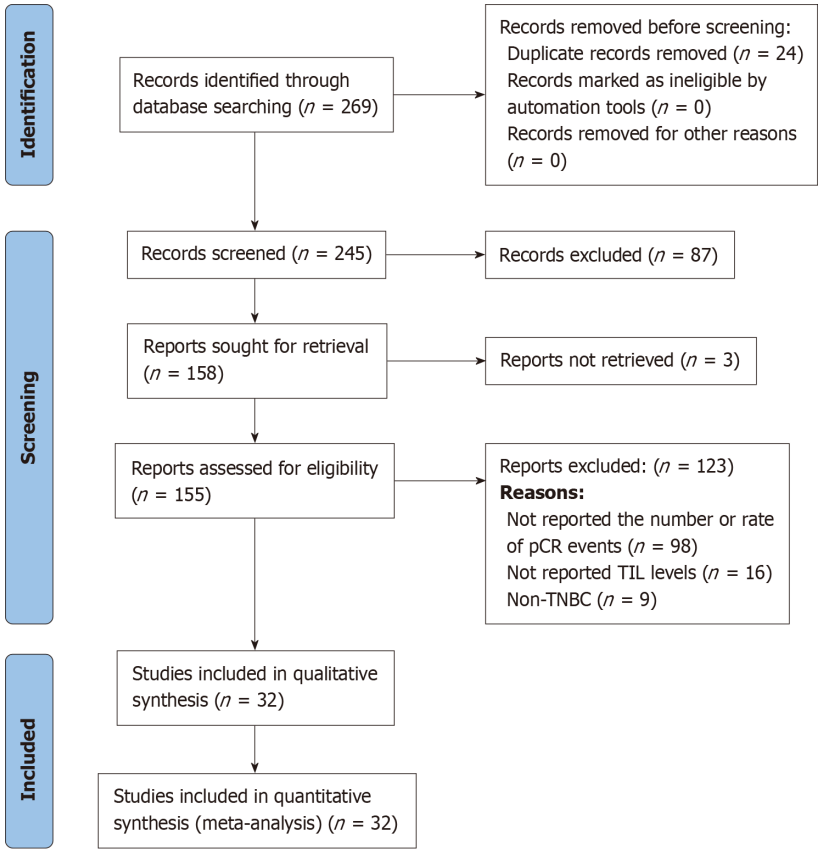

Figure 1 PRISMA flow diagram for study selection of systematic review and meta-analysis. pCR: Pathological complete response; TIL: Tumor-infiltrating lymphocyte; TNBC: Triple-negative breast cancer.

significant differences in the statistics among the subgroups. However, there were noticeable differences in the heterogeneity among the subgroups. Subgroup analysis revealed that the sources of between-study heterogeneity could stem from the subgroup of retrospective cohort studies (I^2 = 58%) (Figure 4), the subgroups with cutoff values of 40% (I^2 = 78%) and 20% (I^2 = 67%), the subgroup with sample sizes > 80 (I^2 = 69%), and the subgroup with European populations (I^2 = 77%) (Table 2).

Meta-analysis of different NAT regimens

Among the 32 studies, except for five studies[35,41-44] without a description of the NAT regimen, the reported NAT regimens in 27 included 14 studies with anthracycline combined with cyclophosphamide (AC) followed by sequential paclitaxel (T) (AC-T) [15,17,18,32,36,37,39,40,47-50,52,53], three studies with AC followed by sequential T in combination with anti-HER2 targeted therapy (AC-T + targeted therapy)[16,33,38], four studies with AC followed by sequential T in combination with platinum (Cb) agents (AC-TCb)[34,45,46,51], two studies with AC followed by sequential T in combination with fluorouracil (Fu) (AC-T + Fu)[31,54], and four studies with AC combined with T (TAC)[27-30].

The included studies were analyzed according to the NAT regimens, and the results revealed that patients with high TIL levels in different NAT regimens, such as AC-T, AC-TCb, AC-T + targeted therapy, AC-T + FU, and TAC, had 1.57 to 2.75 times greater rates of pCR events than those with low TIL levels. Moreover, there was no significant difference in the statistics among the various NAT regimens (P = 0.29). The detailed meta-analysis data of TILs associated with treatment response to different NAT regimens in TNBC patients are presented in Figure 5 and Table 2.

DISCUSSION

Tumor immunity plays a crucial role in the body's defense against tumors and in mediating the response to anti-cancer treatments. The presence of TILs in breast tumors has been associated with improved clinical outcomes[55]. The role of TILs in the NAT response in TNBC patients has been extensively studied. Based on the existing studies evaluating the correlation between TIL assessment and NAT treatment outcomes in TNBC patients, we conducted a systematic review and meta-analysis of the relationship between TIL status and the response to NAT in TNBC patients. The results showed that TNBC patients with high levels of TILs had greater NAT pCR rates than did those with low TIL levels. Furthermore, analysis based on different NAT regimens revealed that TIL levels were significantly associated with treatment response in all NAT regimens incorporating anthracycline combined with taxane drugs. This suggests that TILs have predictive

Table 2 Subgroup analysis examining heterogeneity among the included studies

Analysis	No. of studies	Risk ratio (95%CI)	I^2 statistic (%)	*P* value for heterogeneity	Analytical model	*P* value for subgroup differences
Study design						
RCT	5	1.42 (1.23-1.64)	41	0.15	FEM	
Prospective cohort	2	2.24 (1.77-2.83)	0	0.64	FEM	
Retrospective cohort	18	2.27 (1.84-2.80)	58	0.01	REM	
Not reported	7	2.05 (1.77-2.36)	45	0.09	FEM	0.02
Cut-off						
60%	2	2.01 (1.57-2.58)	0	0.90	FEM	
50%	8	2.31 (1.95-2.74)	0	0.71	FEM	
40%	3	3.06 (1.60-5.84)	78	0.01	REM	
30%	3	2.33 (1.61-3.37)	46	0.16	FEM	
20%	4	1.68 (1.29-2.20)	67	0.03	REM	
10%	10	1.63 (1.24-2.15)	49	0.04	REM	
Locations						
Asia	12	1.90 (1.62-2.24)	46	0.04	FEM	
Europe	11	2.07 (1.58-2.71)	77	0.01	REM	
Americas	9	2.01 (1.76-2.30)	34	0.14	FEM	0.35
Sample size						
$n \leq 80$	16	2.62 (2.14-3.20)	35	0.08	FEM	
$n > 80$	16	1.82 (1.56-2.12)	69	0.01	REM	0.04
NAT regimens						
AC-T	14	2.13 (1.72-2.63)	56	0.01	REM	
TAC	4	1.99 (1.43-2.75)	0	0.44	FEM	
AC-T + targeted therapy	3	1.73 (1.12-2.67)	82	0.01	REM	
AC-TCb	4	1.57 (1.31-1.90)	43	0.15	FEM	
AC-T + Fu	2	2.75 (1.28-5.92)	0	0.61	FEM	0.02

AC: Anthracycline combined with cyclophosphamide; AC-T: Anthracycline combined with cyclophosphamide followed by paclitaxel or docetaxel; AC-T + Fu: Anthracycline combined with cyclophosphamide followed by paclitaxel or docetaxel, and fluorouracil; AC-TCb: Anthracycline combined with cyclophosphamide followed by paclitaxel or docetaxel, and platinum; FEM: Fixed-effects model; NAT: Neoadjuvant therapy; TAC: Paclitaxel or docetaxel combined with anthracycline, and cyclophosphamide; REM: Random-effects model.

value for treatment response in these NAT regimens. To our knowledge, this is the first comprehensive and specific evaluation of the ability of TILs to predict the response of TNBC patients to NAT, which offers important insights into predicting treatment response based on pretreatment tumor immune status in TNBC patients.

TILs play a vital role in the surveillance and defense against tumors within the tumor immune microenvironment. The positioning, clustering, interaction, and costimulation of TIL subgroups are crucial for effective antitumor immune responses[13]. TILs can directly eliminate cancer cells through various mechanisms, including the specific recognition of endogenous antigen peptide-MHC class I molecule complexes by CD8+ T cells, the secretion of substances such as perforin and granzymes to induce tumor cell death through proteolytic activity, and the expression of FasL or the secretion of tumor necrosis factor (TNF)-alpha to induce apoptosis in cancer cells by binding to the death receptor Fas and TNF receptor on the surface of target cells[56]. Studies have shown that chemotherapy drugs can not only directly kill cancer cells through cytotoxic effects but also regulate TILs to eliminate cancer cells. For example, T cells pretreated with doxorubicin, cyclophosphamide, and paclitaxel in a coculture system with tumor organoids showed a greater proportion of cancer cell apoptosis than did T cells that were only pretreated with doxorubicin and cyclophosphamide and cocultured with tumor organoids. In another study, no significant difference was observed in T-cell pretreatment between doxorubicin, cyclophosphamide, and carboplatin combination therapy and doxorubicin and cyclophosphamide alone.

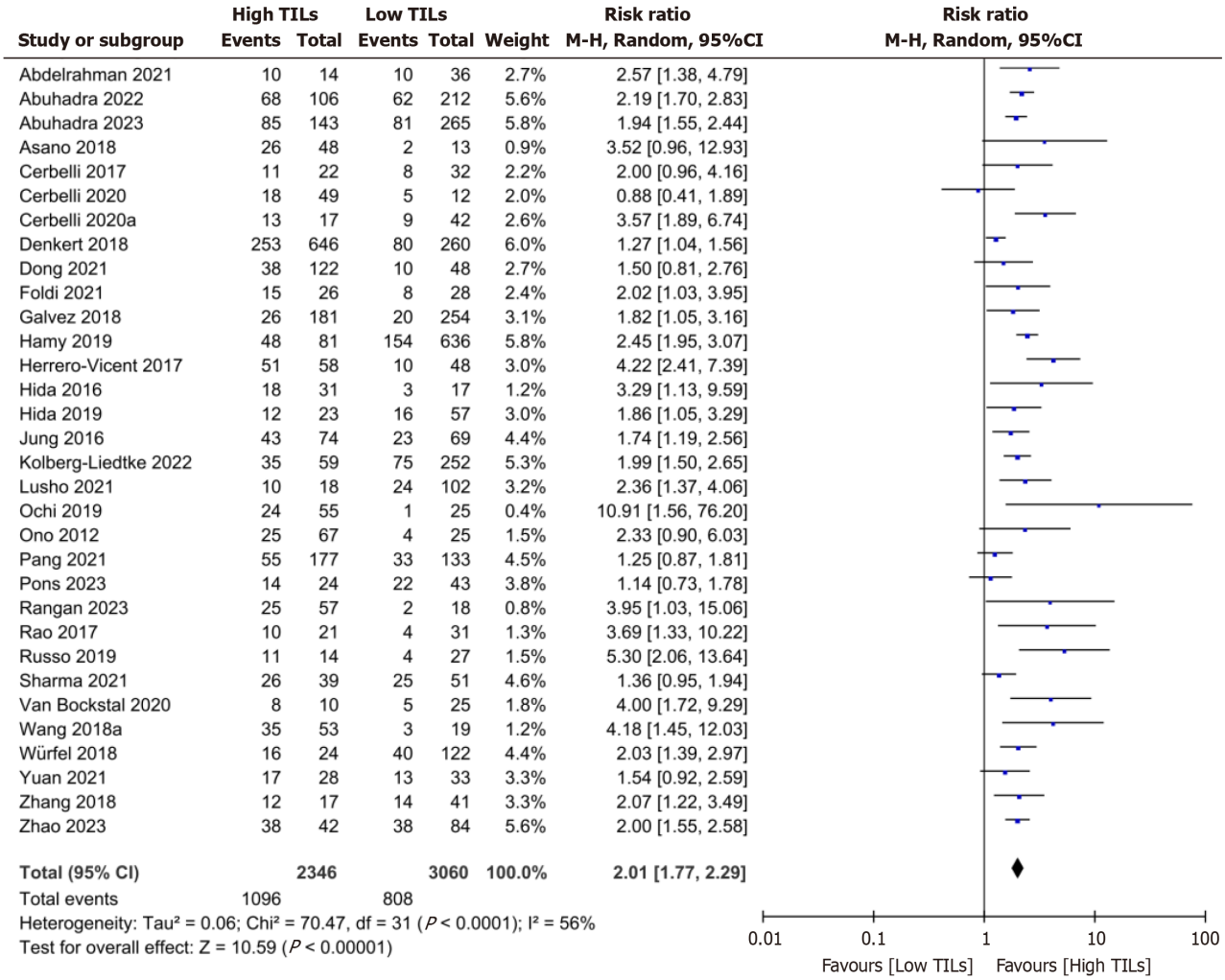

Figure 2 Forest plot demonstrating the correlation between tumor-infiltrating lymphocyte levels and the pathological complete response rate in triple-negative breast cancer patients receiving neoadjuvant therapy. TIL: Tumor-infiltrating lymphocyte.

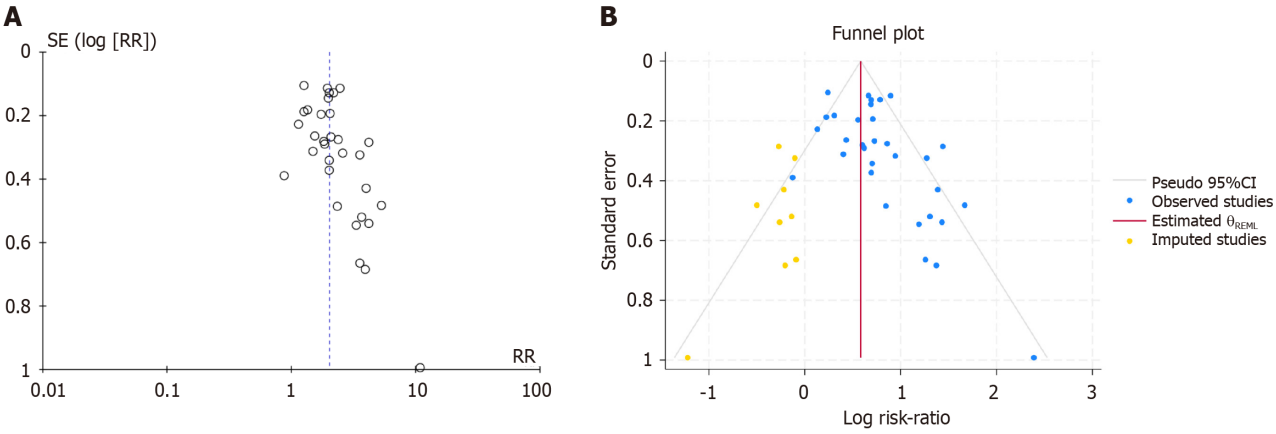

Figure 3 Funnel plot illustrating the correlation between tumor-infiltrating lymphocyte levels and the pathological complete response rate in studies investigating neoadjuvant therapy in triple-negative breast cancer patients. A: An asymmetric funnel plot and Egger's test *P* value (*P* = 0.001) less than 0.05 suggested potential publication bias in the included studies of overall meta-analysis; B: Trim-and-fill method showed that there was no significant asymmetry in the trimmed funnel plot and still significant overall meta-analytical effect size after adjusting for publication bias, suggesting that there was limited or insignificant publication bias.

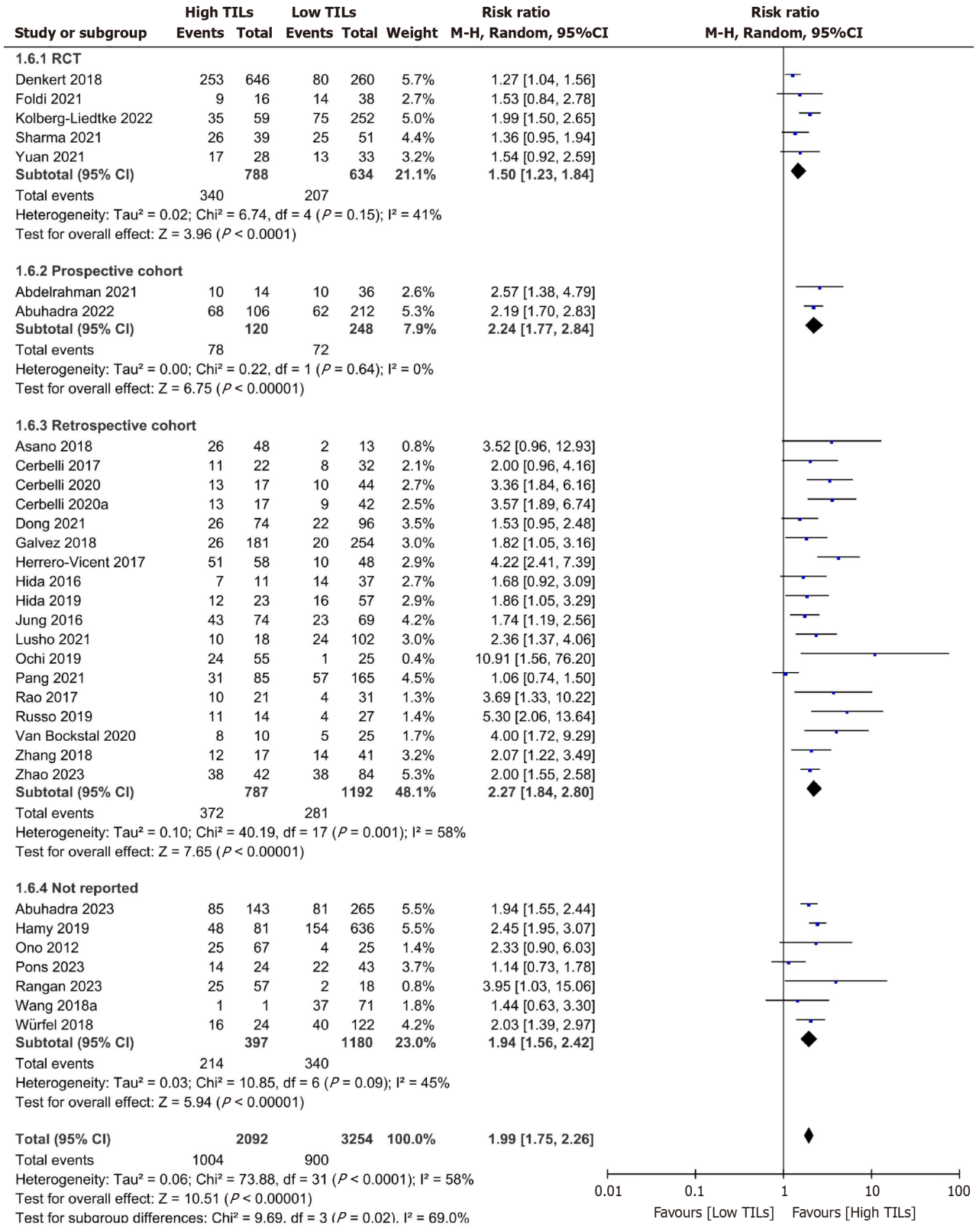

Figure 4 Forest plot illustrating subgroup analysis based on study design of included meta-analysis. TIL: Tumor-infiltrating lymphocyte; RCT: Randomized controlled trial.

This suggests that paclitaxel can modulate the cytotoxicity of T cells and exert an antitumor effect[57]. Furthermore, research has shown that BC patients with higher levels of TILs have better clinical responses to chemotherapy containing paclitaxel than to adjuvant chemotherapy regimens without taxanes, confirming this concept at the clinical level[58].

The systematic assessment and meta-analysis conducted herein provide substantial evidence that TNBC patients exhibiting high TIL levels exhibit superior treatment responses regardless of the specific NAT scheme employed, particularly in terms of higher pCR rates. Moreover, an increase in the TIL level following NAT treatment is associated with improved therapeutic outcomes in BC patients. The study findings indicate that the administration of anthracycline-

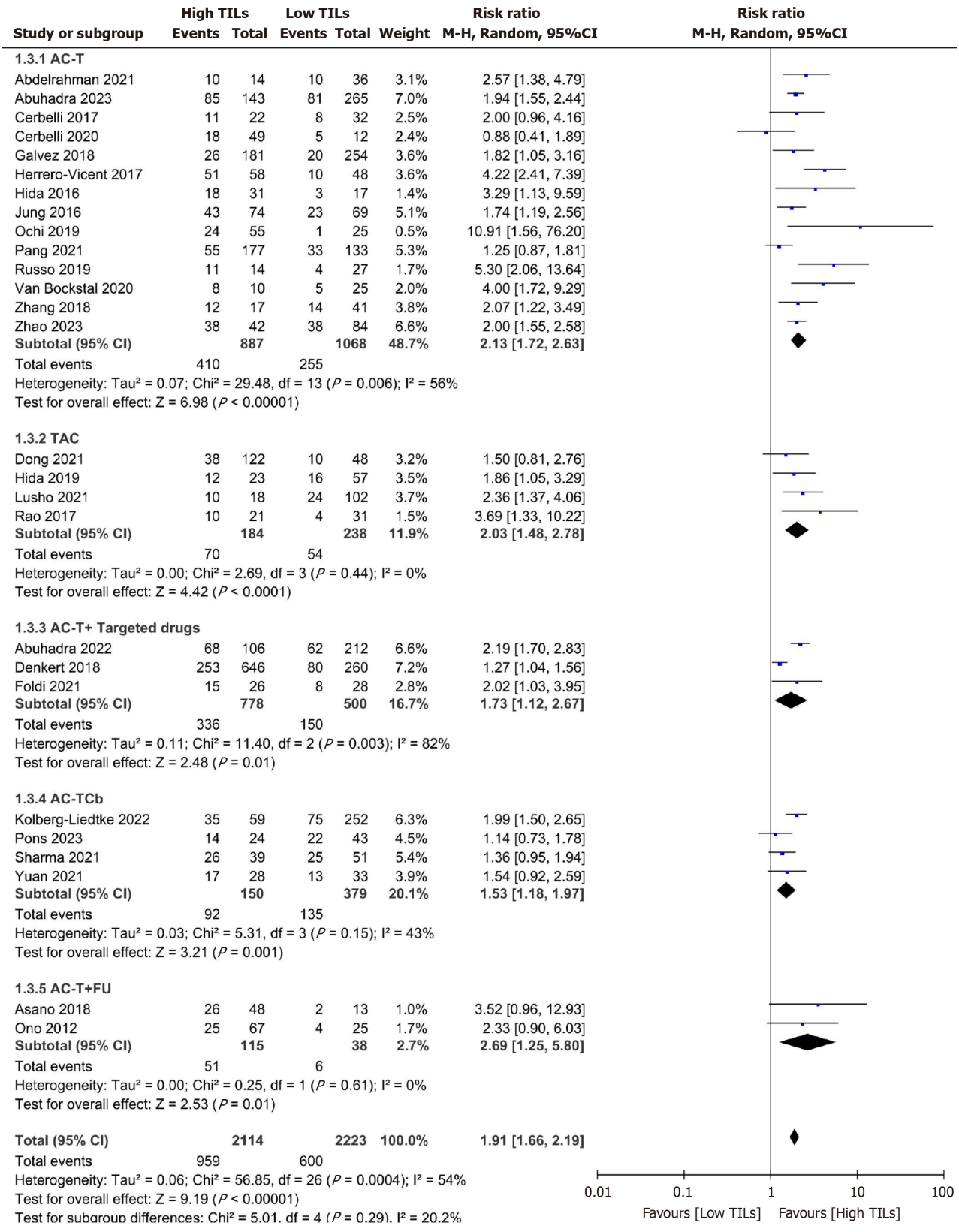

Figure 5 Forest plot illustrating the correlation between tumor-infiltrating lymphocyte levels and pathological complete response rates across various neoadjuvant therapy regimens. TIL: Tumor-infiltrating lymphocyte; AC: Anthracycline combined with cyclophosphamide; AC-T: Anthracycline combined with cyclophosphamide followed by paclitaxel or docetaxel; TAC: Paclitaxel or docetaxel combined with anthracycline, and cyclophosphamide; AC-TCb: Anthracycline combined with cyclophosphamide followed by paclitaxel or docetaxel, and platinum; AC-T + Fu: Anthracycline combined with cyclophosphamide followed by paclitaxel or docetaxel, and fluorouracil.

based chemotherapy drugs along with cyclophosphamide augments TIL levels in BC patients receiving NAT, and this increase in TIL levels is positively correlated with an improved pCR rate[33,59]. A study that stratified TNBC cohorts into lymphocyte-predominant BC (LPBC) and non-LPBC based on stromal TIL levels revealed that higher levels of stromal TILs in TNBC patients not only correlated with a greater pCR rate but also supported a greater pCR rate in LPBC patients than in non-LPBC patients. Additionally, even within the LPBC subgroup, the inclusion of platinum-based drugs in anthracycline-based chemotherapy followed by sequential paclitaxel yielded more significant benefits than in non-LPBC patients[60]. These clinical findings have been validated in various established experimental models of carcinogen-induced BC. In these animal models, the administration of doxorubicin amplifies the tumor antigen-specific proliferation of CD8+ T cells in tumor-draining lymph nodes in a homologous antigen-specific manner. Furthermore, it augments the ratio of CD8+ T cells infiltrating the tumor tissue and elicits tumor antigen-specific interferon-gamma production by these CD8+ TILs. Ultimately, the therapeutic effects of doxorubicin are mediated through these two mechanisms[61].

Due to the substantial heterogeneity observed in the meta-analysis of the 32 eligible studies, we performed subgroup analysis to investigate the sources of heterogeneity. The subgroup analysis showed that TNBC patients with high preoperative TIL counts exhibited increased pCR rates, irrespective of the study design. However, there were significant variations in heterogeneity among the different subgroups. In particular, the subgroup of randomized controlled trials and prospective cohort studies showed no interstudy heterogeneity, whereas the subgroup of retrospective cohort studies demonstrated considerable interstudy heterogeneity. Therefore, the primary contributor to the interstudy heterogeneity among the overall meta-analysis was attributed to the included retrospective cohort studies. These findings highlight the essential requirement for rigorous and well-designed research, including prospective designs and/or randomized controlled designs in future research protocols, to ensure the consistency and accuracy of clinical trial outcomes. Consequently, when assessing the predictive value of TILs for TNBC NAT treatment response, the meta-analysis results from the subgroup of randomized controlled trials and prospective cohort studies, which exhibit good consistency, can be considered robust evidence for clinical decision-making. Additionally, subgroup analysis was performed to explore the influence of high TIL cutoff values, the source of the study population, and the median sample size on the heterogeneity observed in the current meta-analysis. The analytical results presented that the differences in the cutoff values and the source of the study population were also potential sources of interstudy heterogeneity. Sensitivity analysis, carried out by sequentially excluding individual studies from the overall meta-analysis results, showed that the overall findings were not affected by any single study, but the heterogeneity varied. Notably, exclusion of the study conducted by Denkert *et al* [33] resulted in the lowest level of heterogeneity (I^2 = 45%).

Despite our comprehensive evaluation of the association between TIL levels in preoperative BC tissue treated with NATs and pCR in TNBC patients, our systematic review and meta-analysis has several limitations. First, the assessment of TILs is subjective, and there may be substantial variations in determining TIL levels among different studies due to the subjective judgments of various pathology experts. This subjectivity may impact the true relationship between TIL levels and treatment response and introduce heterogeneity across studies. Additionally, the analysis was limited by the paucity of studies that examined the correlation between TIL levels and NAT treatment response according to different molecular marker types of TILs. Consequently, it was not possible to more comprehensively conduct a subgroup analysis based on TIL molecular subtypes to explore the relationship between TIL levels and NAT treatment response. Finally, the restriction to studies published in English or Chinese may introduce language bias in this analysis. Therefore, given these considerations, it is advisable to interpret the results of this meta-analysis with caution.

CONCLUSION

In summary, this systematic review and meta-analysis indicated that TNBC patients with elevated TILs exhibited significantly greater pCR after NAT than those with low TILs, even among different NAT regimens and in TNBC patients from diverse populations. Therefore, it can be concluded that high TIL levels in preoperative TNBC tissue have the potential to predict treatment response to various NAT regimens in all TNBC patients. Additionally, the subgroup analysis results of homogeneous randomized controlled trials support the use of high TIL levels as Class Ia clinical evidence to predict NAT treatment response in TNBC patients, and the results of homogeneous prospective cohort studies are classified as class 2a evidence. Therefore, in clinical practice, adopting appropriate threshold to define high levels of TILs can effectively predict the response to NAT and aid in making NAT decisions for TNBC patients.

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FOOTNOTES

Author contributions: Sun HK and Jiang WL acquisition of data, analysis, and interpretation of data, drafting the article, final approval; Zhang SL, Xu PC, and Wei LM interpretation of data, revising the article, final approval; Liu JB conception and design of the study, critical revision, final approval. Sun HK and Jiang WL contributed equally to this work as co-first authors. The reasons for designating

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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 **Garrido-Castro AC**, Lin NU, Polyak K. Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment. *Cancer Discov* 2019; **9**: 176-198 [PMID: 30679171 DOI: 10.1158/2159-8290.CD-18-1177]
3 **Dent R**, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P, Narod SA. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007; **13**: 4429-4434 [PMID: 17671126 DOI: 10.1158/1078-0432.CCR-06-3045]
4 **Bianchini G**, De Angelis C, Licata L, Gianni L. Treatment landscape of triple-negative breast cancer - expanded options, evolving needs. *Nat Rev Clin Oncol* 2022; **19**: 91-113 [PMID: 34754128 DOI: 10.1038/s41571-021-00565-2]
5 **Denkert C**, Liedtke C, Tutt A, von Minckwitz G. Molecular alterations in triple-negative breast cancer-the road to new treatment strategies. *Lancet* 2017; **389**: 2430-2442 [PMID: 27939063 DOI: 10.1016/S0140-6736(16)32454-0]
6 **Leon-Ferre RA**, Goetz MP. Advances in systemic therapies for triple negative breast cancer. *BMJ* 2023; **381**: e071674 [PMID: 37253507 DOI: 10.1136/bmj-2022-071674]
7 **Vaidya JS**, Massarut S, Vaidya HJ, Alexander EC, Richards T, Caris JA, Sirohi B, Tobias JS. Rethinking neoadjuvant chemotherapy for breast cancer. *BMJ* 2018; **360**: j5913 [PMID: 29326104 DOI: 10.1136/bmj.j5913]
8 **Cortazar P**, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, Bonnefoi H, Cameron D, Gianni L, Valagussa P, Swain SM, Prowell T, Loibl S, Wickerham DL, Bogaerts J, Baselga J, Perou C, Blumenthal G, Blohmer J, Mamounas EP, Bergh J, Semiglazov V, Justice R, Eidtmann H, Paik S, Piccart M, Sridhara R, Fasching PA, Slaets L, Tang S, Gerber B, Geyer CE Jr, Pazdur R, Ditsch N, Rastogi P, Eiermann W, von Minckwitz G. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* 2014; **384**: 164-172 [PMID: 24529560 DOI: 10.1016/S0140-6736(13)62422-8]
9 **Savas P**, Salgado R, Denkert C, Sotiriou C, Darcy PK, Smyth MJ, Loi S. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. *Nat Rev Clin Oncol* 2016; **13**: 228-241 [PMID: 26667975 DOI: 10.1038/nrclinonc.2015.215]
10 **Denkert C**, Loibl S, Noske A, Roller M, Müller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C, von Törne C, Weichert W, Engels K, Solbach C, Schrader I, Dietel M, von Minckwitz G. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. *J Clin Oncol* 2010; **28**: 105-113 [PMID: 19917869 DOI: 10.1200/JCO.2009.23.7370]
11 **Schmid P**, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, Denkert C, Park YH, Hui R, Harbeck N, Takahashi M, Foukakis T, Fasching PA, Cardoso F, Untch M, Jia L, Karantza V, Zhao J, Aktan G, Dent R, O'Shaughnessy J; KEYNOTE-522 Investigators. Pembrolizumab for Early Triple-Negative Breast Cancer. *N Engl J Med* 2020; **382**: 810-821 [PMID: 32101663 DOI: 10.1056/NEJMoa1910549]
12 **Schmid P**, Cortes J, Dent R, Pusztai L, McArthur H, Kümmel S, Bergh J, Denkert C, Park YH, Hui R, Harbeck N, Takahashi M, Untch M, Fasching PA, Cardoso F, Andersen J, Patt D, Danso M, Ferreira M, Mouret-Reynier MA, Im SA, Ahn JH, Gion M, Baron-Hay S, Boileau JF, Ding Y, Tryfonidis K, Aktan G, Karantza V, O'Shaughnessy J; KEYNOTE-522 Investigators. Event-free Survival with Pembrolizumab in Early Triple-Negative Breast Cancer. *N Engl J Med* 2022; **386**: 556-567 [PMID: 35139274 DOI: 10.1056/NEJMoa2112651]
13 **Paijens ST**, Vledder A, de Bruyn M, Nijman HW. Tumor-infiltrating lymphocytes in the immunotherapy era. *Cell Mol Immunol* 2021; **18**: 842-859 [PMID: 33139907 DOI: 10.1038/s41423-020-00565-9]
14 **Loi S**, Michiels S, Adams S, Loibl S, Budczies J, Denkert C, Salgado R. The journey of tumor-infiltrating lymphocytes as a biomarker in breast cancer: clinical utility in an era of checkpoint inhibition. *Ann Oncol* 2021; **32**: 1236-1244 [PMID: 34311075 DOI: 10.1016/j.annonc.2021.07.007]

15 **Abuhadra N**, Sun R, Yam C, Rauch GM, Ding Q, Lim B, Thompson AM, Mittendorf EA, Adrada BE, Damodaran S, Virani K, White J, Ravenberg E, Sun J, Choi J, Candelaria R, Arun B, Ueno NT, Santiago L, Saleem S, Abouharb S, Murthy RK, Ibrahim N, Sahin A, Valero V, Symmans WF, Litton JK, Tripathy D, Moulder S, Huo L. Predictive Roles of Baseline Stromal Tumor-Infiltrating Lymphocytes and Ki-67 in Pathologic Complete Response in an Early-Stage Triple-Negative Breast Cancer Prospective Trial. *Cancers (Basel)* 2023; **15** [PMID: 37444385 DOI: 10.3390/cancers15133275]

16 **Abuhadra N**, Sun R, Litton JK, Rauch GM, Yam C, Chang JT, Seth S, Bassett R Jr, Lim B, Thompson AM, Mittendorf E, Adrada BE, Damodaran S, White J, Ravenberg E, Candelaria R, Arun B, Ueno NT, Santiago L, Saleem S, Abouharb S, Murthy RK, Ibrahim N, Sahin AA, Valero V, Symmans WF, Tripathy D, Moulder S, Huo L. Prognostic Impact of High Baseline Stromal Tumor-Infiltrating Lymphocytes in the Absence of Pathologic Complete Response in Early-Stage Triple-Negative Breast Cancer. *Cancers (Basel)* 2022; **14** [PMID: 35267631 DOI: 10.3390/cancers14051323]

17 **Galvez M**, Castaneda CA, Sanchez J, Castillo M, Rebaza LP, Calderon G, Cruz M, Cotrina JM, Abugattas J, Dunstan J, Guerra H, Mejia O, Gomez HL. Clinicopathological predictors of long-term benefit in breast cancer treated with neoadjuvant chemotherapy. *World J Clin Oncol* 2018; **9**: 33-41 [PMID: 29651385 DOI: 10.5306/wjco.v9.i2.33]

18 **Pang J**, Zhou H, Dong X, Wang S, Xiao Z. Relationship Between the Neutrophil to Lymphocyte Ratio, Stromal Tumor-infiltrating Lymphocytes, and the Prognosis and Response to Neoadjuvant Chemotherapy in Triple-negative Breast Cancer. *Clin Breast Cancer* 2021; **21**: e681-e687 [PMID: 34001439 DOI: 10.1016/j.clbc.2021.04.004]

19 **Loi S**, Drubay D, Adams S, Pruneri G, Francis PA, Lacroix-Triki M, Joensuu H, Dieci MV, Badve S, Demaria S, Gray R, Munzone E, Lemonnier J, Sotiriou C, Piccart MJ, Kellokumpu-Lehtinen PL, Vingiani A, Gray K, Andre F, Denkert C, Salgado R, Michiels S. Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers. *J Clin Oncol* 2019; **37**: 559-569 [PMID: 30650045 DOI: 10.1200/JCO.18.01010]

20 **Telli ML**, Chu C, Badve SS, Vinayak S, Silver DP, Isakoff SJ, Kaklamani V, Gradishar W, Stearns V, Connolly RM, Ford JM, Gruber JJ, Adams S, Garber J, Tung N, Neff C, Bernhisel R, Timms KM, Richardson AL. Association of Tumor-Infiltrating Lymphocytes with Homologous Recombination Deficiency and BRCA1/2 Status in Patients with Early Triple-Negative Breast Cancer: A Pooled Analysis. *Clin Cancer Res* 2020; **26**: 2704-2710 [PMID: 31796517 DOI: 10.1158/1078-0432.CCR-19-0664]

21 **Mao Y**, Qu Q, Zhang Y, Liu J, Chen X, Shen K. The value of tumor infiltrating lymphocytes (TILs) for predicting response to neoadjuvant chemotherapy in breast cancer: a systematic review and meta-analysis. *PLoS One* 2014; **9**: e115103 [PMID: 25501357 DOI: 10.1371/journal.pone.0115103]

22 **Page MJ**, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; **372**: n71 [PMID: 33782057 DOI: 10.1136/bmj.n71]

23 **Wells GA**, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses. [cited 30 August 2023]. Available from: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

24 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]

25 **Duval S**, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000; **56**: 455-463 [PMID: 10877304 DOI: 10.1111/j.0006-341x.2000.00455.x]

26 **Salgado R**, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, Wienert S, Van den Eynden G, Baehner FL, Penault-Llorca F, Perez EA, Thompson EA, Symmans WF, Richardson AL, Brock J, Criscitiello C, Bailey H, Ignatiadis M, Floris G, Sparano J, Kos Z, Nielsen T, Rimm DL, Allison KH, Reis-Filho JS, Loibl S, Sotiriou C, Viale G, Badve S, Adams S, Willard-Gallo K, Loi S; International TILs Working Group 2014. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol* 2015; **26**: 259-271 [PMID: 25214542 DOI: 10.1093/annonc/mdu450]

27 **Hida AI**, Watanabe T, Sagara Y, Kashiwaba M, Sagara Y, Aogi K, Ohi Y, Tanimoto A. Diffuse distribution of tumor-infiltrating lymphocytes is a marker for better prognosis and chemotherapeutic effect in triple-negative breast cancer. *Breast Cancer Res Treat* 2019; **178**: 283-294 [PMID: 31402409 DOI: 10.1007/s10549-019-05390-x]

28 **Lusho S**, Durando X, Mouret-Reynier MA, Kossai M, Lacrampe N, Molnar I, Penault-Llorca F, Radosevic-Robin N, Abrial C. Platelet-to-Lymphocyte Ratio Is Associated With Favorable Response to Neoadjuvant Chemotherapy in Triple Negative Breast Cancer: A Study on 120 Patients. *Front Oncol* 2021; **11**: 678315 [PMID: 34367964 DOI: 10.3389/fonc.2021.678315]

29 **Dong X**, Liu C, Yuan J, Wang S, Ding N, Li Y, Wu Y, Xiao Z. Prognostic Roles of Neutrophil-to-Lymphocyte Ratio and Stromal Tumor-Infiltrating Lymphocytes and Their Relationship in Locally Advanced Triple-Negative Breast Cancer Treated with Neoadjuvant Chemotherapy. *Breast Care (Basel)* 2021; **16**: 328-334 [PMID: 34602938 DOI: 10.1159/000509498]

30 **Rao N**, Qiu J, Wu J, Zeng H, Su F, Qiu K, Wu J, Yao H. Significance of Tumor-Infiltrating Lymphocytes and the Expression of Topoisomerase IIα in the Prediction of the Clinical Outcome of Patients with Triple-Negative Breast Cancer after Taxane-Anthracycline-Based Neoadjuvant Chemotherapy. *Chemotherapy* 2017; **62**: 246-255 [PMID: 28472798 DOI: 10.1159/000470900]

31 **Asano Y**, Kashiwagi S, Goto W, Takada K, Takahashi K, Hatano T, Takashima T, Tomita S, Motomura H, Ohsawa M, Hirakawa K, Ohira M. Prediction of Treatment Response to Neoadjuvant Chemotherapy in Breast Cancer by Subtype Using Tumor-infiltrating Lymphocytes. *Anticancer Res* 2018; **38**: 2311-2321 [PMID: 29599354 DOI: 10.21873/anticanres.12476]

32 **Ochi T**, Bianchini G, Ando M, Nozaki F, Kobayashi D, Criscitiello C, Curigliano G, Iwamoto T, Niikura N, Takei H, Yoshida A, Takei J, Suzuki K, Yamauchi H, Hayashi N. Predictive and prognostic value of stromal tumour-infiltrating lymphocytes before and after neoadjuvant therapy in triple negative and HER2-positive breast cancer. *Eur J Cancer* 2019; **118**: 41-48 [PMID: 31302586 DOI: 10.1016/j.ejca.2019.05.014]

33 **Denkert C**, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, Budczies J, Huober J, Klauschen F, Furlanetto J, Schmitt WD, Blohmer JU, Karn T, Pfitzner BM, Kümmel S, Engels K, Schneeweiss A, Hartmann A, Noske A, Fasching PA, Jackisch C, van Mackelenbergh M, Sinn P, Schem C, Hanusch C, Untch M, Loibl S. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol* 2018; **19**: 40-50 [PMID: 29233559 DOI: 10.1016/S1470-2045(17)30904-X]

34 **Yuan Y**, Lee JS, Yost SE, Li SM, Frankel PH, Ruel C, Schmolze D, Robinson K, Tang A, Martinez N, Stewart D, Waisman J, Kruper L, Jones V, Menicucci A, Uygun S, Yoder E, van der Baan B, Yim JH, Yeon C, Somlo G, Mortimer J. Phase II Trial of Neoadjuvant Carboplatin and

Nab-Paclitaxel in Patients with Triple-Negative Breast Cancer. *Oncologist* 2021; **26**: e382-e393 [PMID: 33098195 DOI: 10.1002/onco.13574]

35 **Wang Y**, Brodsky AS, Xiong J, Lopresti ML, Yang D, Resnick MB. Stromal Clusterin Expression Predicts Therapeutic Response to Neoadjuvant Chemotherapy in Triple Negative Breast Cancer. *Clin Breast Cancer* 2018; **18**: e373-e379 [PMID: 28890185 DOI: 10.1016/j.clbc.2017.08.007]

36 **Cerbelli B**, Botticelli A, Pisano A, Pernazza A, Campagna D, De Luca A, Ascierto PA, Pignataro MG, Pelullo M, Rocca CD, Marchetti P, Fortunato L, Costarelli L, d'Amati G. CD73 expression and pathologic response to neoadjuvant chemotherapy in triple negative breast cancer. *Virchows Arch* 2020; **476**: 569-576 [PMID: 31853625 DOI: 10.1007/s00428-019-02722-6]

37 **Hida AI**, Sagara Y, Yotsumoto D, Kanemitsu S, Kawano J, Baba S, Rai Y, Oshiro Y, Aogi K, Sagara Y, Ohi Y. Prognostic and predictive impacts of tumor-infiltrating lymphocytes differ between Triple-negative and HER2-positive breast cancers treated with standard systemic therapies. *Breast Cancer Res Treat* 2016; **158**: 1-9 [PMID: 27260189 DOI: 10.1007/s10549-016-3848-2]

38 **Foldi J**, Silber A, Reisenbichler E, Singh K, Fischbach N, Persico J, Adelson K, Katoch A, Horowitz N, Lannin D, Chagpar A, Park T, Marczyk M, Frederick C, Burrello T, Ibrahim E, Qing T, Bai Y, Blenman K, Rimm DL, Pusztai L. Neoadjuvant durvalumab plus weekly nab-paclitaxel and dose-dense doxorubicin/cyclophosphamide in triple-negative breast cancer. *NPJ Breast Cancer* 2021; **7**: 9 [PMID: 33558513 DOI: 10.1038/s41523-021-00219-7]

39 **Abdelrahman AE**, Rashed HE, MostafaToam, Omar A, Abdelhamid MI, Matar I. Clinicopathological significance of the immunologic signature (PDL1, FOXP3+ Tregs, TILs) in early stage triple-negative breast cancer treated with neoadjuvant chemotherapy. *Ann Diagn Pathol* 2021; **51**: 151676 [PMID: 33360026 DOI: 10.1016/j.anndiagpath.2020.151676]

40 **Cerbelli B**, Pernazza A, Botticelli A, Fortunato L, Monti M, Sciattella P, Campagna D, Mazzuca F, Mauri M, Naso G, Marchetti P, d'Amati G, Costarelli L. PD-L1 Expression in TNBC: A Predictive Biomarker of Response to Neoadjuvant Chemotherapy? *Biomed Res Int* 2017; **2017**: 1750925 [PMID: 29387716 DOI: 10.1155/2017/1750925]

41 **Cerbelli B**, Scagnoli S, Mezi S, De Luca A, Pisegna S, Amabile MI, Roberto M, Fortunato L, Costarelli L, Pernazza A, Strigari L, Della Rocca C, Marchetti P, d'Amati G, Botticelli A. Tissue Immune Profile: A Tool to Predict Response to Neoadjuvant Therapy in Triple Negative Breast Cancer. *Cancers (Basel)* 2020; **12** [PMID: 32947953 DOI: 10.3390/cancers12092648]

42 **Hamy AS**, Bonsang-Kitzis H, De Croze D, Laas E, Darrigues L, Topciu L, Menet E, Vincent-Salomon A, Lerebours F, Pierga JY, Brain E, Feron JG, Benchimol G, Lam GT, Laé M, Reyal F. Interaction between Molecular Subtypes and Stromal Immune Infiltration before and after Treatment in Breast Cancer Patients Treated with Neoadjuvant Chemotherapy. *Clin Cancer Res* 2019; **25**: 6731-6741 [PMID: 31515462 DOI: 10.1158/1078-0432.CCR-18-3017]

43 **Rangan R**, Kanetkar SR, Bhosale SJ, Mane DA, Patil NJ, Gudur RA. Assessment of Intratumoural and Stromal Infiltrating Lymphocytes In The Various Subtypes of Breast Carcinoma Patients who have Received Neoadjuvant Chemotherapy. *Asian Pac J Cancer Prev* 2023; **24**: 2347-2352 [PMID: 37505765 DOI: 10.31557/APJCP.2023.24.7.2347]

44 **Würfel F**, Erber R, Huebner H, Hein A, Lux MP, Jud S, Kremer A, Kranich H, Mackensen A, Häberle L, Hack CC, Rauh C, Wunderle M, Gaß P, Rabizadeh S, Brandl AL, Langemann H, Volz B, Nabieva N, Schulz-Wendtland R, Dudziak D, Beckmann MW, Hartmann A, Fasching PA, Rübner M. TILGen: A Program to Investigate Immune Targets in Breast Cancer Patients - First Results on the Influence of Tumor-Infiltrating Lymphocytes. *Breast Care (Basel)* 2018; **13**: 8-14 [PMID: 29950961 DOI: 10.1159/000486949]

45 **Pons L**, Hernández L, Urbizu A, Osorio P, Rodríguez-Martínez P, Castella E, Muñoz A, Sanz C, Arnaldo L, Felip E, Quiroga V, Tapia G, Margelí M, Fernandez PL. Pre- and Post-Neoadjuvant Clinicopathological Parameters Can Help in the Prognosis and the Prediction of Response in HER2+ and Triple Negative Breast Cancer. *Cancers (Basel)* 2023; **15** [PMID: 37370679 DOI: 10.3390/cancers15123068]

46 **Sharma P**, Kimler BF, O'Dea A, Nye L, Wang YY, Yoder R, Staley JM, Prochaska L, Wagner J, Amin AL, Larson K, Balanoff C, Elia M, Crane G, Madhusudhana S, Hoffmann M, Sheehan M, Rodriguez R, Finke K, Shah R, Satelli D, Shrestha A, Beck L, McKittrick R, Pluenneke R, Raja V, Beeki V, Corum L, Heldstab J, LaFaver S, Prager M, Phadnis M, Mudaranthakam DP, Jensen RA, Godwin AK, Salgado R, Mehta K, Khan Q. Randomized Phase II Trial of Anthracycline-free and Anthracycline-containing Neoadjuvant Carboplatin Chemotherapy Regimens in Stage I-III Triple-negative Breast Cancer (NeoSTOP). *Clin Cancer Res* 2021; **27**: 975-982 [PMID: 33208340 DOI: 10.1158/1078-0432.CCR-20-3646]

47 **Russo L**, Maltese A, Betancourt L, Romero G, Cialoni D, De la Fuente L, Gutierrez M, Ruiz A, Agüero E, Hernández S. Locally advanced breast cancer: Tumor-infiltrating lymphocytes as a predictive factor of response to neoadjuvant chemotherapy. *Eur J Surg Oncol* 2019; **45**: 963-968 [PMID: 30745134 DOI: 10.1016/j.ejso.2019.01.222]

48 **Herrero-Vicent C**, Guerrero A, Gavilá J, Gozalbo F, Hernández A, Sandiego S, Algarra MA, Calatrava A, Guillem-Porta V, Ruiz-Simón A. Predictive and prognostic impact of tumour-infiltrating lymphocytes in triple-negative breast cancer treated with neoadjuvant chemotherapy. *Ecancermedicalscience* 2017; **11**: 759 [PMID: 28900472 DOI: 10.3332/ecancer.2017.759]

49 **Van Bockstal MR**, Noel F, Guiot Y, Duhoux FP, Mazzeo F, Van Marcke C, Fellah L, Ledoux B, Berlière M, Galant C. Predictive markers for pathological complete response after neo-adjuvant chemotherapy in triple-negative breast cancer. *Ann Diagn Pathol* 2020; **49**: 151634 [PMID: 32987254 DOI: 10.1016/j.anndiagpath.2020.151634]

50 **Zhao M**, Xing H, He J, Wang X, Liu Y. Tumor infiltrating lymphocytes and neutrophil-to-lymphocyte ratio in relation to pathological complete remission to neoadjuvant therapy and prognosis in triple negative breast cancer. *Pathol Res Pract* 2023; **248**: 154687 [PMID: 37478522 DOI: 10.1016/j.prp.2023.154687]

51 **Kolberg-Liedtke C**, Feuerhake F, Garke M, Christgen M, Kates R, Grischke EM, Forstbauer H, Braun M, Warm M, Hackmann J, Uleer C, Aktas B, Schumacher C, Kuemmel S, Wuerstlein R, Graeser M, Nitz U, Kreipe H, Gluz O, Harbeck N. Impact of stromal tumor-infiltrating lymphocytes (sTILs) on response to neoadjuvant chemotherapy in triple-negative early breast cancer in the WSG-ADAPT TN trial. *Breast Cancer Res* 2022; **24**: 58 [PMID: 36056374 DOI: 10.1186/s13058-022-01552-w]

52 **Zhang L**, Wang XI, Zhang S. Tumor-infiltrating lymphocyte volume is a better predictor of neoadjuvant therapy response and overall survival in triple-negative invasive breast cancer. *Hum Pathol* 2018; **80**: 47-54 [PMID: 29883779 DOI: 10.1016/j.humpath.2018.05.024]

53 **Jung YY**, Hyun CL, Jin MS, Park IA, Chung YR, Shim B, Lee KH, Ryu HS. Histomorphological Factors Predicting the Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer. *J Breast Cancer* 2016; **19**: 261-267 [PMID: 27721875 DOI: 10.4048/jbc.2016.19.3.261]

54 **Ono M**, Tsuda H, Shimizu C, Yamamoto S, Shibata T, Yamamoto H, Hirata T, Yonemori K, Ando M, Tamura K, Katsumata N, Kinoshita T, Takiguchi Y, Tanzawa H, Fujiwara Y. Tumor-infiltrating lymphocytes are correlated with response to neoadjuvant chemotherapy in triple-negative breast cancer. *Breast Cancer Res Treat* 2012; **132**: 793-805 [PMID: 21562709 DOI: 10.1007/s10549-011-1554-7]

55 **Cao B**, Zhang Z, Wang C, Lv X. Prognostic relevance of tumorinfiltrating lymphocytes in residual tumor tissue from patients with triplenegative breast cancer following neoadjuvant chemotherapy: A systematic review and metaanalysis. *Oncol Lett* 2023; **26**: 441 [PMID:

37664648 DOI: 10.3892/ol.2023.14028]

56 **Heckler M**, Ali LR, Clancy-Thompson E, Qiang L, Ventre KS, Lenehan P, Roehle K, Luoma A, Boelaars K, Peters V, McCreary J, Boschert T, Wang ES, Suo S, Marangoni F, Mempel TR, Long HW, Wucherpfennig KW, Dougan M, Gray NS, Yuan GC, Goel S, Tolaney SM, Dougan SK. Inhibition of CDK4/6 Promotes CD8 T-cell Memory Formation. *Cancer Discov* 2021; **11**: 2564-2581 [PMID: 33941591 DOI: 10.1158/2159-8290.CD-20-1540]

57 **Vennin C**, Cattaneo CM, Bosch L, Vegna S, Ma X, Damstra HGJ, Martinovic M, Tsouri E, Ilic M, Azarang L, van Weering JRT, Pulver E, Zeeman AL, Schelfhorst T, Lohuis JO, Rios AC, Dekkers JF, Akkari L, Menezes R, Medema R, Baglio SR, Akhmanova A, Linn SC, Lemeer S, Pegtel DM, Voest EE, van Rheenen J. Taxanes trigger cancer cell killing in vivo by inducing non-canonical T cell cytotoxicity. *Cancer Cell* 2023; **41**: 1170-1185.e12 [PMID: 37311414 DOI: 10.1016/j.ccell.2023.05.009]

58 **Kester L**, Seinstra D, van Rossum AGJ, Vennin C, Hoogstraat M, van der Velden D, Opdam M, van Werkhoven E, Hahn K, Nederlof I, Lips EH, Mandjes IAM, van Leeuwen-Stok AE, Canisius S, van Tinteren H, Imholz ALT, Portielje JEA, Bos MEMM, Bakker SD, Rutgers EJ, Horlings HM, Wesseling J, Voest EE, Wessels LFA, Kok M, Oosterkamp HM, van Oudenaarden A, Linn SC, van Rheenen J. Differential Survival and Therapy Benefit of Patients with Breast Cancer Are Characterized by Distinct Epithelial and Immune Cell Microenvironments. *Clin Cancer Res* 2022; **28**: 960-971 [PMID: 34965952 DOI: 10.1158/1078-0432.CCR-21-1442]

59 **Loi S**, Sirtaine N, Piette F, Salgado R, Viale G, Van Eenoo F, Rouas G, Francis P, Crown JP, Hitre E, de Azambuja E, Quinaux E, Di Leo A, Michiels S, Piccart MJ, Sotiriou C. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. *J Clin Oncol* 2013; **31**: 860-867 [PMID: 23341518 DOI: 10.1200/JCO.2011.41.0902]

60 **Denkert C**, von Minckwitz G, Brase JC, Sinn BV, Gade S, Kronenwett R, Pfitzner BM, Salat C, Loi S, Schmitt WD, Schem C, Fisch K, Darb-Esfahani S, Mehta K, Sotiriou C, Wienert S, Klare P, André F, Klauschen F, Blohmer JU, Krappmann K, Schmidt M, Tesch H, Kümmel S, Sinn P, Jackisch C, Dietel M, Reimer T, Untch M, Loibl S. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. *J Clin Oncol* 2015; **33**: 983-991 [PMID: 25534375 DOI: 10.1200/JCO.2014.58.1967]

61 **Mattarollo SR**, Loi S, Duret H, Ma Y, Zitvogel L, Smyth MJ. Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. *Cancer Res* 2011; **71**: 4809-4820 [PMID: 21646474 DOI: 10.1158/0008-5472.CAN-11-0753]

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CASE REPORT

Rare primary squamous cell carcinoma of the intrahepatic bile duct: A case report and review of literature

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Abstract

BACKGROUND

Cholangiocarcinoma is the most common malignancy of the biliary tree and has a poor prognosis. Adenocarcinoma is the most common pathological type of cholangiocarcinomas, but rare squamous, adenosquamous, and mucinous variants have been reported without adequate clinical data.

CASE SUMMARY

This report describes a rare case of primary squamous cell carcinoma (SCC) of the intrahepatic bile duct. The patient was admitted with a tumor in the hepatic caudate lobe with no obvious clinical symptoms. Examination revealed hepatitis B surface antigen positivity, a slight increase in alfa-fetoprotein to 16.34 ng/mL, and an irregular slightly heterogeneous enhancing lesion in the hepatic caudate lobe, which was initially thought to be hepatocellular carcinoma. Laparoscopic resection was performed, and the final pathology suggested a rare primary SCC of the intrahepatic bile duct. Immunohistochemistry indicated positivity for villin, partial positivity for p63, and negativity for hepatocyte, CK7, CK8, CK19, and CK20. The Ki-67 index was approximately 60%. The patient received six cycles of Tegio chemotherapy. A new lesion was detected in the liver after 15 months. The surgery was performed, and the patient was followed-up at a local hospital. To date, no new lesions have been observed.

CONCLUSION

Surgery is the first choice for resectable lesions, and combined chemotherapy based on pathology is essential for increasing overall survival.

Key Words: Squamous cell carcinoma; Bile duct; Cholangiocarcinoma; Clinical characteristics; Treatment; Case report

Core Tip: We report a case of primary intrahepatic biliary squamous cell carcinoma (SCC) that was initially considered hepatocellular carcinoma. Intrahepatic biliary SCC is a rare pathological type without typical imaging features or serum markers. Its diagnosis depends on biopsy or postoperative pathology. Surgical resection is still considered the first choice for resectable lesions, but the intraoperative pathology of atypical liver lesions is essential for radical resection. Combined chemotherapy or chemoradiotherapy is beneficial for prolonging overall survival and decreasing the risk of recurrence. This case report and related literature review provide a valuable reference for the diagnosis and treatment of this disease.

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INTRODUCTION

Liver cancer is a malignancy that has a severe negative impact on human health worldwide. According to global cancer statistics for 2020, in men, the incidence rate of liver cancer was the sixth highest rate among cancers, and its mortality rate was the third highest. These rates were tenth and seventh highest, respectively, in women[1]. The pathological classification of primary liver cancer includes mainly hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and combined hepatocellular cholangiocarcinoma. The prognosis of ICC is particularly dismal owing to its highly malignant behavior, propensity for lymph node metastasis, and postoperative recurrence[2].

ICC is a cholangiocarcinoma that is located in a secondary or more proximal bile duct and is derived from epithelial cells in the duct. ICC accounts for approximately 10%-15% of primary liver cancers and < 10% of cholangiocarcinomas[3]. The main morphological growth patterns of ICCs are the formation of a mass, periductal and intraductal infiltration, and superficial spread[4]. Adenocarcinoma is the main pathological type, but there are some rare variants, including squamous, adenosquamous, mucinous, signet ring, clear, and undifferentiated cells[5]. Surgery is still the preferred treatment for ICC. However, unlike surgery for HCC, hepatic hilar lymph node dissection following resection of the primary lesion is recommended to improve the prognosis of ICC and prolong overall survival (OS). Chemotherapy, radiotherapy, and targeted or local treatment after pathological diagnosis could achieve a better prognosis for inoperable ICC[6]. However, the diagnosis and treatment strategies for some rare pathological types of ICC might be different from those used for biliary adenocarcinoma and need further exploration and discussion by clinicians.

This report describes a rare case of primary squamous cell carcinoma (SCC) of the intrahepatic bile duct that was initially misdiagnosed as atypical HCC based on imaging and laboratory examinations. The final pathology was determined after resection and guided subsequent chemotherapy. This report summarizes this case and discusses the key points of clinical diagnosis and related therapeutic strategies for biliary SCC, including surgery and chemotherapy regimens.

CASE PRESENTATION

Chief complaints

A 52-year-old man with a tumor of the hepatic caudate lobe that had been detected 14 days earlier in Changguo Hospital of Zibo (Shandong, China) was admitted on June 8, 2021.

History of present illness

Enhanced magnetic resonance imaging of the upper abdomen performed at a local hospital revealed a lesion in the hepatic caudate lobe that was thought to be a malignant tumor. Moreover, gastroscopy and colonoscopy revealed gastric and colonic polyps but did not detect a tumor. No treatment was started at that time.

History of past illness

The patient had a history of hepatitis B virus infection and was hepatitis B surface antigen-positive. However, he was not receiving treatment or undergoing regular examinations.

Personal and family history

The patient denied any family history of malignant tumors.

Physical examination

Physical examination did not reveal any obvious positive signs.

Laboratory examinations

Laboratory investigations confirmed hepatitis B surface antigen, hepatitis B e-antibody, and hepatitis B core antibody positivity without abnormal hepatitis B virus DNA or liver dysfunction. The alfa-fetoprotein level was slightly increased at 16.34 ng/mL (normal range: 0-8.78 ng/mL), and the carcinoembryonic antigen (normal range: 0-5 ng/mL) and carbohydrate antigen (CA) 19-9 (normal range: 0-27 U/mL) levels were 2.31 ng/mL and 6.1 U/mL, respectively. The patients' preoperative alanine aminotransferase and aspartate aminotransferase levels were 18.30 U/L (normal reference range: < 50 U/L) and 23.30 U/L (normal reference range: < 40 U/L), respectively. The levels of gamma-glutamyl transferase and alkaline phosphatase were 16.30 U/L (normal reference range: < 60 U/L) and 71.00 U/L (normal reference range: 45-125 U/L), respectively. The total bilirubin level was 7.70 μmol/L (normal reference range: 5.0-24.0 μmol/L). The albumin level was 38.30 g/L (normal reference range: 40-55 g/L). The white blood cell and red blood cell counts were 6.05 × 10^9/L [normal reference range: (3.5-9.5) × 10^9/L] and 4.13 × 10^{12}/L [normal reference range: (4.3-5.8) × 10^{12}/L], respectively.

Imaging examinations

An enhanced computed tomography (CT) scan indicated liver cirrhosis and showed an irregular lesion with a maximum diameter of 2.5 cm in the hepatic caudate lobe. The tumor showed slightly heterogeneous enhancement (Figure 1).

Further diagnostic work-up

Three days after admission, the patient underwent laparoscopic liver tumor resection at the First Affiliated Hospital of Shandong First Medical University. He recovered well following treatment with hepatoprotective medication, nutritional support, and human albumin and was discharged on postoperative day 10. Pathologic examination revealed a pale nodular mass measuring 4.0 cm × 3.0 cm × 2.2 cm that had a soft texture, was partially necrotic, had a clear boundary, and had invaded the liver capsule. According to the China Liver Cancer Staging, the tumor stage was I a, and the vascular invasion showed venous invasion. The background liver tissue exhibited hepatitis and cirrhosis, with no presence of cholelithiasis. Hematoxylin-eosin (HE) staining revealed atypical cells arranged in cords, and no keratin pearls were observed (Figure 2). Immunohistochemical examination revealed that the patient was positive for villin, partially positive for p63, and negative for glypican, hepatocyte, CK7, CK8, CK19, and CK20 (Figure 3). The Ki-67 index was approximately 60%.

FINAL DIAGNOSIS

The final pathological examination was considered as a rare primary SCC of the intrahepatic bile duct.

TREATMENT

Six cycles of Tegio (50 mg orally twice daily) were recommended after consultation with an oncology expert, and the patient opted for postoperative follow-up at a local hospital.

OUTCOME AND FOLLOW-UP

A new lesion was detected in the liver after 15 months, and surgery was performed at a local hospital without post-operative chemotherapy. To date, no new lesions have been detected.

DISCUSSION

Cholangiocarcinoma is a relatively rare malignant tumor of the digestive tract that may arise anywhere in the biliary duct and can be divided into intrahepatic, perihilar, or distal depending on the primary site[7]. Based on clinical data from single-center and multicenter analyses, the incidence of perihilar and distal disease is > 90%, and that of ICC is < 10%[3, 8]. Based on the above classification, the corresponding operations and complete lymph node dissection, such as hepatectomy and duodenopancreatectomy, are the first choices for prolonging OS. For advanced cholangiocarcinoma, chemotherapy consisting of gemcitabine and cisplatin is recommended as the standard first-line treatment for improving progression-free survival and OS[9]. Folinic acid, fluorouracil, and oxaliplatin (FOLFOX) plus active symptom control is considered the second-line choice because a study showed that the median OS and the 6-month and 12-month OS rates were better in patients who received FOLFOX plus active symptom control than in those who received active symptom control alone[10]. In addition to chemotherapy and radiotherapy, immunotherapy in combination with targeted therapy (*e.g.*, pembrolizumab + lenvatinib) and immunotherapy alone are being explored for treating cholangiocarcinoma[11].

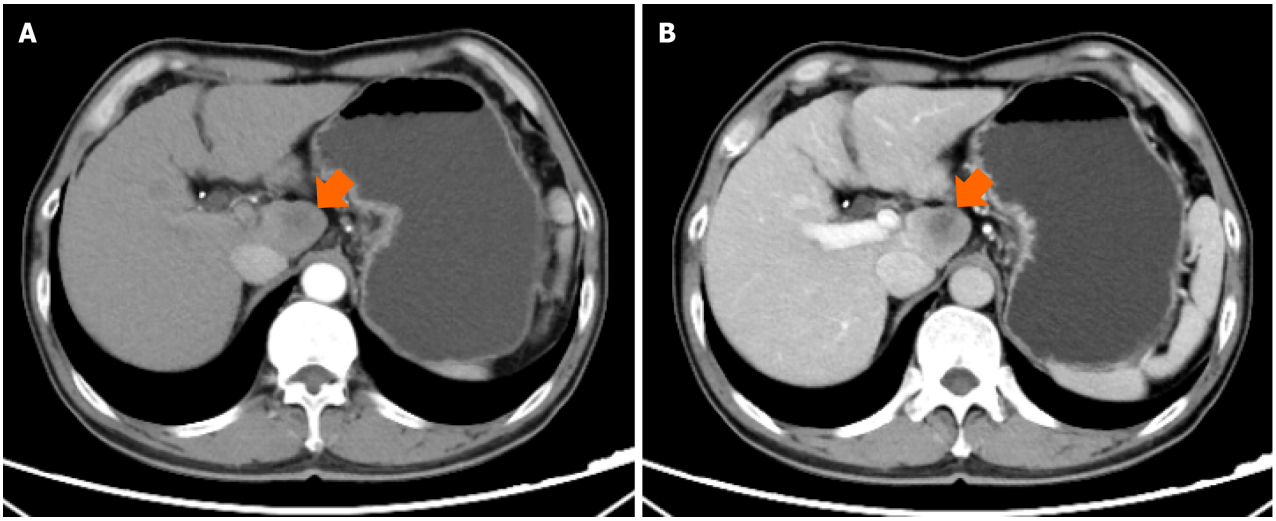

Figure 1 Computed tomography imaging of liver lesion. A: The arterial phase of computed tomography (CT) showed no obvious enhancement (orange arrow); B: The venous phase of CT showed slightly heterogeneous enhancement (orange arrow).

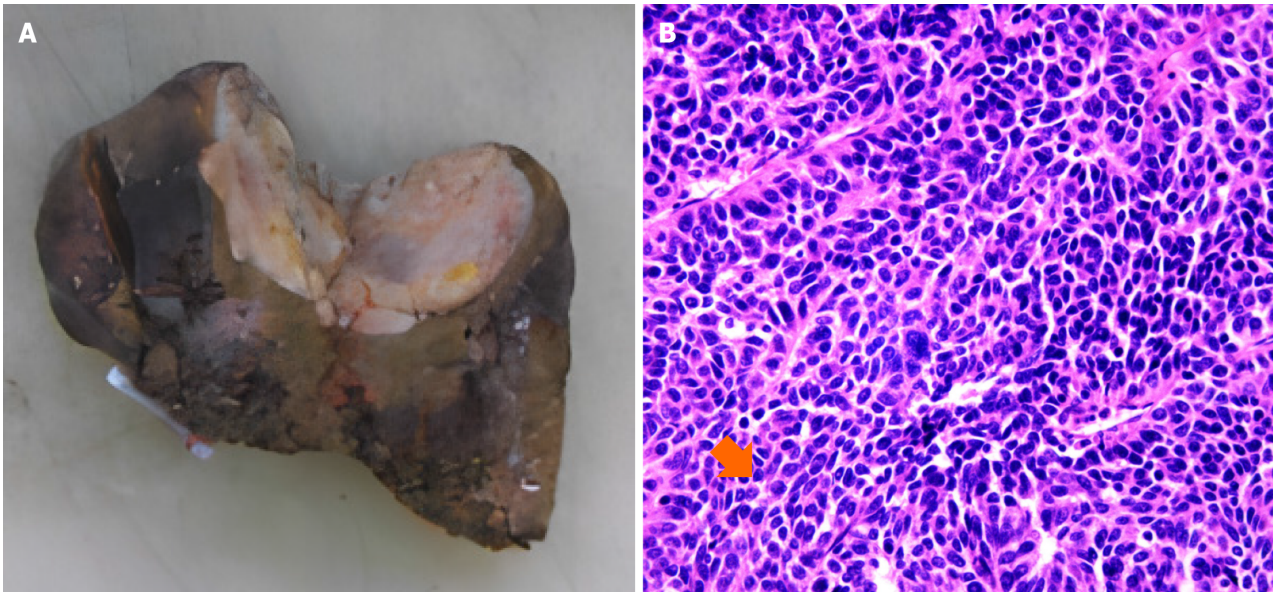

Figure 2 The pathology of liver lesion. A: The lesion owned soft texture, partially necrosis, clear boundary, and invasion of liver capsule; B: Hematoxylin and eosin staining indicated atypical cell arranged in nests (orange arrow), and no obvious keratin pearl was observed.

The main risk factors for cholangiocarcinoma are biliary diseases, biliary malformations/choledochal cysts, cholelithiasis, cholecystitis/cholecystectomy, liver flukes, hepatitis C virus infection, and type 2 diabetes[12]. The prognosis of biliary SCC is worse than that of intrahepatic bile duct adenocarcinoma or HCC because of lymph node metastasis, irregular margins, and vascular invasion. The most common pathologic type is adenocarcinoma, and exploration of therapeutic strategies is focused mainly on this type[13]. However, we sometimes encounter rare types of cholangiocarcinoma, such as biliary SCC, which has a poor prognosis owing to a lack of effective clinical therapies and limited relevant research data[14]. Therefore, we explored the published literature on the epidemiology, clinical features, and diagnosis and treatment of SCC of the bile duct.

The pathogenesis of SCC of the bile duct remains unclear, and we explored it mainly *via* case reports because of the lack of clinical studies. Several theories have been suggested. First, anaplastic carcinoma of the bile duct could differentiate into adenocarcinoma or SCC[15]. Second, SCC could derive from adenocarcinoma, and Iemura *et al*[16] described the possible transformation from adenocarcinoma to SCC in a mouse model of cholangiocarcinoma. Third, the metaplastic squamous epithelium of the bile duct has the potential to transform into SCC, and the inflammation associated with choledochal cysts, cholelithiasis, sclerosing cholangitis, and Caroli disease could be an important cause of metaplasia[17]. Some experts have suggested that the "inflammation-cancer" transformation is an inescapable risk factor for the occurrence of cholangiocarcinoma, although SCC is rarer than adenocarcinoma[18]. Fourth, ectopic squamous epithelium may be another etiology. In our patient, the final pathology was considered to be poorly differentiated biliary SCC possibly derived from anaplastic carcinoma. The positive expression of p63 and the arrangement of atypical cells in

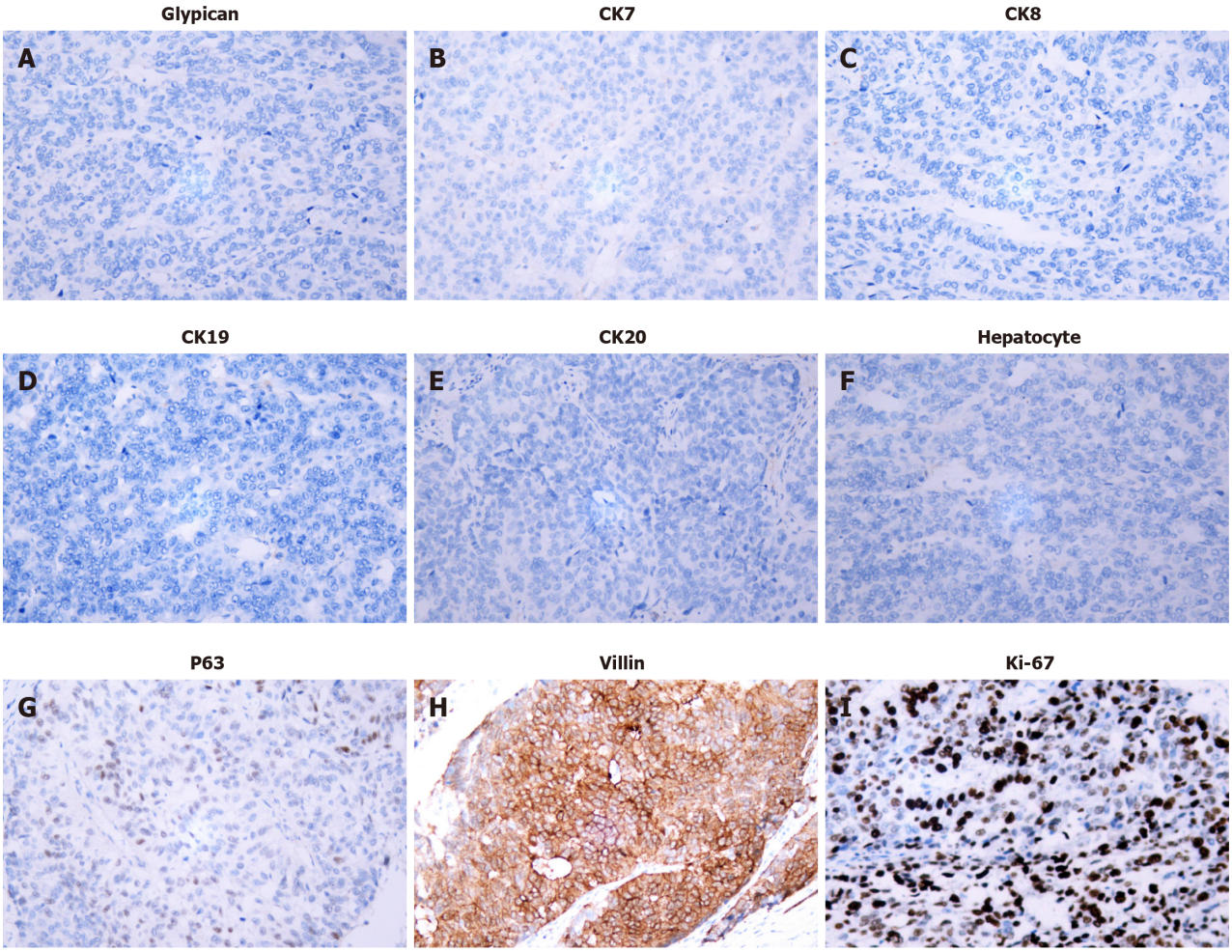

Figure 3 Immunohistochemistry of liver lesion. The immunohistochemical examination was positive for villin, p63, and negative for glypican, hepatocyte, CK7, CK8, CK19, and CK20, the Ki-67 index was about 60% (× 200). A: Glypican; B: CK7; C: CK8; D: CK19; E: CK20; F: Hepatocyte; G: P63; H: Villin; I: Ki-67.

nests were the basis for the diagnosis of SCC (Figures 2 and 3).

According to our literature review, biliary SCC is most common in the extrahepatic bile duct, and clinical reports of the intrahepatic type are rare. Unlike for that of cholangioadenocarcinoma, an accurate diagnosis of biliary SCC is difficult owing to its rarity and lack of typical manifestations. We found that the common clinical symptoms of biliary SCC were abdominal pain, jaundice, and gastrointestinal abnormalities, which could be caused by biliary obstruction, inflammation, neuronal invasion, or biliary spasm[14]. However, some patients have no significant symptoms, especially if they have the intrahepatic type, as in our case. Laboratory examinations indicated impaired liver function with elevated CA19-9 levels. However, only five of the 14 patients listed in Table 1 had elevated CA19-9 levels, suggesting that the specificity of CA 19-9 is lower for SCC than for cholangioadenocarcinoma[19]. However, the relevant data are limited. Analysis of large-scale multicenter clinical data remains important, as is examination of the levels of the hepatobiliary tumor markers carcinoembryonic antigen, CA 19-9, and alfa-fetoprotein. On imaging, HCC is always presented as markedly enhanced in the arterial phase and has low-density contrast in the venous phase. Moreover, ICC adenocarcinoma has delayed enhancement in the venous phase, sometimes accompanied by dilation of the distal bile duct on CT or magnetic resonance imaging. However, biliary SCC may present as an avascular and delayed or heterogeneous enhancement mass similar to that of the ICC adenocarcinoma[18,20] (Table 1). Our patient's CT manifestations revealed an avascular and heterogeneous enhancement mass. The above data indicate that biliary SCC has no classical serologic or radiologic findings. Biopsy is essential for advanced hepatobiliary tumors; needle biopsy is suitable for intrahepatic lesions. However, surgery remains the first option for resectable lesions because of the risk of needle transfer during biopsy. Rapid intraoperative pathology is required for accurate diagnosis and complete resection. Endoscopic retrograde cholangiopancreatography, intraductal ultrasonography, or spy glass are valuable for the biopsy of extrahepatic lesions[14]. Even if SCC of the bile duct has been confirmed, gastroenteroscopy and positron emission tomography-CT are still necessary in view of reports of biliary metastasis of esophageal SCC[21,22]. The immunohistochemistry results vary widely among differentiated cases. Keratin pearls and CK5/6 positivity are consistently observed, but the final pathology should include both clinical features and cytologic morphology[23] (Table 1). In our case, the patient tested positive for villin, which was not previously observed in SCC patients. However, we cannot deny that villin is not a specific marker for SCC. The detection of villin was used to identify the source of the tumor, and the final diagnosis should be determined by its combination with other markers, such as p63[24].

Table 1 The review of literature for squamous cell carcinoma of bile duct

Ref.	Case	Symptoms	Serum study	CT/MR	Pathology	Treatments
Gatof *et al*[14], 2004	A 86-year-old female	Abdominal pain and jaundice	-	Cholelithiasis of CBD and dilated bile duct	SCC with pancytokeratin stains (+)	Chemotherapy and high-dose radiation
Sewkani, *et al*[17], 2005	A 60-year-old male	Jaundice	-	A thickening of the distal BD	keratin pearl (+)	Surgery
Abbas *et al*[27], 2008	A 28-year-old female	Abdominal pain and jaundice	-	An avascular liver mass	SCC with dysplasia of the bile-duct epithelium	Extended left lobectomy and image guided external beam radiation
Price *et al*[31], 2008	A 41-year-old female	Painless jaundice, diarrhea	ALP↑, CA199↑	Choledochal cysts with cholelithiasis and a mass	SCC with keratin pearl	Chemotherapy and radiation
Avezbadalov *et al*[29], 2014	A 78-year-old Hispanic woman	Somnolent	ALP↑, GGTP↑	Liver carcinoma with 17.5 cm invading colon–hepatic flexure	poorly diferentiated SCC: CK5/6(+), p63(+), CK19(+), CK20(-), CK7(-), HMB-45(-), TTF-I(-)	Home hospice
Goto *et al*[23], 2016	A 77-year-old female	Jaundice with fatigue	DBIL↑, TBIL↑, ALP↑, normal CA199	A solid mass in the junction of the cystic and common bile ducts	Keratinization, CK5/6(+), p53(+), MIB-1(+), periodic acid staining(-)	Surgery and adjuvant chemotherapy with gemcitabine
Nishiguchi *et al*[26], 2016	A 78-year-old male	Brown urine	Normal CA199 and CEA, elevated hepatobiliary enzyme	The thickening and enhancement of distal BD	keratin pearl(+)	Surgery and chemotherapy with cisplatin and tegafur/gimeracil/oteracil
Tamaoka *et al*[20], 2018	A 82-year-old male	No significant symptoms	CA199↑	Liver mass with homogeneous density and delayed enhancement	CK 5/6(+), Keratin pearls(+), p40(-)	Extended right lobectomy
Knudsen *et al*[28], 2019	A 66-year-old female	Dark-colored urine	ALT↑, ALP↑, GGT	CBD dilation with a tumor in distal BD	Keratin pearl(+), CK5/6(+), p40(+)	Surgery
Wang *et al*[18], 2020	A 32-year-old female with choledochal cyst	Nausea, abdominal pain	ALP↑, DBIL↑, TBIL↑	Choledochal cyst complicated a tumor	SCC with scattered distribution of heteromorphic epithelial cells	Surgery and postoperative chemotherapy with gemcitabine and cisplatin
Bacha *et al*[30], 2021	A 35-year-old male	Epigastric pain, jaundice and fatigue	DBIL↑, TBIL↑, GGTP↑, ALP↑, normal CA199 and CEA	Irregular wall thickening in the distal CBD	Keratinization, CK 19(+)	Biliary drainage
Zhong *et al*[19], 2021	A 76-year-old male	Jaundice	TBIL↑, GGTP↑, ALP↑, CA199↑	A low signal intensity area in the middle CBD	CK5/6(+), p63(+), CK20(-), no keratin pearls	Surgery and postoperative chemotherapy with gemcitabine and cisplatin
Shrestha *et al*[32], 2021	A 21-year-old male	Abdominal pain and jaundice	DBIL↑, ALP↑, CA199↑	Choledochal cysts with cholelithiasis	SCC with atypical cells arranged in cords and nests	Surgery and paclitaxel-based chemotherapy

SCC: Squamous cell carcinoma; ALP: Alkaline phosphatase; GGTP: Gamma-glutamyl transpeptidase; DBIL: Direct bilirubin; TBIL: Total bilirubin; PGE 1: Prostaglandin E 1; DVT: Deep venous thrombosis; TIPS: Transjugular intrahepatic portosystemic shunt; TACE: Transarterial chemoembolization; DIC: Disseminated intravascular coagulation; AST: Aspartate aminotransferase; ALT: Alanine transaminase; CA199: Carbohydrate antigen 199; CEA: Carcinoembryonic antigen; CBD: Common bile duct; GGT: Gamma-glutamyl transferase; CT: Computed tomography; MR: Magnetic resonance.

Due to its highly fibroproliferative nature, complex tumor microenvironment, and genetic heterogeneity, cholangiocarcinoma is prone to developing drug resistance[25]. Owing to its rarity, the clinical behavior of biliary SCC is still poorly understood, and targeted treatment options are inadequate. Moreover, SCC is likely to progress to an advanced stage, with a short survival time, large tumor size, aggressive intrahepatic spread, and frequent metastasis[15]. Surgical resection remains the preferred treatment for biliary SCC depending on the primary site. For patients with advanced disease and those who have undergone surgery, the recommended chemotherapy strategy is gemcitabine plus oxaliplatin or gemcitabine plus cisplatin. Chemotherapy with S1 plus cisplatin or docetaxel plus cisplatin plus 5-fluorouracil has also been reported to be helpful[26]. SCC has mainly been reported *via* case reports and treated by surgery, radiation, and chemotherapy. However, advanced SCC patients only receive relieving treatment, such as biliary drainage and analgesic treatment (Table 1), and these chemotherapy regimens mainly refer to the Guidelines of Chinese Society of Clinical Oncology (CSCO) Biliary Tract Cancer 2020 and Guidelines of Chinese Society of Clinical Oncology (CSCO) Biliary Tract Cancer 2023[6,7,12]. Intraoperative radiation to the surgical margins combined with postoperative external beam radiation is an option for decreasing the risk of local recurrence[27]. The limited data available suggest that targeted chemotherapy or chemoradiotherapy has prognostic benefits, including prolonged OS and decreased risk of recurrence, but confirmation is required in multicenter clinical studies. The importance of intraoperative pathology for all liver lesions should be recognized, and a standardized diagnostic process could decrease the rate of misdiagnosis, allow more precise treatment strategies, improve prognosis, and prolong OS.

Furthermore, some genetic polymorphisms have been shown to increase the risk of cholangiocarcinoma, to be involved in DNA repair (MTHFR, TYMS, GSTO1, and XRCC1), to protect cells from toxins (ABCC2, CYP1A2, and NAT2), and to play a role in immune surveillance (KLRK1, MICA, and PTGS2)[7]. After encountering the case reported here, we reviewed the clinical features and treatment options for biliary SCC. Knudsen *et al*[28] reported that alterations in FBXW7, CREBBP, CTCF, FAT1, MAGI2, MLL2, and NOTCH1 can predict the risk of extrahepatic biliary SCC.

CONCLUSION

In conclusion, the preoperative diagnosis of biliary SCC is difficult, and surgery is still the first choice for treating resectable lesions. Postoperative chemotherapy treatment requires multidisciplinary consultation, and Tegio therapy alone could be considered to improve postoperative outcomes. Furthermore, whole-genome sequencing of tumor specimens may be necessary to achieve precise and personalized molecular targeted therapy and improve OS. Since SCC is rare, its clinical, pathological and therapeutic outcomes still need to be further explored *via* multicenter cooperation.

FOOTNOTES

Author contributions: Ma QJ and Yang NN designed the report and wrote the paper, they contributed equally to this manuscript; Wang FH and Liu F performed the surgery; Wei HL collected the patient's clinical data; Wang FH analyzed the data and revised the paper; all authors have read and approved the final version of this manuscript.

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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 **El-Diwany R**, Pawlik TM, Ejaz A. Intrahepatic Cholangiocarcinoma. *Surg Oncol Clin N Am* 2019; **28**: 587-599 [PMID: 31472907 DOI: 10.1016/j.soc.2019.06.002]

3 **DeOliveira ML**, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; **245**: 755-762 [PMID: 17457168 DOI: 10.1097/01.sla.0000251366.62632.d3]

4 **Razumilava N**, Gores GJ. Classification, diagnosis, and management of cholangiocarcinoma. *Clin Gastroenterol Hepatol* 2013; **11**: 13-21.e1; quiz e3 [PMID: 22982100 DOI: 10.1016/j.cgh.2012.09.009]

5 **Washington MK**, Berlin J, Branton PA, Burgart LJ, Carter DK, Compton CC, Fitzgibbons PL, Frankel WL, Jessup JM, Kakar S, Minsky B, Nakhleh RE, Vauthey JN; Members of the Cancer Committee, College of American Pathologists. Protocol for the examination of specimens from patients with carcinoma of the distal extrahepatic bile ducts. *Arch Pathol Lab Med* 2010; **134**: e8-13 [PMID: 20367298 DOI: 10.5858/134.4.e8]

6 **Khan AS**, Dageforde LA. Cholangiocarcinoma. *Surg Clin North Am* 2019; **99**: 315-335 [PMID: 30846037 DOI: 10.1016/j.suc.2018.12.004]

7 **Tyson GL**, El-Serag HB. Risk factors for cholangiocarcinoma. *Hepatology* 2011; **54**: 173-184 [PMID: 21488076 DOI: 10.1002/hep.24351]

8 **de Jong MC**, Marques H, Clary BM, Bauer TW, Marsh JW, Ribero D, Majno P, Hatzaras I, Walters DM, Barbas AS, Mega R, Schulick RD, Choti MA, Geller DA, Barroso E, Mentha G, Capussotti L, Pawlik TM. The impact of portal vein resection on outcomes for hilar cholangiocarcinoma: a multi-institutional analysis of 305 cases. *Cancer* 2012; **118**: 4737-4747 [PMID: 22415526 DOI: 10.1002/cncr.27492]

9 **Valle JW**, Furuse J, Jitlal M, Beare S, Mizuno N, Wasan H, Bridgewater J, Okusaka T. Cisplatin and gemcitabine for advanced biliary tract cancer: a meta-analysis of two randomised trials. *Ann Oncol* 2014; **25**: 391-398 [PMID: 24351397 DOI: 10.1093/annonc/mdt540]

10 **Lamarca A**, Palmer DH, Wasan HS, Ross PJ, Ma YT, Arora A, Falk S, Gillmore R, Wadsley J, Patel K, Anthoney A, Maraveyas A, Iveson T, Waters JS, Hobbs C, Barber S, Ryder WD, Ramage J, Davies LM, Bridgewater JA, Valle JW; Advanced Biliary Cancer Working Group. Second-line FOLFOX chemotherapy versus active symptom control for advanced biliary tract cancer (ABC-06): a phase 3, open-label, randomised, controlled trial. *Lancet Oncol* 2021; **22**: 690-701 [PMID: 33798493 DOI: 10.1016/S1470-2045(21)00027-9]

11 **Kam AE**, Masood A, Shroff RT. Current and emerging therapies for advanced biliary tract cancers. *Lancet Gastroenterol Hepatol* 2021; **6**: 956-969 [PMID: 34626563 DOI: 10.1016/S2468-1253(21)00171-0]

12 **Krasinskas AM**. Cholangiocarcinoma. *Surg Pathol Clin* 2018; **11**: 403-429 [PMID: 29751883 DOI: 10.1016/j.path.2018.02.005]

13 **Chen R**, Zheng D, Li Q, Xu S, Ye C, Jiang Q, Yan F, Jia Y, Zhang X, Ruan J. Immunotherapy of cholangiocarcinoma: Therapeutic strategies and predictive biomarkers. *Cancer Lett* 2022; **546**: 215853 [PMID: 35921970 DOI: 10.1016/j.canlet.2022.215853]

14 **Gatof D**, Chen YK, Shah RJ. Primary squamous cell carcinoma of the bile duct diagnosed by transpapillary cholangioscopy: case report and review. *Gastrointest Endosc* 2004; **60**: 300-304 [PMID: 15278069 DOI: 10.1016/s0016-5107(04)01561-5]

15 **Nakajima T**, Kondo Y. A clinicopathologic study of intrahepatic cholangiocarcinoma containing a component of squamous cell carcinoma. *Cancer* 1990; **65**: 1401-1404 [PMID: 2155056 DOI: 10.1002/1097-0142(19900315)65:6<1401::aid-cncr2820650626>3.0.co;2-k]

16 **Iemura A**, Yano H, Mizoguchi A, Kojiro M. A cholangiocellular carcinoma nude mouse strain showing histologic alteration from adenocarcinoma to squamous cell carcinoma. *Cancer* 1992; **70**: 415-422 [PMID: 1319814 DOI: 10.1002/1097-0142(19920715)70:2<415::aid-cncr2820700208>3.0.co;2-9]

17 **Sewkani A**, Kapoor S, Sharma S, Naik S, Juneja M, Jain A, Varshney S. Squamous cell carcinoma of the distal common bile duct. *JOP* 2005; **6**: 162-165 [PMID: 15767732]

18 **Wang L**, Ma X, Chen D, Ren J, Cheng H, Huang G, Wang R, Cheng J. Primary congenital choledochal cyst with squamous cell carcinoma: a case report. *J Int Med Res* 2020; **48**: 300060520946871 [PMID: 32962499 DOI: 10.1177/0300060520946871]

19 **Zhong L**, Qi W, Tao F, Zhang L, Kong J. Primary squamous cell carcinoma of the common bile duct. *Indian J Pathol Microbiol* 2021; **64**: S182-S183 [PMID: 34135167 DOI: 10.4103/IJPM.IJPM_633_20]

20 **Tamaoka K**, Tanemura M, Furukawa K, Mikamori M, Saito T, Ohtsuka M, Suzuki Y, Tei M, Kishi K, Yasuoka H, Tsujimoto M, Akamatsu H. Primary Intrahepatic Squamous Cell Carcinoma with Histological Collision of Adenocarcinoma and Squamous Cell Carcinoma: A Case Report. *Am J Case Rep* 2018; **19**: 1184-1191 [PMID: 30287803 DOI: 10.12659/AJCR.910676]

21 **Rana SS**, Kapoor R, Gupta P, Gupta R. Extrahepatic biliary obstruction due to bile duct metastasis from primary esophageal squamous cell carcinoma: a rare cause of jaundice. *Ann Gastroenterol* 2019; **32**: 528 [PMID: 31474804 DOI: 10.20524/aog.2019.0397]

22 **Yoo Y**, Mun S. Synchronous double primary squamous cell carcinoma and adenocarcinoma of the extrahepatic bile duct: a case report. *J Med Case Rep* 2015; **9**: 116 [PMID: 25986701 DOI: 10.1186/s13256-015-0600-1]

23 **Goto T**, Sasajima J, Koizumi K, Sugiyama Y, Kawamoto T, Fujibayashi S, Moriichi K, Yamada M, Fujiya M, Kohgo Y. Primary Poorly Differentiated Squamous Cell Carcinoma of the Extrahepatic Bile Duct. *Intern Med* 2016; **55**: 1581-1584 [PMID: 27301509 DOI: 10.2169/internalmedicine.55.6226]

24 **Dum D**, Lennartz M, Menz A, Kluth M, Hube-Magg C, Weidemann S, Fraune C, Luebke AM, Hornsteiner L, Bernreuther C, Simon R, Clauditz TS, Sauter G, Uhlig R, Hinsch A, Kind S, Jacobsen F, Möller K, Wilczak W, Steurer S, Minner S, Burandt E, Marx AH, Krech T, Lebok P. Villin expression in human tumors: a tissue microarray study on 14,398 tumors. *Expert Rev Mol Diagn* 2022; **22**: 665-675 [PMID: 35866621 DOI: 10.1080/14737159.2022.2104122]

25 **Razumilava N**, Gores GJ. Cholangiocarcinoma. *Lancet* 2014; **383**: 2168-2179 [PMID: 24581682 DOI: 10.1016/S0140-6736(13)61903-0]

26 **Nishiguchi R**, Kim DH, Honda M, Sakamoto T. Squamous cell carcinoma of the extrahepatic bile duct with metachronous para-aortic lymph node metastasis successfully treated with S-1 plus cisplatin. *BMJ Case Rep* 2016; **2016** [PMID: 27932438 DOI: 10.1136/bcr-2016-218177]

27 **Abbas R**, Willis J, Kinsella T, Siegel C, Sanabria J. Primary squamous cell carcinoma of the main hepatic bile duct. *Can J Surg* 2008; **51**: E85-E86 [PMID: 18815636]

28 **Knudsen KN**, Mortensen MB, Detlefsen S. Squamous cell carcinoma of the common bile duct: A case report with genomic profiling. *Pathol Int* 2019; **69**: 427-431 [PMID: 31286611 DOI: 10.1111/pin.12806]

29 **Avezbadalov A**, Aksenov S, Kaplan B, Jung G. Asymptomatic primary squamous cell carcinoma of the liver. *J Community Support Oncol* 2014; **12**: 75-76 [PMID: 24971409 DOI: 10.12788/jcso.0019]

30 **Bacha D**, Hajri M, Ferjaoui W, Talbi G, Gharbi L, Khalfallah MT, Slama SB, Lahmar A. Primary squamous cell carcinoma of the common bile duct with liver metastases. *Arq Bras Cir Dig* 2021; **34**: e1564 [PMID: 34008708 DOI: 10.1590/0102-672020190004e1564]

31 **Price L**, Kozarek R, Agoff N. Squamous cell carcinoma arising within a choledochal cyst. *Dig Dis Sci* 2008; **53**: 2822-2825 [PMID: 18274902 DOI: 10.1007/s10620-007-0182-x]

32 **Shrestha S**, Bhandari RS, Shrestha BM, Shrestha S, Subedi N, Pradhan S. Squamous cell carcinoma arising within choledochal cyst managed by pancreaticoduodenectomy: A case report. *Int J Surg Case Rep* 2021; **82**: 105919 [PMID: 33932816 DOI: 10.1016/j.ijscr.2021.105919]

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CASE REPORT

Concomitant epidermal growth factor receptor mutation/c-ros oncogene 1 rearrangement in non-small cell lung cancer: A case report

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Abstract

BACKGROUND

Epidermal growth factor receptor (*EGFR*) mutation and c-ros oncogene 1 (*ROS1*) rearrangement are key genetic alterations and predictive tumor markers for non-small cell lung cancer (NSCLC) and are typically considered to be mutually exclusive. *EGFR/ROS1* co-mutation is a rare event, and the standard treatment approach for such cases is still equivocal.

CASE SUMMARY

Herein, we report the case of a 64-year-old woman diagnosed with lung adenocarcinoma, with concomitant *EGFR* L858R mutation and *ROS1* rearrangement. The patient received two cycles of chemotherapy after surgery, but the disease progressed. Following 1-month treatment with gefitinib, the disease progressed again. However, after switching to crizotinib, the lesion became stable. Currently, crizotinib has been administered for over 53 months with a remarkable treatment effect.

CONCLUSION

The efficacy of *EGFR* tyrosine kinase inhibitors and crizotinib was vastly different in this NSCLC patient with *EGFR/ROS1* co-mutation. This report will aid future treatment of such patients.

Key Words: Non-small cell lung cancer; Epidermal growth factor receptor; C-ros oncogene 1; Co-mutation; Treatment strategies; Case report

Core Tip: Over the past two decades, molecular targeted therapies have improved clinical outcomes significantly for non-small cell lung cancer (NSCLC) patients with either epidermal growth factor receptor (*EGFR*) mutation or c-ros oncogene 1 (*ROS1*) fusion. Nevertheless, *EGFR*/*ROS1* co-mutation is a rare event in NSCLC, and the standard treatment for such cases is still equivocal. We report an *EGFR*/*ROS1* co-mutation in an NSCLC patient who remained clinically stable after 53 months of treatment with crizotinib. This is the longest progression-free survival reported in the literature. This case suggested that crizotinib may be a potential choice for NSCLC patients with such *EGFR*/*ROS1* co-mutations.

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INTRODUCTION

Lung cancer has the second-highest incidence of all cancers and the highest cancer-related death rate in the world[1]. Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancers. Lung adenocarcinoma is the most common histopathological subtype of NSCLC, accounting for about 55% of cases. The mutation rate of epidermal growth factor receptor (*EGFR*) in Asian patients with lung adenocarcinoma is approximately 51%, and the mutation rate of c-ros oncogene 1 (*ROS1*) in NSCLC is 2%-3%[2-4]. *EGFR/ROS1* co-mutation is relatively rare, and the incidence of concomitant *EGFR* mutations in patients with *ROS1* fusion genes is less than 24%[5-7].

Early studies have shown that tyrosine kinase inhibitors (TKIs) targeting *EGFR* and *ROS1* are the first-line treatment for patients with *EGFR* mutations or *ROS1* rearrangement[8-11]. However, there is currently no consensus on the optimal management of patients with these co-mutations. This article reports the treatment of an NSCLC patient with *EGFR* and *ROS1* co-mutation to provide a reference for the treatment and management of other NSCLC patients with these co-mutations.

CASE PRESENTATION

Chief complaints

A 64-year-old Chinese woman presented on November 1, 2018 with a complaint of cough and sputum production for 1 wk.

History of present illness

Symptoms started 1 wk before presentation, with cough and sputum production.

History of past illness

An appendectomy due to appendicitis had been performed approximately 30 years prior to the presentation. Cholecystectomy due to cholelithiasis had been performed 10 years after the appendectomy.

Personal and family history

The patient denied any family history of malignant tumors. The patient also had no history of smoking.

Physical examination

On physical examination, the vital signs were as follows: Body temperature, 36.6 °C; blood pressure, 136/75 mmHg; heart rate, 74 beats per min; and respiratory rate, 20 breaths per min.

Laboratory examinations

Levels of serum tumor markers were normal. Carcinoembryonic antigen was < 0.5 ng/mL (normal range: 0-5 ng/mL), squamous cell carcinoma antigen was 0.6 ng/mL (normal range: 0-1.5 ng/mL), and cytokeratin 19 fragment was 1.57 ng/mL (normal range: 0-2.08 ng/mL). No abnormality was found in the routine blood and urine analyses.

Imaging examinations

On November 2, 2018, chest computed tomography (CT) showed a 2.8 cm × 1.8 cm patchy shadow in the inferior lobe of the right lung. The scan also showed multiple ground-glass nodules in bilateral lungs. The patient was prescribed oral anti-tuberculosis drugs. She discontinued the medication after 1 month due to intolerance. On March 25, 2019, the patient visited our hospital again. Chest CT showed a 4.0 cm × 2.7 cm shadow in the lower lobe of the right lung. Due to the significant growth, the possibility of a neoplastic lesion was considered.

FINAL DIAGNOSIS

Combined with the patient's medical history, the final diagnosis was determined to be NSCLC.

TREATMENT

On April 3, 2019, thoracoscopic radical resection of the lower right lung cancer and pleural adhesion cauterization was performed under general anesthesia. Postoperative pathological diagnosis was acinar adenocarcinoma grade III, micropapillary adenocarcinoma (accounting for about 5% of tumor tissue) in the local area, and tumor thrombus in the alveolar cavity (Figure 1). *ROS1* rearrangement and *EGFR* L858R mutation were detected by next-generation sequencing of 13 lung cancer driver genes. The frequency of the *EGFR* mutation found in this patient was only 1.3% (Figure 2).

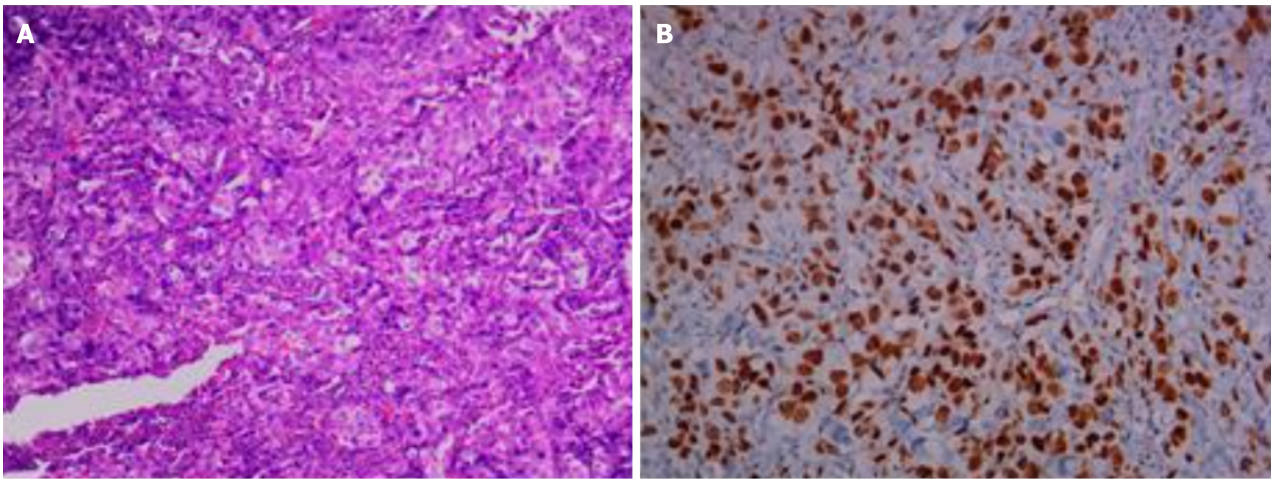

Figure 1 Histological and immunohistochemical features. A: Histology of lung biopsy; B: Expression pattern of thyroid transcription factor 1.

After thoracoscopic radical resection, the patient received two cycles of chemotherapy (pemetrexed 690 mg D1 + cisplatin 50 mg D1-2; on May 14, 2019 and June 5, 2019). Comparison of the CT film obtained on June 25, 2019 with the previous film (May 7, 2019) showed progression of the lesion. Multiple lymph nodes in the mediastinum and right hilum were also enlarged, suggesting metastasis (Figure 3).

Gefitinib was administered for 1 month starting on June 26, 2019. During this period, the patient developed drug-induced diarrhea, which improved after treatment with loperamide. Chest CT on July 30, 2019 revealed that multiple lymph nodes in the mediastinum, right cardiophrenic angle, and right hilum were enlarged, indicating progression of the disease (Figure 3).

On August 7, 2019, crizotinib was administered. The efficacy of crizotinib was evaluated after 1 month, which revealed stable disease. Since then, the patient has been treated with crizotinib for over 53 months and continues to have stable disease (Figure 3).

OUTCOME AND FOLLOW-UP

After 53 months of treatment with crizotinib, the patient is still alive.

DISCUSSION

Genetic mutations are typically considered to be mutually exclusive[12-15]. A recent study[16] demonstrated that patients with co-mutations have a poor progression-free survival. Patients with both *ROS1* fusion and *EGFR* mutations are exceedingly rare in comparison to the co-occurrence of *EGFR/PIK3CA*, *ALK/KRAS*, and *EGFR/MET* mutations[16-18]. However, using highly sensitive methods such as the amplification-refractory mutation system analysis leads to an increase in the detection rate of co-mutations of *KRAS* or *BRAF* mutations and *ALK* or *ROS1* rearrangements[13]. Studies [13-15] have shown that the incidence of concomitant gene mutations in lung adenocarcinoma was 3.8%, and *EGFR/ROS1* and *EML4-ALK* were among the mutations detected. There is no consensus on the clinical characteristics, treatment options, and prognosis of NSCLC patients with co-mutations.

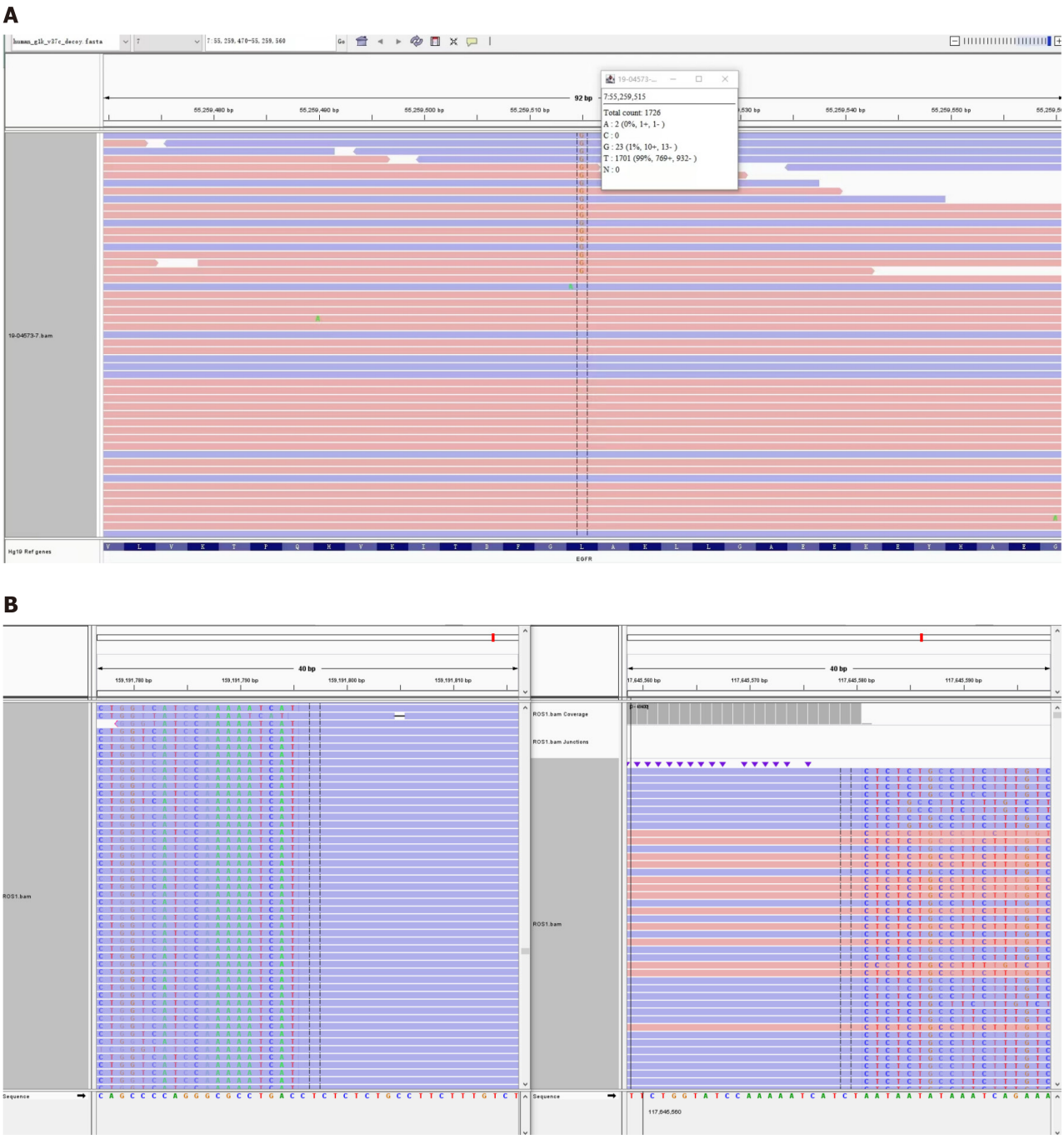

Figure 2 Detection of epidermal growth factor receptor mutation and c-ros oncogene 1 rearrangement using high sensitivity next-generation sequencing. A: Epidermal growth factor receptor L858R mutation; B: *EZR-ROS1* E10R34 fusion.

The mechanism of *EGFR/ROS1* co-mutation remains unclear. Wu *et al*[19] hypothesized that two gene alterations can be detected within the same tumor cells in NSCLC, which might result in cancer development through co-action. Won *et al*[20] proposed that genetic instability in cancer cells leads to genetic and phenotypic heterogeneity in tumors, indicating that different genetic mutations might occur in tumor cells rather than in a single clone. Accumulating evidence shows co-existence of classical oncogenes, including *EGFR*, *ALK*, *ROS1*, and *MET*, in lung adenocarcinoma patients, especially in young females with no history of smoking[21]. In this case report, the patient was an elderly woman and the pathology suggested adenocarcinoma, which was consistent with the literature reports.

Cases of *EGFR/ROS1* co-mutation have been infrequently reported in NSCLC, creating a lack of clear treatment standards. Zhang *et al*[22] reported a patient with an *EGFR* exon 21 L858R mutation. The disease progressed after gefitinib treatment for 11 months. The gene test showed *EGFR* exon 21 L858R mutation (abundance 36.62%), *EGFR* exon 20 T790M (7.95%), and *ROS1* fusion (15.81%), as well as *EGFR*, *HER2*, and *BRAF* amplification. The second-line treatment included osimertinib, and the disease progressed after 7 months. The third-line chemotherapy included two cycles of pemetrexed and carboplatin, which did not prevent disease progression.

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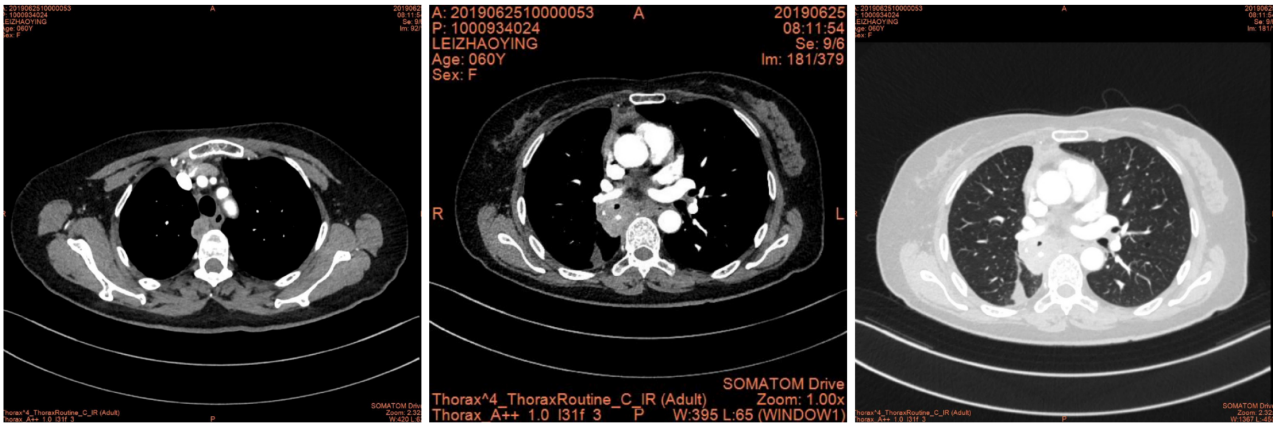
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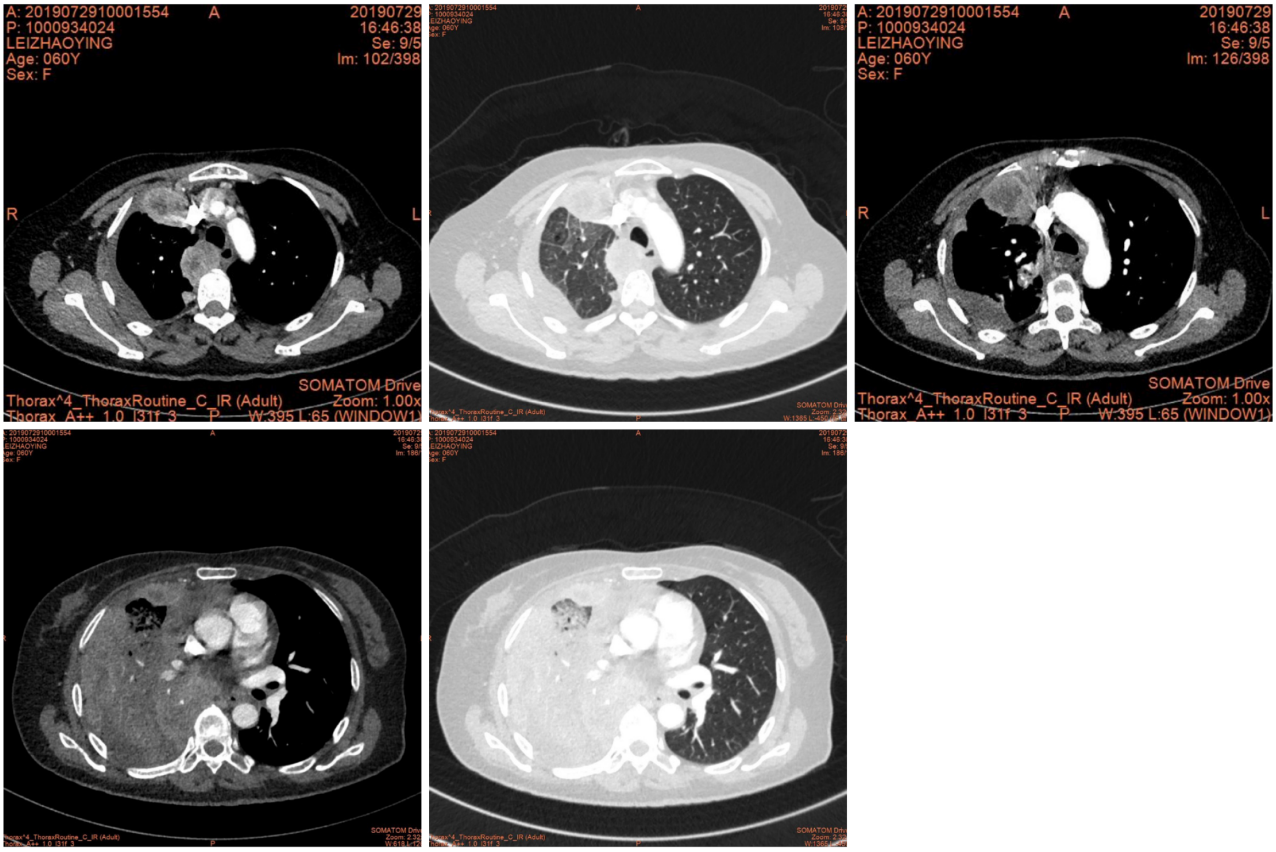
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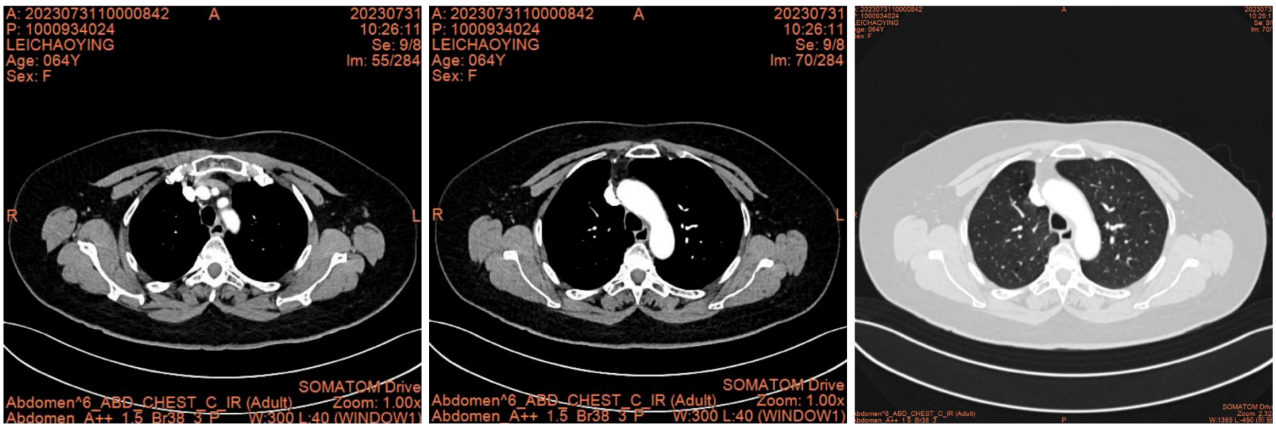
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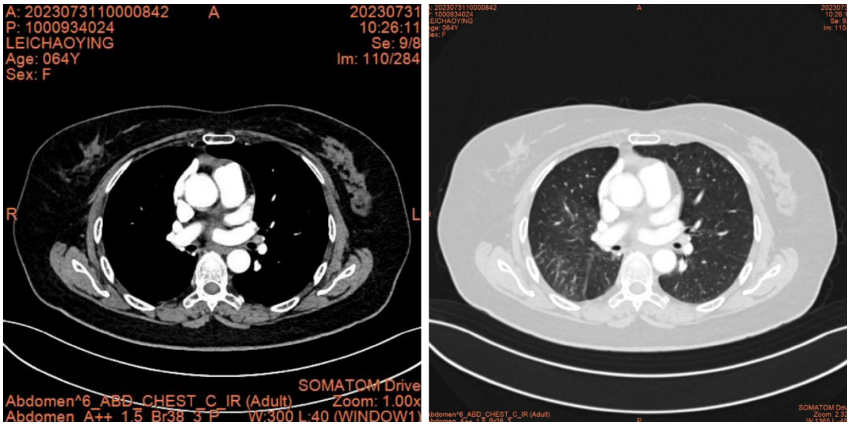

Figure 3 Serial computed tomography images. A: Computed tomography (CT) after postoperative recurrence; B: CT after 1 month of gefitinib therapy. Progressive disease of the lung was detected; C: CT after 53 months of crizotinib therapy. Stable disease was detected.

Another study reported the cases of three stage IA NSCLC patients with *EGFR/ROS1* co-mutation who received chemotherapy (regimen not reported) for 3 months after surgery[23]. During the 12-month follow-up, one patient developed relapse and the other two were in stable condition. In another case report, a 53-year-old man was diagnosed with lung adenocarcinoma with concomitant *EGFR* and *KRAS* mutations and *ROS1* rearrangement[24]. He was treated with crizotinib as the first-line treatment, but the disease progressed after 1 month. After icotinib was administered for 1 month, a partial curative effect was observed. The condition of that patient was stable after 8 months of maintenance treatment.

According to Zhuang *et al*[21], the progression-free survival of patients with an *EGFR* co-mutation treated with first-line TKIs was better than that of patients treated with first-line chemotherapy. A recent study described the case of a 48-year-old woman diagnosed with NSCLC with an *EGFR* 19 deletion/*ROS1* rearrangement who achieved a favorable outcome[19]. Following 1 month of treatment with a combination of almonertinib and crizotinib, there was a significant reduction in the primary mass and all lymph nodes. Almonertinib was subsequently replaced by furmonertinib due to elevated levels of creatine kinase. The patient received furmonertinib and crizotinib treatment for 7 months, and stable disease was achieved throughout the follow-up period.

It appears that the clinical efficacy of *EGFR* TKIs and crizotinib treatment is quite different based on these clinical reports and our experience. The best choice for first-line treatment remains unclear. It has been reported, though, that the efficacy of TKIs can be predicted by the phosphorylation levels of EGFR and ALK in patients with *EGFR/ALK* co-mutation[25].

Nevertheless, there are still several challenges in predicting the efficacy of treatment in patients with *EGFR/ROS1* co-mutations. First, current genetic testing is unable to determine the dominant oncogene aberration in a single tumor cell. Second, there is limited research on the relationship between the abundance of a *ROS1* mutation and lung cancer prognosis. Finally, there have been no reports on whether EGFR phosphorylation levels and *ROS1* mutation abundance can predict the effectiveness of TKIs. In our report, we found that the patient had a very low *EGFR* mutation frequency (1.3%), which may explain the poor response to the gefitinib treatment. Therefore, it is possible that low EGFR phosphorylation levels could be associated with a poorer prognosis when treated with TKI therapy.

Liu *et al*[26] observed that TKI combination therapy was more effective than single TKI treatment in patients with *EGFR/ALK* co-mutations. Wu *et al*[19] demonstrated similar outcome in one patient with an *EFGR* 19 deletion/*ROS1* rearrangement upon treatment with a combination of third-generation EGFR TKIs and crizotinib. Combination therapy appears to exhibit a favorable efficacy. However, not all patients are able to tolerate the toxicity associated with TKI combination therapy. Therefore, further investigation is required to determine the necessity of combination therapy.

There is no overall survival difference between patients with single *EGFR* mutations and those with concomitant *ALK/ROS1* mutations (21.0 months *vs* 23.0 months, respectively, $P = 0.196$)[27]. However, concomitant *EGFR* mutation and *ALK/ROS1* mutation reduced the therapeutic effect of EGFR TKIs in patients. Patients with co-mutations had a significantly shorter progression-free survival than those with a single *EGFR* mutation (6.6 months *vs* 10.7 months, respectively, $P = 0.004$)[27]. In our report, the patient remained stable after 53 months of crizotinib treatment, which is the longest reported progression-free survival of a patient with an *EGFR/ROS1* co-mutation.

CONCLUSION

EGFR/ROS1 co-mutation is rare in patients with NSCLC. Due to its rarity, the best treatment approach is unclear. TKI therapy can be used as the first-line option, but the clinical efficacy of EGFR-TKIs and crizotinib therapy appears to vary significantly between patients. The level of *EGFR* phosphorylation may play a crucial role in the selection of therapeutic drugs. Further investigation is required to examine the correlation between *ROS1* mutation frequency and the prognosis of lung cancer.

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FOOTNOTES

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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 **Shi Y**, Au JS, Thongprasert S, Srinivasan S, Tsai CM, Khoa MT, Heeroma K, Itoh Y, Cornelio G, Yang PC. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol* 2014; **9**: 154-162 [PMID: 24419411 DOI: 10.1097/JTO.0000000000000033]

3 **Han B**, Tjulandin S, Hagiwara K, Normanno N, Wulandari L, Laktionov K, Hudoyo A, He Y, Zhang YP, Wang MZ, Liu CY, Ratcliffe M, McCormack R, Reck M. EGFR mutation prevalence in Asia-Pacific and Russian patients with advanced NSCLC of adenocarcinoma and non-adenocarcinoma histology: The IGNITE study. *Lung Cancer* 2017; **113**: 37-44 [PMID: 29110846 DOI: 10.1016/j.lungcan.2017.08.021]

4 **Kohno T**, Nakaoku T, Tsuta K, Tsuchihara K, Matsumoto S, Yoh K, Goto K. Beyond ALK-RET, ROS1 and other oncogene fusions in lung cancer. *Transl Lung Cancer Res* 2015; **4**: 156-164 [PMID: 25870798 DOI: 10.3978/j.issn.2218-6751.2014.11.11]

5 **Lin JJ**, Ritterhouse LL, Ali SM, Bailey M, Schrock AB, Gainor JF, Ferris LA, Mino-Kenudson M, Miller VA, Iafrate AJ, Lennerz JK, Shaw AT. ROS1 Fusions Rarely Overlap with Other Oncogenic Drivers in Non-Small Cell Lung Cancer. *J Thorac Oncol* 2017; **12**: 872-877 [PMID: 28088512 DOI: 10.1016/j.jtho.2017.01.004]

6 **Scheffler M**, Schultheis A, Teixido C, Michels S, Morales-Espinosa D, Viteri S, Hartmann W, Merkelbach-Bruse S, Fischer R, Schildhaus HU, Fassunke J, Sebastian M, Serke M, Kaminsky B, Randerath W, Gerigk U, Ko YD, Krüger S, Schnell R, Rothe A, Kropf-Sanchen C, Heukamp L, Rosell R, Büttner R, Wolf J. ROS1 rearrangements in lung adenocarcinoma: prognostic impact, therapeutic options and genetic variability. *Oncotarget* 2015; **6**: 10577-10585 [PMID: 25868855 DOI: 10.18632/oncotarget.3387]

7 **Wiesweg M**, Eberhardt WEE, Reis H, Ting S, Savvidou N, Skiba C, Herold T, Christoph DC, Meiler J, Worm K, Kasper S, Theegarten D, Hense J, Hager T, Darwiche K, Oezkan F, Aigner C, Welter S, Kühl H, Stuschke M, Schmid KW, Schuler M. High Prevalence of Concomitant Oncogene Mutations in Prospectively Identified Patients with ROS1-Positive Metastatic Lung Cancer. *J Thorac Oncol* 2017; **12**: 54-64 [PMID: 27575422 DOI: 10.1016/j.jtho.2016.08.137]

8 **Mok TS**, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; **361**: 947-957 [PMID: 19692680 DOI: 10.1056/NEJMoa0810699]

9 **Zhou C**, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, Lu S, Zhang L, Hu C, Hu C, Luo Y, Chen L, Ye M, Huang J, Zhi X, Zhang Y, Xiu Q, Ma J, Zhang L, You C. Erlotinib *versus* chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. *Lancet Oncol* 2011; **12**: 735-742 [PMID: 21783417 DOI: 10.1016/S1470-2045(11)70184-X]

10 **Shaw AT**, Ou SH, Bang YJ, Camidge DR, Solomon BJ, Salgia R, Riely GJ, Varella-Garcia M, Shapiro GI, Costa DB, Doebele RC, Le LP, Zheng Z, Tan W, Stephenson P, Shreeve SM, Tye LM, Christensen JG, Wilner KD, Clark JW, Iafrate AJ. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med* 2014; **371**: 1963-1971 [PMID: 25264305 DOI: 10.1056/NEJMoa1406766]

11 **Soria JC**, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, Dechaphunkul A, Imamura F, Nogami N, Kurata T, Okamoto I, Zhou C, Cho BC, Cheng Y, Cho EK, Voon PJ, Planchard D, Su WC, Gray JE, Lee SM, Hodge R, Marotti M, Rukazenkov Y, Ramalingam SS; FLAURA Investigators. Osimertinib in Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer. *N Engl J Med* 2018; **378**: 113-125 [PMID: 29151359 DOI: 10.1056/NEJMoa1713137]

12 **Cardarella S**, Ortiz TM, Joshi VA, Butaney M, Jackman DM, Kwiatkowski DJ, Yeap BY, Jänne PA, Lindeman NI, Johnson BE. The introduction of systematic genomic testing for patients with non-small-cell lung cancer. *J Thorac Oncol* 2012; **7**: 1767-1774 [PMID: 23154547 DOI: 10.1097/JTO.0b013e3182745bcb]

13 **Takeuchi K**, Soda M, Togashi Y, Suzuki R, Sakata S, Hatano S, Asaka R, Hamanaka W, Ninomiya H, Uehara H, Lim Choi Y, Satoh Y, Okumura S, Nakagawa K, Mano H, Ishikawa Y. RET, ROS1 and ALK fusions in lung cancer. *Nat Med* 2012; **18**: 378-381 [PMID: 22327623 DOI: 10.1038/nm.2658]

14 **Sun Y**, Ren Y, Fang Z, Li C, Fang R, Gao B, Han X, Tian W, Pao W, Chen H, Ji H. Lung adenocarcinoma from East Asian never-smokers is a disease largely defined by targetable oncogenic mutant kinases. *J Clin Oncol* 2010; **28**: 4616-4620 [PMID: 20855837 DOI: 10.1200/JCO.2010.29.6038]

15 **Yoshida A**, Kohno T, Tsuta K, Wakai S, Arai Y, Shimada Y, Asamura H, Furuta K, Shibata T, Tsuda H. ROS1-rearranged lung cancer: a clinicopathologic and molecular study of 15 surgical cases. *Am J Surg Pathol* 2013; **37**: 554-562 [PMID: 23426121 DOI: 10.1097/PAS.0b013e3182758fe6]

16 **Li W**, Chang F, Zhang H, Meng F, Ke Z, Zhang Y. Clinical Pathological Characteristics and Prognosis of Multigene Co-Mutations in Elderly Patients With Non-Small Cell Lung Cancer: A Retrospective Analysis. *Clin Med Insights Oncol* 2023; **17**: 11795549231211505 [PMID: 38033742 DOI: 10.1177/11795549231211505]

17 **Sakamoto T**, Matsubara T, Takahama T, Yokoyama T, Nakamura A, Tokito T, Okamoto T, Akamatsu H, Oki M, Sato Y, Tobino K, Ikeda S, Mori M, Mimura C, Maeno K, Miura S, Harada T, Nishimura K, Hiraoka M, Kenmotsu H, Fujimoto J, Shimokawa M, Yamamoto N, Nakagawa K. Biomarker Testing in Patients With Unresectable Advanced or Recurrent Non-Small Cell Lung Cancer. *JAMA Netw Open* 2023; **6**: e2347700 [PMID: 38100106 DOI: 10.1001/jamanetworkopen.2023.47700]

18 **Chen H**, Liu M, Dai Z, Li S, Luo Y, Wang Y, Su W, Cai W, Yang D, Huang J, Yang Z. Concomitant genetic alterations are associated with response to EGFR targeted therapy in patients with lung adenocarcinoma. *Transl Lung Cancer Res* 2020; **9**: 1225-1234 [PMID: 32953500 DOI: 10.21037/tlcr-20-679]

19 **Wu Z**, Zhang Z, Zhang D, Li Z. Remarkable response to third-generation EGFR-TKI plus crizotinib in a patient with pulmonary adenocarcinoma harboring EGFR and ROS1 co-mutation: a case report. *Front Oncol* 2024; **14**: 1357230 [PMID: 38476366 DOI: 10.3389/fonc.2024.1357230]

20 **Won JK**, Keam B, Koh J, Cho HJ, Jeon YK, Kim TM, Lee SH, Lee DS, Kim DW, Chung DH. Concomitant ALK translocation and EGFR mutation in lung cancer: a comparison of direct sequencing and sensitive assays and the impact on responsiveness to tyrosine kinase inhibitor. *Ann Oncol* 2015; **26**: 348-354 [PMID: 25403583 DOI: 10.1093/annonc/mdu530]

21 **Zhuang X**, Zhao C, Li J, Su C, Chen X, Ren S, Li X, Zhou C. Clinical features and therapeutic options in non-small cell lung cancer patients with concomitant mutations of EGFR, ALK, ROS1, KRAS or BRAF. *Cancer Med* 2019; **8**: 2858-2866 [PMID: 31016879 DOI: 10.1002/cam4.2183]

22 **Zhang S**, Xu Z, Zhang W. OPRM1-ROS1 Fusion Detected by Next-Generation Sequencing with Circulating DNA in a Patient with EGFR Mutated Advanced NSCLC: A Case Report. *Case Rep Oncol* 2022; **15**: 700-704 [PMID: 36157687 DOI: 10.1159/000507980]

23 **Zhang X**, Jiang Y, Yu H, Xia H, Wang X. A comprehensive study on the oncogenic mutation and molecular pathology in Chinese lung adenocarcinoma patients. *World J Surg Oncol* 2020; **18**: 172 [PMID: 32677962 DOI: 10.1186/s12957-020-01947-z]

24 **Ju L**, Han M, Zhao C, Li X. EGFR, KRAS and ROS1 variants coexist in a lung adenocarcinoma patient. *Lung Cancer* 2016; **95**: 94-97 [PMID: 27040858 DOI: 10.1016/j.lungcan.2016.03.005]

25 **Yang JJ**, Zhang XC, Su J, Xu CR, Zhou Q, Tian HX, Xie Z, Chen HJ, Huang YS, Jiang BY, Wang Z, Wang BC, Yang XN, Zhong WZ, Nie Q, Liao RQ, Mok TS, Wu YL. Lung cancers with concomitant EGFR mutations and ALK rearrangements: diverse responses to EGFR-TKI and crizotinib in relation to diverse receptors phosphorylation. *Clin Cancer Res* 2014; **20**: 1383-1392 [PMID: 24443522 DOI: 10.1158/1078-0432.CCR-13-0699]

26 **Liu J**, Mu Z, Liu L, Li K, Jiang R, Chen P, Zhou Q, Jin M, Ma Y, Xie Y, Xiang J, Li B, Ma Y, Mao X, Zhang L, Zhang T, Wu D. Frequency, clinical features and differential response to therapy of concurrent ALK/EGFR alterations in Chinese lung cancer patients. *Drug Des Devel Ther* 2019; **13**: 1809-1817 [PMID: 31213769 DOI: 10.2147/DDDT.S196189]

27 **Mao Y**, Wu S. ALK and ROS1 concurrent with EGFR mutation in patients with lung adenocarcinoma. *Onco Targets Ther* 2017; **10**: 3399-3404 [PMID: 28744144 DOI: 10.2147/OTT.S133349]

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CASE REPORT

Amelanotic primary cervical malignant melanoma: A case report and review of literature

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Abstract

BACKGROUND

Primary malignant melanoma of the cervix (PMMC) is an extremely rare disease that originates from primary cervical malignant melanoma and frequently represents a challenge in disease diagnosis due to unclarified clinical and histological presentations, particularly those without melanin.

CASE SUMMARY

Here, we report a case of amelanotic PMMC, with a history of breast cancer and thyroid carcinoma. The patient was finally diagnosed by immunohistochemical staining and staged as IB2 based on the International Federation of Gynecology and Obstetrics with reference to National Comprehensive Cancer Network guidelines and was treated with radical hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy. She then received combination therapy consisting of immunotherapy with tislelizumab and radiofrequency hyperthermia. She has remained free of disease for more than 1 year.

CONCLUSION

The differential diagnosis process reenforced the notion that immunohistochemical staining is the most reliable approach for amelanotic PMMC diagnosis. Due to the lack of established therapeutic guidelines, empirical information from limited available studies does not provide the rationale for treatment-decision making. By integrating 'omics' technologies and patient-derived xenografts or mini-patient-derived xenograft models this will help to identify selective therapeutic window(s) and screen the appropriate therapeutics for targeted therapies, immune checkpoint blockade or combination therapy strategies effectively and precisely that will ultimately improve patient survival.

Key Words: Primary cervical malignant melanoma; MelanA; Immunotherapy; Patient management; Case report

Core Tip: We report a case of unsuspected amelanotic primary malignant melanoma of the cervix (PMMC) with a history of breast cancer. The patient underwent radical hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy, and then received radiotherapy combined with immunotherapy. She has remained free of disease for more than 1 year. The successful management of this patient underscores the critical role of routine immunohistochemical staining during cervical cancer diagnosis to exclude unsuspected PMMC, and adjuvant immunotherapy may be an option for PMMC.

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INTRODUCTION

Primary malignant melanoma of the cervix (PMMC) is extremely rare. Due to a lack of melanocytes in the cervix, PMMC represents a challenge in clinical diagnosis. Currently, there is no consensus or guidelines for the treatment and management of PMMC. In most cases, treatment follows the surgical criteria for cervical squamous cell carcinoma. PMMC can be managed postoperatively or preoperatively.

CASE PRESENTATION

Chief complaints

A 56-year-old woman presented to our hospital in December 2022 with one-day postmenopausal bleeding.

History of present illness

The surgery was planned by a multidisciplinary team and she underwent radical hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy, and one lesion (2.8 cm × 1.5 cm × 1.3 cm) was observed in the lower end of the cervix and its section appeared white in color. Biopsies were further evaluated by pathological examination. The tumor had invaded into 1/2 layer of the cervical muscle wall, and the depth of tumor invasion in the cervix was approximately 6 mm. The endometrium, bilateral adnexa, lymph nodes, and vaginal stump were free of tumors.

History of past illness

Eighteen years ago, she was diagnosed with breast duct carcinoma, and she underwent radical left unilateral mastectomy and then right unilateral mastectomy in 2014. She additionally underwent thyroidectomy two years later due to thyroid carcinoma.

Personal and family history

Eighteen years ago, she was diagnosed with breast duct carcinoma, and underwent radical left unilateral mastectomy and then right unilateral mastectomy in 2014. She additionally underwent thyroidectomy two years later due to thyroid carcinoma.

Physical examination

Slight bulging of the anterior vaginal wall and posterior vaginal fornix was observed.

Laboratory examinations

Human papillomavirus screening was negative. Quantitative DNA ploidy analysis identified at least 3 heterotypic cells and the DNA index value was over 2.5. Unexpectedly, a routine serum chemistry panel and plasma tumor biomarker examination, including squamous cell carcinoma antigen, were all within normal limits.

Imaging examinations

Ultrasound findings revealed a hypoechoic area measuring approximately 14 mm × 15 mm × 12 mm with clear boundaries and irregular internal echoes were seen in the cervix. Magnetic resonance imaging suggested abnormal cervical morphology, with a high signal intensity of mixed T1WI (T1 weighted image) and T2WI (Figure 1A and B). Uneven reinforcement was observed after enhanced scanning (Figure 1C and D). Furthermore, colposcopy and cervical biopsy

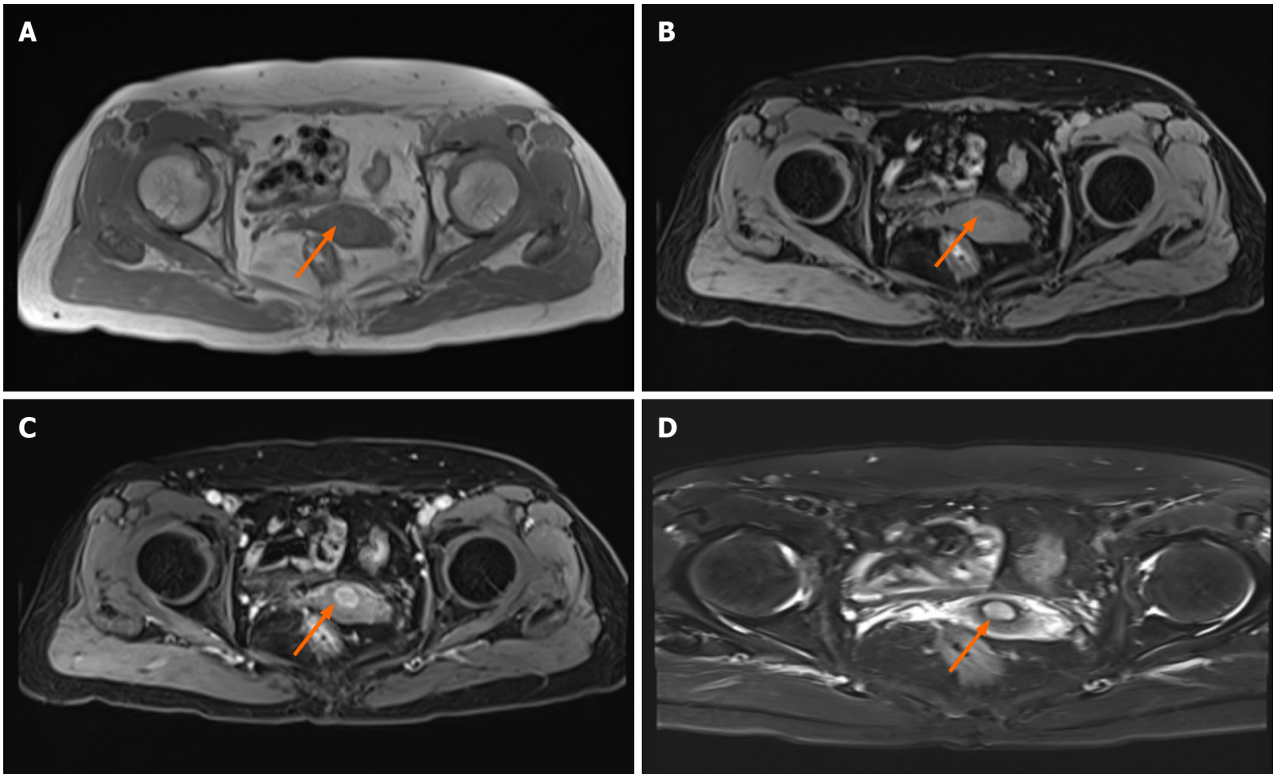

Figure 1 Pelvic magnetic resonance imaging. A: T1 weighted image (arrow); B: Fat-suppression T1-weighted image (arrow); C: T1WI lipocompression enhanced scan (arrow); D: T2WI lipocompression enhanced scan (arrow).

examination suggested small round cell malignancy, of which the tumor cells were arranged in a nest-like and cord-like pattern, with an epithelial-like morphology, significant atypia, minimal cytoplasm, deep staining and frequent mitotic activity (Figure 2).

MULTIDISCIPLINARY EXPERT CONSULTATION

The surgery was planned by a multidisciplinary team and the patient underwent radical hysterectomy, bilateral salpingo-oophorectomy and pelvic lymphadenectomy.

FINAL DIAGNOSIS

Considering the negative expression of pan-Keratin (AE1/AE3) and GATA3, as well the histological findings, this generally rules out the possibility that the tumor originated from primary breast cancer. Negative p40 and p16 reactivity in resected tumors on immunohistochemical (IHC) staining excluded the possibility of primary cervical cancer. Stepwise serial diagnostic IHC staining of biomarkers related to common cancers was performed. Surprisingly, the cells were strongly positive for MelanA, S-100, SOX-10 and HMB45, the biomarker for cervical melanoma (Figure 3). Due to the lack of melanin observed in the lesion, primary cervical malignant melanoma was not considered initially. The patient was finally diagnosed with primary cervical malignant melanoma.

TREATMENT

Multiplex gene-panel testing indicated a genetic mutation of BRCA2 (exon11). She then received combination therapy consisting of the anti-PD1 antibody tislelizumab (200 mg, d1, q3w) and radiofrequency hyperthermia for 1.5 years.

OUTCOME AND FOLLOW-UP

The patient has undergone monthly follow-up visits. To date, she remains free of disease, without evidence of disease recurrence or metastasis for 1 year.

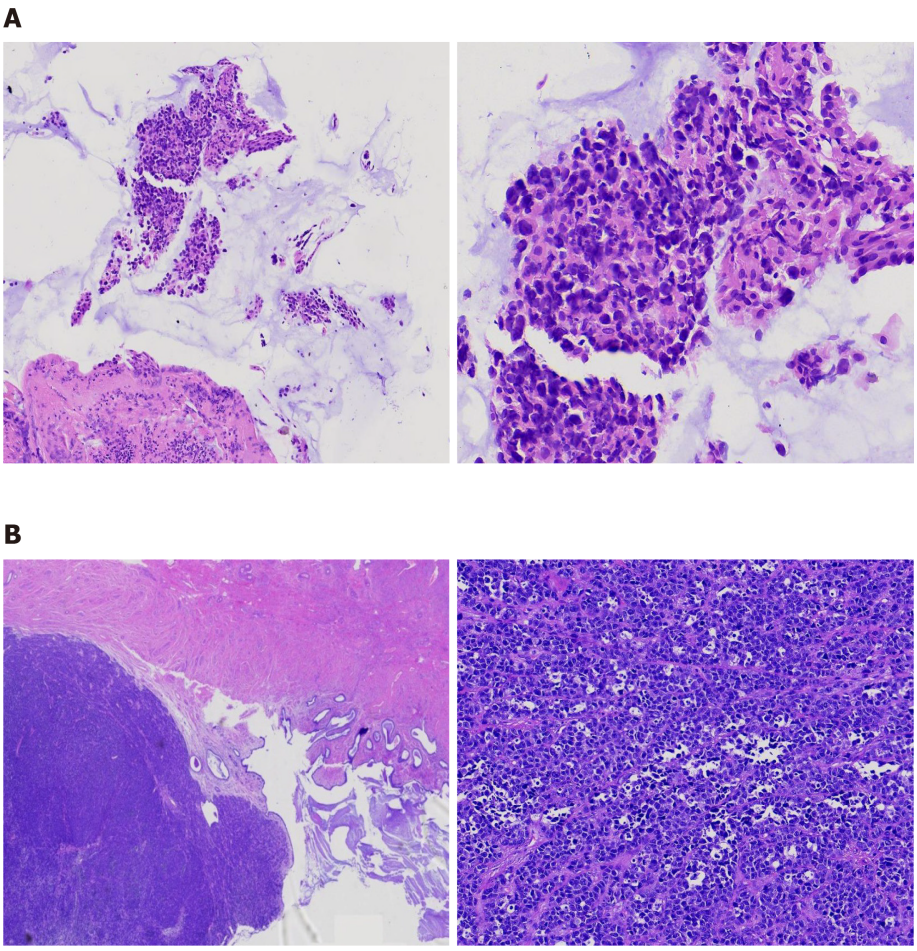

Figure 2 Histological examination showed small round cell malignancy with a high mitotic activity. A: Colposcopy and cervical biopsy; B: Resected biopsy specimen.

DISCUSSION

Primary cervical malignant melanoma represents an exceedingly uncommon tumor that can occur in the uterine cervix[1-4]. Since it was initially described as macroscopically "black cancer" of the cervix in 1889, only 149 cases have been reported to date[5]. The presence of melanin is one of the four criteria for the diagnosis of cervical melanoma[6]. Less than 20% of cases are, however, amelanotic and 3.5% cells in the cervical melanoma are melanin-containing cells compared with normal cervical epithelia[7,8]. Therefore, routine inclusion of IHC staining of combined S100 sensitivity, HBM45 specificity and MelanA staining is of great significance in facilitating the differential diagnosis of cervical malignancies without delays in situations where there is a lack of pigment. This is probably why IHC staining is more specific than Masson–Fontana staining[1,3]. Given that primary malignant melanoma frequently undergoes distant metastasis, excluding its origin from a primary cutaneous melanoma is a top priority for cervical melanoma diagnosis[9-12]. Both scanning and later positron emission tomography/computed tomography ruled out the presence of melanoma in other anatomic structures due to a distinct signal pattern from the paramagnetic properties of melanin[11,13]. This case was staged as IB2, without lymph node and distant metastasis, and she underwent regional lymph node dissection, although dissection of clinically negative regional lymph nodes is still controversial[14-18], indicating that a future study involving a larger sample size is necessary to determine the value of lymph node dissection in patients with PMMC.

Although melanoma is considered radio-resistant, the combination of ionizing radiation with hyperthermia provokes a systemic immune response and potentiates the efficacy of immunotherapy[19,20]. This case therefore received radiotherapy combined with immunotherapy, and the long-term effect is yet to be evaluated although she has been free of disease for 1 year. Notably, the combination of chemotherapy with either immunotherapy or radiotherapy has demonstrated a limited effect in patients with PMMC as previously reported[11,13,21,22]. Similar to melanoma, PMMC bears common driver mutations, such as BRAF and BRCA2, which can be therapeutically targeted. Our patient was found to have a BRAC2 mutation (exon11), ideally providing a therapeutic opportunity for treating this patient with the PARP inhibitor olaparib, which has been approved for BRCA1/2-mutated metastatic ovarian cancer and showed considerable survival benefit[23-26]. This agent may be an option if tumor relapse occurs in this patient in the future. Given the lack of consensus with respect to the management of MM due to disease rarity, it is impossible to conduct clinical trials with sufficient cases, and anecdotal evidence and empirical information from previous studies do not provide enough evidence on the impact of available treatment options[1,5]. With this in mind, it is therefore important to take advantage of new 'omics' technologies that lead to the understanding of the genetic and epigenetic landscape of individual PMMC to

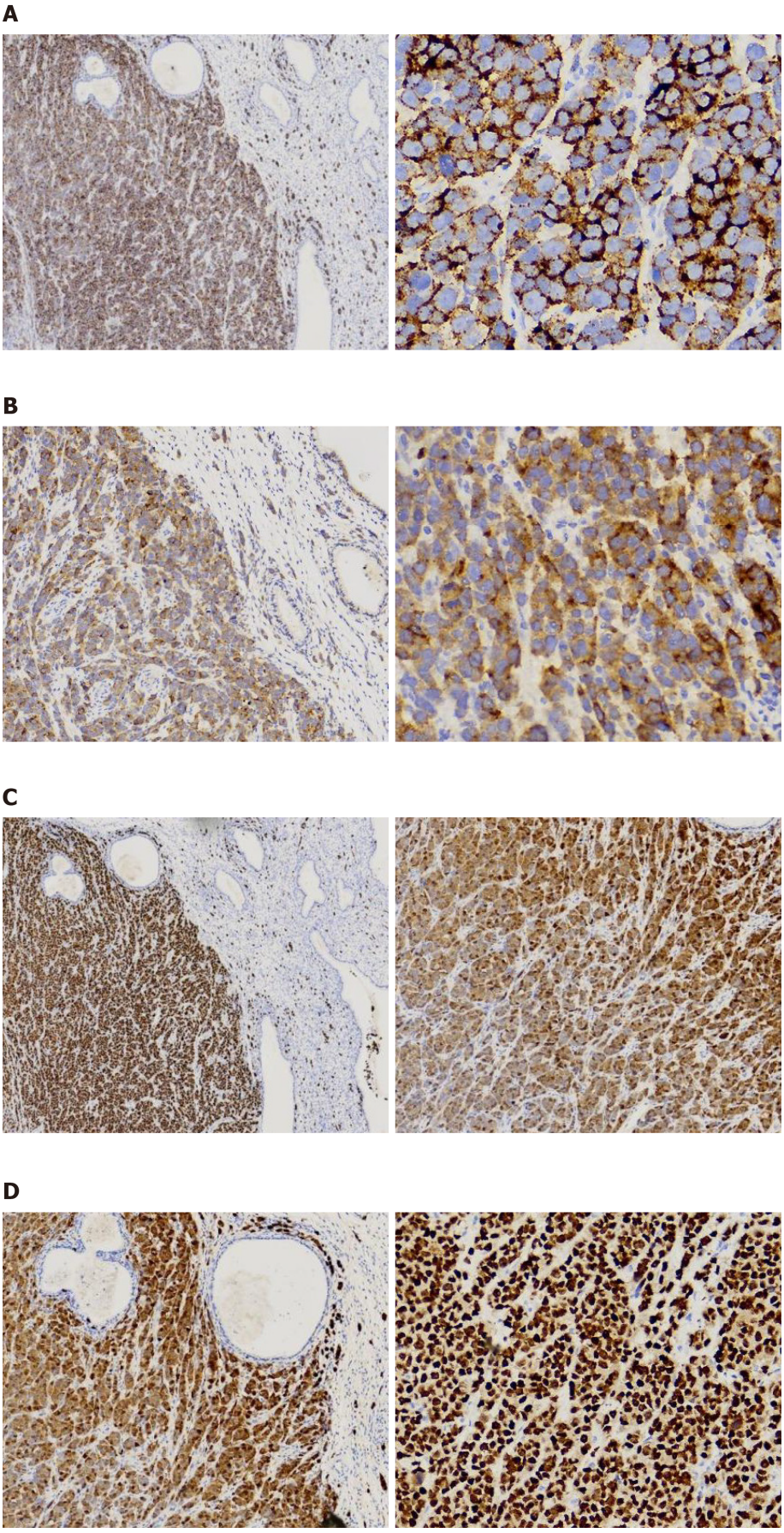

Figure 3 Immunohistochemical staining of resected biopsy specimen. A: HMB45; B: MelanA; C: S-100; D: SOX10. The tumors were positive for HMB45, MelanA, S-100 and SOX10. Scale bar, left, 100 μm; right, 400 μm.

optimize the potential of personalized medicine. On the other hand, patient-derived xenografts (PDXs) are powerful models in screening and selecting the correct therapeutics in the clinic[27-32]. A mini patient-derived xenograft (MiniPDX), which is based on capsule implantation in nude mice, can rapidly test drug efficacy within 7 days[33-37]. The application of these new models to PMMC will be extremely helpful in strengthening personalized treatment of PMMC.

CONCLUSION

The differential diagnosis process reenforced the notion that immunohistochemical staining is the most reliable approach for amelanotic PMMC diagnosis. Due to the lack of established therapeutic guidelines, empirical information from limited previous studies does not provide the rationale for treatment-decision making. By integrating 'omics' technologies and PDXs or mini-PDX models this will help to identify selective therapeutic window(s) and screen the correct therapeutics for targeted therapies, immune checkpoint blockade or combination therapy strategies effectively and precisely that will ultimately improve patient survival.

FOOTNOTES

Author contributions: Duan JL and Yang Y contributed to the histological examinations and collection of patient data; Duan JL and Yang Y assembled the data; Huang WT and Zhang YL performed data analysis and interpretation; Huang WT, Duan JL, and Zhang YL wrote the manuscript; All authors read and approved the final manuscript; Huang WT and Zhang YL confirm the authenticity of all the raw data; Duan JL and Yang J contributed equally to this work.

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REFERENCES

1 **Pusceddu S**, Bajetta E, Carcangiu ML, Formisano B, Ducceschi M, Buzzoni R. A literature overview of primary cervical malignant melanoma: an exceedingly rare cancer. *Crit Rev Oncol Hematol* 2012; **81**: 185-195 [PMID: 21515070 DOI: 10.1016/j.critrevonc.2011.03.008]

2 **Shi YF**, Chen YQ, Chen HF, Hu X. An atypical primary malignant melanoma arising from the cervical nerve root: A case report and review of literture. *World J Clin Cases* 2022; **10**: 381-387 [PMID: 35071542 DOI: 10.12998/wjcc.v10.i1.381]

3 **Singh N**, Tripathi R, Mala YM. Primary malignant melanoma of uterine cervix with probable origin from benign cervical melanosis. *BMJ Case Rep* 2013; **2013** [PMID: 23737592 DOI: 10.1136/bcr-2013-010042]

4 **Yin C**, Yang A, Zhang Y, Tao L, Zou H, Ren Y, Liang W, Jiang J, Zhao J, Zhang W, Li F, Jia W. Primary Cervical Malignant Melanoma: 2 Cases and a Literature Review. *Int J Gynecol Pathol* 2019; **38**: 196-203 [PMID: 29474317 DOI: 10.1097/PGP.0000000000000480]

5 **Min A**, Fu A, Huang M, Wang H, Chen H. Primary Malignant Melanoma of the Cervix: An Integrated Analysis of Case Reports and Series. *Front Oncol* 2022; **12**: 913964 [PMID: 35814437 DOI: 10.3389/fonc.2022.913964]

6 **Norris HJ**, Taylor HB. Melanomas of the vagina. *Am J Clin Pathol* 1966; **46**: 420-426 [PMID: 5924009 DOI: 10.1093/ajcp/46.4.420]

7 **CID JM**. Melanoid pigmentation of the endocervix: a neurogenic visceral argument. *Ann Anat Pathol (Paris)* 1959; **4**: 617-628 [PMID: 13849392]

8 **Nigogosyan G**, Delapava S, Pickren JW. Melanoblasts in vaginal mucosa. Origin for primary malignant melanoma. *Cancer* 1964; **17**: 912-913 [PMID: 14179553 DOI: 10.1002/1097-0142(196407)17:7<912::AID-CNCR2820170711>3.0.CO;2-F]

9 **Goldman RL**. Melanomas of vagina. *N Engl J Med* 1970; **282**: 1492 [PMID: 5419307 DOI: 10.1056/NEJM197006252822618]

10 **Ohtsuka M**, Miura T, Mori T, Ishikawa M, Yamamoto T. Occurrence of Psoriasiform Eruption During Nivolumab Therapy for Primary Oral Mucosal Melanoma. *JAMA Dermatol* 2015; **151**: 797-799 [PMID: 25875052 DOI: 10.1001/jamadermatol.2015.0249]

11 **Piura B**. Management of primary melanoma of the female urogenital tract. *Lancet Oncol* 2008; **9**: 973-981 [PMID: 19071254 DOI: 10.1016/S1470-2045(08)70254-7]

12 **Yuan-Mou Yang J**, Krishna GS, Macleod C, Oosthuysen W. Primary gastric mucosal melanoma. *N Z Med J* 2008; **121**: 96-99 [PMID: 18709052]

13 **Sugiyama VE**, Chan JK, Kapp DS. Management of melanomas of the female genital tract. *Curr Opin Oncol* 2008; **20**: 565-569 [PMID: 19106662 DOI: 10.1097/CCO.0b013e32830b0dda]

14 **Furuya M**, Shimizu M, Nishihara H, Ito T, Sakuragi N, Ishikura H, Yoshiki T. Clear cell variant of malignant melanoma of the uterine cervix: a case report and review of the literature. *Gynecol Oncol* 2001; **80**: 409-412 [PMID: 11263942 DOI: 10.1006/gyno.2000.6091]

15 **Jones HW 3rd**, Droegemueller W, Makowski EL. A primary melanocarcinoma of the cervix. *Am J Obstet Gynecol* 1971; **111**: 959-963 [PMID: 5118034 DOI: 10.1016/0002-9378(71)90953-7]

16 **Cantuaria G**, Angioli R, Fernandez-Abril A, Penalver M. Primary malignant melanoma of the uterine cervix: case report and review of the literature. *Prim Care Update Ob Gyns* 1998; **5**: 159-160 [PMID: 10838297 DOI: 10.1016/S1068-607X(98)00052-3]

17 **Kim MS**, Choi CH, Kim TJ, Lee JW, Lee J, Bae DS, Kim BG. Primary malignant melanoma of the uterine cervix treated with pembrolizumab after radical surgery: a case report and literature review. *Obstet Gynecol Sci* 2018; **61**: 524-528 [PMID: 30018908 DOI: 10.5468/ogs.2018.61.4.524]

18 **Mousavi AS**, Fakor F, Nazari Z, Ghaemmaghami F, Hashemi FA, Jamali M. Primary malignant melanoma of the uterine cervix: case report and review of the literature. *J Low Genit Tract Dis* 2006; **10**: 258-263 [PMID: 17012994 DOI: 10.1097/01.lgt.0000229564.11741.4e]

19 **Tagliaferri L**, Lancellotta V, Fionda B, Mangoni M, Casà C, Di Stefani A, Pagliara MM, D'Aviero A, Schinzari G, Chiesa S, Mazzarella C, Manfrida S, Colloca GF, Marazzi F, Morganti AG, Blasi MA, Peris K, Tortora G, Valentini V. Immunotherapy and radiotherapy in melanoma: a multidisciplinary comprehensive review. *Hum Vaccin Immunother* 2022; **18**: 1903827 [PMID: 33847208 DOI: 10.1080/21645515.2021.1903827]

20 **Zhang Z**, Liu X, Chen D, Yu J. Radiotherapy combined with immunotherapy: the dawn of cancer treatment. *Signal Transduct Target Ther* 2022; **7**: 258 [PMID: 35906199 DOI: 10.1038/s41392-022-01102-y]

21 **Wydra D**, Sawicki S, Ciach K, Emerich J. Malignant melanoma of the uterine cervix. *Eur J Obstet Gynecol Reprod Biol* 2006; **124**: 257-258 [PMID: 16099578 DOI: 10.1016/j.ejogrb.2005.06.024]

22 **Garbe C**, Eigentler TK, Keilholz U, Hauschild A, Kirkwood JM. Systematic review of medical treatment in melanoma: current status and future prospects. *Oncologist* 2011; **16**: 5-24 [PMID: 21212434 DOI: 10.1634/theoncologist.2010-0190]

23 **Antonarakis ES**, Abida W. Combining Poly(ADP)-Ribose Polymerase Inhibitors With Abiraterone in Castration-Resistant Prostate Cancer: Is Biomarker Testing Necessary? *J Clin Oncol* 2023; **41**: 3291-3294 [PMID: 36952642 DOI: 10.1200/JCO.23.00270]

24 **Bryant HE**, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005; **434**: 913-917 [PMID: 15829966 DOI: 10.1038/nature03443]

25 **Konstantinopoulos PA**, Cannistra SA. Comparing poly (ADP-ribose) polymerase inhibitors with standard chemotherapy in BRCA-mutated, recurrent ovarian cancer: lessons learned from a negative trial. *J Clin Oncol* 2012; **30**: 347-350 [PMID: 22203759 DOI: 10.1200/JCO.2011.40.1489]

26 **Walsh C**, Cass I. Poly(ADP-Ribose) Polymerase Inhibitors and Myeloid Neoplasm Risk-Clues to a Mechanistic Connection? *JAMA Oncol* 2021; **7**: 1763-1765 [PMID: 34647967 DOI: 10.1001/jamaoncol.2021.4639]

27 **Gao H**, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, Zhang C, Schnell C, Yang G, Zhang Y, Balbin OA, Barbe S, Cai H, Casey F, Chatterjee S, Chiang DY, Chuai S, Cogan SM, Collins SD, Dammassa E, Ebel N, Embry M, Green J, Kauffmann A, Kowal C, Leary RJ, Lehar J, Liang Y, Loo A, Lorenzana E, Robert McDonald E 3rd, McLaughlin ME, Merkin J, Meyer R, Naylor TL, Patawaran M, Reddy A, Röelli C, Ruddy DA, Salangsang F, Santacroce F, Singh AP, Tang Y, Tinetto W, Tobler S, Velazquez R, Venkatesan K, Von Arx F, Wang HQ, Wang Z, Wiesmann M, Wyss D, Xu F, Bitter H, Atadja P, Lees E, Hofmann F, Li E, Keen N, Cozens R, Jensen MR, Pryer NK, Williams JA, Sellers WR. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nat Med* 2015; **21**: 1318-1325 [PMID: 26479923 DOI: 10.1038/nm.3954]

28 **Garman B**, Anastopoulos IN, Krepler C, Brafford P, Sproesser K, Jiang Y, Wubbenhorst B, Amaravadi R, Bennett J, Beqiri M, Elder D, Flaherty KT, Frederick DT, Gangadhar TC, Guarino M, Hoon D, Karakousis G, Liu Q, Mitra N, Petrelli NJ, Schuchter L, Shannan B, Shields CL, Wargo J, Wenz B, Wilson MA, Xiao M, Xu W, Xu X, Yin X, Zhang NR, Davies MA, Herlyn M, Nathanson KL. Genetic and Genomic Characterization of 462 Melanoma Patient-Derived Xenografts, Tumor Biopsies, and Cell Lines. *Cell Rep* 2017; **21**: 1936-1952 [PMID: 29141224 DOI: 10.1016/j.celrep.2017.10.052]

29 **Gris-Oliver A**, Palafox M, Monserrat L, Brasó-Maristany F, Òdena A, Sánchez-Guixé M, Ibrahim YH, Villacampa G, Grueso J, Parés M, Guzmán M, Rodríguez O, Bruna A, Hirst CS, Barnicle A, de Bruin EC, Reddy A, Schiavon G, Arribas J, Mills GB, Caldas C, Dienstmann R, Prat A, Nuciforo P, Razavi P, Scaltriti M, Turner NC, Saura C, Davies BR, Oliveira M, Serra V. Genetic Alterations in the PI3K/AKT Pathway and Baseline AKT Activity Define AKT Inhibitor Sensitivity in Breast Cancer Patient-derived Xenografts. *Clin Cancer Res* 2020; **26**: 3720-3731 [PMID: 32220884 DOI: 10.1158/1078-0432.CCR-19-3324]

30 **Shattuck-Brandt RL**, Chen SC, Murray E, Johnson CA, Crandall H, O'Neal JF, Al-Rohil RN, Nebhan CA, Bharti V, Dahlman KB, Ayers GD, Yan C, Kelley MC, Kauffmann RM, Hooks M, Grau A, Johnson DB, Vilgelm AE, Richmond A. Metastatic Melanoma Patient-Derived Xenografts Respond to MDM2 Inhibition as a Single Agent or in Combination with BRAF/MEK Inhibition. *Clin Cancer Res* 2020; **26**: 3803-3818 [PMID: 32234759 DOI: 10.1158/1078-0432.CCR-19-1895]

31 **Woo XY**, Giordano J, Srivastava A, Zhao ZM, Lloyd MW, de Bruijn R, Suh YS, Patidar R, Chen L, Scherer S, Bailey MH, Yang CH, Cortes-Sanchez E, Xi Y, Wang J, Wickramasinghe J, Kossenkov AV, Rebecca VW, Sun H, Mashl RJ, Davies SR, Jeon R, Frech C, Randjelovic J, Rosains J, Galimi F, Bertotti A, Lafferty A, O'Farrell AC, Modave E, Lambrechts D, Ter Brugge P, Serra V, Marangoni E, El Botty R, Kim H, Kim JI, Yang HK, Lee C, Dean DA 2nd, Davis-Dusenbery B, Evrard YA, Doroshow JH, Welm AL, Welm BE, Lewis MT, Fang B, Roth JA,

Meric-Bernstam F, Herlyn M, Davies MA, Ding L, Li S, Govindan R, Isella C, Moscow JA, Trusolino L, Byrne AT, Jonkers J, Bult CJ, Medico E, Chuang JH; PDXNET Consortium; EurOPDX Consortium. Conservation of copy number profiles during engraftment and passaging of patient-derived cancer xenografts. *Nat Genet* 2021; **53**: 86-99 [PMID: 33414553 DOI: 10.1038/s41588-020-00750-6]

32 **Basak A**, Lotfipour F. Modulating furin activity with designed mini-PDX peptides: synthesis and *in vitro* kinetic evaluation. *FEBS Lett* 2005; **579**: 4813-4821 [PMID: 16102752 DOI: 10.1016/j.febslet.2005.07.062]

33 **Long Z**, Lu Y, Li M, Ji C, Chen G, Li J, Xiang L, Yu H, Wang Q, Wang Z. Predicting chemosensitivity based on mini patient-derived xenografts in osteosarcoma patients: A retrospective study. *J Cancer Res Ther* 2023; **19**: 71-77 [PMID: 37006045 DOI: 10.4103/jcrt.jcrt_825_22]

34 **Zhang F**, Wang W, Long Y, Liu H, Cheng J, Guo L, Li R, Meng C, Yu S, Zhao Q, Lu S, Wang L, Wang H, Wen D. Characterization of drug responses of mini patient-derived xenografts in mice for predicting cancer patient clinical therapeutic response. *Cancer Commun (Lond)* 2018; **38**: 60 [PMID: 30257718 DOI: 10.1186/s40880-018-0329-5]

35 **Zhao P**, Chen H, Wen D, Mou S, Zhang F, Zheng S. Personalized treatment based on mini patient-derived xenografts and WES/RNA sequencing in a patient with metastatic duodenal adenocarcinoma. *Cancer Commun (Lond)* 2018; **38**: 54 [PMID: 30139386 DOI: 10.1186/s40880-018-0323-y]

36 **Zhu X**, Xu X, Zhang B, Dong Y, Gong S, Gong T, Zhang F, Jin C. Individualized therapy based on the combination of mini-PDX and NGS for a patient with metastatic AFP-producing and HER-2 amplified gastric cancer. *Oncol Lett* 2022; **24**: 411 [PMID: 36245818 DOI: 10.3892/ol.2022.13531]

37 **Kai M**, Tamura K, Ohno M, Ohkura Y. Simple determination of forphenicinol in human plasma and erythrocytes by HPLC with native fluorescence detection. *Biomed Chromatogr* 1986; **1**: 143-146 [PMID: 3506826 DOI: 10.1177/10781552221074973]

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