ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2020 January 15; 12(1): 1-123

Published by Baishideng Publishing Group Inc

WJGO

World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 12 Number 1 January 15, 2020

EVIDENCE-BASED MEDICINE

Contents

World Journal of Gastrointestinal Oncology

Volume 12 Number 1 January 15, 2020

ABOUT COVER

Editorial Board Member of *World Journal of Gastrointestinal Oncology*, Xin-Zu Chen, MD, PhD, Associate Professor, Department of Gastrointestinal Surgery and Laboratory of Gastric Cancer, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

AIMS AND SCOPE

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

WJGO mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal oncology and covering a wide range of topics including islet cell adenoma, liver cell adenoma, adenomatous polyposis coli, appendiceal neoplasms, bile duct neoplasms, biliary tract neoplasms, hepatocellular carcinoma, islet cell carcinoma, pancreatic ductal carcinoma, cecal neoplasms, colonic neoplasms, colorectal neoplasms, hereditary nonpolyposis colorectal neoplasms, common bile duct neoplasms, duodenal neoplasms, esophageal neoplasms, gallbladder neoplasms, *etc*.

INDEXING/ABSTRACTING

The *WJGO* is now indexed in Science Citation Index Expanded (also known as SciSearch®), PubMed, and PubMed Central. The 2019 edition of Journal Citation Reports® cites the 2018 impact factor for *WJGO* as 2.758 (5-year impact factor: 3.220), ranking *WJGO* as 52 among 84 journals in gastroenterology and hepatology (quartile in category Q3), and 131 among 229 journals in oncology (quartile in category Q3).

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Lu-Lu Qi*

Proofing Production Department Director: *Yun-Xiaojian Wu*

NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
February 15, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Monjur Ahmed, Rosa M Jimenez Rodriguez, Pashtoon Kasi

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5204/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director

PUBLICATION DATE
January 15, 2020

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

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

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

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

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

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

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 1-20

DOI: 10.4251/wjgo.v12.i1.1

ISSN 1948-5204 (online)

REVIEW

Precision medicine for gastrointestinal cancer: Recent progress and future perspective

Tasuku Matsuoka, Masakazu Yashiro

ORCID number: Tasuku Matsuoka (0000-0001-5019-8519); Masakazu Yashiro (0000-0001-5743-7228).

Author contributions: Matsuoka T and Yahiro M performed literature research; Matsuoka T wrote the manuscript and performed the revision and approval of the final version; Yahiro M designed research, coordinated and corrected the writing of the paper.

Supported by KAKENHI (Grant-in-Aid for Scientific Research), No. 18H02883.

Conflict-of-interest statement: There are not any financial or other interests regarding the submitted manuscript that might be construed as a conflict of interest.

Manuscript source: Invited manuscript

Received: March 14, 2019
Peer-review started: March 15, 2019
First decision: July 31, 2019

Tasuku Matsuoka, Masakazu Yashiro, Department of Gastroenterological Surgery, Osaka City University Graduate School of Medicine, Osaka 5458585, Japan

Masakazu Yashiro, Oncology Institute of Geriatrics and Medical Science, Osaka City University Graduate School of Medicine, Osaka 5458585, Japan

Corresponding author: Masakazu Yashiro, MD, PhD, Associate Professor, Department of Surgical Oncology, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 5458585, Japan. m9312510@med.osaka-cu.ac.jp

Abstract

Gastrointestinal (GI) cancer has a high tumor incidence and mortality rate worldwide. Despite significant improvements in radiotherapy, chemotherapy, and targeted therapy for GI cancer over the last decade, GI cancer is characterized by high recurrence rates and a dismal prognosis. There is an urgent need for new diagnostic and therapeutic approaches. Recent technological advances and the accumulation of clinical data are moving toward the use of precision medicine in GI cancer. Here we review the application and status of precision medicine in GI cancer. Analyses of liquid biopsy specimens provide comprehensive real-time data of the tumor-associated changes in an individual GI cancer patient with malignancy. With the introduction of gene panels including next-generation sequencing, it has become possible to identify a variety of mutations and genetic biomarkers in GI cancer. Although the genomic aberration of GI cancer is apparently less actionable compared to other solid tumors, novel informative analyses derived from comprehensive gene profiling may lead to the discovery of precise molecular targeted drugs. These progressions will make it feasible to incorporate clinical, genome-based, and phenotype-based diagnostic and therapeutic approaches and apply them to individual GI cancer patients for precision medicine.

Key words: Gastrointestinal cancer; Esophageal cancer; Gastric cancer; Colorectal cancer; Precision medicine; Liquid biopsy; Gene panel; Precision surgery; Biomarkers

Core tip: Gastrointestinal (GI) cancer is one of the most common leading causes of cancer death worldwide. Hence, any effort in early diagnosis, choice of appropriate therapeutic strategies can have a pivotal role in reducing the disease related mortalities.

Revised: October 12, 2019
Accepted: November 4, 2019
Article in press: November 4, 2019
Published online: January 15, 2020

P-Reviewer: Friedel D, Usta J
S-Editor: Dou Y
L-Editor: A
E-Editor: Liu MY

Our review purpose to clarify the current advancement for precision medicine in GI cancer by elucidating the benefit of liquid biopsy, multiple gene panel, novel biomarkers and surgery in GI cancer.

Citation: Matsuoka T, Yashiro M. Precision medicine for gastrointestinal cancer: Recent progress and future perspective. *World J Gastrointest Oncol* 2020; 12(1): 1-20
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/1.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.1

INTRODUCTION

Precision medicine is a strategy designed to treat individual patients with the most suitable therapy at the most appropriate time based on the patient's biologic and molecular features, using the analyses of genes of the patient's cancer cells with next-generation sequencing (NGS). Such analyses can detect cancer-specific gene mutations, and molecular targeted drugs can be designed to be effective for one or more specific gene mutations. Precision medicine is thus a type of tailor-made and personalized therapy. The use of inappropriate medicine may not only do not benefit, but lead to cancer progression. As the accessibility to tumor genome sequencing technologies increases, genome-driven cancer treatment has emerged as a favorable approach[1]. The increasing number of patients who undergo multigene sequencing of their cancer can thus expect to be informed of their genomic alterations that could effectively be targeted with corresponding drugs[2].

Gastrointestinal (GI) cancer has a high tumor incidence and mortality rate worldwide[3]. Although colorectal cancer (CRC) could be largely managed, which results in long-term survival by a combination of drugs even in patients with widespread stage and GI lymphoma (*e.g.*, MALT) may also be associated with good response and prolonged survival, the overall prognosis of patients with advanced GI cancer remains poor. Precision medicine approaches are currently being applied with molecular targeted and immune-based therapeutics across a variety of malignancies, such as advanced melanoma and non-small-cell lung cancer (NSCLC)[4,5]. Although GI cancer has been investigated with biomarkers (*e.g.*, Ras and HER2 status), the development of biomarkers as well as targeted therapies for GI cancer has fallen behind compared to those developed for other malignancies. Analyses of liquid biopsies, multiple gene panels, and well-designed prospective trials are necessary to move the treatment of GI cancer forward. In this review, we summarize the progression of precision medicine in GI cancer in terms of specimens, assays, further biomarker information, surgery, and future perspectives.

LITERATURE SEARCH

We first conducted a search of the PubMed database for English articles using the medical subject heading terms in combination with "gastrointestinal cancer", "esophageal cancer", "gastric cancer", "colorectal cancer", "precision medicine", "liquid biopsy", "gene expression profiling", "biomarker", "molecular targeted therapy", and "gene panel". Relevant articles which were chosen from experimental studies and clinical trials since 1989 were involved as well as articles which were related to the disease processes. Articles which did not deal with the precision medicine of GI cancer were excluded from this review. Liver and pancreatic cancer and GI stromal tumor were not covered in this review due to the limited scope of the topic.

LIQUID BIOPSY

Conventionally, tissue biopsies have been used to access the molecular information of tumors, such as the histology and gene mutation[6]. However, the practical use of consecutive tissue biopsies to monitor for mutations is limited due to patient discomfort, pain, and risks associated with repeat tissue biopsies, and difficulty in capturing intra-tumor heterogeneity[6]. These shortcomings highlight the need for more innovative screening. One promising alternative to tissue biopsy is a new

approach that may change the principles of cancer treatment. The term 'liquid biopsy' refers to the analysis of tumor-derived biomarkers identified from biological fluids of patients with malignancies. Even though peripheral blood is the major specimen for the liquid biopsy approach, tumor biomarkers can be isolated from various body fluids including urine, pleural effusions, ascites, and cerebrospinal fluid[7].

The liquid biopsy technique been studied to a great extent and is attracting further attention as it leads to efficient therapeutic interventions, reducing the therapeutic cost and significantly improving patient outcomes and overall survival[8]. Analyses of liquid biopsy specimens can provide comprehensive real-time data of the tumor-associated changes in an individual patient with a malignancy. These data can be used for cancer screening, the detection of minimal residual disease, drug selection (including sensitivity to anticancer agents), monitoring recurrence, and monitoring the patient's response to targeted agents (including drug resistance)[9]. For example, an analysis of NSCLC patients' plasma for epidermal growth factor receptor (EGFR) to determine the existence of a T790M mutation is widely used[10]. Liquid biopsies could become a new tool with a significant impact on cancer therapy.

Studies of liquid biopsy methodology have focused on the analysis of circulating tumor cells (CTCs), circulating tumor free (cf) DNA or RNA, and tumor-derived extracellular vesicles (exosomes)[11]. For the most effective discussion of the details of liquid biopsy methodology, it is essential to understand the different types of cancer-related biomarkers and their respective molecular aspects.

CTCs

CTCs are tumor cells that are mainly detached from primary or metastatic lesions. They circulate through the body fluid to metastatic sites, either as a single cell or in clusters, which lead to the establishment of one or more secondary tumor foci[12]. The United States Food and Drug Administration (FDA)-cleared CellSearch system has enabled the enumeration of CTCs in cancer patients, and this has made it possible to determine disease activity and patients' treatment responses, which rely on the expressions of epithelial cell adhesion molecule and cytokeratin on cancer cells in blood[13]. The authors of a previous study described the establishment of colon CTC cultures and permanent cell lines which provided *in vivo* experimental models. These experiments may provide genetic and epigenetic information on tumor biology, and they may help assess the cells' sensitivity to anticancer drugs[13]. However, the number of CTCs is generally low in patients with GI cancer[14], and this limits the clinical applications of CTC analyses in site of the progression of various methods[14].

Circulating tumor DNA (ctDNA)

ctDNA has emerged as another component of liquid biopsies as a quantitative marker of tumor DNA, reflecting genomic alterations in the blood[15,16]. Compared to the detection of CTCs, the ctDNA-based approach provides more information about a patient-specific disease and treatment. Further benefits of the use of ctDNA as a marker is that ctDNA measurements can provide the real-time pathology of the patient's disease and higher sensitivity for the early detection of cancers[17]. A previous study showed a significantly broad range for ctDNA among patients with CRC (22–3922 ng/mL of blood) compared to healthy subjects (5-16 ng/mL of blood)[18]. Liquid biopsy analyses may take the place of tissue testing for assessing the mutational status of RAS in patients with CRC. The OncoBEAM RAS CRC Assay identifies the cfDNA of the most frequent *KRAS* and *NRAS* mutations by using BEAMing technology[19].

MicroRNAs (miRNAs)

In addition to the quantification of cfDNA, circulating transcriptome is also detectable in the serum of individuals with malignancies. The circulating transcriptome consists of both coding and noncoding RNAs, such as miRNAs or long noncoding RNAs (lncRNAs)[20]. Although RNA is generally unstable in blood, microRNA (miRNA) comprises stable, short, noncoding molecules made of 18-25 nucleotides. This endogenous, single-stranded RNA mediates the expression of nearly 30% of protein-encoding genes in humans[21]. MiRNAs can be analyzed by targeted or RNA sequencing methods, with miRNA signatures observed to be significantly deregulated in cancer patients compared to healthy parsons, and these analyses may become useful in cancer diagnosis and prognosis.

Exosomes

Exosomes are nanosized vesicles (40-150 nm)[22]. These small, membrane-bound vesicles can transport a number of biomolecules which lead to the modification of the activity of recipient cells[22]. Compared to CTCs and ctDNA, exosomes have advantages in several aspects, including their homogeneous size distribution. In

addition, due to the particular form of exosomes, they can be distinguishable by electron microscopy. Previous studies have obtained evidence that the exosome-mediated recruitment and manipulation of the tumor microenvironment is a critical step in the formation process of metastasis[23].

Liquid biopsy in GI cancer: Toward clinical applications

The clinical utility of a liquid biopsy has been studied in different clinical phases of GI cancer, from the screening for this disease to the identification of outcome factors in early GI cancer, the detection of minimal residual tumor, drug selection, and monitoring for recurrence and the patients' response to targeted agents. Current advances of liquid biopsy as diagnostic, monitoring and predictive markers in GI cancer are summarized in Table 1 and Table 2.

Cancer screening: The noninvasive nature of a liquid biopsy makes this approach ideal for the early detection of cancer. The evaluation of molecular biomarkers in early-stage cancer patients is necessary for the development of more personalized monitoring and treatment schedules. However, the possibility of detecting a malignancy at an early stage with a liquid biopsy is somewhat limited by the low concentration of circulating biomarkers associated with the low tumor burden. With respect to CRC, screening has been impacted using colonoscopy as the gold standard, mainly because of its high sensitivity and specificity for detecting cancerous and precancerous lesions. Despite its strengths, colonoscopy has certain disadvantages and limitations (*e.g.*, bowel preparation, sedation, aspiration, perforation, and splenic injury).Therefore, continued progress in novel assays, such as fecal immunochemical test, fecal DNA and other molecular markers, can be expected to further displace screening colonoscopy[24]. The Epi proColon® 2.0 assay (also referred to as the mSEPT9 assay), which was FDA-approved for CRC screening in April 2016, is a qualitative in vitro diagnostic polymerase chain reaction (PCR) test for the detection of mutated methylated septin9 DNA in EDTA plasma derived from patient whole-blood specimens[25].

Detection of minimal residual disease: One of the major fields of the application of liquid biopsy would be the detection of minimal residual disease in patients with surgically treatable tumors. The tumor burden of GI cancer at diagnosis is acknowledged as a pivotal factor of disease assessment before the beginning of treatment. A recent study indicated that somatic *KRAS*- and *BRAF*-mutated DNA in the peripheral blood of CRC patients may be a good estimate of CTCs and of surgical clearance of the disease[26].

Drug selection: Chemotherapy is often administered for patients with metastatic disease (*e.g.*, metastasis of regional lymph nodes) in a resected tumor specimen. Although there are a number of different chemotherapeutic agents that can be combined in a variety of chemotherapeutic regimens, the effect of chemotherapy on a specific patient cannot be predicted. Specific ctDNA identification has also been used as guidance for specific systemic chemotherapy and targeted agents. For instance, emerging RAS mutations during therapy with anti-EGFR antibody revealed resistance in patients with metastatic CRC (mCRC)[27]. Some studies found that undetectable low-frequency KRAS-mutant clones may be selected for anti-EGFR treatment by assessing ctDNA in the blood of mCRC patients during anti-EGFR therapy[28,29]. In similar, resistance to crizotinib has been emerged by using serial ctDNA measurements in gastric cancer (GC)[30].

Monitoring recurrence: One of the most challenging tasks in GI oncology is the identification of patients who will benefit from postoperative adjuvant chemotherapy after curative surgery. The histopathologic and molecular tumor features correlated with greater relapse risk (*e.g.*, the TNM classification) only imply a tendency for metastasis; they do not reveal whether metastatic cells were seeded during surgery. The identification of postoperative ctDNA is a definite sign that occult tumor cells remain in the patient.

The authors of a recent study proposed that in patients with CRC, the postoperative detection of ctDNA can be used to monitor the patients for residual disease and predict their future relapse risk with high probability[31]. Moreover, serial ctDNA serves as a tool for the early detection of recurrence during patient follow-up and for the patient's response to relapse intervention[31]. In CRC, the novel BCAT1/IKZF1 blood test was found to be more sensitive for recurrence compared to carcinoembryonic antigen (CEA) as a marker, and the likelihood of recurrence given a positive BCAT1/IKZF1 result was twice that compared to a positive CEA result[32].

Monitoring patients' responses to cytotoxic and targeted agents: The most

Table 1 Current progress of circulating tumor cells, circulating tumor DNA and stool DNA as diagnostic, monitoring and predictive markers in gastrointestinal cancer

Liquid biopsy	Patients/ controls	Organs	Source of fluid	Abnormalities	Technology	Target	Clinical setting	Ref.
CTCs	140/0	EC	B		FIHC	CK19, CD45	Prognosis	Li *et al*[82], 2016
CTCs	NA	EC	B		ISET	NA	Prognosis	Han *et al*[83], 2019
CTCs	116/31	GC	B		FAST-disc	EpCAM, CK, CD45-	Diagnostic	Kang *et al*[84], 2017
CTCs	81/31	GC	B		ISET	CK8/18/19, Vimentin, CD45	Prognostic	Zheng *et al*, 2017
CTCs	101/31	GC	B		CellSearch and IF-FISH	EpCAM, CK8, CK18, CK19, CD45-, HER2	Predictive	Mishima *et al*[86], 2017
CTCs	121/0	CRC	B		Cyttel method/imFISH	CD45	Prognostic	Wang *et al*[87], 2019
ctDNA	11/0	EC	P, T, NT	Mutation	WES and NGS panel		Diagnostic /Therapeutic	Luo *et al*[88], 2016
ctDNA	13/0	EC	P, T	Mutation	NGS panel		Predictive	Ueda *et al*[89], 2016
ctDNA	63/0	EC	P	Copy number status	qPCR	*CCND1*	Predictive	Komatsu *et al*[90], 2014
cfDNA	32/0	GC	P	Copy number status	cfDNA NGS testing	*ERBBB2*	Therapeutic	Kim *et al*[91], 2018
ctDNA	277/0	EC/GC	P, T	Mutation	MassARRAY	*TP53, PIK3CA, ERBB2, KRAS*	Diagnostic /Prognostic	Kato *et al*, 2018
ctDNA	70/0	GC	P, T	Mutation	NGS panel	*HER2*	Therapeutic	Gao *et al*[93], 2017
cfDNA	60/30	GC	P	Mutation	Droplet digital PCR	*HER2*	Therapeutic	Shoda *et al*[94], 2017
ctDNA	1/0	GC	P, T	Mutation	NGS panel	*MET*	Therapeutic	Du *et al*[30], 2017
ctDNA	230/0	CRC	B	Mutation	Safe-SeqS assay	NA	Prognostic	Tie *et al*[95], 2016
cfDNA	22/0	CRC	S	Mutation	NGS/dPCR	*TP53, KRAS, APC, PIK3CA, BRAF, FBXW7, NRAS*	Diagnostic /Prognostic	Furuki *et al*[96], 2018
cfDNA	3/0	CRC	P	Mutation	BEAMing	*RAS, BRAF, PIK3CA*	Predictive	Klein-Scory *et al*, 2018
cfDNA	20/0	CRC	P	Mutation	Droplet digital PCR	*APC, TP53, KRAS, PI3CA*	Predictive	Vandeputte *et al*[97], 2018
Stool DNA	71/22	CRC	Stool	Methylation	QIAamp DNA Stool Mini Kit	*SDC2*	Diagnostic	Oh *et al*[98], 2017

EC: Esophageal cancer; GC: Gastric cancer; CRC: Colorectal cancer; NT: Normal tissue; B: Blood; P: Plasma; S: Serum; T: Tumor tissue; PLF: Peritoneal lavage fluid; NGS: Next-generation sequencing; WES: Whole exome sequencing; FIHC: Fluorescent immunohistochemistry; NA: Not avaibable; EpCAM: Epithelial cell adhesion molecule.

potentially beneficial application of the liquid biopsy approach is the possibility of using this approach to monitor patients' therapeutic responses. In general, ctDNA has seemed to be an early biomarker that can be used to deduce the tumor burden of patients with CRC during chemotherapy and to predict the early therapeutic reaction. Molecular alterations that are related to drug resistance can be identified at an early stage by evaluating ctDNA, and this evaluation can be performed easily for the same patient at different time intervals.

A single-arm phase II trial (Erbitux Study of CPT11, Oxaliplatin, UFToral Targeted-therapy) was carried out in patients with previously untreated *KRAS* wild-type advanced CRC, using a regimen of irinotecan, oxaliplatin, and tegafur-uracil with leucovorin and cetuximab. The stratification of patients by the CTC count can identify the patients who might benefit the most from an intensive four-drug regimen, avoiding the use of high-toxicity regimens in low-CTC groups[33].

Table 2 Current progress of microRNAs and exosome as diagnostic, monitoring and predictive markers in gastrointestinal cancer

Liquid biopsy	Patients/ controls	Organs	Source of fluid	Abnormalities	Technology	Target	Clinical setting	Ref.
MiRNAs	231/0	EC	Peripheral blood lymphocytes	Polymorphism	SNPShot	KIAA0423 rs1053667, GEMIN3 rs197412	Prognostic	Faluyi *et al*[99], 2017
MiRNAs	3156/0	EC	S/P	Upregulation/ Downregulation	NA	miR-15a, miR-22, miR-31, miR-451, miR-506, miR-613, miR-1297	Diagnostic /Prognostic	Yao *et al*[100], 2018
MiRNAs	125/0	EC	S/P	Upregulation/ Downregulation	RT-PCR	miR-21, miR-223, miR-100, miR-25, miR-375	Diagnostic /Prognostic	Zhang *et al*[101], 2018
MiRNAs	250/538	GC	Gastric juice	Upregulation	miScript RT kit	miR-421, miR-21, miR-106a, miR-129	Diagnostic	Virgilio *et al*[102], 2018
MiRNAs	20/20	GC	S	Upregulation	TaqMan OpenArray assays	miR-331 and miR-21	Diagnostic	Sierzega *et al*[103], 2017
MiRNAs	The miRNA expression profile (GSE29298)	CRC	NA -	Upregulation	NA	miR-198, miR-765, miR-630, miR-371-5p, miR-575, miR-202, miR-513a-5p	Predictive	Zhu *et al*[104], 2017
MiRNAs	232/0	CRC	S	Upregulation	NA	miR-21, miR-29b, miR-92.	Diagnostic	Carter *et al*[105], 2017
MiRNAs	61/0	CRC	P	Upregulation	miRVANA PARIS kit	miR-20b, miR-29b, miR-155	Prognosis /Predivtive	Ulivi *et al*[106], 2018
Exosome	66/20	EC	P	Upregulation	AChE activity	Exosomes	Prognostic	Matsumoto *et al*[107], 2016
Exosome	30/0	GC	PLF	Upregulation	MiRNA microarray	miR-21, miR-1225-5p	Diagnostic /Therapeutic	Tokuhisa *et al*[108], 2015
Exosome	232/20	GC	P	Downregulation	Taqman microRNA assays	miR-23b	Prediction /Prognostic	Kumata *et al*[109], 2018
Exosome	227/28	CRC	S	Upregulation/ Downregulation	qRT-PCR microarray	miR-17, miR-18a, miR-19a, miR-19b, miR20a, miR-92a, hsa-miR-25-106b, hsa-miR-17-92a	Predictive /Prognosis	Matsumura *et al*[110], 2015
Exosome	108/0	CRC	S	Downregulation	The total exosome isolation kit	miR-548c-5p	Prognosis	Peng *et al*[111], 2018

EC: Esophageal cancer; GC: Gastric cancer; CRC: Colorectal cancer; NT: Normal tissue; B: Blood; P: Plasma; S: Serum; T: Tumor tissue; PLF: Peritoneal lavage fluid; NGS: Next-generation sequencing; WES: Whole exome sequencing; FIHC: Fluorescent immunohistochemistry; NA: Not avaibable.

GENE PANEL SEQUENCING IN GI CANCER

Sequencing is often performed to identify cancer-associated gene mutations in patients with advanced cancer. Sequencing panels allow the targeting of multiple genes simultaneously, quickly and accurately through comprehensive bioinformatics in order to exploit the useful information from a single study. The NGS of tumor sample DNA can lead to the optimal clinical treatment by offering diagnostic and/or prognostic data and by contributing to the selection of potential treatment regimens (*e.g.*, molecular-targeted and immune checkpoint blockade therapies). Recent advances in NGS has enabled the performance of whole-genome sequencing, whole-exome sequencing, whole-transcriptome sequencing and RNA sequencing, as well as the detection of enormous genetic aberrations[34].

Due to the progress in sequencing technologies, tissue comprehensive genome profiling has become more widely available in clinical practice. For example, the

current National Comprehensive Cancer Network guidelines recommend comprehensive genome profiling in patients with advanced non-small-cell lung adenocarcinoma[4]. Currently, NGS provides faster, cheaper, and more accurate whole-genome sequencing. The Cancer Genome Atlas has revealed the genome profiles of many cancers, including GI cancer[35,36]. Current progress of multiplex gene panels in GI cancer is summarized in Table 3.

Gene panaels contains the most commonly mutated genes or candidate actionable genes in many cancers. In CRC, *KRAS*, *BRAF*, *PIK3CA*, *TP53*, *CTNNB1, APC, SMAD4*, and *PTEN* are among the most commonly altered genes[37,38]. Patients with CRC in Japan were recently studied using an NGS - based comprehensive genomic panel test[39]. Significant differences in *ERBB2, APC, TP53, CDKN2A*, and *NRAS* mutations were identified in the Japanese patients compared to United States patients. Genomic alterations in DNA repair genes (*e.g., ATM, BLM, BRCA2, NBN, NRE11A*), which are observed in a significant proportion of CRC patients, were also detected. A novel, positive correlation between *APC* and *TP53* mutations with tumors that presented on the left side was reported. A study through deep sequencing in patients with mCRC presented that mutations in *TP53*, *KRAS*, *APC, KRAS*, *GNAS*, and *SMAD4* genes were detected in 69.3%, 39.6%, 23.7%, 16.8% and 13.8% patients, respectively. The mutations in *KRAS*, *GNAS*, and *SMAD4* were significantly associated with lung metastasis[40].

In GC, comprehensive genomic sequencing using a 435-gene panel in Japanese gastric cancers (GCs) showed that the most frequently mutated gene was *TP53* (53.1%), followed by *ARID1A* (15.9%) and *CDH1* (14.0%); *ERBB2* amplification (12.1%) was the most frequently observed somatic copy number alteration, followed by *CCNE1* (7.2%) and *KRAS* (5.8%) amplification[41]. Specific subcategories of GCs harbor characteristic genetic aberrations, such as somatic mutations in *RHOA* and a chimeric gene fusion of *CLDN18-ARHGAP26* in diffuse-type GCs[42,43]. The landscape of esophageal cancer (EC)-related gene mutations that regulate the cell cycle (*TP53, CCND1, CDKN2A, FBXW7*), epigenetic processes (*MLL2, EP300, CREBBP, TET2*), and the signaling pathways involving NOTCH (*NOTCH1, NOTCH3*), WNT (*FAT1, YAP1, AJUBA*) and receptor-tyrosine kinase-phosphoinositide 3-kinase (*PIK3CA, EGFR, ERBB2*) has been described[44].

Current advances in cancer genome analyses using NGS have revealed an increased mutation burden (a high rate of somatic mutation) in some solid tumors. In GI cancers, one of the leading causes of hypermutation-which is closely related to the generation of neo-antigens-is a defect in DNA mismatch repair (MMR), leading to microsatellite instability (MSI). Several research groups have stated that the tumor mutated burden correlates with the clinical response to immunotherapy[45,46]. GI cancer patients with MMR deficiency and a subsequent hypermutated phenotype achieved outstanding outcomes after anti-PD-1 therapy[47]. This highlights the clinical significance of identifying hypermutated tumors for immunotherapy treatment.

In CRC, mutations in transforming growth factor-beta (TGF-β) signaling genes and *BRAF* were markedly increased in hypermutated tumors[35]. Mutations in DNA polymerase D1 (*POLD1*) and DNA polymerase E (*POLE*) genes have also been described as a cause of hypermutated CRC[48]. The mutation rate of MSI-High GCs was significantly higher than that of MSS tumors[41]. *TGFBR2*, *ACVR2A*, *SMAD4*, and *ELF3* as well as the TGF-β pathway are frequently mutated, suggesting a pivotal role in GC pathogenesis, including MSI[43,49].

Given the advances in NGS, it may well become possible in the near future to identify the predominant cancer genes and pathways and tumor-specific genes and pathways. Several multigene assays are available to estimate the risk of relapse after definitive surgery, including the MSK-IMPACT, NCC Oncopanel, Todai OncoPanel, Oncomine Dx Target test, Foundation OneCDx, and CANCERPLEX.

A recent study using the Exiqon panel identified miR-20b-5p, miR-28-3p, miR-192-5p, miR-223-3p, and miR-296-5p as significantly upregulated in the serum of patients with EC, suggesting that these 5-miRNA signatures may serve as potential diagnostic biomarkers for ECs[50]. Similarly, the expressions of seven miRNAs (miR-103a-3p, miR-127-3p, miR-151a-5p, miR-17-5p, miR-181a-5p, miR-18a-5p, and miR-18b-5p) were significantly higher in CRC compared to normal controls[51].

BIOMARKERS FOR GI CANCER

Convincing biomarkers are a crucial aspect of precision medicine, used to match appropriate patients with the right treatment at the right time. Clinically relevant biomarkers are genetic, epigenetic, proteinic, or cellular alterations that are intrinsic to cancer cells. These biomarkers can be used to predict patients' responses to

Table 3 Current progress of multiplex gene panels in gastrointestinal cancer

Organs	Panel tested	Number of genes tested	Number of patients	The type of sample	Companion diagnostic indications	Ref.
EC	HiSeq2000	N/A	144	Tumor tissue DNA	*CCND1, CDKN2A, FBXW7, MLL2, EP300, CREBBP, TET2, NOTCH1, NOTCH3, FAT1, YAP1, AJUBA, PIK3CA, EGFR, ERBB2*	Sawada *et al*[44], 2016
EC	Exiqon miRNA qPCR panel	168miRNA	140	Serum miRNA	miR-20b-5p, miR-28-3p, miR-192-5p, miR-223-3p, and miR-296-5p	Huang *et al*[50], 2017
EC	Ion AmpliSeq Custom DNA Panel	12	27	Tumor tissue/Serum DNA	*BRAF, DDR2, ERBB2, HRAS, KEAP1, KRAS, NFE2L2, NRAS, PIK3CA, PTEN, RHOA*	Pasternack *et al*[112], 2018
GC	Illumina HiSeq 2000	38	138	Tumor tissue DNA	*RHOA, CDH1, PIK3CA, CTNNB1, APC, ARID1A, KMT2C, KRAS*	Kakiuchi *et al*[42], 2014
GC	Illumina HiSeq 2000	N/A	100	Tumor tissue DNA	*ARID1A, CDH1, MUC6, CTNNA2, GLI3, RNF43, RHOA*	Wang *et al*[43], 2014
GC	CANCERPLEX	435	207	Tumor tissue DNA	*ARID1A, CDH1, ERBB2, CCNE1, KRAS*	Ichikawa *et al*[41], 2017
GC	Ion-Proton sequencer	50	29	Tumor tissue DNA	*APC, CTNNB, KRAS, NPM1, FBXW7 ERBB2, FGFR2, KIT*	Yoshida *et al*[113], 2019
CRC	CANCERPLEX	415	201	Tumor tissue DNA	*ERBB2, APC, CDKN2A, NRAS, ATM, BLM, BRCA2, NBN, NRE11A*	Nagahashi *et al*[39], 2016
CRC	IT-PGM seqencing	22	77	Tumor tissue DNA	*RAS, PIK3CA, FBXW7, BRAF, SMAD4, MET, FGFR1*	Capalbo *et al*[114], 2019
CRC	OncoAim™ DNA panel	39	648	Tumor tissue DNA	*KRAS, APC, PIK3CA, SMAD4, BRAF, FBXW7, NRAS*	Wang *et al*[115], 2018
CRC	MiSeq	207	22	Tumor tissue DNA	*KRAS, PIK3CA, FBXW7, PTEN, SMAD4, BRAF, CTNNB1, NRAS*	Gao *et al*[116], 2019
CRC	cfDNA panel	14	101	Plasma cfDNA	*AKT1, BRAF, CTNNB1, EGFR, ERBB2, FBXW7, GNAS, KRAS, MAP2K1, NRAS, PIK3CA, SMAD4, APC,*	Osumi *et al*, 2018
CRC	TruSight Cancer Sequencing Panel	42	N/A	Blood ctDNA	*MLH1, MSH6, PMS2 APC, SMAD4, TP53, BRIP1, CHEK2, MUTYH, HNF1A, XPC*	Seifert *et al*[117], 2019

TP53 was commonly implicated in all references except 28035762, 30297788 and 30523343 (PMID)[50,112,117]. EC: Esophageal cancer; GC: Gastric cancer; CRC: Colorectal cancer; NGS: Next-generation sequencing; FFPE: Formalin-fixed paraffin-embedded; N/A: Not available; EBV: Epstein-Barr virus; MSI: Microsatellite instability; NGS: Next-generation sequencing.

chemotherapy, targeted therapy, or immune checkpoint inhibitors. To date, the most

reliable molecular marker in clinical practice is the *KRAS* gene for patients receiving EGFR - targeted therapy for CRC metastatic disease and HER2 overexpression for patients with HER2-positive GC[52,53]. Detection of BRAF mutation status was also recommended due to the ineffectiveness of anti-EGFR therapy for CRC patients with BRAF mutations[54]. Although there is a crucial need for novel diagnostic and prognostic biomarkers to improve GI cancer prognosis, these tools are still being investigated. In this section, we summarize the current advances of biomarkers in GI cancer, with a focus on the development of new biomarkers that are of predictive and/or prognostic values.

Another biomarker for therapeutic target in GI cancer may be *MET*. A multicenter phase II study demonstrated antitumor activity of small-molecule MET inhibitor was shown in MRT-amplifier gastric/gastroesophageal/esophageal adenocarcinoma[55]. A recent study using whole-exome sequencing characterized KDR/VEGFR2 somatic mutations as potential genetic biomarkers of patients' responses to antiangiogenic cancer therapies[56]. Interestingly, a recent cohort study presented that ALK, ROS1, and NTRK rearrangements classified a new subtype of mCRC with particularly poor outcome[57]. Rearrangements of ALK, ROS1, and NTRK were more frequently observed in elderly patients with right-sided tumors and node-spreading, RAS wild-type, and MSI-high cancers. As noted above, ctDNA and RNA-based biomarkers provide high specificity and are ideal as predictive markers for monitoring patients' responses to chemotherapy as well as tumor progression[52]. MMR-deficiency deficiency has emerged as another meaningful biomarker. MMR deficiency has been shown to be positively prognostic for outcome in patients with GC and CRC[58,59]. Notably, MMR deficiency is a variety of cancer predictor for response to anti-PD-1/PD-L1 blockade therapies[60]. Tumor-infiltrating lymphocytes (TILs) are the major type of infiltrating immune cells[36]. The density of TILs is considered to be an indication of the host immune response against tumor cells. To date, the density of TILs have been investigated as a useful prognostic factor in GI cancer[61]. Collectively, research has moved towards the identification of mutations in key genes involved in the progression of GI cancer. In the meanwhile, large-scale prospective clinical studies for evaluating the sensitivity and specificity of these biomarkers are required before their application in clinical practice, due to their low mutational burden and insufficient specificity. The approved biomarkers and candidate biomarkers of GI cancer are summarized in Table 4.

Future research may identify biomarkers that enable cost-effective and noninvasiveness treatments for GI cancer. It is also necessary to determine the best prognostic panel of biomarkers and to find predictive biomarkers to help in the selection of the most suitable therapy.

PRECISION SURGERY IN GI CANCER

Precision medicine is a general concept and is thus not limited to genetic detection. Although surgery is the most effective treatment for localized GI cancer and is often curative, an insufficient removal of a tumor results in secondary tumor foci for which the existing chemotherapeutics and/or radiation would be ineffective. In this finally section, we would like to discuss the progress of the precision treatment of GI cancers through surgery.

Fluorescence-guided surgery for GI cancer

Surgery has been said to provide the most benefit for patients with GI cancer. When R0 resection was carried out in a series of GI cancer patients, the local 5-year relapse rate was significantly improved[62]. The reported rates of local recurrence and distant metastasis were high at 2.6% and 30% of patients who underwent an R0 resection[63,64]. Real-time imaging to find positive surgical margins during a surgical procedure may be useful to diminish the rates of recurrence. Intraoperative fluorescence imaging, or fluorescence-guided surgery (FGS), can offer highly reliable tumor visualization for localization and margin identification[65]. The targeted fluorescent labeling of cancer cells may therefore alter the ways we detect and treat cancer.

Indocyanine green (ICG) is applied clinically to define liver tumor margins and biliary anatomy. The authors of a recent meta-analysis stated that intraoperative ICG fluorescence angiography has been demonstrated to reduce anastomotic leakage rates after colorectal resection[66]. In CRC, ICG fluorescence lymphangiography can be used to detect the primary tumor, its lymphatic drainage, and potentially malignant nodes, which may change the operative plan[67]. FGS can thus serve as a surgical guide with the potential to provide benefits for patients with GI cancer.

Sentinel node navigation surgery

Table 4 Current progress of biomarkers associated with diagnosis, prognosis, prediction of therapeutic response in gastrointestinal cancer (excluding liquid biopsy)

Market	Tumor type	Alteration	Clinical setting	Ref.
HER2	GC, CRC	Amplification, Overexpression	Predictive	Bang *et al*[118], 2010; Sartore-Bianchi *et al*[119], 2016
KRAS	CRC	Activating mutation within catalytic RAS domain	Predictive	Wormald *et al*, 2013; Febbo *et al*, 2011; Schmoll *et al*[122], 2012; Locker *et al*[123], 2006
NRAS,	CRC	Overexpression	Prognostic/Predictive	Hu *et al*[124], 2018
BRAF	CRC	Mutation	Prognostic/Therapeutic	Tie *et al*[54], 2011
KDR	CRC	Mutation	Predictive	Loaiza-Bonilla *et al*[125], 2016
VEGF-D	CRC	Overexpression	Predictive	Tabernero *et al*[126], 2018
AKT	GC	Activation	Predictive	Ito *et al*[127], 2017
PTEN	GC	Downregulation	Predictive	Kim *et al*, 2017
NTRK fusion	CRC	Overexpression	Predictive	Drilon *et al*[129], 2018
ALK	CRC	Rearrangement	Prognostic	Pietrantonio *et al*[57], 2017
POLE	CRC	Mutation	Predictive	Domingo *et al*[130], 2016
MMR	GC, CRC		Predictive	Llosa *et al*[131], 2015
PD-L1	CRC	Mutatoin	Prognostic	Eriksen *et al*[132], 2019
Tumor infiltrating lymphocyte	GC, CRC	Overexpression	Prognostic	Iseki *et al*[133], 2018
CagA	GC	Upregulated	Diagnostic	Saju *et al*[134], 2016
Gastrokine 1	GC	Downregulated	Diagnostic	Altieri *et al*[135], 2017
MEK	CRC	Activation	Predictive	Martinelli *et al*[136], 2017
PIK3CA	CRC	Mutation	Prognostic/ Therapeutic	Jehan *et al*[137], 2019; Schmoll *et al*[122], 2012
TP53	EC, GC, CRC	Mutation	Prognostic	Schmoll *et al*[122], Guo *et al*[138], 2017
CTNNB1	CRC EC, GC	Mutation Overexpression	Prognostic Prognostic	Gao *et al*[116], 2019; Szász *et al*[139], 2016; Ishiguro *et al*[140], 2016
APC	CRC	Mutation	Prognostic	Liang *et al*[141], 2017; Chen *et al*[142], 2013
IGFR-!R	CRC	Upregulation	Prognostic	Codony-Servat *et al*[143], 2017
SFRP2	CRC	Hypermethylation	Diagnostic/Prognostic	Tang *et al*, 2011
UGT1A1	CRC	Hypermethylation	Predictive	Crea *et al*[145], 2011
SMAD4,	EC, GC, CRC	Downregulation	Prognostic/Predictive	Salem *et al*[146], 2018; Wasserman *et al*[147], 2019
MET	EC, GC	Amplificatoin	Predictive	Van Cutsem *et al*[55], 2018
CDKN2A	EC,	Methylation	Diagnostic	Zhou *et al*, 2017
ATM	GC, CRC	Mutaion/Downregulation	Prognostic	Randon *et al*[149], 2019; Han *et al*[83], 2017
BLM,	CRC	Mutaion/Polymorphisms	Diagnostic	de Voer *et al*[150], 2015; Frank *et al*[151], 2010
BRCA1/2,	CRC	Mutaion	Diagnostic	Oh *et al*[152], 2018
ARID1A	GC	Mutation	Predictive	Wei *et al*[153], 2014
	CRC	Overexpresion	Prognostic	Ronchetti *et al*[154], 2017
CDH1	GC	Mutation	Diagnostic	Hansford *et al*[155], 2015
	CRC	Polymorphism	Diagnostic	Grünhage *et al*[156], 2008
CCNE1	GC	Amplification	Therapeutic	Ooi *et al*[157], 2017
RHOA	GC, CRC	Overexpression	Prognostic	Chang *et al*[158], 2016
CCND1	EC	Amplification/Overexpression	Diagnostic	Hu *et al*[159], 2016
	CRC	Polymorphism	Diagnostic	Grünhage *et al*[156], 2008
FBXW7	CRC	Mutation	Prognostic	Korphaisarn *et al*[160], 2017
NOTCH1	EC	Mutation	Prognostic	Song *et al*[161], 2016
	CRC	Gene copy number	Prognostic	Arcaroli *et al*[162], 2016
NOTCH3	CRC	Overexpression	Predictive	Ozawa *et al*[163], 2014

YAP1	EC, GC, CRC	Overexpression	Prognostic	Zhang *et al*[164], 2018

EC: Esophageal cancer; GC: Gastric cancer; CRC: Colorectal cancer; MMR: mismatch repair; PD-L1: programmed death ligand 1; PD-1: programmed death-1; POLE: DNA polymerase 1; HER2: human epidermal growth factor receptor type2; EGFR: epidermal growth factor receptor.

Many investigators have described the potential usage of sentinel node (SN) navigation surgery in patients with early-stage EC and GC who have no lymph node metastasis preoperatively[68,69]. In early stage upper GI cancer, SN mapping provides significant information about an individual patient's metastatic situation and enables the modification of the patient's surgery. Several single-institution investigations have noted pivotal benefits of SN mapping for early EC, especially when using the radio-guided method[70]. Clinically T1 esophageal cancers were suitable targets for SN mapping, because in T3 or T4 tumors as well as those with lymph node metastasis, the original lymphatic routes can be obstructed, which leads to a high rate of false-negative outcomes. SNs were detected in 95% of patients, and the accuracy was as high as 94%[71]. Moreover, SNs were identified widely from the cervical area to the abdominal area, which allows the partial resection of the distal esophagus *via* the laparoscopic trans-hiatal approach without extensive mediastinal lymph node dissection when the SNs are identified only in the abdominal region and are pathologically negative in cT1N0 cases of the distal esophagus[71]. The precise indications for laparoscopic surgeries (*e.g.*, partial resection and segmental gastrectomy for cT1N0 GC) based on the SN status could be individually determined. SN navigation surgery could be a strategy to ensure a better prognosis than conventional operative strategies.

FUTURE PERSPECTIVES

Precision medicine is the application of the latest biological technology that takes into account the patient's living environment along with the patient's clinical data (as well as molecular imaging techniques and bioinformatics technology) to achieve accurate diagnoses and treatments. It is difficult to determine the precise clinical and biological significance for each individual patient because of the inconsistency in biological features on the human genome[71]. Moreover, the complexity of the NGS data-analysis process makes it impractical for oncologists to understand the meanings and uncertainties of the results easily. A systematic and easily interpreted system with an accessible database is immediately necessary for detecting specific genomic alterations and genotype-matched therapeutic options with clinical practice. Although it would be impossible to completely prepare a treatment plan for each individual case, more suitable treatment based on the unique genomic changes of each patient's tumor could be adapted.

The recent progress in the use of precision medicine in GI cancer was summarized in this review. Regarding treatment, we expect that the narrowing down of the number of eligible patients in accord with dose setting, schedule setting, and the selection of concomitant drugs based on the mechanism of molecular targeted agents will lead to effective therapy customized to each individual. For GI cancers, there is an urgent need for preclinical models to identify and select suitable target for therapy. Recent developments in stem cell biology have enabled the in vitro generation of complex three-dimensional (3D) multicellular stem cell-derived constructs that mimic their corresponding organ *in vivo*[72]. These organ-like structures denoted as organoids. Patient-derived organoids (PDOs) may be an attractive candidate for an appropriate cancer model that is able to identify the most effective therapy for individual patients with currently available drugs in a timely manner, but also the future of regenerative medicine. therapies, 3D organoids have been advanced for several cancer types and been shown to effectively recapitulate tumor specific characteristics, which may lead to facilitate the development of precision medicine[73]. A recent study demonstrated that the feasibility of GC PODs from endoscopic biopsies and also suggest that endoscopic-derived PDOs may serve as an precise surrogates of the primary lesion of tumor, which may lead to possess the superiority to drug sensitivity screening and precision therapies[74]. Other study using patient-derived CRC organoids presented that of all RASGTPases activating proteins, only neurofibromin (NF1) deficiency facilitate cell survival and prompted EGF-independent tumor cell growth in human CRC samples, suggesting that NF1 protein levels should be measured in CRCs prior to initiate of targeted therapy against the MAPK pathway[75].

Our understanding of the fundamental biology of GI cancer is continually advancing. GI cancer is a heterogeneous disease with significant differences between

patients in prognosis and therapeutic response. Part of these differences can be explained by the molecular diversity detected in GI cancer. So as to provide a more overall insight into this complexity, biologically distinct molecular subtypes of GI cancer based on gene expression analyses were defined and validated. EC is classified into three distinct molecular subgroups based on gene analysis findings[76]. The first subgroup (ESCC1) includes tumors that respond poorly to chemoradiotherapy, leading to poor prognoses. The principal gene alteration identified is NRF2 pathway disruption. The second subgroup is ESCC2, characterized by the mutation of *NOTCH1*, *ZNF750*, *KDM6A*, *KDM2D*, *PTEN*, *PIK3R1*, and *CDK6* amplification. This subgroup is also associated with white blood cell infiltration. The last molecular subgroup (ESCC3) is characterized by PI3K pathway disruption. Similarly, GC is subclassified into four major subtypes based on the molecular pattern; the EBV group, MSI group, chromosomal instability group, and genomically stable group[36]. In CRC, four consensus molecular subtypes (CMS) were shown. CMS1 is enriched for MSI tumors that reveal marked immune activation. CMS2 reflects the classical subtype encompassing higher CIN and strong WNT/MYC-driven tumors with epithelial characteristics, whereas CMS3 is enriched for *KRAS*-mutated tumors with activation of metabolic pathways. CMS4 has mesenchymal features, shows a high stromal content and activation of TGF-β and VEGFR pathways[77]. Apparent clinical distinctions are distinct with poor prognosis for CMS4 and a relatively good prognosis for CMS1. A study classifying CRC by both tumor side and location using NGS panel presented that RAS mutations are seen in 70% of cecal tumors but only 57% of ascending colon and 43% of hepatic flexure tumors. BRAFV600 mutations occur in 10% of cecal, 16% of ascending colon, and 22% of hepatic flexure tumors. PIK3CA mutations are seen in 26% of descending colon but only 14% of sigmoid and 9% of rectosigmoid tumors. CTNNB1 mutations are almost absent in the sigmoid (1%), rectosigmoid junction (0%), and rectum (1%), but are still present in the descending colon (6%). This study also revealed increasing rates of CMS2 moving from right to left, accompanied by a fall in CMS1, while CMS3 and CMS4 were relatively stable when we compared CMS by tumor side[78]. In summary, the region from the sigmoid colon to the rectum appears unique and the transverse colon appears distinct from other right sided locations.

Another study define the colorectal cancer intrinsic subtypes (CRIS) distinguished by specific molecular, functional and pathogenic features; (1) CRIS-A: Mucinous subtype, glycolytic metabolism, with marked MSI, mutated *BRAF* or *KRAS*; (2) CRIS-B: Active TGF-β signaling, epithelial–mesenchymal transition, bad prognosis; (3) CRIS-C: High EGFR signaling, and to EGFR inhibitors (*i.e.*, cetuximab); (4) CRIS-D: High WNT signaling, *IGF2* gene amplification/ overexpression; and (5) CRIS-E: Paneth-like phenotype and *TP53*-mutated genotype[79]. Recent work revealed that subtype-specific analysis can be used to predict therapy response, which provides a great opportunity to improve patients' management regarding precision medicine[80,81].

Although subclassification systems proposed for each GI cancer type have also possessed major challenges and caused important questions that need to be further investigated still it is applied for patient care timely, there is the possibility that these subgroup analyses revolutionize our approach towards precision medicine. Advances in tumor genomics and the immunologic landscape based on "big data" will allow the identification of expanding indications for molecular target drugs and chemotherapy in GI cancer and its predictive biomarkers. Clinical trials for targeted therapies, coupled with genomic profiling for optimum patient selection, are required to demonstrate clinical utility, including treatment outcomes and cost-effectiveness. Investigations of the safety and efficacy of clinical cancer therapies may reveal novel research directions for treating GI cancer. Increasing our knowledge of the signaling that mediates the driver mutations in GI cancer will improved our understanding of GI cancer and serve to guide future precision medicine applications for this disease. At present, we are in the very early phases of this transition towards precision and personalized medicine. We hope that this review can be a guideline for clinical and bench investigators to further develop precision medicine.

REFERENCES

1 **Hyman DM**, Taylor BS, Baselga J. Implementing Genome-Driven Oncology. *Cell* 2017; **168**: 584-599 [PMID: 28187282 DOI: 10.1016/j.cell.2016.12.015]

2 **Zehir A**, Benayed R, Shah RH, Syed A, Middha S, Kim HR, Srinivasan P, Gao J, Chakravarty D, Devlin SM, Hellmann MD, Barron DA, Schram AM, Hameed M, Dogan S, Ross DS, Hechtman JF. DeLair DF, Yao J, Mandelker DL, Cheng DT, Chandramohan R, Mohanty AS, Ptashkin RN, Jayakumaran G, Prasad M, Syed MH, Rema AB, Liu ZY, Nafa K, Borsu L, Sadowska J, Casanova J, Bacares R, Kiecka IJ,

Razumova A, Son JB, Stewart L, Baldi T, Mullaney KA, Al-Ahmadie H, Vakiani E, Abeshouse AA, Penson AV, Jonsson P, Camacho N, Chang MT, Won HH, Gross BE, Kundra R, Heins ZJ, Chen HW, Phillips S, Zhang H, Wang J, Ochoa A, Wills J, Eubank M, Thomas SB, Gardos SM, Reales DN, Galle J, Durany R, Cambria R, Abida W, Cercek A, Feldman DR, Gounder MM, Hakimi AA, Harding JJ, Iyer G, Janjigian YY, Jordan EJ, Kelly CM, Lowery MA, Morris LGT, Omuro AM, Raj N, Razavi P, Shoushtari AN, Shukla N, Soumerai TE, Varghese AM, Yaeger R, Coleman J, Bochner B, Riely GJ, Saltz LB, Scher HI, Sabbatini PJ, Robson ME, Klimstra DS, Taylor BS, Baselga J, Schultz N, Hyman DM, Arcila ME, Solit DB, Ladanyi M, Berger MF. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017; **23**: 703-713 [PMID: 28481359 DOI: 10.1038/nm.4333]

3 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

4 **Ettinger DS**, Wood DE, Aisner DL, Akerley W, Bauman J, Chirieac LR, D'Amico TA, DeCamp MM, Dilling TJ, Dobelbower M, Doebele RC, Govindan R, Gubens MA, Hennon M, Horn L, Komaki R, Lackner RP, Lanuti M, Leal TA, Leisch LJ, Lilenbaum R, Lin J, Loo BW, Martins R, Otterson GA, Reckamp K, Riely GJ, Schild SE, Shapiro TA, Stevenson J, Swanson SJ, Tauer K, Yang SC, Gregory K, Hughes M. Non-Small Cell Lung Cancer, Version 5.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2017; **15**: 504-535 [PMID: 28404761 DOI: 10.6004/jnccn.2017.0050]

5 **Gladfelter P**, Darwish NHE, Mousa SA. Current status and future direction in the management of malignant melanoma. *Melanoma Res* 2017; **27**: 403-410 [PMID: 28800028 DOI: 10.1097/CMR.0000000000000379]

6 **Crowley E**, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; **10**: 472-484 [PMID: 23836314 DOI: 10.1038/nrclinonc.2013.110]

7 **Mader S**, Pantel K. Liquid Biopsy: Current Status and Future Perspectives. *Oncol Res Treat* 2017; **40**: 404-408 [PMID: 28693023 DOI: 10.1159/000478018]

8 **Gao Y**, Zhang K, Xi H, Cai A, Wu X, Cui J, Li J, Qiao Z, Wei B, Chen L. Diagnostic and prognostic value of circulating tumor DNA in gastric cancer: a meta-analysis. *Oncotarget* 2017; **8**: 6330-6340 [PMID: 28009985 DOI: 10.18632/oncotarget.14064]

9 **Cohen JD**, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, Douville C, Javed AA, Wong F, Mattox A, Hruban RH, Wolfgang CL, Goggins MG, Dal Molin M, Wang TL, Roden R, Klein AP, Ptak J, Dobbyn L, Schaefer J, Silliman N, Popoli M, Vogelstein JT, Browne JD, Schoen RE, Brand RE, Tie J, Gibbs P, Wong HL, Mansfield AS, Jen J, Hanash SM, Falconi M, Allen PJ, Zhou S, Bettegowda C, Diaz LA, Tomasetti C, Kinzler KW, Vogelstein B, Lennon AM, Papadopoulos N. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* 2018; **359**: 926-930 [PMID: 29348365 DOI: 10.1126/science.aar3247]

10 **Díaz-Serrano A**, Gella P, Jiménez E, Zugazagoitia J, Paz-Ares Rodríguez L. Targeting EGFR in Lung Cancer: Current Standards and Developments. *Drugs* 2018; **78**: 893-911 [PMID: 29915896 DOI: 10.1007/s40265-018-0916-4]

11 **Zhang W**, Xia W, Lv Z, Ni C, Xin Y, Yang L. Liquid Biopsy for Cancer: Circulating Tumor Cells, Circulating Free DNA or Exosomes? *Cell Physiol Biochem* 2017; **41**: 755-768 [PMID: 28214887 DOI: 10.1159/000458736]

12 **Ferreira MM**, Ramani VC, Jeffrey SS. Circulating tumor cell technologies. *Mol Oncol* 2016; **10**: 374-394 [PMID: 26897752 DOI: 10.1016/j.molonc.2016.01.007]

13 **Riethdorf S**, Fritsche H, Müller V, Rau T, Schindlbeck C, Rack B, Janni W, Coith C, Beck K, Jänicke F, Jackson S, Gornet T, Cristofanilli M, Pantel K. Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system. *Clin Cancer Res* 2007; **13**: 920-928 [PMID: 17289886 DOI: 10.1158/1078-0432.CCR-06-1695]

14 **Sumanasuriya S**, Lambros MB, de Bono JS. Application of Liquid Biopsies in Cancer Targeted Therapy. *Clin Pharmacol Ther* 2017; **102**: 745-747 [PMID: 28755443 DOI: 10.1002/cpt.764]

15 **Wan JCM**, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, Pacey S, Baird R, Rosenfeld N. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer* 2017; **17**: 223-238 [PMID: 28233803 DOI: 10.1038/nrc.2017.7]

16 **Diaz LA**, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol* 2014; **32**: 579-586 [PMID: 24449238 DOI: 10.1200/JCO.2012.45.2011]

17 **Bettegowda C**, Sausen M, Leary RJ, Kinde I, Wang Y. Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SK, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih lM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Diaz LA JrDetection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014; **6**: 224ra24 [PMID: 24553385 DOI: 10.1126/scitranslmed.3007094]

18 **Schwarzenbach H**, Stoehlmacher J, Pantel K, Goekkurt E. Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. *Ann N Y Acad Sci* 2008; **1137**: 190-196 [PMID: 18837946 DOI: 10.1196/annals.1448.025]

19 **García-Foncillas J**, Alba E, Aranda E, Díaz-Rubio E, López-López R, Tabernero J, Vivancos A. Incorporating BEAMing technology as a liquid biopsy into clinical practice for the management of colorectal cancer patients: an expert taskforce review. *Ann Oncol* 2017; **28**: 2943-2949 [PMID: 28945877 DOI: 10.1093/annonc/mdx501]

20 **Tóth K**, Barták BK, Tulassay Z, Molnár B. Circulating cell-free nucleic acids as biomarkers in colorectal cancer screening and diagnosis. *Expert Rev Mol Diagn* 2016; **16**: 239-252 [PMID: 26652067 DOI: 10.1586/14737159.2016.1132164]

21 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438 DOI: 10.1016/S0092-8674(04)00045-5]

22 **Munson P**, Shukla A. Exosomes: Potential in Cancer Diagnosis and Therapy. *Medicines (Basel)* 2015; **2**: 310-327 [PMID: 27088079 DOI: 10.3390/medicines2040310]

23 **Rahbari M**, Rahbari N, Reissfelder C, Weitz J, Kahlert C. Exosomes: novel implications in diagnosis and treatment of gastrointestinal cancer. *Langenbecks Arch Surg* 2016; **401**: 1097-1110 [PMID: 27342853 DOI: 10.1007/s00423-016-1468-2]

24 **van Lanschot MC**, Carvalho B, Coupé VM, van Engeland M, Dekker E, Meijer GA. Molecular stool

testing as an alternative for surveillance colonoscopy: a cross-sectional cohort study. *BMC Cancer* 2017; **17**: 116 [PMID: 28173852 DOI: 10.1186/s12885-017-3078-y]

25 **Issa IA**, Noureddine M. Colorectal cancer screening: An updated review of the available options. *World J Gastroenterol* 2017; **23**: 5086-5096 [PMID: 28811705 DOI: 10.3748/wjg.v23.i28.5086]

26 **Norcic G**, Jelenc F, Cerkovnik P, Stegel V, Novakovic S. Role of specific DNA mutations in the peripheral blood of colorectal cancer patients for the assessment of tumor stage and residual disease following tumor resection. *Oncol Lett* 2016; **12**: 3356-3362 [PMID: 27900004 DOI: 10.3892/ol.2016.5078]

27 **Klein-Scory S**, Maslova M, Pohl M, Eilert-Micus C, Schroers R, Schmiegel W, Baraniskin A. Significance of Liquid Biopsy for Monitoring and Therapy Decision of Colorectal Cancer. *Transl Oncol* 2018; **11**: 213-220 [PMID: 29367069 DOI: 10.1016/j.tranon.2017.12.010]

28 **Morelli MP**, Overman MJ, Dasari A, Kazmi SM, Mazard T, Vilar E, Morris VK, Lee MS, Herron D, Eng C, Morris J, Kee BK, Janku F, Deaton FL, Garrett C, Maru D, Diehl F, Angenendt P, Kopetz S. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. *Ann Oncol* 2015; **26**: 731-736 [PMID: 25628445 DOI: 10.1093/annonc/mdv005]

29 **Diaz LA**, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, Allen B, Bozic I, Reiter JG, Nowak MA, Kinzler KW, Oliner KS, Vogelstein B. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 2012; **486**: 537-540 [PMID: 22722843 DOI: 10.1038/nature11219]

30 **Du J**, Wu X, Tong X, Wang X, Wei J, Yang Y, Chang Z, Mao Y, Shao YW, Liu B. Circulating tumor DNA profiling by next generation sequencing reveals heterogeneity of crizotinib resistance mechanisms in a gastric cancer patient with MET amplification. *Oncotarget* 2017; **8**: 26281-26287 [PMID: 28460431 DOI: 10.18632/oncotarget.15457]

31 **Schøler LV**, Reinert T, Ørntoft MW, Kassentoft CG, Árnadóttir SS, Vang S, Nordentoft I, Knudsen M, Lamy P, Andreasen D, Mortensen FV, Knudsen AR, Stribolt K, Sivesgaard K, Mouritzen P, Nielsen HJ, Laurberg S, Ørntoft TF, Andersen CL. Clinical Implications of Monitoring Circulating Tumor DNA in Patients with Colorectal Cancer. *Clin Cancer Res* 2017; **23**: 5437-5445 [PMID: 28600478 DOI: 10.1158/1078-0432.CCR-17-0510]

32 **Young GP**, Pedersen SK, Mansfield S, Murray DH, Baker RT, Rabbitt P, Byrne S, Bambacas L, Hollington P, Symonds EL. A cross-sectional study comparing a blood test for methylated BCAT1 and IKZF1 tumor-derived DNA with CEA for detection of recurrent colorectal cancer. *Cancer Med* 2016; **5**: 2763-2772 [PMID: 27726312 DOI: 10.1002/cam4.868]

33 **Krebs MG**, Renehan AG, Backen A, Gollins S, Chau I, Hasan J, Valle JW, Morris K, Beech J, Ashcroft L, Saunders MP, Dive C. Circulating Tumor Cell Enumeration in a Phase II Trial of a Four-Drug Regimen in Advanced Colorectal Cancer. *Clin Colorectal Cancer* 2015; **14**: 115-22.e1-2 [PMID: 25680623 DOI: 10.1016/j.clcc.2014.12.006]

34 **Mäbert K**, Cojoc M, Peitzsch C, Kurth I, Souchelnytskyi S, Dubrovska A. Cancer biomarker discovery: current status and future perspectives. *Int J Radiat Biol* 2014; **90**: 659-677 [PMID: 24524284 DOI: 10.3109/09553002.2014.892229]

35 **Cancer Genome Atlas Network**. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

36 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]

37 **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]

38 **Ciombor KK**, Wu C, Goldberg RM. Recent therapeutic advances in the treatment of colorectal cancer. *Annu Rev Med* 2015; **66**: 83-95 [PMID: 25341011 DOI: 10.1146/annurev-med-051513-102539]

39 **Nagahashi M**, Wakai T, Shimada Y, Ichikawa H, Kameyama H, Kobayashi T, Sakata J, Yagi R, Sato N, Kitagawa Y, Uetake H, Yoshida K, Oki E, Kudo SE, Izutsu H, Kodama K, Nakada M, Tse J, Russell M, Heyer J, Powers W, Sun R, Ring JE, Takabe K, Protopopov A, Ling Y, Okuda S, Lyle S. Genomic landscape of colorectal cancer in Japan: clinical implications of comprehensive genomic sequencing for precision medicine. *Genome Med* 2016; **8**: 136 [PMID: 28007036 DOI: 10.1186/s13073-016-0387-8]

40 **Osumi H**, Shinozaki E, Takeda Y, Wakatsuki T, Ichimura T, Saiura A, Yamaguchi K, Takahashi S, Noda T, Zembutsu H. Clinical relevance of circulating tumor DNA assessed through deep sequencing in patients with metastatic colorectal cancer. *Cancer Med* 2019; **8**: 408-417 [PMID: 30575318 DOI: 10.1002/cam4.1913]

41 **Ichikawa H**, Nagahashi M, Shimada Y, Hanyu T, Ishikawa T, Kameyama H, Kobayashi T, Sakata J, Yabusaki H, Nakagawa S, Sato N, Hirata Y, Kitagawa Y, Tanahashi T, Yoshida K, Nakanishi R, Oki E, Vuzman D, Lyle S, Takabe K, Ling Y, Okuda S, Akazawa K, Wakai T. Actionable gene-based classification toward precision medicine in gastric cancer. *Genome Med* 2017; **9**: 93 [PMID: 29089060 DOI: 10.1186/s13073-017-0484-3]

42 **Kakiuchi M**, Nishizawa T, Ueda H, Gotoh K, Tanaka A, Hayashi A, Yamamoto S, Tatsuno K, Katoh H, Watanabe Y, Ichimura T, Ushiku T, Funahashi S, Tateishi K, Wada I, Shimizu N, Nomura S, Koike K, Seto Y, Fukayama M, Aburatani H, Ishikawa S. Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. *Nat Genet* 2014; **46**: 583-587 [PMID: 24816255 DOI: 10.1038/ng.2984]

43 **Wang K**, Yuen ST, Xu J, Lee SP, Yan HH, Shi ST, Siu HC, Deng S, Chu KM, Law S, Chan KH, Chan AS, Tsui WY, Ho SL, Chan AK, Man JL, Foglizzo V, Ng MK, Chan AS, Ching YP, Cheng GH, Xie T, Fernandez J, Li VS, Clevers H, Rejto PA, Mao M, Leung SY. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat Genet* 2014; **46**: 573-582 [PMID: 24816253 DOI: 10.1038/ng.2983]

44 **Sawada G**, Niida A, Uchi R, Hirata H, Shimamura T, Suzuki Y, Shiraishi Y, Chiba K, Imoto S, Takahashi Y, Iwaya T, Sudo T, Hayashi T, Takai H, Kawasaki Y, Matsukawa T, Eguchi H, Sugimachi K, Tanaka F, Suzuki H, Yamamoto K, Ishii H, Shimizu M, Yamazaki H, Yamazaki M, Tachimori Y, Kajiyama Y, Natsugoe S, Fujita H, Mafune K, Tanaka Y, Kelsell DP, Scott CA, Tsuji S, Yachida S, Shibata T, Sugano S, Doki Y, Akiyama T, Aburatani H, Ogawa S, Miyano S, Mori M, Mimori K. Genomic Landscape of Esophageal Squamous Cell Carcinoma in a Japanese Population. *Gastroenterology* 2016; **150**: 1171-1182 [PMID: 26873401 DOI: 10.1053/j.gastro.2016.01.035]

45 **Rizvi NA**, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS, Miller ML, Rekhtman N, Moreira AL, Ibrahim F, Bruggeman C, Gasmi B, Zappasodi R, Maeda Y, Sander C, Garon EB, Merghoub T, Wolchok JD, Schumacher TN, Chan TA. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; **348**: 124-

128 [PMID: 25765070 DOI: 10.1126/science.aaa1348]
46 **McGranahan N**, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, Jamal-Hanjani M, Wilson GA, Birkbak NJ, Hiley CT, Watkins TB, Shafi S, Murugaesu N, Mitter R, Akarca AU, Linares J, Marafioti T, Henry JY, Van Allen EM, Miao D, Schilling B, Schadendorf D, Garraway LA, Makarov V, Rizvi NA, Snyder A, Hellmann MD, Merghoub T, Wolchok JD, Shukla SA, Wu CJ, Peggs KS, Chan TA, Hadrup SR, Quezada SA, Swanton C. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 2016; **351**: 1463-1469 [PMID: 26940869 DOI: 10.1126/science.aaf1490]
47 **Le DT**, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, Wong F, Azad NS, Rucki AA, Laheru D, Donehower R, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Greten TF, Duffy AG, Ciombor KK, Eyring AD, Lam BH, Joe A, Kang SP, Holdhoff M, Danilova L, Cope L, Meyer C, Zhou S, Goldberg RM, Armstrong DK, Bever KM, Fader AN, Taube J, Housseau F, Spetzler D, Xiao N, Pardoll DM, Papadopoulos N, Kinzler KW, Eshleman JR, Vogelstein B, Anders RA, Diaz LA. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017; **357**: 409-413 [PMID: 28596308 DOI: 10.1126/science.aan6733]
48 **Bourdais R**, Rousseau B, Pujals A, Boussion H, Joly C, Guillemin A, Baumgaertner I, Neuzillet C, Tournigand C. Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. *Crit Rev Oncol Hematol* 2017; **113**: 242-248 [PMID: 28427513 DOI: 10.1016/j.critrevonc.2017.03.027]
49 **Nagarajan N**, Bertrand D, Hillmer AM, Zang ZJ, Yao F, Jacques PÉ, Teo AS, Cutcutache I, Zhang Z, Lee WH, Sia YY, Gao S, Ariyaratne PN, Ho A, Woo XY. Veeravali L, Ong CK, Deng N, Desai KV, Khor CC, Hibberd ML, Shahab A, Rao J, Wu M, Teh M, Zhu F, Chin SY, Pang B, So JB, Bourque G, Soong R, Sung WK, Tean Teh B, Rozen S, Ruan X, Yeoh KG, Tan PB, Ruan Y. Whole-genome reconstruction and mutational signatures in gastric cancer. *Genome Biol* 2012; **13**: R115 [PMID: 23237666 DOI: 10.1186/gb-2012-13-12-r115]
50 **Huang Z**, Zhang L, Zhu D, Shan X, Zhou X, Qi LW, Wu L, Zhu J, Cheng W, Zhang H, Chen Y, Zhu W, Wang T, Liu P. A novel serum microRNA signature to screen esophageal squamous cell carcinoma. *Cancer Med* 2017; **6**: 109-119 [PMID: 28035762 DOI: 10.1002/cam4.973]
51 **Zhang H**, Zhu M, Shan X, Zhou X, Wang T, Zhang J, Tao J, Cheng W, Chen G, Li J, Liu P, Wang Q, Zhu W. A panel of seven-miRNA signature in plasma as potential biomarker for colorectal cancer diagnosis. *Gene* 2019; **687**: 246-254 [PMID: 30458288 DOI: 10.1016/j.gene.2018.11.055]
52 **Vacante M**, Borzì AM, Basile F, Biondi A. Biomarkers in colorectal cancer: Current clinical utility and future perspectives. *World J Clin Cases* 2018; **6**: 869-881 [PMID: 30568941 DOI: 10.12998/wjcc.v6.i15.869]
53 **Matsuoka T**, Yashiro M. Biomarkers of gastric cancer: Current topics and future perspective. *World J Gastroenterol* 2018; **24**: 2818-2832 [PMID: 30018477 DOI: 10.3748/wjg.v24.i26.2818]
54 **Tie J**, Gibbs P, Lipton L, Christie M, Jorissen RN, Burgess AW, Croxford M, Jones I, Langland R, Kosmider S, McKay D, Bollag G, Nolop K, Sieber OM, Desai J. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. *Int J Cancer* 2011; **128**: 2075-2084 [PMID: 20635392 DOI: 10.1002/ijc.25555]
55 **Van Cutsem E**, Karaszewska B, Kang YK, Chung HC, Shankaran V, Siena S, Go NF, Yang H, Schupp M, Cunningham D. A Multicenter Phase II Study of AMG 337 in Patients with *MET*-Amplified Gastric/Gastroesophageal Junction/Esophageal Adenocarcinoma and Other *MET*-Amplified Solid Tumors. *Clin Cancer Res* 2019; **25**: 2414-2423 [PMID: 30366938 DOI: 10.1158/1078-0432.CCR-18-1337]
56 **Toledo RA**, Garralda E, Mitsi M, Pons T, Monsech J, Vega E, Otero Á, Albarran MI, Baños N, Durán Y, Bonilla V. Sarno F, Camacho-Artacho M, Sanchez-Perez T, Perea S, Álvarez R, De Martino A, Lietha D, Blanco-Aparicio C, Cubillo A, Domínguez O, Martínez-Torrecuadrada JL, Hidalgo M. Exome Sequencing of Plasma DNA Portrays the Mutation Landscape of Colorectal Cancer and Discovers Mutated VEGFR2 Receptors as Modulators of Antiangiogenic Therapies. *Clin Cancer Res* 2018; **24**: 3550-3559 [PMID: 29588308 DOI: 10.1158/1078-0432.CCR-18-0103]
57 **Pietrantonio F**, Di Nicolantonio F, Schrock AB, Lee J, Tejpar S, Sartore-Bianchi A, Hechtman JF, Christiansen J, Novara L, Tebbutt N, Fucà G, Antoniotti C, Kim ST, Murphy D, Berenato R, Morano F, Sun J, Min B, Stephens PJ, Chen M, Lazzari L, Miller VA, Shoemaker R, Amatu A, Milione M, Ross JS, Siena S, Bardelli A, Ali SM, Falcone A, de Braud F, Cremolini C. ALK, ROS1, and NTRK Rearrangements in Metastatic Colorectal Cancer. *J Natl Cancer Inst* 2017; 109 [PMID: 29370427 DOI: 10.1093/jnci/djx089]
58 **Papadopoulos N**, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD. Mutation of a mutL homolog in hereditary colon cancer. *Science* 1994; **263**: 1625-1629 [PMID: 8128251 DOI: 10.1126/science.8128251]
59 **Smyth EC**, Wotherspoon A, Peckitt C, Gonzalez D, Hulkki-Wilson S, Eltahir Z, Fassan M, Rugge M, Valeri N, Okines A, Hewish M, Allum W, Stenning S, Nankivell M, Langley R, Cunningham D. Mismatch Repair Deficiency, Microsatellite Instability, and Survival: An Exploratory Analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) Trial. *JAMA Oncol* 2017; **3**: 1197-1203 [PMID: 28241187 DOI: 10.1001/jamaoncol.2016.6762]
60 **Viale G**, Trapani D, Curigliano G. Mismatch Repair Deficiency as a Predictive Biomarker for Immunotherapy Efficacy. *Biomed Res Int* 2017; **2017**: 4719194 [PMID: 28770222 DOI: 10.1155/2017/4719194]
61 **Yu PC**, Long D, Liao CC, Zhang S. Association between density of tumor-infiltrating lymphocytes and prognoses of patients with gastric cancer. *Medicine (Baltimore)* 2018; **97**: e11387 [PMID: 29979429 DOI: 10.1097/MD.0000000000011387]
62 **Hohenberger W**, Weber K, Matzel K, Papadopoulos T, Merkel S. Standardized surgery for colonic cancer: complete mesocolic excision and central ligation--technical notes and outcome. *Colorectal Dis* 2009; **11**: 354-64; discussion 364-5 [PMID: 19016817 DOI: 10.1111/j.1463-1318.2008.01735.x]
63 **Campos FG**, Calijuri-Hamra MC, Imperiale AR, Kiss DR, Nahas SC, Cecconello I. Locally advanced colorectal cancer: results of surgical treatment and prognostic factors. *Arq Gastroenterol* 2011; **48**: 270-275 [PMID: 22147133 DOI: 10.1590/S0004-28032011000400010]
64 **Shao H**, Ma X, Gao Y, Wang J, Wu J, Wang B, Li J, Tian J. Comparison of the diagnostic efficiency for local recurrence of rectal cancer using CT, MRI, PET and PET-CT: A systematic review protocol. *Medicine (Baltimore)* 2018; **97**: e12900 [PMID: 30508883 DOI: 10.1097/MD.0000000000012900]
65 **Olson MT**, Ly QP, Mohs AM. Fluorescence Guidance in Surgical Oncology: Challenges, Opportunities, and Translation. *Mol Imaging Biol* 2019; **21**: 200-218 [PMID: 29942988 DOI: 10.1007/s11307-018-1239-2]

66 **Shen R**, Zhang Y, Wang T. Indocyanine Green Fluorescence Angiography and the Incidence of Anastomotic Leak After Colorectal Resection for Colorectal Cancer: A Meta-analysis. *Dis Colon Rectum* 2018; **61**: 1228-1234 [PMID: 30192332 DOI: 10.1097/DCR.0000000000001123]

67 **Chand M**, Keller DS, Joshi HM, Devoto L, Rodriguez-Justo M, Cohen R. Feasibility of fluorescence lymph node imaging in colon cancer: FLICC. *Tech Coloproctol* 2018; **22**: 271-277 [PMID: 29551004 DOI: 10.1007/s10151-018-1773-6]

68 **Takeuchi H**, Kitagawa Y. Sentinel lymph node biopsy in gastric cancer. *Cancer J* 2015; **21**: 21-24 [PMID: 25611776 DOI: 10.1097/PPO.0000000000000088]

69 **Yashiro M**, Matsuoka T. Sentinel node navigation surgery for gastric cancer: Overview and perspective. *World J Gastrointest Surg* 2015; **7**: 1-9 [PMID: 25625004 DOI: 10.4240/wjgs.v7.i1.1]

70 **Takeuchi H**, Kawakubo H, Nakamura R, Fukuda K, Takahashi T, Wada N, Kitagawa Y. Clinical Significance of Sentinel Node Positivity in Patients with Superficial Esophageal Cancer. *World J Surg* 2015; **39**: 2941-2947 [PMID: 26296842 DOI: 10.1007/s00268-015-3217-z]

71 **Takeuchi M**, Takeuchi H, Kawakubo H, Kitagawa Y. Update on the indications and results of sentinel node mapping in upper GI cancer. *Clin Exp Metastasis* 2018; **35**: 455-461 [PMID: 30132238 DOI: 10.1007/s10585-018-9934-6]

72 **Roth AD**, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol* 2010; **28**: 466-474 [PMID: 20008640 DOI: 10.1200/JCO.2009.23.3452]

73 **Lin M**, Gao M, Cavnar MJ, Kim J. Utilizing gastric cancer organoids to assess tumor biology and personalize medicine. *World J Gastrointest Oncol* 2019; **11**: 509-517 [PMID: 31367270 DOI: 10.4251/wjgo.v11.i7.509]

74 **Gao M**, Lin M, Rao M, Thompson H, Hirai K, Choi M, Georgakis GV, Sasson AR, Bucobo JC, Tzimas D, D'Souza LS, Buscaglia JM, Davis J, Shroyer KR, Li J, Powers S, Kim J. Development of Patient-Derived Gastric Cancer Organoids from Endoscopic Biopsies and Surgical Tissues. *Ann Surg Oncol* 2018; **25**: 2767-2775 [PMID: 30003451 DOI: 10.1245/s10434-018-6662-8]

75 **Post JB**, Hami N, Mertens AEE, Elfrink S, Bos JL, Snippert HJG. CRISPR-induced RASGAP deficiencies in colorectal cancer organoids reveal that only loss of NF1 promotes resistance to EGFR inhibition. *Oncotarget* 2019; **10**: 1440-1457 [PMID: 30858928 DOI: 10.18632/oncotarget.26677]

76 **Xiong T**, Wang M, Zhao J, Liu Q, Yang C, Luo W, Li X, Yang H, Kristiansen K, Roy B, Zhou Y. An esophageal squamous cell carcinoma classification system that reveals potential targets for therapy. *Oncotarget* 2017; **8**: 49851-49860 [PMID: 28591712 DOI: 10.18632/oncotarget.17989]

77 **Guinney J**, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, Marisa L, Roepman P, Nyamundanda G, Angelino P, Bot BM, Morris JS, Simon IM, Gerster S, Fessler E, De Sousa E Melo F, Missiaglia E, Ramay H, Barras D, Homicsko K, Maru D, Manyam GC, Broom B, Boige V, Perez-Villamil B, Laderas T, Salazar R, Gray JW, Hanahan D, Tabernero J, Bernards R, Friend SH, Laurent-Puig P, Medema JP, Sadanandam A, Wessels L, Delorenzi M, Kopetz S, Vermeulen L, Tejpar S. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; **21**: 1350-1356 [PMID: 26457759 DOI: 10.1038/nm.3967]

78 **Loree JM**, Pereira AAL, Lam M, Willauer AN, Raghav K, Dasari A, Morris VK, Advani S, Menter DG, Eng C, Shaw K, Broaddus R, Routbort MJ, Liu Y, Morris JS, Luthra R, Meric-Bernstam F, Overman MJ, Maru D, Kopetz S. Classifying Colorectal Cancer by Tumor Location Rather than Sidedness Highlights a Continuum in Mutation Profiles and Consensus Molecular Subtypes. *Clin Cancer Res* 2018; **24**: 1062-1072 [PMID: 29180604 DOI: 10.1158/1078-0432.CCR-17-2484]

79 **Isella C**, Brundu F, Bellomo SE, Galimi F, Zanella E, Porporato R, Petti C, Fiori A, Orzan F, Senetta R, Boccaccio C, Ficarra E, Marchionni L, Trusolino L, Medico E, Bertotti A. Selective analysis of cancer-cell intrinsic transcriptional traits defines novel clinically relevant subtypes of colorectal cancer. *Nat Commun* 2017; **8**: 15107 [PMID: 28561063 DOI: 10.1038/ncomms15107]

80 **Linnekamp JF**, Hooff SRV, Prasetyanti PR, Kandimalla R, Buikhuisen JY, Fessler E, Ramesh P, Lee KAST, Bochove GGW, de Jong JH, Cameron K, Leersum RV, Rodermond HM, Franitza M, Nürnberg P, Mangiapane LR, Wang X, Clevers H, Vermeulen L, Stassi G, Medema JP. Consensus molecular subtypes of colorectal cancer are recapitulated in in vitro and in vivo models. *Cell Death Differ* 2018; **25**: 616-633 [PMID: 29305587 DOI: 10.1038/s41418-017-0011-5]

81 **Okita A**, Takahashi S, Ouchi K, Inoue M, Watanabe M, Endo M, Honda H, Yamada Y, Ishioka C. Consensus molecular subtypes classification of colorectal cancer as a predictive factor for chemotherapeutic efficacy against metastatic colorectal cancer. *Oncotarget* 2018; **9**: 18698-18711 [PMID: 29721154 DOI: 10.18632/oncotarget.24617]

82 **Li SP**, Guan QL, Zhao D, Pei GJ, Su HX, Du LN, He JX, Liu ZC. Detection of Circulating Tumor Cells by Fluorescent Immunohistochemistry in Patients with Esophageal Squamous Cell Carcinoma: Potential Clinical Applications. *Med Sci Monit* 2016; **22**: 1654-1662 [PMID: 27184872 DOI: 10.12659/msm.898335]

83 **Han L**, Li YJ, Zhang WD, Song PP, Li H, Li S. Clinical significance of tumor cells in the peripheral blood of patients with esophageal squamous cell carcinoma. *Medicine (Baltimore)* 2019; **98**: e13921 [PMID: 30732126 DOI: 10.1097/MD.0000000000013921]

84 **Kang HM**, Kim GH, Jeon HK, Kim DH, Jeon TY, Park DY, Jeong H, Chun WJ, Kim MH, Park J, Lim M, Kim TH, Cho YK. Circulating tumor cells detected by lab-on-a-disc: Role in early diagnosis of gastric cancer. *PLoS One* 2017; **12**: e0180251 [PMID: 28662130 DOI: 10.1371/journal.pone.0180251]

85 **Zheng X**, Fan L, Zhou P, Ma H, Huang S, Yu D, Zhao L, Yang S, Liu J, Huang A, Cai C, Dai X, Zhang T. Detection of Circulating Tumor Cells and Circulating Tumor Microemboli in Gastric Cancer. *Transl Oncol* 2017; **10**: 431-441 [PMID: 28448959 DOI: 10.1016/j.tranon.2017.02.007]

86 **Mishima Y**, Matsusaka S, Chin K, Mikuniya M, Minowa S, Takayama T, Shibata H, Kuniyoshi R, Ogura M, Terui Y, Mizunuma N, Hatake K. Detection of HER2 Amplification in Circulating Tumor Cells of HER2-Negative Gastric Cancer Patients. *Target Oncol* 2017; **12**: 341-351 [PMID: 28508152 DOI: 10.1007/s11523-017-0493-6]

87 **Wang L**, Zhou S, Zhang W, Wang J, Wang M, Hu X, Liu F, Zhang Y, Jiang B, Yuan H. Circulating tumor cells as an independent prognostic factor in advanced colorectal cancer: a retrospective study in 121 patients. *Int J Colorectal Dis* 2019; **34**: 589-597 [PMID: 30627849 DOI: 10.1007/s00384-018-03223-9]

88 **Luo H**, Li H, Hu Z, Wu H, Liu C, Li Y, Zhang X, Lin P, Hou Q, Ding G, Wang Y, Li S, Wei D, Qiu F, Li Y, Wu S. Noninvasive diagnosis and monitoring of mutations by deep sequencing of circulating tumor

DNA in esophageal squamous cell carcinoma. *Biochem Biophys Res Commun* 2016; **471**: 596-602 [PMID: 26876573 DOI: 10.1016/j.bbrc.2016.02.011]

89 **Ueda M**, Iguchi T, Masuda T, Nakahara Y, Hirata H, Uchi R, Niida A, Momose K, Sakimura S, Chiba K, Eguchi H, Ito S, Sugimachi K, Yamasaki M, Suzuki Y, Miyano S, Doki Y, Mori M, Mimori K. Somatic mutations in plasma cell-free DNA are diagnostic markers for esophageal squamous cell carcinoma recurrence. *Oncotarget* 2016; **7**: 62280-62291 [PMID: 27556701 DOI: 10.18632/oncotarget.11409]

90 **Komatsu S**, Ichikawa D, Hirajima S, Takeshita H, Shiozaki A, Fujiwara H, Kawaguchi T, Miyamae M, Konishi H, Kubota T, Okamoto K, Yagi N, Otsuji E. Clinical impact of predicting CCND1 amplification using plasma DNA in superficial esophageal squamous cell carcinoma. *Dig Dis Sci* 2014; **59**: 1152-1159 [PMID: 24458211 DOI: 10.1007/s10620-013-3005-2]

91 **Kim ST**, Banks KC, Pectasides E, Kim SY, Kim K, Lanman RB, Talasaz A, An J, Choi MG, Lee JH, Sohn TS, Bae JM, Kim S, Park SH, Park JO, Park YS, Lim HY, Kim NKD, Park W, Lee H, Bass AJ, Kim K, Kang WK, Lee J. Impact of genomic alterations on lapatinib treatment outcome and cell-free genomic landscape during HER2 therapy in HER2+ gastric cancer patients. *Ann Oncol* 2018; **29**: 1037-1048 [PMID: 29409051 DOI: 10.1093/annonc/mdy034]

92 **Kato S**, Okamura R, Baumgartner JM, Patel H, Leichman L, Kelly K, Sicklick JK, Fanta PT, Lippman SM, Kurzrock R. Analysis of Circulating Tumor DNA and Clinical Correlates in Patients with Esophageal, Gastroesophageal Junction, and Gastric Adenocarcinoma. *Clin Cancer Res* 2018; **24**: 6248-6256 [PMID: 30348637 DOI: 10.1158/1078-0432.CCR-18-1128]

93 **Gao J**, Wang H, Zang W, Li B, Rao G, Li L, Yu Y, Li Z, Dong B, Lu Z, Jiang Z, Shen L. Circulating tumor DNA functions as an alternative for tissue to overcome tumor heterogeneity in advanced gastric cancer. *Cancer Sci* 2017; **108**: 1881-1887 [PMID: 28677165 DOI: 10.1111/cas.13314]

94 **Shoda K**, Ichikawa D, Fujita Y, Masuda K, Hiramoto H, Hamada J, Arita T, Konishi H, Komatsu S, Shiozaki A, Kakihara N, Okamoto K, Taniguchi H, Imoto I, Otsuji E. Monitoring the HER2 copy number status in circulating tumor DNA by droplet digital PCR in patients with gastric cancer. *Gastric Cancer* 2017; **20**: 126-135 [PMID: 26874951 DOI: 10.1007/s10120-016-0599-z]

95 **Tie J**, Wang Y, Tomasetti C, Li L, Springer S, Kinde I, Silliman N, Tacey M, Wong HL, Christie M, Kosmider S, Skinner I, Wong R, Steel M, Tran B, Desai J, Jones I, Haydon A, Hayes T, Price TJ, Strausberg RL, Diaz LA, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. *Sci Transl Med* 2016; **8**: 346ra92 [PMID: 27384348 DOI: 10.1126/scitranslmed.aaf6219]

96 **Furuki H**, Yamada T, Takahashi G, Iwai T, Koizumi M, Shinji S, Yokoyama Y, Takeda K, Taniai N, Uchida E. Evaluation of liquid biopsies for detection of emerging mutated genes in metastatic colorectal cancer. *Eur J Surg Oncol* 2018; **44**: 975-982 [PMID: 29452859 DOI: 10.1016/j.ejso.2018.01.224]

97 **Vandeputte C**, Kehagias P, El Housni H, Ameye L, Laes JF, Desmedt C, Sotiriou C, Deleporte A, Puleo F, Geboes K, Delaunoit T, Demolin G, Peeters M, D'Hondt L, Janssens J, Carrasco J, Marechal R, Galdon MG, Heimann P, Paesmans M, Flamen P, Hendlisz A. Circulating tumor DNA in early response assessment and monitoring of advanced colorectal cancer treated with a multi-kinase inhibitor. *Oncotarget* 2018; **9**: 17756-17769 [PMID: 29707145 DOI: 10.18632/oncotarget.24879]

98 **Oh TJ**, Oh HI, Seo YY, Jeong D, Kim C, Kang HW, Han YD, Chung HC, Kim NK, An S. Feasibility of quantifying *SDC2* methylation in stool DNA for early detection of colorectal cancer. *Clin Epigenetics* 2017; **9**: 126 [PMID: 29225717 DOI: 10.1186/s13148-017-0426-3]

99 **Faluyi OO**, Eng L, Qiu X, Che J, Zhang Q, Cheng D, Ying N, Tse A, Kuang Q, Dodbiba L, Renouf DJ, Marsh S, Savas S, Mackay HJ, Knox JJ, Darling GE, Wong RK, Xu W, Azad AK, Liu G. Validation of microRNA pathway polymorphisms in esophageal adenocarcinoma survival. *Cancer Med* 2017; **6**: 361-373 [PMID: 28074552 DOI: 10.1002/cam4.989]

100 **Yao C**, Liu HN, Wu H, Chen YJ, Li Y, Fang Y, Shen XZ, Liu TT. Diagnostic and Prognostic Value of Circulating MicroRNAs for Esophageal Squamous Cell Carcinoma: a Systematic Review and Meta-analysis. *J Cancer* 2018; **9**: 2876-2884 [PMID: 30123356 DOI: 10.7150/jca.25351]

101 **Zhang L**, Dong B, Ren P, Ye H, Shi J, Qin J, Wang K, Wang P, Zhang J. Circulating plasma microRNAs in the detection of esophageal squamous cell carcinoma. *Oncol Lett* 2018; **16**: 3303-3318 [PMID: 30127929 DOI: 10.3892/ol.2018.8995]

102 **Virgilio E**, Giarnieri E, Giovagnoli MR, Montagnini M, Proietti A, D'Urso R, Mercantini P, Balducci G, Cavallini M. Gastric Juice MicroRNAs as Potential Biomarkers for Screening Gastric Cancer: A Systematic Review. *Anticancer Res* 2018; **38**: 613-616 [PMID: 29374683 DOI: 10.21873/anticanres.12265]

103 **Sierzega M**, Kaczor M, Kolodziejczyk P, Kulig J, Sanak M, Richter P. Evaluation of serum microRNA biomarkers for gastric cancer based on blood and tissue pools profiling: the importance of miR-21 and miR-331. *Br J Cancer* 2017; **117**: 266-273 [PMID: 28641313 DOI: 10.1038/bjc.2017.190]

104 **Zhu Y**, Peng Q, Lin Y, Zou L, Shen P, Chen F, Min M, Shen L, Chen J, Shen B. Identification of biomarker microRNAs for predicting the response of colorectal cancer to neoadjuvant chemoradiotherapy based on microRNA regulatory network. *Oncotarget* 2017; **8**: 2233-2248 [PMID: 27903980 DOI: 10.18632/oncotarget.13659]

105 **Carter JV**, Galbraith NJ, Yang D, Burton JF, Walker SP, Galandiuk S. Blood-based microRNAs as biomarkers for the diagnosis of colorectal cancer: a systematic review and meta-analysis. *Br J Cancer* 2017; **116**: 762-774 [PMID: 28152545 DOI: 10.1038/bjc.2017.12]

106 **Ulivi P**, Canale M, Passardi A, Marisi G, Valgiusti M, Frassineti GL, Calistri D, Amadori D, Scarpi E. Circulating Plasma Levels of miR-20b, miR-29b and miR-155 as Predictors of Bevacizumab Efficacy in Patients with Metastatic Colorectal Cancer. *Int J Mol Sci* 2018; 19 [PMID: 29361687 DOI: 10.3390/ijms19010307]

107 **Matsumoto Y**, Kano M, Akutsu Y, Hanari N, Hoshino I, Murakami K, Usui A, Suito H, Takahashi M, Otsuka R, Xin H, Komatsu A, Iida K, Matsubara H. Quantification of plasma exosome is a potential prognostic marker for esophageal squamous cell carcinoma. *Oncol Rep* 2016; **36**: 2535-2543 [PMID: 27599779 DOI: 10.3892/or.2016.5066]

108 **Tokuhisa M**, Ichikawa Y, Kosaka N, Ochiya T, Yashiro M, Hirakawa K, Kosaka T, Makino H, Akiyama H, Kunisaki C, Endo I. Exosomal miRNAs from Peritoneum Lavage Fluid as Potential Prognostic Biomarkers of Peritoneal Metastasis in Gastric Cancer. *PLoS One* 2015; **10**: e0130472 [PMID: 26208314 DOI: 10.1371/journal.pone.0130472]

109 **Kumata Y**, Iinuma H, Suzuki Y, Tsukahara D, Midorikawa H, Igarashi Y, Soeda N, Kiyokawa T, Horikawa M, Fukushima R. Exosomeencapsulated microRNA23b as a minimally invasive liquid biomarker for the prediction of recurrence and prognosis of gastric cancer patients in each tumor stage.

Oncol Rep 2018; **40**: 319-330 [PMID: 29749537 DOI: 10.3892/or.2018.6418]
110 **Matsumura T**, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, Sawada G, Ueda M, Uchi R, Ueo H, Takano Y, Shinden Y, Eguchi H, Yamamoto H, Doki Y, Mori M, Ochiya T, Mimori K. Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. *Br J Cancer* 2015; **113**: 275-281 [PMID: 26057451 DOI: 10.1038/bjc.2015.201]
111 **Peng ZY**, Gu RH, Yan B. Downregulation of exosome-encapsulated miR-548c-5p is associated with poor prognosis in colorectal cancer. *J Cell Biochem* 2018 [PMID: 30171732 DOI: 10.1002/jcb.27291]
112 **Pasternack H**, Fassunke J, Plum PS, Chon SH, Hescheler DA, Gassa A, Merkelbach-Bruse S, Bruns CJ, Perner S, Hallek M, Büttner R, Bollschweiler E, Hölscher AH, Quaas A, Zander T, Weiss J, Alakus H. Somatic alterations in circulating cell-free DNA of oesophageal carcinoma patients during primary staging are indicative for post-surgical tumour recurrence. *Sci Rep* 2018; **8**: 14941 [PMID: 30297788 DOI: 10.1038/s41598-018-33027-4]
113 **Yoshida T**, Yamaguchi T, Maekawa S, Takano S, Kuno T, Tanaka K, Iwamoto F, Tsukui Y, Kobayashi S, Asakawa Y, Shindo H, Fukasawa M, Nakayama Y, Inoue T, Uetake T, Ohtaka M, Sato T, Mochizuki K, Enomoto N. Identification of early genetic changes in well-differentiated intramucosal gastric carcinoma by target deep sequencing. *Gastric Cancer* 2019; **22**: 742-750 [PMID: 30756200 DOI: 10.1007/s10120-019-00926-y]
114 **Capalbo C**, Belardinilli F, Raimondo D, Milanetti E, Malapelle U, Pisapia P, Magri V, Prete A, Pecorari S, Colella M, Coppa A, Bonfiglio C, Nicolussi A, Valentini V, Tessitore A, Cardinali B, Petroni M, Infante P, Santoni M, Filetti M, Colicchia V, Paci P, Mezi S, Longo F, Cortesi E, Marchetti P, Troncone G, Bellavia D, Canettieri G, Giannini G. A Simplified Genomic Profiling Approach Predicts Outcome in Metastatic Colorectal Cancer. *Cancers (Basel)* 2019; 11 [PMID: 30691222 DOI: 10.3390/cancers11020147]
115 **Wang Y**, Liu H, Hou Y, Zhou X, Liang L, Zhang Z, Shi H, Xu S, Hu P, Zheng Z, Liu R, Tang T, Ye F, Liang Z, Bu H. Performance validation of an amplicon-based targeted next-generation sequencing assay and mutation profiling of 648 Chinese colorectal cancer patients. *Virchows Arch* 2018; **472**: 959-968 [PMID: 29705968 DOI: 10.1007/s00428-018-2359-4]
116 **Gao XH**, Yu GY, Hong YG, Lian W, Chouhan H, Xu Y, Liu LJ, Bai CG, Zhang W. Clinical significance of multiple gene detection with a 22-gene panel in formalin-fixed paraffin-embedded specimens of 207 colorectal cancer patients. *Int J Clin Oncol* 2019; **24**: 141-152 [PMID: 30612269 DOI: 10.1007/s10147-018-1377-1]
117 **Seifert BA**, McGlaughon JL, Jackson SA, Ritter DI, Roberts ME, Schmidt RJ, Thompson BA, Jimenez S, Trapp M, Lee K, Plon SE, Offit K, Stadler ZK, Zhang L, Greenblatt MS, Ferber MJ. Determining the clinical validity of hereditary colorectal cancer and polyposis susceptibility genes using the Clinical Genome Resource Clinical Validity Framework. *Genet Med* 2019; **21**: 1507-1516 [PMID: 30523343 DOI: 10.1038/s41436-018-0373-1]
118 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]
119 **Sartore-Bianchi A**, Trusolino L, Martino C, Bencardino K, Lonardi S, Bergamo F, Zagonel V, Leone F, Depetris I, Martinelli E, Troiani T, Ciardiello F, Racca P, Bertotti A, Siravegna G, Torri V, Amatu A, Ghezzi S, Marrapese G, Palmeri L, Valtorta E, Cassingena A, Lauricella C, Vanzulli A, Regge D, Veronese S, Comoglio PM, Bardelli A, Marsoni S, Siena S. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016; **17**: 738-746 [PMID: 27108243 DOI: 10.1016/S1470-2045(16)00150-9]
120 **Wormald S**, Milla L, O'Connor L. Association of candidate single nucleotide polymorphisms with somatic mutation of the epidermal growth factor receptor pathway. *BMC Med Genomics* 2013; **6**: 43 [PMID: 24152305 DOI: 10.1186/1755-8794-6-43]
121 **Febbo PG**, Ladanyi M, Aldape KD, De Marzo AM, Hammond ME, Hayes DF, Iafrate AJ, Kelley RK, Marcucci G, Ogino S, Pao W, Sgroi DC, Birkeland ML. NCCN Task Force report: Evaluating the clinical utility of tumor markers in oncology. *J Natl Compr Canc Netw* 2011; **9** Suppl 5: S1-32; quiz S33 [PMID: 22138009 DOI: 10.6004/jnccn.2011.0137]
122 **Schmoll HJ**, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, Nordlinger B, van de Velde CJ, Balmana J, Regula J, Nagtegaal ID, Beets-Tan RG, Arnold D, Ciardiello F, Hoff P, Kerr D, Köhne CH, Labianca R, Price T, Scheithauer W, Sobrero A, Tabernero J, Aderka D, Barroso S, Bodoky G, Douillard JY, El Ghazaly H, Gallardo J, Garin A, Glynne-Jones R, Jordan K, Meshcheryakov A, Papamichail D, Pfeiffer P, Souglakos I, Turhal S, Cervantes A. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. *Ann Oncol* 2012; **23**: 2479-2516 [PMID: 23012255 DOI: 10.1093/annonc/mds236]
123 **Locker GY**, Hamilton S, Harris J, Jessup JM, Kemeny N, Macdonald JS, Somerfield MR, Hayes DF, Bast RC; ASCO. ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J Clin Oncol* 2006; **24**: 5313-5327 [PMID: 17060676 DOI: 10.1200/JCO.2006.08.2644]
124 **Hu Y**, Tao SY, Deng JM, Hou ZK, Liang JQ, Huang QG, Li LH, Li HB, Chen YM, Yi H, Chen XL, Liu H. Prognostic Value of NRAS Gene for Survival of Colorectal Cancer Patients: A Systematic Review and Meta-Analysis. *Asian Pac J Cancer Prev* 2018; **19**: 3001-3008 [PMID: 30484984 DOI: 10.31557/APJCP.2018.19.11.3001]
125 **Loaiza-Bonilla A**, Jensen CE, Shroff S, Furth E, Bonilla-Reyes PA, Deik AF, Morrissette J. KDR Mutation as a Novel Predictive Biomarker of Exceptional Response to Regorafenib in Metastatic Colorectal Cancer. *Cureus* 2016; **8**: e478 [PMID: 27004155 DOI: 10.7759/cureus.478]
126 **Tabernero J**, Hozak RR, Yoshino T, Cohn AL, Obermannova R, Bodoky G, Garcia-Carbonero R, Ciuleanu TE, Portnoy DC, Prausová J, Muro K, Siegel RW, Konrad RJ, Ouyang H, Melemed SA, Ferry D, Nasroulah F, Van Cutsem E. Analysis of angiogenesis biomarkers for ramucirumab efficacy in patients with metastatic colorectal cancer from RAISE, a global, randomized, double-blind, phase III study. *Ann Oncol* 2018; **29**: 602-609 [PMID: 29228087 DOI: 10.1093/annonc/mdx767]
127 **Ito C**, Nishizuka SS, Ishida K, Uesugi N, Sugai T, Tamura G, Koeda K, Sasaki A. Analysis of PIK3CA mutations and PI3K pathway proteins in advanced gastric cancer. *J Surg Res* 2017; **212**: 195-204 [PMID: 28550907 DOI: 10.1016/j.jss.2017.01.018]

128 **Kim C**, Lee CK, Chon HJ, Kim JH, Park HS, Heo SJ, Kim HJ, Kim TS, Kwon WS, Chung HC, Rha SY. PTEN loss and level of HER2 amplification is associated with trastuzumab resistance and prognosis in HER2-positive gastric cancer. *Oncotarget* 2017; **8**: 113494-113501 [PMID: 29371924 DOI: 10.18632/oncotarget.23054]

129 **Drilon A**, Laetsch TW, Kummar S, DuBois SG, Lassen UN, Demetri GD, Nathenson M, Doebele RC, Farago AF, Pappo AS, Turpin B, Dowlati A, Brose MS, Mascarenhas L, Federman N, Berlin J, El-Deiry WS, Baik C, Deeken J, Boni V, Nagasubramanian R, Taylor M, Rudzinski ER, Meric-Bernstam F, Sohal DPS, Ma PC, Raez LE, Hechtman JF, Benayed R, Ladanyi M, Tuch BB, Ebata K, Cruickshank S, Ku NC, Cox MC, Hawkins DS, Hong DS, Hyman DM. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *N Engl J Med* 2018; **378**: 731-739 [PMID: 29466156 DOI: 10.1056/NEJMoa1714448]

130 **Domingo E**, Freeman-Mills L, Rayner E, Glaire M, Briggs S, Vermeulen L, Fessler E, Medema JP, Boot A, Morreau H, van Wezel T, Liefers GJ, Lothe RA, Danielsen SA, Sveen A, Nesbakken A, Zlobec I, Lugli A, Koelzer VH, Berger MD, Castellví-Bel S, Muñoz J; Epicolon consortium, de Bruyn M, Nijman HW, Novelli M, Lawson K, Oukrif D, Frangou E, Dutton P, Tejpar S, Delorenzi M, Kerr R, Kerr D, Tomlinson I, Church DN. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. *Lancet Gastroenterol Hepatol* 2016; **1**: 207-216 [PMID: 28404093 DOI: 10.1016/S2468-1253(16)30014-0]

131 **Llosa NJ**, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan H, Wang H, Luber BS, Zhang M, Papadopoulos N, Kinzler KW, Vogelstein B, Sears CL, Anders RA, Pardoll DM, Housseau F. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 2015; **5**: 43-51 [PMID: 25358689 DOI: 10.1158/2159-8290.CD-14-0863]

132 **Eriksen AC**, Sørensen FB, Lindebjerg J, Hager H, dePont Christensen R, Kjær-Frifeldt S, Hansen TF. Programmed Death Ligand-1 expression in stage II colon cancer - experiences from a nationwide populationbased cohort. *BMC Cancer* 2019; **19**: 142 [PMID: 30755167 DOI: 10.1186/s12885-019-5345-6]

133 **Iseki Y**, Shibutani M, Maeda K, Nagahara H, Fukuoka T, Matsutani S, Kashiwagi S, Tanaka H, Hirakawa K, Ohira M. A new method for evaluating tumor-infiltrating lymphocytes (TILs) in colorectal cancer using hematoxylin and eosin (H-E)-stained tumor sections. *PLoS One* 2018; **13**: e0192744 [PMID: 29698402 DOI: 10.1371/journal.pone.0192744]

134 **Saju P**, Murata-Kamiya N, Hayashi T, Senda Y, Nagase L, Noda S, Matsusaka K, Funata S, Kunita A, Urabe M, Seto Y, Fukayama M, Kaneda A, Hatakeyama M. Host SHP1 phosphatase antagonizes Helicobacter pylori CagA and can be downregulated by Epstein-Barr virus. *Nat Microbiol* 2016; **1**: 16026 [PMID: 27572445 DOI: 10.1038/nmicrobiol.2016.26]

135 **Altieri F**, Di Stadio CS, Federico A, Miselli G, De Palma M, Rippa E, Arcari P. Epigenetic alterations of gastrokine 1 gene expression in gastric cancer. *Oncotarget* 2017; **8**: 16899-16911 [PMID: 28129645 DOI: 10.18632/oncotarget.14817]

136 **Martinelli E**, Morgillo F, Troiani T, Ciardiello F. Cancer resistance to therapies against the EGFR-RAS-RAF pathway: The role of MEK. *Cancer Treat Rev* 2017; **53**: 61-69 [PMID: 28073102 DOI: 10.1016/j.ctrv.2016.12.001]

137 **Jehan Z**, Bavi P, Sultana M, Abubaker J, Bu R, Hussain A, Alsbeih G, Al-Sanea N, Abduljabbar A, Ashari LH, Alhomoud S, Al-Dayel F, Uddin S, Al-Kuraya KS. Frequent PIK3CA gene amplification and its clinical significance in colorectal cancer. *J Pathol* 2009; **219**: 337-346 [PMID: 19697359 DOI: 10.1002/path.2601]

138 **Guo J**, Yu W, Su H, Pang X. Genomic landscape of gastric cancer: molecular classification and potential targets. *Sci China Life Sci* 2017; **60**: 126-137 [PMID: 27460193 DOI: 10.1007/s11427-016-0034-1]

139 **Szász AM**, Lánczky A, Nagy Á, Förster S, Hark K, Green JE, Boussioutas A, Busuttil R, Szabó A, Győrffy B. Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. *Oncotarget* 2016; **7**: 49322-49333 [PMID: 27384994 DOI: 10.18632/oncotarget.10337]

140 **Ishiguro H**, Wakasugi T, Terashita Y, Sakamoto N, Tanaka T, Mizoguchi K, Sagawa H, Okubo T, Takeyama H. Decreased expression of CDH1 or CTNNB1 affects poor prognosis of patients with esophageal cancer. *World J Surg Oncol* 2016; **14**: 240 [PMID: 27600761 DOI: 10.1186/s12957-016-0956-8]

141 **Liang TJ**, Wang HX, Zheng YY, Cao YQ, Wu X, Zhou X, Dong SX. APC hypermethylation for early diagnosis of colorectal cancer: a meta-analysis and literature review. *Oncotarget* 2017; **8**: 46468-46479 [PMID: 28515349 DOI: 10.18632/oncotarget.17576]

142 **Chen TH**, Chang SW, Huang CC, Wang KL, Yeh KT, Liu CN, Lee H, Lin CC, Cheng YW. The prognostic significance of APC gene mutation and miR-21 expression in advanced-stage colorectal cancer. *Colorectal Dis* 2013; **15**: 1367-1374 [PMID: 23773491 DOI: 10.1111/codi.12318]

143 **Codony-Servat J**, Cuatrecasas M, Asensio E, Montironi C, Martínez-Cardús A, Marín-Aguilera M, Horndler C, Martínez-Balibrea E, Rubini M, Jares P, Reig O, Victoria I, Gaba L, Martín-Richard M, Alonso V, Escudero P, Fernández-Martos C, Feliu J, Méndez JC, Méndez M, Gallego J, Salud A, Rojo F, Castells A, Prat A, Rosell R, García-Albéniz X, Camps J, Maurel J. Nuclear IGF-1R predicts chemotherapy and targeted therapy resistance in metastatic colorectal cancer. *Br J Cancer* 2017; **117**: 1777-1786 [PMID: 29123263 DOI: 10.1038/bjc.2017.279]

144 **Tang D**, Liu J, Wang DR, Yu HF, Li YK, Zhang JQ. Diagnostic and prognostic value of the methylation status of secreted frizzled-related protein 2 in colorectal cancer. *Clin Invest Med* 2011; **34**: E88-E95 [PMID: 21463549 DOI: 10.25011/cim.v34i1.15105]

145 **Crea F**, Nobili S, Paolicchi E, Perrone G, Napoli C, Landini I, Danesi R, Mini E. Epigenetics and chemoresistance in colorectal cancer: an opportunity for treatment tailoring and novel therapeutic strategies. *Drug Resist Updat* 2011; **14**: 280-296 [PMID: 21955833 DOI: 10.1016/j.drup.2011.08.001]

146 **Salem ME**, Puccini A, Xiu J, Raghavan D, Lenz HJ, Korn WM, Shields AF, Philip PA, Marshall JL, Goldberg RM. Comparative Molecular Analyses of Esophageal Squamous Cell Carcinoma, Esophageal Adenocarcinoma, and Gastric Adenocarcinoma. *Oncologist* 2018; **23**: 1319-1327 [PMID: 29866946 DOI: 10.1634/theoncologist.2018-0143]

147 **Wasserman I**, Lee LH, Ogino S, Marco MR, Wu C, Chen X, Datta J, Sadot E, Szeglin B, Guillem JG, Paty PB, Weiser MR, Nash GM, Saltz L, Barlas A, Manova-Todorova K, Uppada SPB, Elghouayel AE, Ntiamoah P, Glickman JN, Hamada T, Kosumi K, Inamura K, Chan AT, Nishihara R, Cercek A, Ganesh K, Kemeny NE, Dhawan P, Yaeger R, Sawyers CL, Garcia-Aguilar J, Giannakis M, Shia J, Smith JJ. SMAD4 Loss in Colorectal Cancer Patients Correlates with Recurrence, Loss of Immune Infiltrate, and Chemoresistance. *Clin Cancer Res* 2019; **25**: 1948-1956 [PMID: 30587545 DOI:

10.1158/1078-0432.CCR-18-1726]
148 **Zhou C**, Li J, Li Q. CDKN2A methylation in esophageal cancer: a meta-analysis. *Oncotarget* 2017; **8**: 50071-50083 [PMID: 28637022 DOI: 10.18632/oncotarget.18412]
149 **Randon G**, Fucà G, Rossini D, Raimondi A, Pagani F, Perrone F, Tamborini E, Busico A, Peverelli G, Morano F, Niger M, Antista M, Corallo S, Saggio S, Borelli B, Zucchelli G, Milione M, Pruneri G, Di Bartolomeo M, Falcone A, de Braud F, Cremolini C, Pietrantonio F. Prognostic impact of ATM mutations in patients with metastatic colorectal cancer. *Sci Rep* 2019; **9**: 2858 [PMID: 30814645 DOI: 10.1038/s41598-019-39525-3]
150 **de Voer RM**, Hahn MM, Mensenkamp AR, Hoischen A, Gilissen C, Henkes A, Spruijt L, van Zelst-Stams WA, Kets CM, Verwiel ET, Nagtegaal ID, Schackert HK, van Kessel AG, Hoogerbrugge N, Ligtenberg MJ, Kuiper RP. Deleterious Germline BLM Mutations and the Risk for Early-onset Colorectal Cancer. *Sci Rep* 2015; **5**: 14060 [PMID: 26358404 DOI: 10.1038/srep14060]
151 **Frank B**, Hoffmeister M, Klopp N, Illig T, Chang-Claude J, Brenner H. Colorectal cancer and polymorphisms in DNA repair genes WRN, RMI1 and BLM. *Carcinogenesis* 2010; **31**: 442-445 [PMID: 19945966 DOI: 10.1093/carcin/bgp293]
152 **Oh M**, McBride A, Yun S, Bhattacharjee S, Slack M, Martin JR, Jeter J, Abraham I. BRCA1 and BRCA2 Gene Mutations and Colorectal Cancer Risk: Systematic Review and Meta-analysis. *J Natl Cancer Inst* 2018; **110**: 1178-1189 [PMID: 30380096 DOI: 10.1093/jnci/djy148]
153 **Wei XL**, Wang DS, Xi SY, Wu WJ, Chen DL, Zeng ZL, Wang RY, Huang YX, Jin Y, Wang F, Qiu MZ, Luo HY, Zhang DS, Xu RH. Clinicopathologic and prognostic relevance of ARID1A protein loss in colorectal cancer. *World J Gastroenterol* 2014; **20**: 18404-18412 [PMID: 25561809 DOI: 10.3748/wjg.v20.i48.18404]
154 **Ronchetti L**, Melucci E, De Nicola F, Goeman F, Casini B, Sperati F, Pallocca M, Terrenato I, Pizzuti L, Vici P, Sergi D, Di Lauro L, Amoreo CA, Gallo E, Diodoro MG, Pescarmona E, Vitale I, Barba M, Buglioni S, Mottolese M, Fanciulli M, De Maria R, Maugeri-Saccà M. DNA damage repair and survival outcomes in advanced gastric cancer patients treated with first-line chemotherapy. *Int J Cancer* 2017; **140**: 2587-2595 [PMID: 28233295 DOI: 10.1002/ijc.30668]
155 **Hansford S**, Kaurah P, Li-Chang H, Woo M, Senz J, Pinheiro H, Schrader KA, Schaeffer DF, Shumansky K, Zogopoulos G, Santos TA, Claro I, Carvalho J, Nielsen C, Padilla S, Lum A, Talhouk A, Baker-Lange K, Richardson S, Lewis I, Lindor NM, Pennell E, MacMillan A, Fernandez B, Keller G, Lynch H, Shah SP, Guilford P, Gallinger S, Corso G, Roviello F, Caldas C, Oliveira C, Pharoah PD, Huntsman DG. Hereditary Diffuse Gastric Cancer Syndrome: CDH1 Mutations and Beyond. *JAMA Oncol* 2015; **1**: 23-32 [PMID: 26182300 DOI: 10.1001/jamaoncol.2014.168]
156 **Grünhage F**, Jungck M, Lamberti C, Berg C, Becker U, Schulte-Witte H, Plassmann D, Rahner N, Aretz S, Friedrichs N, Buettner R, Sauerbruch T, Lammert F. Association of familial colorectal cancer with variants in the E-cadherin (CDH1) and cyclin D1 (CCND1) genes. *Int J Colorectal Dis* 2008; **23**: 147-154 [PMID: 17960397 DOI: 10.1007/s00384-007-0388-6]
157 **Ooi A**, Oyama T, Nakamura R, Tajiri R, Ikeda H, Fushida S, Dobashi Y. Gene amplification of CCNE1, CCND1, and CDK6 in gastric cancers detected by multiplex ligation-dependent probe amplification and fluorescence in situ hybridization. *Hum Pathol* 2017; **61**: 58-67 [PMID: 27864121 DOI: 10.1016/j.humpath.2016.10.025]
158 **Chang HR**, Nam S, Lee J, Kim JH, Jung HR, Park HS, Park S, Ahn YZ, Huh I, Balch C, Ku JL, Powis G, Park T, Jeong JH, Kim YH. Systematic approach identifies RHOA as a potential biomarker therapeutic target for Asian gastric cancer. *Oncotarget* 2016; **7**: 81435-81451 [PMID: 27806312 DOI: 10.18632/oncotarget.12963]
159 **Hu X**, Moon JW, Li S, Xu W, Wang X, Liu Y, Lee JY. Amplification and overexpression of *CTTN* and *CCND1* at chromosome 11q13 in Esophagus squamous cell carcinoma (ESCC) of North Eastern Chinese Population. *Int J Med Sci* 2016; **13**: 868-874 [PMID: 27877079 DOI: 10.7150/ijms.16845]
160 **Korphaisarn K**, Morris VK, Overman MJ, Fogelman DR, Kee BK, Raghav KPS, Manuel S, Shureiqi I, Wolff RA, Eng C, Menter D, Hamilton SR, Kopetz S, Dasari A. FBXW7 missense mutation: a novel negative prognostic factor in metastatic colorectal adenocarcinoma. *Oncotarget* 2017; **8**: 39268-39279 [PMID: 28424412 DOI: 10.18632/oncotarget.16848]
161 **Song B**, Cui H, Li Y, Cheng C, Yang B, Wang F, Kong P, Li H, Zhang L, Jia Z, Bi Y, Wang J, Zhou Y, Liu J, Wang J, Zhao Z, Zhang Y, Hu X, Shi R, Yang J, Liu H, Yan T, Li Y, Xu E, Qian Y, Xi Y, Guo S, Chen Y, Wang J, Li G, Liang J, Jia J, Chen X, Guo J, Wang T, Zhang Y, Li Q, Wang C, Cheng X, Zhan Q, Cui Y. Mutually exclusive mutations in NOTCH1 and PIK3CA associated with clinical prognosis and chemotherapy responses of esophageal squamous cell carcinoma in China. *Oncotarget* 2016; **7**: 3599-3613 [PMID: 26528858 DOI: 10.18632/oncotarget.6120]
162 **Arcaroli JJ**, Tai WM, McWilliams R, Bagby S, Blatchford PJ, Varella-Garcia M, Purkey A, Quackenbush KS, Song EK, Pitts TM, Gao D, Lieu C, McManus M, Tan AC, Zheng X, Zhang Q, Ozeck M, Olson P, Jiang ZQ, Kopetz S, Jimeno A, Keysar S, Eckhardt G, Messersmith WA. A NOTCH1 gene copy number gain is a prognostic indicator of worse survival and a predictive biomarker to a Notch1 targeting antibody in colorectal cancer. *Int J Cancer* 2016; **138**: 195-205 [PMID: 26152787 DOI: 10.1002/ijc.29676]
163 **Ozawa T**, Kazama S, Akiyoshi T, Murono K, Yoneyama S, Tanaka T, Tanaka J, Kiyomatsu T, Kawai K, Nozawa H, Kanazawa T, Yamaguchi H, Ishihara S, Sunami E, Kitayama J, Morikawa T, Fukayama M, Watanabe T. Nuclear Notch3 expression is associated with tumor recurrence in patients with stage II and III colorectal cancer. *Ann Surg Oncol* 2014; **21**: 2650-2658 [PMID: 24728738 DOI: 10.1245/s10434-014-3659-9]
164 **Zhang L**, Song X, Li X, Wu C, Jiang J. Yes-Associated Protein 1 as a Novel Prognostic Biomarker for Gastrointestinal Cancer: A Meta-Analysis. *Biomed Res Int* 2018; **2018**: 4039173 [PMID: 30539010 DOI: 10.1155/2018/4039173]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 21-36

DOI: 10.4251/wjgo.v12.i1.21

ISSN 1948-5204 (online)

REVIEW

Digestive tract reconstruction options after laparoscopic gastrectomy for gastric cancer

Jian Shen, Xiang Ma, Jing Yang, Jian-Ping Zhang

ORCID number: Jian Shen (0000-0002-3160-5315); Xiang Ma (0000-0001-7246-3360); Jing Yang (0000-0002-3125-8691); Jian-Ping Zhang (0000-0002-1929-792X).

Author contributions: Shen J contributed to the study design, literature search, data analysis, and the writing of the manuscript; Ma X contributed to the literature search and data analysis of the study; Yang J contributed to the literature search and sketch drawing; Zhang JP participated in the study design, supervised the study, and wrote the manuscript. All authors read and approved the final manuscript.

Conflict-of-interest statement: The authors declare they have no competing financial interests.

Manuscript source: Invited manuscript

Received: March 19, 2019
Peer-review started: March 19, 2019

Jian Shen, Xiang Ma, Jian-Ping Zhang, Department of General Surgery, The Second Affiliated Hospital of Nanjing Medical University, Nanjing 210011, Jiangsu Province, China

Jing Yang, Cardiovascular Center, The Second Affiliated Hospital of Nanjing Medical University, Nanjing 210011, Jiangsu Province, China

Corresponding author: Jian-Ping Zhang, MD, PhD, Professor, Department of General Surgery, The Second Affiliated Hospital of Nanjing Medical University, No. 121, Jiangjiayuan Road, Nanjing 210011, Jiangsu Province, China. drzhangjp@njmu.edu.cn

Abstract

In addition to the popularity of laparoscopic gastrectomy (LG), many reconstructive procedures after LG have been reported. Surgical resection and lymphatic dissection determine long-term survival; however, the election of a reconstruction procedure determines the postoperative quality of life for patients with gastric cancer (GC). Presently, no consensus exists regarding the optimal reconstructive procedure. In this review, the current state of digestive tract reconstruction after LG is reviewed. According to the determining influence of the tumor site on the procedures of surgical resection and reconstruction, we divide these reconstruction procedures into three categories consistent with the resection procedures. We focus on the technical tips of every reconstruction procedure and examine the surgical outcomes (length of surgery and blood loss) and postoperative complications (anastomotic leakage and stricture) to facilitate gastrointestinal surgeons to understand the merits and demerits of every reconstruction procedure.

Key words: Digestive tract reconstruction; Laparoscopic gastrectomy; Gastric cancer; Quality of life

Core tip: This article systematically reviews almost all the reconstruction methods currently used and divides them into three categories according to the method of resection (laparoscopic distal gastrectomy, laparoscopic total gastrectomy, and laparoscopic proximal gastrectomy). This review clearly demonstrates the key steps, merits, and demerits of every reconstruction method *via* drawing schematics based on the authors' personal experience.

First decision: July 31, 2019
Revised: October 9, 2019
Accepted: November 4, 2019
Article in press: November 4, 2019
Published online: January 15, 2020

P-Reviewer: Akbulut S, Lazăr DC, Trkulja V
S-Editor: Dou Y
L-Editor: Wang TQ
E-Editor: Liu MY

Citation: Shen J, Ma X, Yang J, Zhang JP. Digestive tract reconstruction options after laparoscopic gastrectomy for gastric cancer. *World J Gastrointest Oncol* 2020; 12(1): 21-36
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/21.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.21

INTRODUCTION

Gastric cancer (GC) remains a disease with high incidence and mortality worldwide[1,2]. GC patients demonstrate reliable survival results due to the implementation of D2 lymphadenectomy, which has become the cornerstone of GC treatment in the past decades[3-5]. Kitano *et al*[6] first reported a case of laparoscopic-assisted distal gastrectomy in 1994. GC surgery has gradually changed from open to laparoscopic-assisted and ultimately to total laparoscopic during the past 20 years. Presently, the main indication for laparoscopic gastrectomy (LG) is early GC because recent studies have shown that the oncologic outcomes of LG were comparable to those of open surgery[7-9]. Three multicenter trials, the JLSSG 0901[10], CLASS-01[11], and KLASS-02[12,13] trials, are current large-scale randomized controlled trials (RCTs) to obtain evidence-based oncological outcomes of LG for advanced GC (AGC)[14]. The results of these RCTs were expected to establish concrete evidence of the widely carried out LG in the treatment of AGC. Various new laparoscopic lymph node dissection procedures were reported and have been shown to achieve pathologically reliable lymphadenectomy during this development process. These technical summaries based on the surgeon's clinical experience made lymph node dissection standardized with reliable quality[15-17].

In addition to the improved survival, quality of life (QoL) attracted more attention, and total laparoscopic surgery has gained widespread global popularity owing to its well-known benefits, such as reduced surgical trauma, decreased pain, low rates of morbidities, and a shorter length of hospital stay[14,18]. Digestive tract reconstruction is a key technique in laparoscopic surgery. However, no definitive consensus is currently available regarding how to choose among the various methods. This review focuses on describing technical tips and discussing the merits and demerits of commonly used laparoscopic reconstruction procedures at present.

LITERATURE SEARCH

To eliminate the influence of the learning curve on complications, a literature search was performed using the terms "laparoscopic gastrectomy", "digestive tract reconstruction", and "gastric cancer" along with their synonyms or abbreviations after 2015. Studies on different reconstructive procedures including less than 10 patients were excluded. The length of surgery, intraoperative blood loss, anastomotic leakage and stricture, esophagitis reflux, and gastric stasis were examined. Data extraction was confirmed manually.

DIGESTIVE TRACT RECONSTRUCTION AFTER LAPAROSCOPIC DISTAL GASTRECTOMY (LDG)

Billroth-I (B-I), Billroth-II (B-II), Roux-en-Y, and uncut Roux-en-Y reconstruction are the most popular methods of reconstruction after LDG for GC.

B-I reconstruction

B-I reconstruction, one of the most popular procedures of reconstruction after distal gastrectomy, is associated with the physiological anatomy and involves only one anastomosis site without stump nor input loop. It is often performed using an extracorporeal procedure with a mini-laparotomy scar in laparoscopy-assisted distal gastrectomy or an intracorporeal procedure in total LDG. The delta-shaped anastomosis (DA) is the most common intracorporeal B-I anastomosis for LDG that was first reported by Kanaya *et al*[19] in 2002. DA is completed by side-to-side gastroduodenostomy with laparoscopic linear staplers (LS) intracorporeally (Figure 1A). This procedure is becoming widely used due to its simplicity and safety[20], and it can be performed safely even by an inexperienced surgeon[21]. Several studies have demonstrated that DA resulted in less blood loss and faster recovery than B-I,

especially in obese patients. However, no difference was found in the surgical outcomes (operative time, number of harvested lymph nodes, and proximal margin) and postoperative complications (anastomotic leakage, stricture, hemorrhage, and wound complications)[22-24] (Table 1). However, some researchers have indicated that DA may affect the blood supply during cutting and result in an increased risk of anastomosis-related complications[25]. Another limitation of DA is that it is difficult to locate the tumor to obtain a pathologically safe margin; sometimes the tumor location requires being marked preoperatively or intraoperatively[20], a step that is likely the main shortcoming and limitation of the operation. Additionally, the cost of DA procedure is higher as it requires more endoscopic liner stapler cartridges[26].

To improve the disadvantages mentioned above, a modified reconstructive procedure using an overlap method for B-I is developed. In general, the anastomosis is performed by overlapping the remnant stomach and duodenal stump *via* LS[27]. Watanabe *et al*[28] believed that this method is safer and easier because the posterior wall of the remnant stomach and anterior wall of the duodenum are anastomosed, and it is not necessary to create a space around the posterior wall of the duodenum. Accordingly, this procedure reduces the possibility of damage to the surrounding structures and duodenum when compared with the formation of anastomosis on the posterior wall in DA[28]. High technical requirements, sufficiently long duodenal stump dissociation, high anastomotic tension, bile reflux, and gastric stump cancer surgery are the inherent shortcomings of B-I reconstruction, and surgeons should consider these issues when choosing this procedure.

Due to a combination of increased screening and improved diagnostic techniques, the diagnostic rate of early GC has increased in recent years. As a result of the satisfactory survival outcomes achieved in the treatment of early GC, surgeons pay more attention to the postoperative QoL[29,30]. Pylorus-preserving gastrectomy (PPG) is a function-preserving surgery for the treatment of patients with cT1N0 middle third GC, aiming to decrease the complication rate and improve the postoperative QoL. The infrapyloric artery and antral cuff (2 cm length) were preserved, D2 lymph node dissection was performed, and the gastrogastrostomy was similar to B-I anastomosis with LS[31-33].

It was reported that PPG has the benefits in postoperative nutrition and can reduce the incidence of bile reflux, dumping syndrome, and cholelithiasis meanwhile[34]. However, some surgeons worry that the intracorporeal reconstruction may lead to micro-dissemination of free cancer cells left over in the remnant gastric lumen[35].

B-II reconstruction

B-II reconstruction is another frequently used method in total LDG. During the procedure, an LS is used to anastomose the greater curvature side of the remnant gastric stomach with the jejunum approximately 10-15 cm from the Treitz ligament.

The main advantages of this method are that the tension of the anastomotic stoma is small, there is no need to dissociate much duodenum, and there is no specific requirement for the location of the tumors. Therefore, B-II is usually used in cases in which B-I is inappropriate. Nevertheless, this method is limited because of a higher risk of complications such as reflux gastritis[36,37]. Considering these reasons, B-II with Braun anastomosis (side-to-side jejunojejunostomy away from the gastrojejunal anastomosis) was applied in total LDG (Figure 1B). Additionally, it can reduce the aï¬erent loop syndrome compared with B-II without Braun anastomosis[38]. Some researchers have revealed that B-II Braun anastomosis cannot reduce the high incidence of bile reflux[39], and Park *et al*[40] reported a high incidence of bile reflux in B-II Braun anastomosis patients (~43.3%). Therefore, some researchers have proposed that Roux-en-Y or uncut Roux-en-Y reconstruction may be an alternative to B-II reconstruction.

Roux-en-Y and uncut Roux-en-Y reconstructions

Roux-en-Y reconstruction (Figure 1C) is a very common procedure in the West, has gained popularity in Asia, and is often preferred if the remnant stomach is relatively small or the tumor is near the pylorus[41]. Previous studies have reported that Roux-en-Y reconstruction can reduce the incidence of food residues, esophagitis, gastritis, and bile reflux in follow-up endoscopic findings than the B-I and B-II groups[39,42,43] (Table 1). However, Roux-en-Y reconstruction in total LDG for GC is a more complicated procedure than B-I or B-II because it involves two anastomoses. Therefore, the operation time and anastomosis time were significantly longer for RY than for B-I[44], and multiple anastomotic lines could increase the probability of anastomotic leakage. Additionally, Roux-en-Y reconstruction has a specific problem named Roux stasis syndrome, which is characterized by vomiting, swelling, nausea, and postprandial pain. Its incidence rate is approximately 10%-30%[45,46]. To solve this problem, uncut Roux-en-Y reconstruction was first carried out in 1988 by Van

Table 1 Summary of reconstruction procedures after laparoscopic distal gastrectomy

Ref.	Publication year	Reconstruction procedure	*n*	Length of surgery, min (mean ± SD or range)	Blood loss, mL (mean ± SD or range)	Anastomotic leakage (*n*)	Anastomotic stricture (*n*)	Stasis (*n*)
Fukunaga *et al*[51]	2018	B-I (augmented rectangle technique)	160	227 ± 75	47.3 ± 50	0	0	0
Lin *et al*[52]	2016	LTDG BI	158	154.4 ± 30.1	51.1 ± 30.9	5	0	NA
		LADG BI	484	155.6 ± 46.2	61.6 ± 78.3	1	0	NA
Jeong *et al*[20]	2015	Intracorporeal B-I	42	116 ± 23	105 ± 69	0	NA	1
		Extracorporeal B-I	179	142 ± 19	50 ± 39	2	NA	5
Jian-Cheng *et al*[53]	2015	DA	24	175.3 ± 64.7	50.8 ± 25.3	NA	NA	NA
Lee *et al*[24]	2015	DA	138	220.4 ± 70.5	99.8 ± 99.0	2	2	NA
		B-I	100	220.5 ± 64.7	133.3 ± 152.1	0	4	NA
Jang *et al*[27]	2015	Overlap	42	228.3 ± 42.5	NA	0	0	5
Watanabe *et al*[28]	2019	B-I	247	203 (107–418)	10 (0–380)	4	0	3
		R-Y	286	257 (134–495)	27.5 (1–915)	5	3	11
Toyomasu *et al*[54]	2018	B-I	123	191.2 ± 51.6	58.2 ± 45.3	1	0	0
		R-Y	24	244.5 ± 40.2	84.8 ± 60.9	0	0	2
Okuno *et al*[55]	2018	R-Y	159	320 ± 65	61 ± 109	4	1	NA
		B-I (β)	78	250 ± 61	70 ± 100	3	3	NA
Kim *et al*[43]	2015	B-I	165	173.4 ± 44.7	92.1 ± 92.1	3	4	NA
		B-II	371	198.7 ± 48.5	172.2 ± 130.8	2	2	NA
		R-Y	161	185.7 ± 55.5	87.1 ± 65.9	1	3	NA
Kim *et al*[56]	2017	B-II LADG	60	205.0 ± 22.4	117.2 ± 81.6	NA	NA	NA
		B-II LTDG	60	197.3 ± 40.1	100.5 ± 36.8	NA	NA	NA
Cui *et al*[57]	2017	R-Y	30	157.3 ± 33.9	89.2 ± 85.5	1	NA	NA
		B-II + Braun	26	134.6 ± 28.8	96.0 ± 89.8	0	NA	NA
In Choi *et al*[58]	2016	B-II + Braun	26	198.1 ± 33.0	161.7±146.6	NA	1	NA
		R-Y	40	242.3±58.1	245.0±207.0	NA	1	NA
Du *et al*[59]	2019	R-Y	24	203.6±26.2	168.3±83.1	0	0	2
Seo *et al*[60]	2018	Uncut R-Y	30	170.0±26.0	122.8±109.0	0	0	4
Ma *et al*[61]	2017	Uncut R-Y	51	170 (135-210)	60 (30-110)	0	0	0
Zang *et al*[62]	2018	Uncut R-Y (ERAS)	20	217.9 ± 52.5	166.1 ± 12.5	NA	0	0
		Uncut R-Y (control)	22	225.4 ± 61.7	150.9 ± 31.7	NA	0	0
Park *et al*[63]	2018	Uncut R-Y	230	185.0 [150.0; 230.0]	100.0 [50.0; 150.0]	NA	6	2
		R-Y	46	200.0 [180.0; 230.0]	100.0 [50.0; 100.0]	NA	0	3
Yang *et al*[50]	2017	Uncut Roux-en-Y	79	154.8 ± 17.8	74.1 ± 26.7	NA	NA	NA
		B-II	79	145.5 ± 15.1	74.0 ± 36.6	NA	NA	NA

NA: Not available; R-Y: Rox-en-Y reconstruction; Uncut R-Y: Uncut-Rox-en-Y reconstruction; DA: Delta-shaped anastomosis.

Stiegmann *et al*[47]. Uncut Roux-en-Y is a simple modification of the B-II with the Braun anastomosis method, in which the jejunogastric pathway is occluded with a nonbladed LS (Figure 1D). It is believed that the mechanism of uncut Roux-en-Y reconstruction can reduce Roux stagnation syndrome by preserving intestinal integrity to facilitate the conduction of myenteric impulse[48]. Park *et al*[40] reported that there was no difference in the incidence of gastritis and bile reflux between the uncut RY and RY group, while the uncut RY group significantly improved stasis compared with the RY group (5.8% *vs* 35.3%). Accordingly, uncut RY reconstruction could

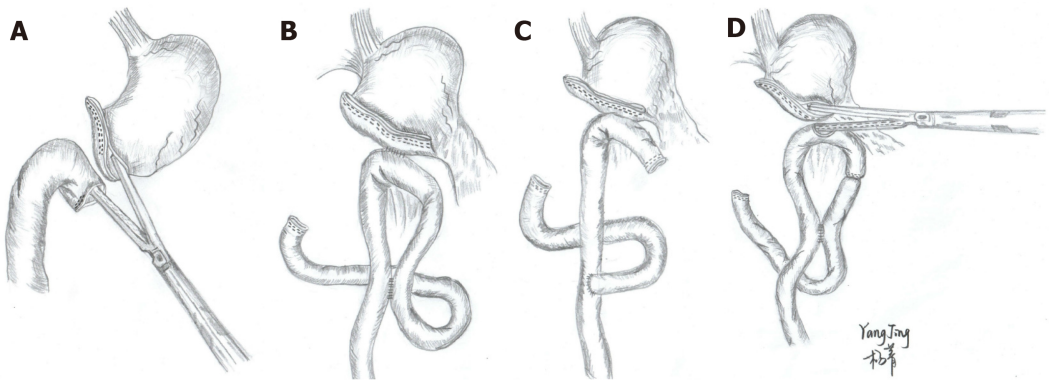

Figure 1 Schematic pictures of digestive tract reconstruction after laparoscopic distal gastrectomy. A: Billroth-I reconstruction; B: Billroth-II reconstruction with Braun anastomosis; C: Roux-en-Y reconstruction; D: Uncut Roux-en-Y reconstruction.

technically overcome the gastric stasis, which is a major drawback of RY reconstruction. However, some studies have reported that the recanalization of the uncut stapled line was relatively high, with a rate of 2.9%-13%[49,50]. Future large-scale prospective randomized clinical trials are needed to evaluate the advantages or disadvantages of uncut RY reconstruction.

DIGESTIVE TRACT RECONSTRUCTION AFTER LAPAROSCOPIC PROXIMAL GASTRECTOMY (LPG)

Proximal gastrectomy (PG) was mainly performed in patients with early GC in the upper-third of the stomach to preserve the physiological function of the remnant stomach[64,65]. Many reconstructive procedures have been reported, including esophagogastrostomy (EGS), jejunal interposition (JI)[66], jejunal pouch interposition (JPI)[67], and double tract reconstruction (DTR)[68,69]. The clinical applications of laparoscopic JI and JPI have not been popularized due to the complexity of the operations, and this review mainly focuses on the methods of EGS and DTR.

EGS is the most popular and a classical reconstruction method because it includes only one anastomosis site and is widely used worldwide. The EGS technique is similar to the esophagojejunostomy (EJS) mentioned above. It was widely recognized that the EGS procedure often leads to severe reflux esophagitis due to resection of the cardiac sphincter and some surgeons choose to perform total gastrectomy (TG)[70,71]. However, patients with early-stage GC usually have good survival outcomes and require higher QoL. Accordingly, there were some improved methods of EGS, such as gastric tube reconstruction after PG. This procedure showed advantages in the operating time and blood loss and could lead to a similar prognosis in patients compared with TG–Roux-en-Y reconstruction. More importantly, preservation of the duodenal passage could contribute to better iron uptake and may ameliorate body weight loss and nutritional status postoperatively[72]. Yamashita *et al*[73] developed a new method of side overlap with fundoplication (SOFY) for EGS that could be easily performed laparoscopically. Reflux esophagitis was rarely observed in the SOFY group (1/14) but was common in the non-SOFY group (5/16). Anastomotic stenosis was also more frequent in the non-SOFY group. One shortcoming was that the number of clinical cases using this method was too small, and a larger sample with higher levels of evidence is needed in the future to observe actual effects[74]. Double-flap (DF), also named Kamikawa's method, is another novel technical development. Briefly, a DF window with a dimension of 2.5 cm × 3.0-3.5 cm (width × height) is created at the anterior wall of gastric remnant. Next, the posterior wall of the esophagus and superior opening of the mucosa on the gastric remnant are manually anastomosed laparoscopically. The anastomotic site is finally covered by the flaps to create the anastomotic valve[74]. Obviously, DF can significantly reduce the symptoms of esophageal reflux. However, a longer operative time is needed and the anastomotic stricture rate remained in a high range from 4.7% to 17.5% in different centers. Otherwise, DF requires complicated intracorporeal suturing and leads to a longer learning curve[75-77]. Additionally, gastric retention was also common in EGS due to vagotomy, and the simple EGS was gradually replaced by other reconstruction procedures.

DTR

DTR is thought to be the best reconstruction procedure with respect to postoperative reflux esophagitis and is commonly used presently. Technically, a conventional Roux-en-Y EJS similar to TG is performed first. A side-to-side gastrojejunostomy is subsequently performed 10-15 cm below the EJS by LS (Figure 2). Reflux esophagitis can be theoretically reduced due to the interposition of the 10- to 15-cm jejunum between the remnant stomach and esophagus[58]. As reported by Aburatani *et al*[78], the DTR group (10.5%) had a lower incidence of reflux symptoms in the first year postoperatively than the EGS group (54.5%). Both EJS and EGS were completed with circular staplers (CS), and the frequency of anastomotic stenosis was also higher in the EG group (27.3% *vs* 0%) in that study[78]. The possible causes of benign anastomotic stenosis were different vascularization and erosive effects of the refluxed duodenal and gastric contents. The distance of anastomosis between gastrojejunostomy and EJS was also considered a risk factor for anastomosis-related late complications[79]. Similar to JI, the DTR procedure was also aimed to maintain gradual intestinal absorption and helped to improve QoL compared with TG. As reported by Nomura *et al*[80], the intestinal absorption and hormonal secretion in the DTR group were largely unaffected by the posture of the meal intake than JI. In the present literature, the DTR did not show a longer operation time and more blood loss. Anastomotic leakage was rarely or even not evident, the incidence of anastomotic stricture ranged from 0% to 6.67%, and the incidence of esophagitis reflux was reported from 0% to 20% (Table 2). These results indicate that DTR is a safe and feasible surgical procedure. In terms of the long-term effects, Cho *et al*[81]'s results showed similar hematologic and nutritional outcomes between the two procedures. However, other studies reported that DTR has advantages in hemoglobin change and vitamin B12 deficiency compared with laparoscopic total gastrectomy (LTG)[79,82,83]. Long-term results in a multicenter study with a larger number of patients should be evaluated in the future to fully elucidate the controversy.

As reviewed, the DTR, improved EGS, and JI methods were used to prevent reflux esophagitis. LPG with DTR maintains comparable oncological safety and anastomosis-related late complications compared with LTG and is preferred as a reasonable alternative to LTG if oncological safety is assured. However, its advantage in nutrition postoperatively remains controversial compared with LTG. Accordingly, surgeons should be aware that LPG should be strictly limited to performance under the premise of radical resection.

DIGESTIVE TRACT RECONSTRUCTION AFTER LAPAROSCOPIC TOTAL GASTRECTOMY (LTG)

The Roux-en-Y procedure is most commonly method for reconstruction between the esophagus and jejunum after LTG. EJS is difficult, and multiple intracorporeal techniques for EJS have been developed that can be divided into two categories: Those using a CS and those using an LS. Only a few reports exist concerning the hand-sewn technique for EJS, which is currently only safely performed in few high-volume centers because it is too difficult to be popularized and is not discussed in this review[87,88].

CS METHOD

Similar to conventional open TG, the CS method of EJS is completed in an end-to-side manner using a CS. In the early LTG surgeries, the anvil was inserted into the esophagus stump using the purse-string instrument[89,90] or hand-sewn method[91,92]. In addition to the improvement in the laparoscopic devices and accumulation of experience, the application of these two methods has decreased and has been gradually replaced by other methods. Presently, the maneuver of inserting the anvil is commonly performed transorally or transabdominally, referred to as the OrVil™ or reverse puncture method (RPM), respectively. The OrVil™ was first reported by Jeong *et al*[93] in 2009. Briefly, the anvil connected to the OrVil™ tube was transorally introduced into the esophagus (Figure 3A) and intracorporeal EJS was consecutively performed with a CS through a minilaparotomy incision that was used to remove the stomach (Figure 3D). The RPM is another common method that was first reported by Omori *et al*[94] in 2009. The main steps of this procedure are as follows: Semicircumferential esophagotomy is performed at the anterior esophageal wall and the anvil secured with a prolene suture that is then inserted into the esophagus.

Table 2 Summary of reconstruction procedures after laparoscopic proximal gastrectomy

Ref.	Publication year	Reconstruction procedure	*n*	Length of surgery, min (mean ± SD or range)	Blood loss, mL (mean ± SD or range)	Esophagitis reflux (*n*)	Anastomotic leakage (*n*)	Anastomotic stricture (*n*)
Nomura *et al*[80]	2019	DTR	15	352.5 ± 67.3	90.5 ± 105.5	1	0	1
		JI	15	322.5 ± 24.2	46.8 ± 69.8	1	0	1
Aburatani *et al*[78]	2017	DTR	19	325.7 ± 66.9	131.4 ± 118.7	2	0	0
		EGS	22	290.3 ± 55.1	132.0 ± 129.7	12	0	6
Tanaka *et al*[84]	2017	DTR	10	285 (146–440)	0 (0–25)	20	0	0
Yang *et al*[85]	2015	DTR	16	219.6 ± 48.6	101.5 ± 71.6	0	0	0
Hong *et al*[86]	2015	DTR	21	173.8 ± 21.8	109.2 ± 96.3	1	0	0
Cho *et al*[81]	2018	DTR	38	217.7 ± 53.0	100.2 ± 92.0	0	1	0
		TG	42	226.9 ± 66.2	118.8 ± 157.2	3	4	2
Park *et al*[82]	2018	DTR	34	212.9 ± 32.6	30 (6-600)	NA	NA	NA
		TG	46	240.7 ± 43.9	59 (20-85)	NA	NA	NA
Jung *et al*[79]	2017	DTR	92	198.3 ± 38.8	84.7 ± 81.7	1	2	3
		TG	156	225.4 ± 51.6	128.3 ± 112.5	3	3	2
Kim *et al*[83]	2016	DTR	17	268.2±40.9	NA	2	0	0
		TG	17	270.2±43.4	NA	1	0	1

NA: Not available; TG: Total gastrectomy; EGS: Esophagogastrostomy; JI: Jejunal interposition; DTR: Double tract reconstruction.

Thereafter, the needle is reversely sutured out and the center rod of the anvil penetrates the esophageal wall by drawing the suture (Figure 3B and C). Finally, the esophagus is transected using a linear cutter, and EJS is achieved with a CS under laparoscopic monitoring.

The CS method has been widespread, especially in the introductory period, because it is similar to the conventional open surgeries. CS also has other merits compared with LS, such as no requirement for intracorporeal suturing and excessive esophageal dissociation[95]. Comparing the two CS methods, the device of OrVil™ is easier and very convenient to perform intracorporeal EJS. Otherwise, the RPM needs laparoscopic suturing and more esophagus may be sacrificed[96]. In terms of the surgical outcomes and postoperative course, no significant difference was found in the surgical time and blood loss between OrVil™ and RPM, and the incidence of anastomotic leakage was also similar. However, the incidence of anastomotic stricture was higher when performing OrVil™, ranging from 0% to 8.3% (Table 2)[97-100]. As reviewed by Inokuchi *et al*[101], the incidence was decreased compared with the results from early surgeries. This progress might be attributed to the standardization of the procedures and accumulation of skills to use the OrVil™ device. Additionally, the anastomotic complications might be related to the insertion site in the abdominal wall for CS[102]. However, higher cost, possibility of bacterial contamination, and injury of the esophageal mucosa are important factors limiting the popularity of the OrVil™ method[96,103]. Many surgeons, including the authors, would choose the RPM after mastering laparoscopic techniques. The determination of the CS method should be selected by the preference and experience of the surgeons.

As described, all CS require a mini incision to finally complete the EJS, and these CS methods are actually laparoscopic-assisted surgeries. Furthermore, it is sometimes difficult to complete anastomosis through this mini incision, especially in obese patients. Additionally, in patients with a small esophageal diameter, the CS method is extremely difficult and would increase the risk of anastomotic complications that could be overcome by LS methods.

LS METHOD

The LS methods are total laparoscopic surgeries because EJS is completed in a side-to-side manner using a LS without any assisted incisions. The main procedures include functional end-to-end anastomosis (FEEA), overlap method, and π-type anastomosis. The FEEA method, also called "inverse-peristaltic anastomosis", was first reported by Uyama *et al*[104] in 1999. First, the distal jejunum loop is pulled to the left side of the

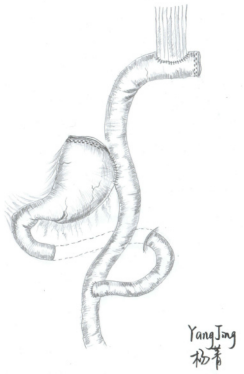

Figure 2 Schematic picture of double tract reconstruction after laparoscopic proximal gastrectomy.

esophageal stump after lymphadenectomy. A functional side-to-side anastomosis is subsequently completed with an LS *via* the stump of the esophagus and jejunum (Figure 4A). The common entry hole is finally closed by LS. The overlap method proposed by Inaba *et al*[105] in 2010 is similar to FEEA. The hole used for overlap anastomosis is not opened at the jejunal stump, and side-to-side anastomosis is completed along the peristaltic direction of the esophagus (Figure 4B). Another difference from FEEA is the closure of the common entry hole that is performed *via* an intracorporeal suture. The π-type anastomosis is an improvement of FEEA. Technically, neither the esophagus nor the jejunum is transected, and a side-to-side EJS is performed with an LS. The esophageal division, common entry hole closure, and jejunal division are subsequently performed using a single 60-mm LS (3-in-1 technique) (Figure 4C)[106].

Comparing these three LS methods, FEEA is time-saving because the common entry hole can be closed with an LS. However, the jejunal limb needs to be lifted further up when performing FEEA, and this step might lead to the tension of the jejunal limb of the mesentery, which might increase the risk of anastomotic leakage[107]. Second, the procedure of FEEA needs more working space than overlap as the jejunum is folded up when performing anastomosis (Figure 4A). No significant difference was found between the two methods in terms of actual anastomotic complications (Table 2). However, the anastomotic oral end tended to have greater tension, which was the high incidence site of anastomotic leakage. Moreover, this site is located in the mediastinum, and it is difficult to strengthen by laparoscopic suture. Accordingly, surgeons should pay more attention to this point, especially for beginners. The surgical procedure is simplified, and the surgical time is reduced by performing π-type anastomosis. However, the largest deficiency of this method is that the margin could not be checked until the reconstruction is completed, limiting its popularity.

Compared with the CS method, LS method shows some merits in surgical outcomes (Table 3): Being time-saving and less blood loss[108-110], with fewer intraoperative events and intraoperative anastomosis events[109]. Regarding anastomotic complications, LS methods seem to have less anastomotic stricture[97,111,112]. Additionally, LS can be performed in the narrow mediastinum because the tips are thinner and can achieve a suitable anastomotic size regardless of the esophageal diameter. Furthermore, the rotary connecter of LS enables the LS method to be performed in real time to reduce the jejunal tension by changing the anastomotic position and direction to improve the quality of anastomosis. Therefore, the LS methods have been favored by more surgeons in the past few years.

CONCLUSION AND PROSPECTS

Almost all the literature included in this review originated from the East and was mainly reported from Japan and Korea. This highlights the prominent position of these two countries in the field of GC treatment, while Western surgeons have less experience in treating GC laparoscopically due to the low incidence and respectability[113]. The results of the RCTs[9,11,12] mentioned above were expected to establish concrete evidence of widely carried out LG in the treatment of AGC. Therefore, LG will encounter a period of rapid development, and controversy concerning reconstruction is expected to be resolved by large-scale and multicenter RCTs in the near future.

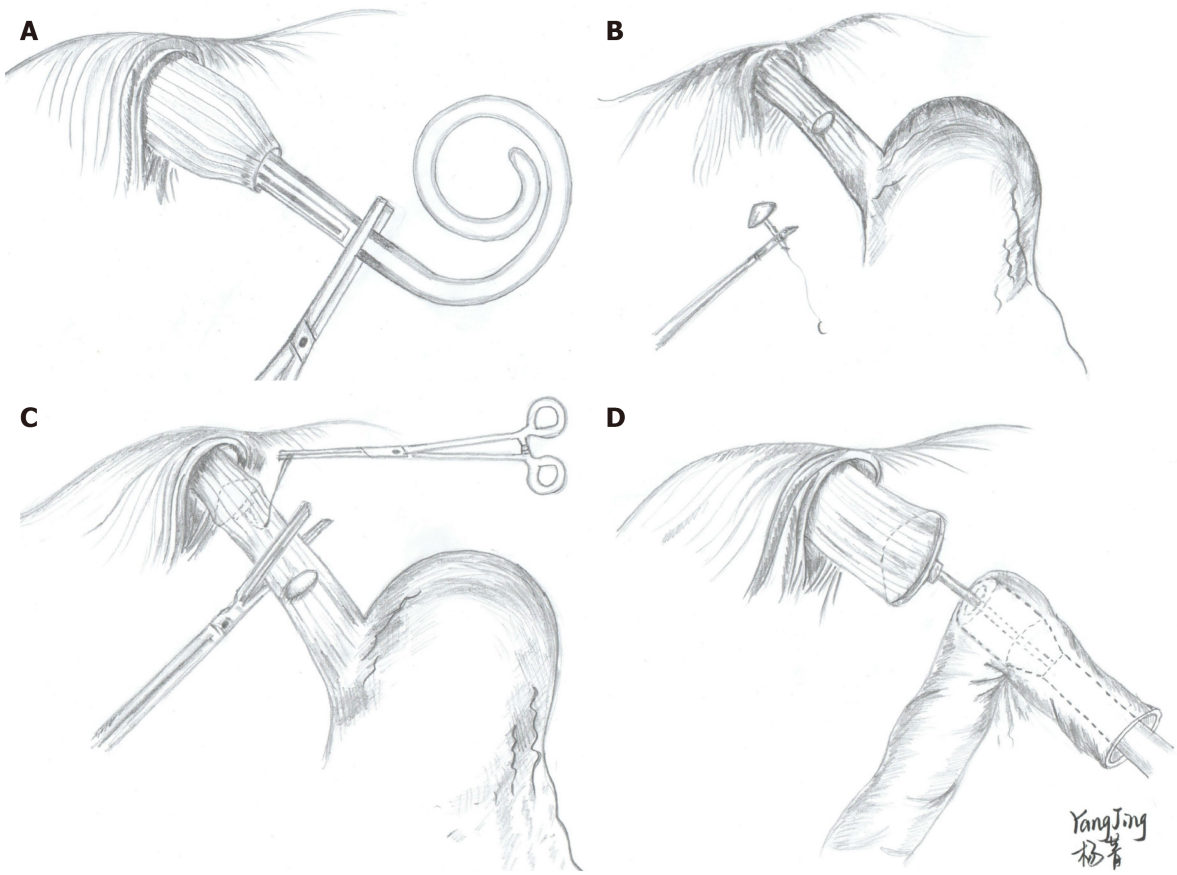

Figure 3 Esophagojejunostomy *via* circular stapler methods. A: OrVil™; B: Semicircumferential esophagotomy performed at the anterior esophageal wall (reverse puncture method); C: The center rod of the anvil penetrates the esophageal wall by drawing the suture; D: Esophagojejunostomy accomplished with a circular stapler under laparoscopic monitoring.

In conclusion, the choice of specific reconstruction method remains unclear presently, and surgeons must understand the merits and demerits of every anastomotic device and procedure. Under the premise of radical gastrectomy and lymphadenectomy, a reasonable reconstruction procedure should be selected to improve the QoL postoperatively by considering the following factors: Safety (anastomosis with sufficient blood supply and free tension), efficiency (simple and time-saving), minimal invasion (less blood loss), stability (surgeon's preference and experience), and QoL (function preservation, if possible, reflux prevention, and nutrition).

Table 3 Summary of reconstruction procedures after laparoscopic total gastrectomy

Ref.	Publication year	LS or CS	EJS procedure	N	Length of surgery, min (mean ± SD or range)	Blood loss, mL (mean ± SD or range)	Anastomotic leakage (*n*)	Anastomotic stricture (*n*)
Tokuhara *et al*[99]	2018	CS	OrVil™	24	NA	NA	1	2
Brenkman *et al*[114]	2016	CS	OrVil™	47	301 (148–454)	300 (30–900)	7	NA
Ali *et al*[115]	2016	CS	RPM	58	199.8 ± 57.0	81.6 ± 40.3	3	5
Wang *et al*[96]	2015	CS	OrVil™	42	287.8 ± 38.4	96.4 ± 32.7	0	2
			RPM	42	271.8 ± 46.1	88.2 ± 36.9	1	2
Li *et al*[103]	2017	CS	OrVil™	19	NA	NA	0	1
			RPM	24	NA	NA	1	0
Lu *et al*[98]	2016	CS	OrVil™	25	216.5 ± 24.9	141.2 ± 121.1	0	0
			LATG-PSI	25	224.0 ± 30.5	138.8 ± 79.9	0	0
Duan *et al*[116]	2017	CS	End-to-side EJS	176	250.0 ± 54.1	114.1 ± 74.0	7	11
			Semi-end-to-end EJS	92	238.0 ± 50.4 0.079	110.5 ± 82.8	1	0
Kyogoku *et al*[108]	2018	CS	OrVil™ / RPM	83	330 (123–627)	100 (0–1108)	3	6
		LS	FEEA/overlap	208	297 (171–553)	23 (0–1070)	4	7
Lee *et al*[117]	2017	LS	Overlap	50	144.6 ± 29.9	NA	0	0
Son *et al*[118]	2017	LS	Overlap	27	171.1 ± 50.9	119.4 ± 107.1	0	0
Kitagami *et al*[119]	2016	LS	Overlap	100	379 (248–649)	65 (5–750)	0	0
Miura *et al*[107]	2017	LS	FEEA	120	350.8	0	2	1
			Overlap	48	402.5	6.5	3	0
Yoshikawa *et al*[112]	2018	CS	OrVil™	36	345 ± 9.9	45 ± 15	0	3
		LS	Overlap	47	398 ± 8	126 ± 13	2	0
Kawamura *et al*[97]	2017	CS	OrVil™	49	259.5 ± 51.4	53.3 ± 70.0	2	2
		LS	Overlap	139	276.5 ± 53.0	69.7 ± 116.6	1	0
Yasukawa *et al*[100]	2017	CS	OrVil™	51	346.1 ± 52.7	34 (10-556)	2	0
		LS	FEEA	18	348.4 ± 53.5	35 (10-750)	0	1
Gong *et al*[109]	2017	CS	NA	266	170 (65-453)	NA	15	3
		LS	NA	421	149 (75-342)	NA	15	2
Huang *et al*[110]	2017	CS	NA	456	203.6 ± 49.3	98.4 ± 149.1	22	4
		LS	IJOM (overlap)	51	209.3 ± 41.0	48.3 ± 38.5	1	0
Chen *et al*[111]	2016	CS	RPM	18	305.6 ± 45.9 (250-380)	80.6 ± 29.4 (50-160)	1	1
		LS	FEEA	22	266.8 ± 38.7 (230-360)	86.4 ± 39.7 (50-200)	0	0
Kim *et al*[120]	2016	CS	PSI	29	230.3 ± 56.5	106.3 ± 70.3	0	1
		LS	Overlap	27	228.9 ± 33.6	90.9 ± 46.0	1	0
Chen *et al*[88]	2016	CS + LS	CS + LS	40	284.3 ± 45.6 (230–380)	83.8 ± 35.2 (30–200)	1	3
			Hand-sewn	59	257.4 ± 47.2 (170–350)	87.6 ± 42.4 (30–200)	0	0

CS: Circular stapler; LS: Linear stapler; RPM: Reverse puncture method; EJS: Esophagojejunostomy; FEEA: Functional end-to-end anastomosis; PSI: Purse-string instrument; IJOM: Isoperistaltic jejunum-later-cut overlap method; NA: Not available.

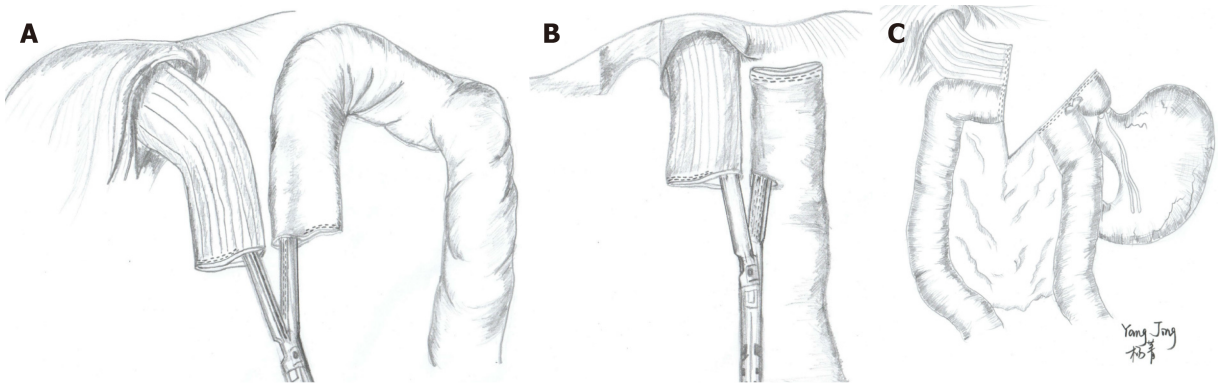

Figure 4 Esophagojejunostomy *via* linear stapler methods. A: Functional end-to-end anastomosis; B: Overlap; C: π-type anastomosis.

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; **68**: 7-30 [PMID: 29313949 DOI: 10.3322/caac.21442]
2 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]
3 **Agolli L**, Nicosia L. Between evidence and new perspectives on the current state of the multimodal approach to gastric cancer: Is there still a role for radiation therapy? *World J Gastrointest Oncol* 2018; **10**: 271-281 [PMID: 30254722 DOI: 10.4251/wjgo.v10.i9.271]
4 **Japanese Gastric Cancer Association**. Japanese gastric cancer treatment guidelines 2014 (ver. 4). *Gastric Cancer* 2017; **20**: 1-19 [PMID: 27342689 DOI: 10.1007/s10120-016-0622-4]
5 **Lee JH**, Kim JG, Jung HK, Kim JH, Jeong WK, Jeon TJ, Kim JM, Kim YI, Ryu KW, Kong SH, Kim HI, Jung HY, Kim YS, Zang DY, Cho JY, Park JO, Lim DH, Jung ES, Ahn HS, Kim HJ. Clinical practice guidelines for gastric cancer in Korea: an evidence-based approach. *J Gastric Cancer* 2014; **14**: 87-104 [PMID: 25061536 DOI: 10.5230/jgc.2014.14.2.87]
6 **Kitano S**, Iso Y, Moriyama M, Sugimachi K. Laparoscopy-assisted Billroth I gastrectomy. *Surg Laparosc Endosc* 1994; **4**: 146-148 [PMID: 8180768]
7 **Kim HH**, Han SU, Kim MC, Hyung WJ, Kim W, Lee HJ, Ryu SW, Cho GS, Kim CY, Yang HK, Park DJ, Song KY, Lee SI, Ryu SY, Lee JH; Korean Laparoscopic Gastrointestinal Surgery Study (KLASS) Group. Prospective randomized controlled trial (phase III) to comparing laparoscopic distal gastrectomy with open distal gastrectomy for gastric adenocarcinoma (KLASS 01). *J Korean Surg Soc* 2013; **84**: 123-130 [PMID: 23396494 DOI: 10.4174/jkss.2013.84.2.123]
8 **Nakamura K**, Katai H, Mizusawa J, Yoshikawa T, Ando M, Terashima M, Ito S, Takagi M, Takagane A, Ninomiya M, Fukushima N, Sasako M. A phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastric Cancer (JCOG0912). *Jpn J Clin Oncol* 2013; **43**: 324-327 [PMID: 23275644 DOI: 10.1093/jjco/hys220]
9 **Katai H**, Mizusawa J, Katayama H, Takagi M, Yoshikawa T, Fukagawa T, Terashima M, Misawa K, Teshima S, Koeda K, Nunobe S, Fukushima N, Yasuda T, Asao Y, Fujiwara Y, Sasako M. Short-term surgical outcomes from a phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastric cancer: Japan Clinical Oncology Group Study JCOG0912. *Gastric Cancer* 2017; **20**: 699-708 [PMID: 27718137 DOI: 10.1007/s10120-016-0646-9]
10 **Inaki N**, Etoh T, Ohyama T, Uchiyama K, Katada N, Koeda K, Yoshida K, Takagane A, Kojima K, Sakuramoto S, Shiraishi N, Kitano S. A Multi-institutional, Prospective, Phase II Feasibility Study of Laparoscopy-Assisted Distal Gastrectomy with D2 Lymph Node Dissection for Locally Advanced Gastric Cancer (JLSSG0901). *World J Surg* 2015; **39**: 2734-2741 [PMID: 26170158 DOI: 10.1007/s00268-015-3160-z]
11 **Hu Y**, Huang C, Sun Y, Su X, Cao H, Hu J, Xue Y, Suo J, Tao K, He X, Wei H, Ying M, Hu W, Du X, Chen P, Liu H, Zheng C, Liu F, Yu J, Li Z, Zhao G, Chen X, Wang K, Li P, Xing J, Li G. Morbidity and Mortality of Laparoscopic Versus Open D2 Distal Gastrectomy for Advanced Gastric Cancer: A Randomized Controlled Trial. *J Clin Oncol* 2016; **34**: 1350-1357 [PMID: 26903580 DOI: 10.1200/JCO.2015.63.7215]
12 **Kim HI**, Hur H, Kim YN, Lee HJ, Kim MC, Han SU, Hyung WJ. Standardization of D2 lymphadenectomy and surgical quality control (KLASS-02-QC): a prospective, observational, multicenter study [NCT01283893]. *BMC Cancer* 2014; **14**: 209 [PMID: 24646327 DOI: 10.1186/1471-2407-14-209]
13 **Lee HJ**, Hyung WJ, Yang HK, Han SU, Park YK, An JY, Kim W, Kim HI, Kim HH, Ryu SW, Hur H, Kong SH, Cho GS, Kim JJ, Park DJ, Ryu KW, Kim YW, Kim JW, Lee JH, Kim MC; Korean Laparo-endoscopic Gastrointestinal Surgery Study (KLASS) Group. Short-term Outcomes of a Multicenter Randomized Controlled Trial Comparing Laparoscopic Distal Gastrectomy With D2 Lymphadenectomy to Open Distal Gastrectomy for Locally Advanced Gastric Cancer (KLASS-02-RCT). *Ann Surg* 2019 [PMID: 30829698 DOI: 10.1097/SLA.0000000000003217]
14 **Huh YJ**, Lee JH. The Advances of Laparoscopic Gastrectomy for Gastric Cancer. *Gastroenterol Res Pract* 2017; **2017**: 9278469 [PMID: 29018482 DOI: 10.1155/2017/9278469]
15 **Shinohara H**, Haruta S, Ohkura Y, Udagawa H, Sakai Y. Tracing Dissectable Layers of Mesenteries Overcomes Embryologic Restrictions when Performing Infrapyloric Lymphadenectomy in Laparoscopic Gastric Cancer Surgery. *J Am Coll Surg* 2015; **220**: e81-e87 [PMID: 25998088 DOI: 10.1016/j.jamcollsurg.2015.02.037]
16 **Huang CM**, Chen QY, Lin JX, Zheng CH, Li P, Xie JW, Wang JB, Lu J, Yang XT. Laparoscopic spleen-preserving no. 10 lymph node dissection for advanced proximal gastric cancer using a left approach. *Ann*

Surg Oncol 2014; **21**: 2051 [PMID: 24590432 DOI: 10.1245/s10434-014-3492-1]
17 **Shen J**, Dong X, Liu Z, Wang G, Yang J, Zhou F, Lu M, Ma X, Li Y, Tang C, Luo X, Zhao Q, Zhang J. Modularized laparoscopic regional en bloc mesogastrium excision (rEME) based on membrane anatomy for distal gastric cancer. *Surg Endosc* 2018; **32**: 4698-4705 [PMID: 30054740 DOI: 10.1007/s00464-018-6375-x]
18 **Best LM**, Mughal M, Gurusamy KS. Laparoscopic versus open gastrectomy for gastric cancer. *Cochrane Database Syst Rev* 2016; **3**: CD011389 [PMID: 27030300 DOI: 10.1002/14651858.CD011389.pub2]
19 **Kanaya S**, Gomi T, Momoi H, Tamaki N, Isobe H, Katayama T, Wada Y, Ohtoshi M. Delta-shaped anastomosis in totally laparoscopic Billroth I gastrectomy: new technique of intraabdominal gastroduodenostomy. *J Am Coll Surg* 2002; **195**: 284-287 [PMID: 12168979]
20 **Jeong O**, Jung MR, Park YK, Ryu SY. Safety and feasibility during the initial learning process of intracorporeal Billroth I (delta-shaped) anastomosis for laparoscopic distal gastrectomy. *Surg Endosc* 2015; **29**: 1522-1529 [PMID: 25294524 DOI: 10.1007/s00464-014-3836-8]
21 **Kanaya S**, Kawamura Y, Kawada H, Iwasaki H, Gomi T, Satoh S, Uyama I. The delta-shaped anastomosis in laparoscopic distal gastrectomy: analysis of the initial 100 consecutive procedures of intracorporeal gastroduodenostomy. *Gastric Cancer* 2011; **14**: 365-371 [PMID: 21573920 DOI: 10.1007/s10120-011-0054-0]
22 **Ding W**, Tan Y, Xue W, Wang Y, Xu XZ. Comparison of the short-term outcomes between delta-shaped anastomosis and conventional Billroth I anastomosis after laparoscopic distal gastrectomy: A meta-analysis. *Medicine (Baltimore)* 2018; **97**: e0063 [PMID: 29489666 DOI: 10.1097/MD.0000000000010063]
23 **Wang SY**, Hong J, Hao HK. A comparative study of delta-shaped and conventional Billroth I anastomosis after laparoscopic distal gastrectomy for gastric cancer. *Surg Endosc* 2017; **31**: 3191-3202 [PMID: 27864720 DOI: 10.1007/s00464-016-5344-5]
24 **Lee HH**, Song KY, Lee JS, Park SM, Kim JJ. Delta-shaped anastomosis, a good substitute for conventional Billroth I technique with comparable long-term functional outcome in totally laparoscopic distal gastrectomy. *Surg Endosc* 2015; **29**: 2545-2552 [PMID: 25427413 DOI: 10.1007/s00464-014-3966-z]
25 **Noshiro H**, Iwasaki H, Miyasaka Y, Kobayashi K, Masatsugu T, Akashi M, Ikeda O. An additional suture secures against pitfalls in delta-shaped gastroduodenostomy after laparoscopic distal gastrectomy. *Gastric Cancer* 2011; **14**: 385-389 [PMID: 21850518 DOI: 10.1007/s10120-011-0082-9]
26 **Matsuhashi N**, Osada S, Yamaguchi K, Saito S, Okumura N, Tanaka Y, Nonaka K, Takahashi T, Yoshida K. Oncologic outcomes of laparoscopic gastrectomy: a single-center safety and feasibility study. *Surg Endosc* 2013; **27**: 1973-1979 [PMID: 23468326 DOI: 10.1007/s00464-012-2696-3]
27 **Jang CE**, Lee SI. Modified intracorporeal gastroduodenostomy in totally laparoscopic distal gastrectomy for gastric cancer: early experience. *Ann Surg Treat Res* 2015; **89**: 306-312 [PMID: 26665125 DOI: 10.4174/astr.2015.89.6.306]
28 **Watanabe Y**, Watanabe M, Suehara N, Saimura M, Mizuuchi Y, Nishihara K, Iwashita T, Nakano T. Billroth-I reconstruction using an overlap method in totally laparoscopic distal gastrectomy: propensity score matched cohort study of short- and long-term outcomes compared with Roux-en-Y reconstruction. *Surg Endosc* 2019 [PMID: 30758666 DOI: 10.1007/s00464-019-06688-z]
29 **Wang Z**, Ma L, Zhang XM, Zhou ZX. Long-term outcomes after D2 gastrectomy for early gastric cancer: survival analysis of a single-center experience in China. *Asian Pac J Cancer Prev* 2014; **15**: 7219-7222 [PMID: 25227817 DOI: 10.7314/APJCP.2014.15.17.7219]
30 **Choi IJ**, Lee JH, Kim YI, Kim CG, Cho SJ, Lee JY, Ryu KW, Nam BH, Kook MC, Kim YW. Long-term outcome comparison of endoscopic resection and surgery in early gastric cancer meeting the absolute indication for endoscopic resection. *Gastrointest Endosc* 2015; **81**: 333-41.e1 [PMID: 25281498 DOI: 10.1016/j.gie.2014.07.047]
31 **Lee SW**, Bouras G, Nomura E, Yoshinaka R, Tokuhara T, Nitta T, Tsunemi S, Tanigawa N. Intracorporeal stapled anastomosis following laparoscopic segmental gastrectomy for gastric cancer: technical report and surgical outcomes. *Surg Endosc* 2010; **24**: 1774-1780 [PMID: 20039069 DOI: 10.1007/s00464-009-0803-x]
32 **Kim JJ**, Song KY, Chin HM, Kim W, Jeon HM, Park CH, Park SM. Totally laparoscopic gastrectomy with various types of intracorporeal anastomosis using laparoscopic linear staplers: preliminary experience. *Surg Endosc* 2008; **22**: 436-442 [PMID: 17593437 DOI: 10.1007/s00464-007-9446-y]
33 **Koeda K**, Chiba T, Noda H, Nishinari Y, Segawa T, Akiyama Y, Iwaya T, Nishizuka S, Nitta H, Otsuka K, Sasaki A. Intracorporeal reconstruction after laparoscopic pylorus-preserving gastrectomy for middle-third early gastric cancer: a hybrid technique using linear stapler and manual suturing. *Langenbecks Arch Surg* 2016; **401**: 397-402 [PMID: 26883539 DOI: 10.1007/s00423-016-1378-3]
34 **Hosoda K**, Yamashita K, Sakuramoto S, Katada N, Moriya H, Mieno H, Watanabe M. Postoperative quality of life after laparoscopy-assisted pylorus-preserving gastrectomy compared With laparoscopy-assisted distal gastrectomy: A cross-sectional postal questionnaire survey. *Am J Surg* 2017; **213**: 763-770 [PMID: 27751530 DOI: 10.1016/j.amjsurg.2016.09.041]
35 **Han TS**, Kong SH, Lee HJ, Ahn HS, Hur K, Yu J, Kim WH, Yang HK. Dissemination of free cancer cells from the gastric lumen and from perigastric lymphovascular pedicles during radical gastric cancer surgery. *Ann Surg Oncol* 2011; **18**: 2818-2825 [PMID: 21455599 DOI: 10.1245/s10434-011-1620-8]
36 **Fukuhara K**, Osugi H, Takada N, Takemura M, Higashino M, Kinoshita H. Reconstructive procedure after distal gastrectomy for gastric cancer that best prevents duodenogastroesophageal reflux. *World J Surg* 2002; **26**: 1452-1457 [PMID: 12370787 DOI: 10.1007/s00268-002-6363-z]
37 **Kumagai K**, Shimizu K, Yokoyama N, Aida S, Arima S, Aikou T; Japanese Society for the Study of Postoperative Morbidity after Gastrectomy. Questionnaire survey regarding the current status and controversial issues concerning reconstruction after gastrectomy in Japan. *Surg Today* 2012; **42**: 411-418 [PMID: 22391980 DOI: 10.1007/s00595-012-0159-z]
38 **Vogel SB**, Drane WE, Woodward ER. Clinical and radionuclide evaluation of bile diversion by Braun enteroenterostomy: prevention and treatment of alkaline reflux gastritis. An alternative to Roux-en-Y diversion. *Ann Surg* 1994; **219**: 458-65; discussion 465-6 [PMID: 8185396 DOI: 10.1097/00000658-199405000-00003]
39 **Lee MS**, Ahn SH, Lee JH, Park DJ, Lee HJ, Kim HH, Yang HK, Kim N, Lee WW. What is the best reconstruction method after distal gastrectomy for gastric cancer? *Surg Endosc* 2012; **26**: 1539-1547 [PMID: 22179454 DOI: 10.1007/s00464-011-2064-8]
40 **Park JY**, Kim YJ. Uncut Roux-en-Y Reconstruction after Laparoscopic Distal Gastrectomy Can Be a

Favorable Method in Terms of Gastritis, Bile Reflux, and Gastric Residue. *J Gastric Cancer* 2014; **14**: 229-237 [PMID: 25580354 DOI: 10.5230/jgc.2014.14.4.229]
41 **He Z**, Zang L. Reconstruction after laparoscopic assisted distal gastrectomy: technical tips and pitfalls. *Transl Gastroenterol Hepatol* 2017; **2**: 66 [PMID: 28905007 DOI: 10.21037/tgh.2017.08.05]
42 **Inokuchi M**, Kojima K, Yamada H, Kato K, Hayashi M, Motoyama K, Sugihara K. Long-term outcomes of Roux-en-Y and Billroth-I reconstruction after laparoscopic distal gastrectomy. *Gastric Cancer* 2013; **16**: 67-73 [PMID: 22467062 DOI: 10.1007/s10120-012-0154-5]
43 **Kim CH**, Song KY, Park CH, Seo YJ, Park SM, Kim JJ. A comparison of outcomes of three reconstruction methods after laparoscopic distal gastrectomy. *J Gastric Cancer* 2015; **15**: 46-52 [PMID: 25861522 DOI: 10.5230/jgc.2015.15.1.46]
44 **An JY**, Cho I, Choi YY, Kim YM, Noh SH. Totally laparoscopic Roux-en-Y gastrojejunostomy after laparoscopic distal gastrectomy: analysis of initial 50 consecutive cases of single surgeon in comparison with totally laparoscopic Billroth I reconstruction. *Yonsei Med J* 2014; **55**: 162-169 [PMID: 24339302 DOI: 10.3349/ymj.2014.55.1.162]
45 **Hoya Y**, Mitsumori N, Yanaga K. The advantages and disadvantages of a Roux-en-Y reconstruction after a distal gastrectomy for gastric cancer. *Surg Today* 2009; **39**: 647-651 [PMID: 19639429 DOI: 10.1007/s00595-009-3964-2]
46 **Pan Y**, Li Q, Wang DC, Wang JC, Liang H, Liu JZ, Cui QH, Sun T, Zhang RP, Kong DL, Hao XS. Beneficial effects of jejunal continuity and duodenal food passage after total gastrectomy: a retrospective study of 704 patients. *Eur J Surg Oncol* 2008; **34**: 17-22 [PMID: 17884327 DOI: 10.1016/j.ejso.2007.08.001]
47 **Van Stiegmann G**, Goff JS. An alternative to Roux-en-Y for treatment of bile reflux gastritis. *Surg Gynecol Obstet* 1988; **166**: 69-70 [PMID: 3336817]
48 **Morrison P**, Miedema BW, Kohler L, Kelly KA. Electrical dysrhythmias in the Roux jejunal limb: cause and treatment. *Am J Surg* 1990; **160**: 252-256 [PMID: 2393051 DOI: 10.1016/S0002-9610(06)80017-6]
49 **Huang Y**, Wang S, Shi Y, Tang D, Wang W, Chong Y, Zhou H, Xiong Q, Wang J, Wang D. Uncut Roux-en-Y reconstruction after distal gastrectomy for gastric cancer. *Expert Rev Gastroenterol Hepatol* 2016; **10**: 1341-1347 [PMID: 27748146 DOI: 10.1080/17474124.2016.1248404]
50 **Yang D**, He L, Tong WH, Jia ZF, Su TR, Wang Q. Randomized controlled trial of uncut Roux-en-Y *vs* Billroth II reconstruction after distal gastrectomy for gastric cancer: Which technique is better for avoiding biliary reflux and gastritis? *World J Gastroenterol* 2017; **23**: 6350-6356 [PMID: 28974902 DOI: 10.3748/wjg.v23.i34.6350]
51 **Fukunaga T**, Ishibashi Y, Oka S, Kanda S, Yube Y, Kohira Y, Matsuo Y, Mori O, Mikami S, Enomoto T, Otsubo T. Augmented rectangle technique for Billroth I anastomosis in totally laparoscopic distal gastrectomy for gastric cancer. *Surg Endosc* 2018; **32**: 4011-4016 [PMID: 29915985 DOI: 10.1007/s00464-018-6266-1]
52 **Lin M**, Zheng CH, Huang CM, Li P, Xie JW, Wang JB, Lin JX, Lu J, Chen QY, Cao LL, Tu RH. Totally laparoscopic versus laparoscopy-assisted Billroth-I anastomosis for gastric cancer: a case-control and case-matched study. *Surg Endosc* 2016; **30**: 5245-5254 [PMID: 27008576 DOI: 10.1007/s00464-016-4872-3]
53 **Jian-Cheng T**, Bo Z, Jian F, Liang Z. Delta-Shaped Gastroduodenostomy in Fully Laparoscopic Distal Gastrectomy: A Retrospective Study. *Medicine (Baltimore)* 2015; **94**: e1153 [PMID: 26181558 DOI: 10.1097/MD.0000000000001153]
54 **Toyomasu Y**, Ogata K, Suzuki M, Yanoma T, Kimura A, Kogure N, Ohno T, Kamiyama Y, Mochiki E, Kuwano H. Comparison of the Physiological Effect of Billroth-I and Roux-en-Y Reconstruction Following Laparoscopic Distal Gastrectomy. *Surg Laparosc Endosc Percutan Tech* 2018; **28**: 328-333 [PMID: 30180143 DOI: 10.1097/SLE.0000000000000575]
55 **Okuno K**, Nakagawa M, Kojima K, Kanemoto E, Gokita K, Tanioka T, Inokuchi M. Long-term functional outcomes of Roux-en-Y versus Billroth I reconstructions after laparoscopic distal gastrectomy for gastric cancer: a propensity-score matching analysis. *Surg Endosc* 2018; **32**: 4465-4471 [PMID: 29654529 DOI: 10.1007/s00464-018-6192-2]
56 **Kim JH**, Jun KH, Chin HM. Short-term surgical outcomes of laparoscopy-assisted versus totally laparoscopic Billroth-II gastrectomy for gastric cancer: a matched-cohort study. *BMC Surg* 2017; **17**: 45 [PMID: 28431531 DOI: 10.1186/s12893-017-0245-7]
57 **Cui LH**, Son SY, Shin HJ, Byun C, Hur H, Han SU, Cho YK. Billroth II with Braun Enteroenterostomy Is a Good Alternative Reconstruction to Roux-en-Y Gastrojejunostomy in Laparoscopic Distal Gastrectomy. *Gastroenterol Res Pract* 2017; **2017**: 1803851 [PMID: 28163716 DOI: 10.1155/2017/1803851]
58 **In Choi C**, Baek DH, Lee SH, Hwang SH, Kim DH, Kim KH, Jeon TY, Kim DH. Comparison Between Billroth-II with Braun and Roux-en-Y Reconstruction After Laparoscopic Distal Gastrectomy. *J Gastrointest Surg* 2016; **20**: 1083-1090 [PMID: 27067234 DOI: 10.1007/s11605-016-3138-7]
59 **Du J**, Xue H, Hua J, Zhao L, Zhang Z. Intracorporeal classic circular-stapled gastrojejunostomy and jejunojejunostomy during laparoscopic distal gastrectomy: A simple, safe "intraluminal poke technique" for anvil placement. *J Surg Oncol* 2019; **119**: 464-471 [PMID: 30582618 DOI: 10.1002/jso.25353]
60 **Seo HS**, Jung YJ, Kim JH, Park CH, Lee HH. Three-Port Right-Side Approach-Duet Totally Laparoscopic Distal Gastrectomy for Uncut Roux-en-Y Reconstruction. *J Laparoendosc Adv Surg Tech A* 2018; **28**: 1109-1114 [PMID: 30088978 DOI: 10.1089/lap.2018.0331]
61 **Ma JJ**, Zang L, Yang A, Hu WG, Feng B, Dong F, Wang ML, Lu AG, Li JW, Zheng MH. A modified uncut Roux-en-Y anastomosis in totally laparoscopic distal gastrectomy: preliminary results and initial experience. *Surg Endosc* 2017; **31**: 4749-4755 [PMID: 28411343 DOI: 10.1007/s00464-017-5551-8]
62 **Zang YF**, Li FZ, Ji ZP, Ding YL. Application value of enhanced recovery after surgery for total laparoscopic uncut Roux-en-Y gastrojejunostomy after distal gastrectomy. *World J Gastroenterol* 2018; **24**: 504-510 [PMID: 29398871 DOI: 10.3748/wjg.v24.i4.504]
63 **Park YS**, Shin DJ, Son SY, Kim KH, Park DJ, Ahn SH, Park DJ, Kim HH. Roux Stasis Syndrome and Gastric Food Stasis After Laparoscopic Distal Gastrectomy with Uncut Roux-en-Y Reconstruction in Gastric Cancer Patients: A Propensity Score Matching Analysis. *World J Surg* 2018; **42**: 4022-4032 [PMID: 29915987 DOI: 10.1007/s00268-018-4715-6]
64 **Shiraishi N**, Adachi Y, Kitano S, Kakisako K, Inomata M, Yasuda K. Clinical outcome of proximal versus total gastrectomy for proximal gastric cancer. *World J Surg* 2002; **26**: 1150-1154 [PMID: 12209245 DOI: 10.1007/s00268-002-6369-6]
65 **Nakamura M**, Yamaue H. Reconstruction after proximal gastrectomy for gastric cancer in the upper third of the stomach: a review of the literature published from 2000 to 2014. *Surg Today* 2016; **46**: 517-527 [PMID: 25987497 DOI: 10.1007/s00595-015-1185-4]

66 **Uyama I**, Sugioka A, Fujita J, Komori Y, Matsui H, Hasumi A. Completely laparoscopic proximal gastrectomy with jejunal interposition and lymphadenectomy. *J Am Coll Surg* 2000; **191**: 114-119 [PMID: 10898192 DOI: 10.1016/S1072-7515(00)00283-0]

67 **Namikawa T**, Oki T, Kitagawa H, Okabayashi T, Kobayashi M, Hanazaki K. Impact of jejunal pouch interposition reconstruction after proximal gastrectomy for early gastric cancer on quality of life: short- and long-term consequences. *Am J Surg* 2012; **204**: 203-209 [PMID: 22813641 DOI: 10.1016/j.amjsurg.2011.09.035]

68 **Ahn SH**, Jung DH, Son SY, Lee CM, Park DJ, Kim HH. Laparoscopic double-tract proximal gastrectomy for proximal early gastric cancer. *Gastric Cancer* 2014; **17**: 562-570 [PMID: 24052482 DOI: 10.1007/s10120-013-0303-5]

69 **Aikou T**, Natsugoe S, Shimazu H, Nishi M. Antrum preserving double tract method for reconstruction following proximal gastrectomy. *Jpn J Surg* 1988; **18**: 114-115 [PMID: 3386066 DOI: 10.1007/BF02470857]

70 **Hosogi H**, Yoshimura F, Yamaura T, Satoh S, Uyama I, Kanaya S. Esophagogastric tube reconstruction with stapled pseudo-fornix in laparoscopic proximal gastrectomy: a novel technique proposed for Siewert type II tumors. *Langenbecks Arch Surg* 2014; **399**: 517-523 [PMID: 24424495 DOI: 10.1007/s00423-014-1163-0]

71 **Chen S**, Li J, Liu H, Zeng J, Yang G, Wang J, Lu W, Yu N, Huang Z, Xu H, Zeng X. Esophagogastrostomy plus gastrojejunostomy: a novel reconstruction procedure after curative resection for proximal gastric cancer. *J Gastrointest Surg* 2014; **18**: 497-504 [PMID: 24163139 DOI: 10.1007/s11605-013-2391-2]

72 **Toyomasu Y**, Ogata K, Suzuki M, Yanoma T, Kimura A, Kogure N, Yanai M, Ohno T, Mochiki E, Kuwano H. Restoration of gastrointestinal motility ameliorates nutritional deficiencies and body weight loss of patients who undergo laparoscopy-assisted proximal gastrectomy. *Surg Endosc* 2017; **31**: 1393-1401 [PMID: 27444825 DOI: 10.1007/s00464-016-5127-z]

73 **Yamashita Y**, Yamamoto A, Tamamori Y, Yoshii M, Nishiguchi Y. Side overlap esophagogastrostomy to prevent reflux after proximal gastrectomy. *Gastric Cancer* 2017; **20**: 728-735 [PMID: 27942874 DOI: 10.1007/s10120-016-0674-5]

74 **Kuroda S**, Nishizaki M, Kikuchi S, Noma K, Tanabe S, Kagawa S, Shirakawa Y, Fujiwara T. Double-Flap Technique as an Antireflux Procedure in Esophagogastrostomy after Proximal Gastrectomy. *J Am Coll Surg* 2016; **223**: e7-e13 [PMID: 27157920 DOI: 10.1016/j.jamcollsurg.2016.04.041]

75 **Muraoka A**, Kobayashi M, Kokudo Y. Laparoscopy-Assisted Proximal Gastrectomy with the Hinged Double Flap Method. *World J Surg* 2016; **40**: 2419-2424 [PMID: 27094564 DOI: 10.1007/s00268-016-3510-5]

76 **Hayami M**, Hiki N, Nunobe S, Mine S, Ohashi M, Kumagai K, Ida S, Watanabe M, Sano T, Yamaguchi T. Clinical Outcomes and Evaluation of Laparoscopic Proximal Gastrectomy with Double-Flap Technique for Early Gastric Cancer in the Upper Third of the Stomach. *Ann Surg Oncol* 2017; **24**: 1635-1642 [PMID: 28130623 DOI: 10.1245/s10434-017-5782-x]

77 **Hosoda K**, Washio M, Mieno H, Moriya H, Ema A, Ushiku H, Watanabe M, Yamashita K. Comparison of double-flap and OrVil techniques of laparoscopy-assisted proximal gastrectomy in preventing gastroesophageal reflux: a retrospective cohort study. *Langenbecks Arch Surg* 2019; **404**: 81-91 [PMID: 30612151 DOI: 10.1007/s00423-018-1743-5]

78 **Aburatani T**, Kojima K, Otsuki S, Murase H, Okuno K, Gokita K, Tomii C, Tanioka T, Inokuchi M. Double-tract reconstruction after laparoscopic proximal gastrectomy using detachable ENDO-PSD. *Surg Endosc* 2017; **31**: 4848-4856 [PMID: 28389804 DOI: 10.1007/s00464-017-5539-4]

79 **Jung DH**, Lee Y, Kim DW, Park YS, Ahn SH, Park DJ, Kim HH. Laparoscopic proximal gastrectomy with double tract reconstruction is superior to laparoscopic total gastrectomy for proximal early gastric cancer. *Surg Endosc* 2017; **31**: 3961-3969 [PMID: 28342130 DOI: 10.1007/s00464-017-5429-9]

80 **Nomura E**, Kayano H, Lee SW, Kawai M, Machida T, Yamamoto S, Nabeshima K, Nakamura K, Mukai M, Uchiyama K. Functional evaluations comparing the double-tract method and the jejunal interposition method following laparoscopic proximal gastrectomy for gastric cancer: an investigation including laparoscopic total gastrectomy. *Surg Today* 2019; **49**: 38-48 [PMID: 30159780 DOI: 10.1007/s00595-018-1699-7]

81 **Cho M**, Son T, Kim HI, Noh SH, Choi S, Seo WJ, Roh CK, Hyung WJ. Similar hematologic and nutritional outcomes after proximal gastrectomy with double-tract reconstruction in comparison to total gastrectomy for early upper gastric cancer. *Surg Endosc* 2019; **33**: 1757-1768 [PMID: 30203207 DOI: 10.1007/s00464-018-6448-x]

82 **Park JY**, Park KB, Kwon OK, Yu W. Comparison of laparoscopic proximal gastrectomy with double-tract reconstruction and laparoscopic total gastrectomy in terms of nutritional status or quality of life in early gastric cancer patients. *Eur J Surg Oncol* 2018; **44**: 1963-1970 [PMID: 30197164 DOI: 10.1016/j.ejso.2018.08.014]

83 **Kim DJ**, Kim W. Laparoscopy-assisted Proximal Gastrectomy with Double Tract Anastomosis Is Beneficial for Vitamin B12 and Iron Absorption. *Anticancer Res* 2016; **36**: 4753-4758 [PMID: 27630323 DOI: 10.21873/anticanres.11031]

84 **Tanaka K**, Ebihara Y, Kurashima Y, Nakanishi Y, Asano T, Noji T, Murakami S, Nakamura T, Tsuchikawa T, Okamura K, Shichinohe T, Hirano S. Laparoscopic proximal gastrectomy with oblique jejunogastrostomy. *Langenbecks Arch Surg* 2017; **402**: 995-1002 [PMID: 28493146 DOI: 10.1007/s00423-017-1587-4]

85 **Yang K**, Bang HJ, Almadani ME, Dy-Abalajon DM, Kim YN, Roh KH, Lim SH, Son T, Kim HI, Noh SH, Hyung WJ. Laparoscopic Proximal Gastrectomy with Double-Tract Reconstruction by Intracorporeal Anastomosis with Linear Staplers. *J Am Coll Surg* 2016; **222**: e39-e45 [PMID: 26968319 DOI: 10.1016/j.jamcollsurg.2016.01.002]

86 **Hong J**, Qian L, Wang YP, Wang J, Hua LC, Hao HK. A novel method of delta-shaped intracorporeal double-tract reconstruction in totally laparoscopic proximal gastrectomy. *Surg Endosc* 2016; **30**: 2396-2403 [PMID: 26416371 DOI: 10.1007/s00464-015-4490-5]

87 **Xu X**, Huang C, Mou Y, Zhang R, Pan Y, Chen K, Lu C. Intra-corporeal hand-sewn esophagojejunostomy is a safe and feasible procedure for totally laparoscopic total gastrectomy: short-term outcomes in 100 consecutive patients. *Surg Endosc* 2018; **32**: 2689-2695 [PMID: 29101569 DOI: 10.1007/s00464-017-5964-4]

88 **Chen K**, Wu D, Pan Y, Cai JQ, Yan JF, Chen DW, Maher H, Mou YP. Totally laparoscopic gastrectomy using intracorporeally stapler or hand-sewn anastomosis for gastric cancer: a single-center experience of

478 consecutive cases and outcomes. *World J Surg Oncol* 2016; **14**: 115 [PMID: 27094509 DOI: 10.1186/s12957-016-0868-7]
89 **Liu W**, Guo Y, Qiu Z, Niu D, Zhang J. Intracorporeal Circular Stapled Esophagojejunostomy Using Conventional Purse-String Suture Instrument After Laparoscopic Total Gastrectomy. *J Laparoendosc Adv Surg Tech A* 2017; **27**: 1299-1304 [PMID: 28414614 DOI: 10.1089/lap.2016.0675]
90 **Usui S**, Nagai K, Hiranuma S, Takiguchi N, Matsumoto A, Sanada K. Laparoscopy-assisted esophagoenteral anastomosis using endoscopic purse-string suture instrument "Endo-PSI (II)" and circular stapler. *Gastric Cancer* 2008; **11**: 233-237 [PMID: 19132486 DOI: 10.1007/s10120-008-0481-8]
91 **Kinoshita T**, Oshiro T, Ito K, Shibasaki H, Okazumi S, Katoh R. Intracorporeal circular-stapled esophagojejunostomy using hand-sewn purse-string suture after laparoscopic total gastrectomy. *Surg Endosc* 2010; **24**: 2908-2912 [PMID: 20383532 DOI: 10.1007/s00464-010-1041-y]
92 **Du J**, Shuang J, Li J, Li J, Hua J. Intracorporeal circular-stapled esophagojejunostomy after laparoscopic total gastrectomy: a novel self-pulling and holding purse-string suture technique. *J Am Coll Surg* 2014; **218**: e67-e72 [PMID: 24559969 DOI: 10.1016/j.jamcollsurg.2013.11.023]
93 **Jeong O**, Park YK. Intracorporeal circular stapling esophagojejunostomy using the transorally inserted anvil (OrVil) after laparoscopic total gastrectomy. *Surg Endosc* 2009; **23**: 2624-2630 [PMID: 19343421 DOI: 10.1007/s00464-009-0461-z]
94 **Omori T**, Oyama T, Mizutani S, Tori M, Nakajima K, Akamatsu H, Nakahara M, Nishida T. A simple and safe technique for esophagojejunostomy using the hemidouble stapling technique in laparoscopy-assisted total gastrectomy. *Am J Surg* 2009; **197**: e13-e17 [PMID: 19101245 DOI: 10.1016/j.amjsurg.2008.04.019]
95 **Kosuga T**, Hiki N, Nunobe S, Ohashi M, Kubota T, Kamiya S, Sano T, Yamaguchi T. Does the Single-Stapling Technique for Circular-Stapled Esophagojejunostomy Reduce Anastomotic Complications After Laparoscopic Total Gastrectomy? *Ann Surg Oncol* 2015; **22**: 3606-3612 [PMID: 25663594 DOI: 10.1245/s10434-015-4417-3]
96 **Wang H**, Hao Q, Wang M, Feng M, Wang F, Kang X, Guan WX. Esophagojejunostomy after laparoscopic total gastrectomy by OrVil™ or hemi-double stapling technique. *World J Gastroenterol* 2015; **21**: 8943-8951 [PMID: 26269685 DOI: 10.3748/wjg.v21.i29.8943]
97 **Kawamura H**, Ohno Y, Ichikawa N, Yoshida T, Homma S, Takahashi M, Taketomi A. Anastomotic complications after laparoscopic total gastrectomy with esophagojejunostomy constructed by circular stapler (OrVil™) versus linear stapler (overlap method). *Surg Endosc* 2017; **31**: 5175-5182 [PMID: 28488177 DOI: 10.1007/s00464-017-5584-z]
98 **Lu X**, Hu Y, Liu H, Mou T, Deng Z, Wang D, Yu J, Li G. Short-term outcomes of intracorporeal esophagojejunostomy using the transorally inserted anvil versus extracorporeal circular anastomosis during laparoscopic total gastrectomy for gastric cancer: a propensity score matching analysis. *J Surg Res* 2016; **200**: 435-443 [PMID: 26421708 DOI: 10.1016/j.jss.2015.08.013]
99 **Tokuhara T**, Nakata E, Tenjo T, Kawai I, Kondo K, Ueda H, Tomioka A. Stenosis after esophagojejunostomy with the hemi-double-stapling technique using the transorally inserted anvil (OrVil™) in Roux-en-Y reconstruction with its efferent loop located on the patient's left side following laparoscopic total gastrectomy. *Surg Endosc* 2019; **33**: 2128-2134 [PMID: 30341648 DOI: 10.1007/s00464-018-6484-6]
100 **Yasukawa D**, Hori T, Kadokawa Y, Kato S, Machimoto T, Hata T, Aisu Y, Sasaki M, Kimura Y, Takamatsu Y, Ito T, Yoshimura T. Impact of stepwise introduction of esophagojejunostomy during laparoscopic total gastrectomy: a single-center experience in Japan. *Ann Gastroenterol* 2017; **30**: 564-570 [PMID: 28845113 DOI: 10.20524/aog.2017.0157]
101 **Inokuchi M**, Otsuki S, Fujimori Y, Sato Y, Nakagawa M, Kojima K. Systematic review of anastomotic complications of esophagojejunostomy after laparoscopic total gastrectomy. *World J Gastroenterol* 2015; **21**: 9656-9665 [PMID: 26327774 DOI: 10.3748/wjg.v21.i32.9656]
102 **Kawaguchi Y**, Shiraishi K, Akaike H, Ichikawa D. Current status of laparoscopic total gastrectomy. *Ann Gastroenterol Surg* 2018; **3**: 14-23 [PMID: 30697606 DOI: 10.1002/ags3.12208]
103 **Li X**, Hong L, Ding D, Liu Y, Niu G, Li L, Wang X, Li X, Ke C. Comparison of OrVil™ and RPD in laparoscopic total gastrectomy for gastric cancer. *Surg Endosc* 2017; **31**: 4773-4779 [PMID: 28409368 DOI: 10.1007/s00464-017-5554-5]
104 **Uyama I**, Sugioka A, Fujita J, Komori Y, Matsui H, Hasumi A. Laparoscopic total gastrectomy with distal pancreatosplenectomy and D2 lymphadenectomy for advanced gastric cancer. *Gastric Cancer* 1999; **2**: 230-234 [PMID: 11957104 DOI: 10.1007/s101209900041]
105 **Inaba K**, Satoh S, Ishida Y, Taniguchi K, Isogaki J, Kanaya S, Uyama I. Overlap method: novel intracorporeal esophagojejunostomy after laparoscopic total gastrectomy. *J Am Coll Surg* 2010; **211**: e25-e29 [PMID: 21036074 DOI: 10.1016/j.jamcollsurg.2010.09.005]
106 **Kwon IG**, Son YG, Ryu SW. Novel Intracorporeal Esophagojejunostomy Using Linear Staplers During Laparoscopic Total Gastrectomy: π-Shaped Esophagojejunostomy, 3-in-1 Technique. *J Am Coll Surg* 2016; **223**: e25-e29 [PMID: 27370184 DOI: 10.1016/j.jamcollsurg.2016.06.011]
107 **Miura S**, Kanaya S, Hosogi H, Kawada H, Akagawa S, Shimoike N, Okumura S, Okada T, Ito T, Arimoto A. Esophagojejunostomy With Linear Staplers in Laparoscopic Total Gastrectomy: Experience With 168 Cases in 5 Consecutive Years. *Surg Laparosc Endosc Percutan Tech* 2017; **27**: e101-e107 [PMID: 28902037 DOI: 10.1097/SLE.0000000000000464]
108 **Kyogoku N**, Ebihara Y, Shichinohe T, Nakamura F, Murakawa K, Morita T, Okushiba S, Hirano S. Circular versus linear stapling in esophagojejunostomy after laparoscopic total gastrectomy for gastric cancer: a propensity score-matched study. *Langenbecks Arch Surg* 2018; **403**: 463-471 [PMID: 29744579 DOI: 10.1007/s00423-018-1678-x]
109 **Gong CS**, Kim BS, Kim HS. Comparison of totally laparoscopic total gastrectomy using an endoscopic linear stapler with laparoscopic-assisted total gastrectomy using a circular stapler in patients with gastric cancer: A single-center experience. *World J Gastroenterol* 2017; **23**: 8553-8561 [PMID: 29358863 DOI: 10.3748/wjg.v23.i48.8553]
110 **Huang ZN**, Huang CM, Zheng CH, Li P, Xie JW, Wang JB, Lin JX, Lu J, Chen QY, Cao LL, Lin M, Tu RH, Lin JL. Digestive tract reconstruction using isoperistaltic jejunum-later-cut overlap method after totally laparoscopic total gastrectomy for gastric cancer: Short-term outcomes and impact on quality of life. *World J Gastroenterol* 2017; **23**: 7129-7138 [PMID: 29093621 DOI: 10.3748/wjg.v23.i39.7129]
111 **Chen K**, Pan Y, Cai JQ, Xu XW, Wu D, Yan JF, Chen RG, He Y, Mou YP. Intracorporeal esophagojejunostomy after totally laparoscopic total gastrectomy: A single-center 7-year experience. *World J Gastroenterol* 2016; **22**: 3432-3440 [PMID: 27022225 DOI: 10.3748/wjg.v22.i12.3432]
112 **Yoshikawa K**, Shimada M, Higashijima J, Tokunaga T, Nishi M, Takasu C, Kashihara H, Ishikawa D.

Usefulness of the Transoral Anvil Delivery System for Esophagojejunostomy After Laparoscopic Total Gastrectomy: A Single-institution Comparative Study of Transoral Anvil Delivery System and the Overlap Method. *Surg Laparosc Endosc Percutan Tech* 2018; **28**: e40-e43 [PMID: 29064880 DOI: 10.1097/SLE.0000000000000495]

113 **Lianos GD**, Hasemaki N, Glantzounis GK, Mitsis M, Rausei S. Assessing safety and feasibility of 'pure' laparoscopic total gastrectomy for advanced gastric cancer in the West. Review article. *Int J Surg* 2018; **53**: 275-278 [PMID: 29602017 DOI: 10.1016/j.ijsu.2018.03.048]

114 **Brenkman HJ**, Correa-Cote J, Ruurda JP, van Hillegersberg R. A Step-Wise Approach to Total Laparoscopic Gastrectomy with Jejunal Pouch Reconstruction: How and Why We Do It. *J Gastrointest Surg* 2016; **20**: 1908-1915 [PMID: 27561635 DOI: 10.1007/s11605-016-3235-7]

115 **Ali B**, Park CH, Song KY. Intracorporeal esophagojejunostomy using hemi-double-stapling technique after laparoscopic total gastrectomy in gastric cancer patients. *Ann Surg Treat Res* 2017; **92**: 30-34 [PMID: 28090503 DOI: 10.4174/astr.2017.92.1.30]

116 **Duan W**, Liu K, Fu X, Shen X, Chen J, Su C, Yu P, Zhao Y. Semi-end-to-end esophagojejunostomy after laparoscopy-assisted total gastrectomy better reduces stricture and leakage than the conventional end-to-side procedure: A retrospective study. *J Surg Oncol* 2017; **116**: 177-183 [PMID: 28420040 DOI: 10.1002/jso.24637]

117 **Lee TG**, Lee IS, Yook JH, Kim BS. Totally laparoscopic total gastrectomy using the overlap method; early outcomes of 50 consecutive cases. *Surg Endosc* 2017; **31**: 3186-3190 [PMID: 27933396 DOI: 10.1007/s00464-016-5343-6]

118 **Son SY**, Cui LH, Shin HJ, Byun C, Hur H, Han SU, Cho YK. Modified overlap method using knotless barbed sutures (MOBS) for intracorporeal esophagojejunostomy after totally laparoscopic gastrectomy. *Surg Endosc* 2017; **31**: 2697-2704 [PMID: 27699517 DOI: 10.1007/s00464-016-5269-z]

119 **Kitagami H**, Morimoto M, Nakamura K, Watanabe T, Kurashima Y, Nonoyama K, Watanabe K, Fujihata S, Yasuda A, Yamamoto M, Shimizu Y, Tanaka M. Technique of Roux-en-Y reconstruction using overlap method after laparoscopic total gastrectomy for gastric cancer: 100 consecutively successful cases. *Surg Endosc* 2016; **30**: 4086-4091 [PMID: 26701704 DOI: 10.1007/s00464-015-4724-6]

120 **Kim EY**, Choi HJ, Cho JB, Lee J. Totally Laparoscopic Total Gastrectomy Versus Laparoscopically Assisted Total Gastrectomy for Gastric Cancer. *Anticancer Res* 2016; **36**: 1999-2003 [PMID: 27069193]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 37-53

DOI: 10.4251/wjgo.v12.i1.37

ISSN 1948-5204 (online)

MINIREVIEWS

Interpretation of the development of neoadjuvant therapy for gastric cancer based on the vicissitudes of the NCCN guidelines

Xian-Ze Wang, Zi-Yang Zeng, Xin Ye, Juan Sun, Zi-Mu Zhang, Wei-Ming Kang

ORCID number: Xian-Ze Wang (0000-0002-7612-0020); Zi-Yang Zeng (0000-0003-0725-142X); Xin Ye (0000-0001-8355-4516); Juan Sun (0000-0003-1351-1208); Zi-Mu Zhang (0000-0001-6901-525X); Wei-Ming Kang (0000-0001-8128-8453).

Author contributions: Wang XZ wrote the manuscript and prepared the tables; Zeng ZY organized the references; Ye X contributed to verifying the accuracy of the manuscript; Sun J and Zhang ZM contributed to the writing of the manuscript; Kang WM designed the aim of the manuscript and wrote the manuscript.

Supported by Beijing Municipal Science and Technology Project, No. D171100006517004; CSCO-ROCHE Research Fund, No. Y-2019Roche-015.

Conflict-of-interest statement: All of the authors declare no conflicts of interest to this work.

Xian-Ze Wang, Zi-Yang Zeng, Xin Ye, Juan Sun, Zi-Mu Zhang, Wei-Ming Kang, Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100730, China

Corresponding author: Wei-Ming Kang, MD, Professor, Department of General Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100730, China. kangweiming@163.com

Abstract

Gastric cancer is one of the most common digestive system tumors in China, and locally advanced gastric cancer (LAGC) accounts for a high proportion of newly diagnosed cases. Although surgery is the main treatment for gastric cancer, surgical excision alone cannot achieve satisfactory outcomes in LAGC patients. Neoadjuvant therapy (NAT) has gradually become the standard treatment for patients with LAGC, and this treatment can not only achieve tumor downstaging and improve surgical rate and the R0 resection rate, but it also significantly improves the long-term prognosis of patients. Peri/preoperative neoadjuvant chemotherapy and preoperative chemoradiotherapy are both recommended according to a large number of studies, and the regimens have also been evolved in the past decades. Since the NCCN guidelines for gastric cancer are one of the most authoritative evidence-based guidelines worldwide, here, we demonstrate the development course and major breakthroughs of NAT for gastric cancer based on the vicissitudes of the NCCN guidelines from 2007 to 2019, and also discuss the future of NAT.

Key words: Gastric cancer; Locally advanced gastric cancer; Neoadjuvant therapy; Neoadjuvant chemotherapy; Neoadjuvant chemoradiotherapy; NCCN guidelines

Core tip: Surgical excision is one of the most effective ways in treating nonmetastatic gastric cancer. However, surgery alone cannot achieve satisfactory therapeutic effects in locally advanced gastric cancer (LAGC), and the 5-year survival rate of LAGC patients is less than 50%. Neoadjuvant therapy (NAT) aims at improving the surgical and R0 resection rate and decreasing the recurrence of micrometastases of LAGC. The strategies of NAT have been continuously developed in the past decades, and the evolvements can be reflected from the vicissitudes of the NCCN guidelines. Moreover, targeted therapy and individualized treatment may be the next hotspots of NAT, and may further improve

Manuscript source: Unsolicited manuscript

Received: August 3, 2019
Peer-review started: August 3, 2019
First decision: August 23, 2019
Revised: September 9, 2019
Accepted: September 26, 2019
Article in press: September 26, 2019
Published online: January 15, 2020

P-Reviewer: Ahmed M, Jeong KY, Mohamed SY, Tanabe S, Tsushima T
S-Editor: Zhang L
L-Editor: Wang TQ
E-Editor: Qi LL

the prognosis of LAGC patients.

Citation: Wang XZ, Zeng ZY, Ye X, Sun J, Zhang ZM, Kang WM. Interpretation of the development of neoadjuvant therapy for gastric cancer based on the vicissitudes of the NCCN guidelines. *World J Gastrointest Oncol* 2020; 12(1): 37-53
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/37.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.37

INTRODUCTION

Gastric cancer (GC) is the most common tumor of the digestive system. GLOBOCAN estimated approximately 1.034 million newly diagnosed GC cases worldwide in 2018, which accounted for 5.7% of all tumors and ranked fifth among all cancers. GC is also the third leading cause of cancer-related deaths, as 0.783 million deaths were caused by GC in 2018, which accounted for 8.2% of all cancer deaths[1]. The incidence of GC in Asia is much higher than that in other countries and regions. The incidence of GC in East Asia is approximately 32.1/100000, and the mortality rate is as high as 13.2/100000[1]. Moreover, the current situation of GC in China is far more serious. First, the number of GC patients in China accounts for a substantial proportion of all GC cases worldwide, with approximately 679000 newly diagnosed cases and 498000 deaths each year[2,3]. Second, the early diagnosis of GC in China is still in its initial stage. Patients with stage II-III GC account for 58.0% of the GC cases in China, while in South Korea and Japan, patients with stage II-III account for only 22.5% and 24.9% of all GC cases, respectively[4,5]. As the 5-year survival rate of patients with locally advanced gastric cancer (LAGC) plunges dramatically, ways to improve the treatment effect and prognosis of these patients have become a primary focus in China and even worldwide.

THE RISE OF NEOADJUVANT THERAPY FOR GC

Surgery is the most effective treatment for nonmetastatic GC, and the cure rate for stage T1 cancer can reach 90% after surgery. However, many patients with LAGC will experience tumor recurrence within 1 year after surgery, even those with R0 resection, and the 5-year survival rate of these patients is less than 50%[6,7]. Most scholars believe that surgical excision alone cannot achieve satisfactory outcomes in LAGC, and thus neoadjuvant therapy (NAT) was developed.

The concept of NAT was first proposed by Frei in 1982[8], and it has also been referred to as preoperative chemotherapy. In the 1990s, Wilke, Plukker, Mai, and other scholars began to apply preoperative chemotherapy in the treatment of GC. They found that preoperative chemotherapy could achieve tumor downstaging, improve the tumor resection rate, and prolong the postoperative survival time of LAGC patients[9-11]. The above study served as the prelude to NAT for LAGC, but conceptually, they should be considered as the conversion therapy. Currently, NAT is applicable to LAGC patients with resectable lesions at initial diagnosis. The purpose of NAT is to further reduce the lesion size, improve the R0 resection rate, inhibit micrometastases, reduce the risk of tumor recurrence, and determine the sensitivity of patients to the corresponding treatment in advance[9,12,13].

NAT strategies for LAGC patients have been developed and continuously improved in recent decades. Studies have mainly focused on the patterns, indications, and the optimal regimens of NAT, as well as the response assessment and additional management after NAT and surgery. We will elaborate on the development and major breakthroughs of NAT for GC based on the vicissitudes of the NCCN guidelines for GC, and assess the future of this therapy.

THE PATTERN OF NAT FOR LAGC

Most NAT schemes referred to adjuvant therapy for gastric cancer. Currently, the NCCN guidelines recommend both perioperative chemotherapy (category 1) and preoperative chemoradiotherapy (category 2B) as alternatives to NAT for LAGC (see related studies and detailed recommendations in Tables 1 and 2).

Table 1 Important studies of neoadjuvant therapy for gastric cancer

Ref.	Number	Characteristics of patients	Arms and interventions	Outcomes and conclusions
FAMTX, Hartgrink *et al*[37], 2004	59	Nonmetastatic resectable cancer of the stomach	Preoperative FAMTX chemotherapy and surgery *vs* surgery alone	FAMTX could not bring benefits to resectability rates or survival
MAGIC, Cunningham *et al*[14], 2006	503	Operable and nonmetastatic cancer of the stomach or lower esophagus, ≥ stage II	Perioperative ECF chemotherapy and surgery *vs* surgery alone	Perioperative ECF decreased tumor sizes and stages and improved PFS and OS
REAL-2, Cunningham *et al*[39], 2008	1002	Inoperable or metastatic cancer of the esophagus, EGJ, or stomach	Randomly received ECF, ECX, EOF, and EOX chemotherapy	Capecitabine and oxaliplatin were as effective as fluorouracil and cisplatin, respectively
EORTC 40954, Schuhmacher *et al*[15], 2010	144	Stages III and IV (cM0) cancer of the EGJ or stomach	Preoperative chemotherapy (cisplatin, leucovorin, and fluorouracil) and surgery *vs* surgery alone	Increased R0 resection rate, failed to demonstrate a survival benefit
FNCLCC and FFCD 9703, Ychou *et al*[16], 2011	224	Resectable lower esophagus, EGJ, or stomach cancer	Perioperative FP chemotherapy and surgery *vs* surgery alone	Perioperative FP improved curative surgical rate, OS, and DFS
V325, van Cutsem *et al*[42], 2006	445	Gastric or EGJ cancer with measurable metastatic disease or locally recurrent disease of lymph nodes	DCF chemotherapy *vs* CF chemotherapy	DCF prolonged the time-to-progression and OS, but associated with more adverse events
FLOT AIO, Al-Batran *et al*[43], 2008	59	Measurable metastatic cancer of the EGJ or stomach	Single arm, biweekly FLOT chemotherapy	Biweekly FLOT was effective and well tolerated
FLOT65+, Al-Batran *et al*[44], 2013	143	Locally advanced or metastatic esophagogastric cancer, age ≥ 65	FLO chemotherapy *vs* FLOT chemotherapy	FLOT improved response rates and PFS, but increased adverse events
Kim *et al*[20], 2012	129	Metastatic or recurrent gastric cancer	SOX chemotherapy *vs* CAPOX chemotherapy	SOX and CAPOX were equally effective and well tolerated
FLOT4, Al-Batran *et al*[17,18], 2016, 2019	300 (phase II), 716 (phase III)	Resectable gastric or EGJ cancer, staged ≥ cT2 and/or cN+	Perioperative ECF/ECX chemotherapy *vs* perioperative FLOT chemotherapy	FLOT achieved more pCR and increased medial survival time and OS than ECF/ECX
CALGB 80403/E1206, Enzinger *et al*[19], 2016	245	Measurable metastatic cancer of the esophagus or EGJ	ECF-C chemotherapy *vs* IC-C chemotherapy *vs* FOLFOX-C chemotherapy	FOLFOX and ECF regimen had similar efficacy, and FOLFOX was better tolerated
ACTS-GC, Sakuramoto *et al*[114], 2011	1059	Nonmetastatic gastric cancer staged as II, IIIA, or IIIB	Surgery and postoperative S-1 chemotherapy *vs* surgery alone	S-1 could prolong the 5-year OS and 5-year RFS rate
FLAGS, Ajani *et al*[102], 2010	1053	Unresectable, locally advanced or metastatic gastric or EGJ cancer	Cisplatin/S-1 chemotherapy *vs* cisplatin/fluorouracil chemotherapy	Cisplatin/S-1 could not prolong the OS but could improve safety profile
INT-0116, Macdonald *et al*[24], 2001	556	Operable cancer of the EGJ or stomach	Surgery and postoperative chemoradiotherapy *vs* surgery alone	Postoperative chemoradiotherapy prolonged the OS and RFS time
RTOG 9904, Ajani *et al*[25], 2006	49	Localized cancer of the EGJ or stomach, staged as T2-3N0-1 or T1N1	Single arm, induction chemotherapy, chemoradiotherapy, and surgery	Achieved a pCR rate of 26% and a R0 resection rate of 77%
CROSS, van Hagen *et al*[27], 2012	368	Resectable cancer of the esophagus or EGJ, staged as T1N1M0 or T2-3N0-1M0	Preoperative chemoradiotherapy and surgery *vs* surgery alone	Preoperative chemoradiotherapy improved survival and was well tolerated
FFCD 9102, Bedenne *et al*[22], 2007	444	Operable T3N0-1M0 cancer of the thoracic esophagus	Additional surgery *vs* additional chemoradiotherapy	Additional surgery had no benefits among patients who responded to chemoradiotherapy
CALGB 9781, Tepper *et al*[26], 2008	56	Operable cancer of the thoracic esophagus or EGJ, staged as T1-3, N1	Preoperative induction chemotherapy, chemoradiotherapy, and surgery *vs* surgery alone	The trimodality therapy improved median survival and 5-year survival

POET, Stahl *et al*[28], 2017	119	Locally advanced cancer of the EGJ, staged as T3 and T4	Chemotherapy and surgery *vs* induction chemotherapy, chemoradiotherapy, and surgery	Induction chemotherapy and chemoradiotherapy could prolong PFS

FAMTX: Fluorouracil, doxorubicin, and methotrexate; ECF: Epirubicin, cisplatin, and fluorouracil; ECX: Epirubicin, cisplatin, and capecitabine; EOF: Epirubicin, oxaliplatin, and fluorouracil; EOX: Epirubicin, oxaliplatin, and capecitabine; DCF: Docetaxel, cisplatin, and fluorouracil; CF: Cisplatin and fluorouracil; FLO: Fluorouracil, leucovorin, and oxaliplatin; FLOT: Docetaxel, fluorouracil, leucovorin, and oxaliplatin; SOX: S-1 and oxaliplatin; CAPOX: Capecitabine and oxaliplatin; ECF-C: ECF and cetuximab; IC-C: Irinotecan, cisplatin, and cetuximab; FOLFOX-C: Oxaliplatin, fluorouracil, leucovorin, and cetuximab; EGJ: Esophagogastric junction; pCR: Pathological complete regression; PFS: Progression-free survival; OS: Overall survival; RFS: Relapse-free survival.

Pre/perioperative neoadjuvant chemotherapy

Although Wilke *et al*[10] have revealed the positive effect of preoperative chemotherapy on LAGC patients through various studies, it was not until 2006 that the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) study in the United Kingdom verified this conclusion through a large-scale randomized controlled trial (RCT). The MAGIC study confirmed that perioperative chemotherapy could achieve tumor downstaging and improve the R0 resection rate in patients with resectable LAGC. Additionally, perioperative chemotherapy and surgery can significantly prolong the progression-free survival (PFS) and overall survival (OS) of patients compared with surgery alone[14]. This landmark study prompted the NCCN guidelines to incorporate preoperative neoadjuvant chemotherapy (NACT) into the standard treatment procedures for LAGC in 2007.

The conclusions of the MAGIC study were subsequently validated by other clinical trials. In 2010, the European Organization for Research and Treatment of Cancer Randomized Trial 40954 (EORTC 40954) study confirmed the significant effect of preoperative chemotherapy in improving the R0 resection rate (81.9% *vs* 66.7%, P = 0.036) and reducing the lymph node metastasis rate (61.4% *vs* 76.5%, P = 0.018) of LAGC patients[15]. The Fédération Nationale des Centres de Lutte contre le Cancer and Fédération Francophone de Cancérologie Digestive 9703 (FNCLCC and FFCD 9703) study published in 2011 not only reached similar conclusions, but also verified the advantages of perioperative chemotherapy in prolonging the 5-year disease-free survival rate (DFS) and OS of patients compared with surgery alone[16]. The FLOT4 (Fluorouracil, leucovorin, oxaliplatin, docetaxel) study published in 2016 and 2019 indicated that NACT can achieve a high pathological complete regression (pCR) rate and significantly prolong the survival of patients[17,18]. At this point, pre/perioperative NACT became a mature scheme with definite efficacy and sufficient evidence and has been listed as a category 1 recommendation in the NCCN guidelines since 2007 (Table 2).

The specific schedules of NACT proposed by the MAGIC, FNCLCC and FFCD 9703, and FLOT4 trials all consist of preoperative and postoperative chemotherapy (also known as perioperative chemotherapy). However, due to the dissatisfactory commencing rates of postoperative chemotherapy in these studies (137/209 (65.6%), 54/109 (49.5%), and 78/119 (65.5%) for MAGIC, FNCLCC and FFCD 9703, and FLOT4 studies, respectively) and even lower completion rates (104/209 (49.8%), 25/109 (22.9%), and 60/119 (55.0%), respectively), the benefits of postoperative chemotherapy were inconclusive. Thus, NCCN guidelines only initially recommended preoperative chemotherapy as the primary treatment for certain LAGC patients, and this recommendation was revised to include perioperative chemotherapy when more evidence became available in 2016.

Although undisputed benefits of perioperative chemotherapy have been presented by many clinical trials (Table 1), the category 1 recommendation made by NCCN guidelines was mainly derived from the above three landmark studies (the MAGIC, FNCLCC and FFCD 9703, and FLOT4 studies)[14,16,17]. Sequentially, the dosing schedules of recommended regimens were also based on these three or their relevant studies (except for fluorouracil and oxaliplatin regimen, Table 2)[19-21].

Preoperative neoadjuvant chemoradiotherapy

Chemoradiotherapy plays an important role in treating esophageal cancer. The Fédération Francophone de Cancérologie Digestive 9102 (FFCD 9102) study reported that, for locally advanced thoracic esophageal cancer patients who responded to chemoradiation, the additional surgery could provide no benefit comparing with the continuation of additional chemoradiation[22]. Due to the successful treatment of esophageal cancer with chemoradiotherapy, scholars attempted to expand this treatment to GC, especially to lower esophageal and esophagogastric junction (EGJ) cancers[23].

Table 2 The vicissitudes of the recommendation categories of different neoadjuvant chemotherapy regimens in the NCCN gastric cancer guidelines

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019.V1	2019. V2
ECF[14]	1	1	1	1	1	1	1	1	1	2B	2B			
ECF modifications[39,40]		1	1	1	1	1	1	2A	2A	2B	2B			
Fluorouracil and cisplatin[16]							1	1	1	1	1	1	1	1
[a]Fluorouracil and oxaliplatin[19-21]											2A	2A[b]	2A[b]	2A[b]
FLOT[17]												1[b]	1[b]	1[b]

References quoted in Table 2 for each regimen were based on NCCN guidelines from 2007 to 2019. 1, 2A, 2B: Categories of recommendations.
[b]: Preferred intervention.
[a]: This regimen was based on extrapolations from literature and clinical practice according to NCCN guidelines, and was revised to fluoropyrimidine and oxaliplatin in 2017 NCCN guidelines. ECF: Epirubicin, cisplatin, and fluorouracil; FLOT: Docetaxel, fluorouracil, leucovorin, and oxaliplatin.

In 2001, the Intergroup-0116 (INT-0116) study found that postoperative chemoradiotherapy could significantly prolong the median OS of patients with EGJ or gastric adenocarcinoma (36 mo *vs* 27 mo, $P = 0.005$) compared with surgery alone[24]. In 2006, the Radiation Therapy Oncology Group 9904 (RTOG 9904) study reported that preoperative induction chemotherapy and sequential chemoradiotherapy could achieve a high pCR rate and R0 resection rate in patients with localized gastric adenocarcinoma[25]. Subsequently, both of the large-scale clinical trials in the United States (Cancer and Leukemia Group B 9781 study, CALGB 9781 study) and the Netherlands (Chemoradiotherapy for Oesophageal Cancer Followed by Surgery Study, CROSS study) confirmed that preoperative chemoradiotherapy could indeed achieve a satisfactory pCR rate and improve the R0 resection rate, and it could also prolong the median survival time and 5-year survival rate of patients with lower esophageal and EGJ cancers[26,27]. As a result, preoperative chemoradiotherapy was recommended as the preferred approach for localized EGJ adenocarcinoma (for Siewert type III EGJ cancer, hereinafter the same) according to the NCCN guidelines from 2012 to 2014[27]. In 2017, the PreOperative therapy in Esophagogastric adenocarcinoma Trial concluded that preoperative induction chemotherapy and chemoradiotherapy might have better therapeutic effects on EGJ cancer than preoperative chemotherapy alone, which would significantly improve the local PFS after resection ($P = 0.01$) and had a trend in prolonging the OS of patients (39.5% *vs* 24.4%, $P = 0.055$)[28].

However, most scholars still believe that, since the incidence, geographical distribution, etiology, disease course, and biological behavior of EGJ cancers are different from those of true gastric (noncardia) cancers, the overall efficacy of neoadjuvant chemoradiotherapy remains inconclusive[29]. Since the effects of preoperative chemoradiotherapy in resectable GC were only proposed by small-scale and single-arm studies, the regimens and dosing schedules listed in NCCN guidelines were based on trials that recruited esophageal and/or EGJ cancers patients[22,25-27,30-35]. Therefore, the recommendation category of preoperative chemoradiotherapy remains in category 2B according to the latest NCCN guidelines. More than that, since there have not been enough studies compared the effect of pre/perioperative chemotherapy with chemoradiotherapy, the preferred recommendation of preoperative chemoradiotherapy for localized EGJ (Siewert type III) adenocarcinoma was also deleted in the 2015 NCCN guidelines. In the following sections, we will focus more on the development of neoadjuvant chemotherapy for LAGC.

THE APPLICABLE POPULATION OF NAT

Studies that specifically focused on the applicable population of NAT are still lacking. However, since NAT aims to improve the surgical outcomes in LAGC patients and the cure rate of T1 gastric cancer could reach 90% after surgery, most clinical trials enrolled patients with tumor ≥ T2/T3 and with/without lymph node metastasis invariably. Meanwhile, cytotoxic agents used in NACT are more efficient for metabolically active and/or proliferating tumor cells. Since the proliferation of tumor cells *in vivo*, which conforms to the Gompertzian model[36], will be retarded along with the growth of tumor and the accumulation of necrosis and metabolites, the sensitivity to chemotherapy will also decline. These concepts serve as the basis for establishing the applications of NAT and reflect its original intention.

The NCCN guidelines have made minor alterations on the applicable population of NAT in the past decade. NAT was initially recommended for patients who are medically fit and with potentially resectable LAGC with clinical stage ≥ T2 or N+. Since 2012, the guidelines have neglected lymph node metastasis and recommend NAT for the abovementioned patients with clinical stage ≥ T2.

THE EVOLUTION OF NEOADJUVANT CHEMOTHERAPY REGIMENS

The efficacy and side effect must be weighted before performing NACT. Two-drug regimens were preferred according to the NCCN guidelines in principle because of their lower toxicity. And three-drug regimens may be applied in medically fit patients with access to frequent evaluation during treatment, to ensure that they can still tolerate surgery after NACT.

ECF and ECF modifications

Fluorouracil, doxorubicin, and methotrexate (FAMTX) was one of the first attempts used in NACT for gastric cancer, but it failed to bring benefits to LAGC patients[37]. Some scholars attributed the failure to the low effectiveness of this regimen, and Webb *et al*[38] did confirm that the efficacy of epirubicin, cisplatin, and fluorouracil (ECF) significantly surpassed that of FAMTX in patients with unresectable GC. With forethought, Cunningham *et al*[39], one of the originators of the ECF regimen, conducted the MAGIC study with landmark significance.

The MAGIC study enrolled 503 patients with nonmetastatic and operable lower esophageal cancer or GC who randomly received perioperative chemotherapy (ECF regimen, 3 cycles before and after surgery) and surgery or surgery alone. The results indicated that preoperative chemotherapy did not increase either postoperative complications or 30-day mortalities. Moreover, NACT resulted in tumor downstaging (T stage, $P = 0.002$; N stage, $P = 0.01$) and a higher R0 resection rate (79.3% *vs* 70.3%, $P = 0.03$). The PFS ($P < 0.001$) and 5-year survival rates (36.3% *vs* 23.0%, $P = 0.009$) were also improved significantly in patients who received NACT. Therefore, the NCCN guidelines began to adopt ECF as the standard regimen for neoadjuvant chemotherapy (category 1) in 2007.

To control the adverse effects and clinical practice difficulties of the ECF regimen, Cunningham *et al*[39] initiated the Randomized ECF for Advanced and Locally Advanced Esophagogastric Cancer 2 (REAL-2) study in 2000[40]. Based on the ECF regimen, the REAL-2 study inspected the substitution of oxaliplatin (O) and capecitabine (X) for cisplatin (C) and fluorouracil (F) in patients with inoperable or metastatic esophageal, EGJ, or gastric cancer. The results confirmed that the incidences of side effects among ECF, ECX, EOF, and EOX (E, epirubicin) were similar ($P > 0.05$); it was also found that the EOX regimen was superior to the ECF regimen in prolonging the OS ($P = 0.02$) of patients. Moreover, the advantages of oral administration of capecitabine and the needlessness of persistent intravenous hydration of oxaliplatin reduce the admission time and frequency for patients. The REAL-2 study was published in 2008, and the three ECF modifications were subsequently adopted by the NCCN as the standard regimens (category 1). In addition, the substitutability between cisplatin and oxaliplatin, as well as infusional fluorouracil and capecitabine, was recognized by the guidelines. At this point, Cunningham *et al*[39] established the first-line status of ECF and ECF modifications in GC NACT, which dominated for a decade (Table 2).

Fluorouracil and platinum-based regimens

Over the next five years, after the rise of the ECF and ECF modifications, few regimens could achieve comparable results or be tested by high-quality clinical trials. This situation finally changed in 2011, when YChou *et al*[16] published the phase III clinical trial FNCLCC and FFCD 9703 and proposed the fluorouracil and cisplatin (FP) regimen.

This two-drug regimen was reported by Rougier *et al*[41] in 1994 and achieved satisfactory results including a 77% surgical rate and a 60% R0 resection rate in patients with nonresectable LAGC. The FNCLCC and FFCD 9703 study further tested the efficacy of the FP regimen as NACT. In this study, 224 patients with resectable lower esophageal, EGJ, or gastric cancer were randomized to receive perioperative FP chemotherapy (2-3 cycles before surgery, 3-4 cycles after surgery) and surgery or surgery alone. The results indicated that preoperative FP chemotherapy can significantly improve the R0 resection rate of patients (84% *vs* 74%, $P = 0.04$) and can achieve downstaging of lymph node metastasis (metastatic lymph node rate, 67% *vs*

80%, P = 0.054). More importantly, the perioperative FP regimen significantly increased the 5-year OS (38% *vs* 24%, log-rank P = 0.02) and 5-year DFS (34% *vs* 19%, log-rank P = 0.003) of patients. Compared with ECF, the two-drug regimen of FP could not only achieve a similar effect in terms of improving the long-term prognosis of patients, but also had the advantages of reducing chemotherapy-related complications, especially grade 3 to 4 leukopenia[16].

In addition, the two-drug regimen of fluorouracil and oxaliplatin also came into view. Kim *et al*[20] verified that both S-1 + oxaliplatin and capecitabine + oxaliplatin had similar efficacy and good tolerance in patients with GC. In the CALGB 80403/E1206 study, Enzinger *et al*[19] also confirmed that the FOLFOX regimen (fluorouracil, leucovorin, and oxaliplatin) had similar effectiveness and better tolerance than the ECF regimen.

Considering the results of the MAGIC, FNCLCC and FFCD 9703, and other studies, as well as the safety priority principle of NACT, the two-drug regimens of fluorouracil and platinum (oxaliplatin/cisplatin) have gradually become the mainstream of neoadjuvant chemotherapy for LAGC. The FP regimen was adopted as a category 1 recommendation in the NCCN guidelines in 2013, and the fluorouracil + oxaliplatin regimen was also adopted in 2017 as a category 2A recommendation, while the recommendation categories of the ECF and ECF modifications were gradually demoted to 2A and 2B (Table 2).

FLOT regimen

After the MAGIC and FNCLCC and FFCD 9703 studies, the FLOT4 study published by German scholars Al-Batran *et al*[21] was considered as another landmark in the history of NACT for LAGC. The highlight of the FLOT regimen was the introduction of docetaxel.

The V325 study published in 2006 was the first large clinical trial that applied docetaxel in GC. Although the DCF regimen (docetaxel, cisplatin, and fluorouracil) used in this study improved the response rate to chemotherapy and prolonged the OS and PFS of patients with metastatic or locally recurrent disease, severe side effects have prevented it from being widely accepted[42]. On this basis, Al-Batran *et al*[43,44] proposed the FLOT (docetaxel, fluorouracil, leucovorin, and oxaliplatin) regimen in 2008, which combined docetaxel with a safer skeleton regimen of FLO (fluorouracil, leucovorin, and oxaliplatin). The effectiveness and safety of the FLOT regimen were then validated through two clinical trials. These results encouraged researchers to further challenge the classical ECF and ECF modifications with the newly developed FLOT regimen.

The FLOT4 phase II study published in 2016 enrolled 300 patients with resectable EGJ or gastric cancer. In that study, patients randomly received perioperative ECF/ECX or FLOT chemotherapy[17]. According to the study, the FLOT regimen not only significantly improved the surgical rate (93% *vs* 81%, P = 0.01) and the R0 resection rate (85% *vs* 74%, P = 0.02), but also promoted the downstaging of tumors (≤ ypT2, 44% *vs* 27%, P = 0.01). Most importantly, the pCR rate (tumor regression grade TRG1a) and the complete or subtotal regression rate (TRG1a/b) of the FLOT group were significantly higher than those of the ECF/ECX group (TRG1a, 16% *vs* 6%, P = 0.02; TRG1a/b, 37% *vs* 23%, P = 0.02). The phase III portion of the FLOT4 study indicated that the incidence of serious side effects of the FLOT regimen was similar to the ECF/ECX regimen (27% *vs* 27%), but the tumor resection rate (94% *vs* 87%, P = 0.001) and the R0 resection rate (85% *vs* 78%, P = 0.0162) of the FLOT group (n = 356) were significantly higher than those of the ECF/ECX group (n = 360). The median OS (50 mo *vs* 35 mo, P = 0.012) and median DFS (30 mo *vs* 18 mo, P = 0.0036) were also significantly longer than those of the ECF/ECX group[18]. In view of the excellent pathological regression rate and the absolute advantages of FLOT over ECF/ECX, the NCCN guidelines adopted FLOT as the preferred regimen with a category 1 recommendation in 2018, and completely removed the ECF regimen and its modifications in the same year (Table 2).

From the domination of ECF and its modifications when NACT was developed in 2007 to the rally of the two-drug regimens of fluorouracil and platinum five years later, and the budding of the FLOT regimen in 2018, the development of chemotherapy drugs and the polishing of chemotherapy regimens have never stopped.

The efficacy of these regimens was further verified in many studies (Table 3). However, the absolute advantages of different regimens can hardly be concluded, because of the different regions, dosing schedules, completion rates, surgery/R0 resection rates and so on. Generally, the fluorouracil plus platinum regimens are more popular in Asia, while the ECF/ECF modifications and the FLOT regimen are widely accepted in Europe[45-61]. An excellent 4-year OS was achieved by Li *et al*[51] with perioperative FOLFOX regimen. In this prospective non-randomized study, LAGC

patients received a total of 6 cycles of FOLFOX chemotherapy perioperatively or postoperatively. The clinical and pathological response rates of FOLFOX were 69.7% and 39.4%, respectively, and the 4-year OS, as well as the 4-year DFS, of the neoadjuvant arm was 78%[51]. Meanwhile, the highest pathological response rate was achieved by Favi *et al*[48] with preoperative FLOT regimen. Patients with advanced distal esophageal and EGJ cancer in this study received 3-6 cycles of FLOT chemotherapy before surgery, the tumor regression rate of Cologne regression grade 1-3 was 52%, and the 3-year OS was 37%[48]. Nevertheless, disease recurrences were still common among all the studies and regimens, with the recurrence rates ranging from 32% to 62.5% (Table 3).

RESPONSE ASSESSMENT AND ADDITIONAL MANAGEMENT FOR NAT

Since more and more patients have received neoadjuvant treatment in the past decade, the 2018 NCCN guidelines proposed a response assessment for those patients in order to improve additional management strategies.

According to the 2018 NCCN guidelines, a chest/abdomen/pelvis CT scan with contrast was used as the method to evaluate disease status. If the outcome showed persistent local disease, surgical treatment was preferred. For patients with unresectable or metastatic disease, and those who were not medically fit for surgery, palliative management was recommended. For patients with no evidence of disease, the guidelines allowed clinicians to perform surveillance on those who refused surgery on the premise that surgery was still preferred.

However, both "surveillance" and "no evidence of disease" are controversial in GC. First, the definition of "no evidence of disease" is vague, and CT scanning with contrast cannot evaluate the disease status accurately[62-64]. Second, although pCR is a predictor of a favorable prognosis, it is still not equivalent to the clinical cure[58,65,66]. Finally, even if patients who achieved pCR after chemotherapy can be screened out by nonsurgical methods, sequential therapy should be recommended as an alternative to surgery[67]. Therefore, the 2019 NCCN guidelines contained major revisions in this chapter, the phrase "no evidence of disease" was deleted, and additional managements were recommended according to the resectability of the lesion. For patients with resectable tumors, surgery was still the preferred treatment, while for other patients, including those with nonresectable/metastatic lesions and those who were not medically fit for surgery, palliative care, but not surveillance, was recommended.

The postoperative treatment strategy for patients who received NAT was based on the cutting-edge of tumors and NAT modes. Due to the lack of direct studies that enrolled post-NAT patients, the recommendations proposed by the NCCN guidelines were derived from indirect studies with a relatively low level of evidence. The vicissitudes of this chapter were focused primarily on four aspects: (1) Before 2016, the stratification of postoperative NAT patients depended on their ypT and ypN stages, and only ypT2 and ypN0 patients were included in the low-risk group. In recent years, the status of lymph nodes has been elevated, and the current stratification is now only based on the presence of metastatic lymph nodes, partially according to the study of Smyth *et al*[68]; (2) The unification of postoperative treatment became a trend, especially for those who achieved R0 resection after NAT. The latest guidelines now do not adhere to the stratification of R0 resected patients and gave highly unified treatment recommendations, partially due to the lack of relevant studies; (3) Chemoradiotherapy is now preferred for non-R0 resected patients after NAT. The INT-0116 study established the "operation and postoperative adjuvant chemoradiotherapy" pattern in North America. Based on this study, the NCCN guidelines recommend that non-R0 resected patients without preoperative chemoradiotherapy should receive postoperative chemoradiotherapy for additional management; and (4) Reconsiderations of selecting the postoperative NACT regimens. The NCCN guidelines previously recommended R1 resected patients who underwent NACT to receive the same NACT regimens after surgery, in order to ensure the integrity and unity of perioperative treatment. However, the 2019 guidelines only recommended those patients with R0 resection to continue their preoperative NACT regimens.

Table 3 Short-term and long-term effects of different pre/perioperative chemotherapy regimens

Ref.	Region	Regimen	*n*	Median age	EGJ/ gastric (%)	pT stage (%)					pN stage (%)				Short-term effect		Long-term effect
						0	1	2	3	4	0	1	2	3	[a]Surgery /R0 (%)	Response	(mo; yr)
Mongan *et al*[60], 2015	EU	EOX	59	65	71/29	6	9	23	58	4	30	44	17	9	80/54	Mandard *et al*[115], TRG 1-3: 34%	Median OS: 22 mo; 4-yr survival: 47%; recurrence rate: 40%; median time to recurrence: 13 mo
Bichev *et al*[59], 2015	EU	ECF/mECF	77	62.1	61/40	0[b]		4	91	5	14	86			88/69	Becker *et al*[116], TRG 1 + 2: 44.2%	5-yr cumulative survival: 36.3%; median OS: 23.7 mo; 5-yr TSS: 42.2%; median TSS: 32.9 mo; recurrence rate: 32%
Mingol *et al*[58], 2015	EU	ECF/ECX	53	64	17/83	33			67		35	17	48		91/72	Becker *et al*[116], TRG 1a + 1b: 17%	5-yr OS: 18%; 5-yr DSS: 22%; recurrence rate: 61.9%
Achilli *et al*[46], 2017	EU	ECF/ECX	67	67	0/100	0	8	18	65	9	NA	NA	73		99/96	CR + PR: 37%; Becker TRG 1: 29%	Median OS: 36.6 mo; median DFS: 25.7 mo; recurrence rate: 54%
REECE-SMITH *et al*[45], 2012	EU	ECF/ECX	100	66	68/32	4	9	32	46	9	50	32	14	5	78/76	Histological regression > 50%: 45.8%	Median survival: 31.7 mo; 2-yr survival: 53%
Favi *et al*[48], 2017	EU	FLOT	40	61.5	100/0	12	15	17	43	10	40	17	28	15	97/85	Cologne Regression Scale 1-3: 52%	1-yr OS: 72%; 2-yr OS: 60%; 3-yr OS: 37%; median OS: 2.4 yr
Al-Batran *et al*[47], 2017	EU	FLOT	51	66	39/61	0[b]		18	69	8	26	75			96/78	NA	Median OS and PFS not achieved
Schulz *et al*[50], 2015	EU	FLOT	58	61	59/38	20	12	16	40	8	54	16	16	10	86/74	Becker *et al*[116], TRG 1a + 1b: 40%	1-yr survival: 79.3%; 1-yr PFS: 67.2%; median DFS: 32.9 mo
Lorenzen *et al*[49], 2013	EU	FLOT	21	69	62/38	10	48		5		29	38			71/67	CR + PR: 59.1%	1.5-yr OS: 78%; 2-yr OS: 78%; median PFS: 21.1 mo
		FLO	22	71.5	41/59	0	68		5		32	41			77/68	CR + PR: 18.2%	1.5-yr OS: 70%; 2-yr OS: 56%; median PFS: 12.0 mo
Yoshikawa *et al*[52], 2016	AS	SC-2	21	66	33/67	0			5	95	5	60	20	15	95/81	NA	3-yr OS: 67%
		SC-4	20	63	25/75	0			5	95	20	40	20	20	90/75	NA	3-yr OS: 55%
Tsuburaya *et al*[55], 2014	AS	SC	53	63	NA	4	14	47	33	2	16	10	43	31	94/82	CR + PR: 65%; JCGC[108] grade 1b-3: 51%	3-yr OS: 59%; 5-yr OS: 53%; 3-yr and 5-yr RFS: 50%
Kochi *et al*[54], 2017	AS	SC	50	64	0/100	NA	12	8	40	36	22	14	32	30	98/88	pCR: 2%; clinical response for LN: 75.5%, for primary tumor: 59.2%	3-yr OS: 48%; 3-yr RFS: 42%
Ott *et al*[53], 2003	EU	FLP	49	58	0/100	0	10	55	24	12	26	36	21	17	86/76	Major pathological tumor regression: 17%	Median survival: 25.4 mo (for ITT patients) and 32 mo (for R0 patients); recurrence rate: 62.5%; median recurrence: 19 mo
Li *et al*[51], 2012	AS	FOLFOX	33	65	0/100	12	6	12	61	9	36	33	27	3	100/91	CR + PR: 69.7%; JRSGC[117] grade 2-3: 39.4%	Mean survival: 74 mo; 4-yr OS: 78%; 4-yr DFS: 78%
Xue *et al*[61], 2018	AS	SOX	25	≥ 65: 48%	0/100	12	8	36	8	36	60	16	8	16	100/100	JCGC[108] grade 2-3: 40%	5-yr OS: 70%
		CAPOX	25	≥ 65: 24%	0/100	4	8	32	20	36	44	20	16	20	100/100	JCGC[108] grade 2-3: 36%	
Yu *et al*[57], 2019	AS	XELOX	54	65	30/70	13	13	17	33	25	31	15	31	23	91/83	CR + PR: 50%; JCGC[108] grade 1b-3: 41.6%	Median OS: 30.77 mo; 3-yr OS: 47.2%; disease progression: 55.6%; median PFS: 20.1 mo; 3-yr PFS: 43.8%
Feng *et al*[56], 2015	AS	SOX	80	60	40/60	15	11	30	19	25	59	16	14	11	100/95	CR + PR: 68.8%; pCR: 12.5%	NA

[a]: Some data were amended in order to increase comparability.

[b]: T and N stages in this study were clinical stages. EU: Europe; AS: Asia; EOX: Epirubicin, oxaliplatin and capecitabine; ECF: epirubicin, cisplatin and fluorouracil; mECF: ECF modifications; ECX: Epirubicin, cisplatin and capecitabine; FLOT: Docetaxel, fluorouracil, leucovorin and oxaliplatin; FLO: Fluorouracil, leucovorin and oxaliplatin; SC: S-1 and cisplatin; SC-2/4: S-1 and cisplatin for 2 cycles/4 cycles; FLP: 5-FU, cisplatin and leucovorin; FOLFOX: Oxaliplatin, fluorouracil and leucovorin; SOX: S-1 and oxaliplatin; CAPOX: Capecitabine and oxaliplatin; XELOX: Capecitabine and oxaliplatin; EGJ: Esophagogastric junction; LN: Lymph node; TRG: Tumor regression grade; JRSGC: Japanese research society for gastric cancer; JCGC: Japanese classification of gastric cancer; CR: Complete regression; PR: Partial regression; pCR: Pathological complete regression; OS: Overall survival; TSS: Tumor specific survival; DSS: Disease specific survival; DFS: Disease free survival; PFS: Progression-free survival; RFS: Relapse-free survival; ITT: Intention to treat; NA: Not available.

THE FUTURE OF NAT FOR GC

NAT is one of the breakthroughs of GC treatment in recent decades, and has the trend to become the standard strategy of this disease. However, the indications and strategies of NAT still need to be perfected, and researchers may gain ground in the following aspects in the future.

Above all, the validation of NAT in a wider range is necessary. The NCCN guidelines may only reflect a corner of NAT from the Western view, and the acceptability of NAT worldwide is still improving, especially in Asia. Chinese GC guidelines recommended that patients with advanced resectable GC (clinical stage III or above) could either receive surgery directly (Grade I recommendations) or receive neoadjuvant chemotherapy (Grade II recommendations)[69]. In Japan, preoperative chemotherapy has just been accepted in the latest guidelines for LAGC patients with bulky lymph nodes[70]. And in South Korea, the efficacy of preoperative chemotherapy and chemoradiotherapy for potentially resectable GC patients remains inconclusive[71]. Meanwhile, numerous trials in Asia, such as JCOG0405, JCOG1002, NCT01515748, NCT01534546, NCT02555358, and NCT00252161[55,72,73], have provided or will provide more evidence about the best indications for NAT, and physicians should always be critical when adopting the recommendations from foreign guidelines.

Second, the enhancement and delicacy management of NACT are required. Fluorouracil and platinum have been used as skeleton regimens of NACT for years, and their efficiency and tolerance in patients have been tested. However, it is an eternal rule that old regimens will be eliminated and that the development of new drugs may further improve the prognosis of patients[74,75]. Besides traditional cytotoxic regimens, the development of targeted therapy, immunotherapy, and metabolism based anticancer therapy may help us usher in a new era of LAGC treatment. Targeted drugs such as trastuzumab (anti-HER2) and ramucirumab (anti-VEGF2) have shown potential in improving clinical outcomes for late staged patients[74-85]. Immunotherapy, such as anti-PD-1/PD-L1 and anti-CTLA-4 drugs (nivolumab, pembrolizumab, avelumab, tremelimumab, *etc*.), adoptive cell therapy, and VEGF related cancer vaccine have also been evaluated in gastric cancer and have shown promising effects[86-92]. Studies about cancer metabolomics also provided new insights in cancer treatment. Drugs targeting at hexokinase II may intervene the glycolysis of tumor cells[93], and others that altered the metabolism of lipid, amino acid, *etc*. also presented exciting prospects in treating GC *in vitro*[94-96]. In addition, the continuous monitoring of NACT efficacy can also help to clarify the optimal operation timing for chemotherapy-sensitive patients, or it can encourage the termination of unnecessary treatment for chemotherapy-resistant patients in advance to avoid disease progression[97,98].

Besides, the individualized treatment and efficacy prediction of neoadjuvant chemotherapy may be a trend. It is true that the antitumor effects of cytotoxic drugs are extensive and without high selection, but the correlation between genetic traits and chemosensitivity may also be underestimated. Polymorphisms, gene mutations, and unique genetic backgrounds may lead to different response rates to the same chemotherapy regimen[99,100]. The advantages of the S-1 and cisplatin regimens reported by the SPIRITS (S-1 Plus cisplatin versus S-1 In RCT In the Treatment for Stomach cancer) study in Japan were not consistently concluded in the non-Asian trial of the First-Line Advanced Gastric Cancer Study study (median OS, 13.0 mo *vs* 8.6 mo, respectively)[101,102]. Scholars have also found that genetic polymorphisms play an important role in selecting NAT for each patient[103]. Additionally, the Trastuzumab for Gastric Cancer study confirmed that chemotherapy combined with HER-2 targeted therapy resulted in a better therapeutic effect than chemotherapy alone for patients with high HER-2 expression[76], which may enlighten us about the possibility of neoadjuvant chemotherapy plus targeted therapy. The heterogeneity of histopathology in GC also results in different response rates to the same regimen. Although the latest NCCN guidelines of GC (2019.V2) did not recommend the best regimen for each pathological type, clinical trials such as the FLOT study have proposed different histopathological regression rates among different histology types. We should never handle GC as one kind of disease, and preoperative treatment will eventually be recommended based on the histopathology types (Lauren, JGCA, WHO classification, *etc*.) and/or the molecular types (TCGA, ACRG classification, *etc*.)[104-108]. In the future, the individual differences of patients may be carefully considered before performing NACT, and cytotoxic regimens combined with targeted therapy may be a new option for certain patients[79,81,82,109-111].

Finally, the strategic flow of NAT will be continuously perfected. The booming of NAT in the past decade benefited from abundant high-quality clinical trials, while the decision-making process of NAT still needs to be perfected. For example, there is still no consensus on whether surgery can bring absolute benefits to patients who exhibit

an excellent response to NACT. And for patients who have received NACT but did not achieve R0 resection, which treatment (either chemoradiotherapy or alternative chemotherapy) should be administered remains unclear. The clarity of such decisions will have substantial impacts on patients' prognosis and quality of life. We believe that the NCCN guidelines will continue perfecting the strategic flow to allow better choices for patients base on future studies and trials.

CONCLUSION

NAT is becoming the standard treatment for patients with resectable, nonmetastatic LAGC. Although the universality of present evidence is insufficient, and the frontier of NAT is still led by Western scholars, we are always confident in Asian researchers for their unremitting efforts[112,113]. We are also looking forward to more high-quality studies such as the NCT01534546, NCT02555358, and NCT00252161, which will help to establish a characteristic NAT strategy that is more appropriate for Asian populations.

REFERENCES

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

2 **Chen W**, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115-132 [PMID: 26808342 DOI: 10.3322/caac.21338]

3 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

4 **Jeong O**, Park YK. Clinicopathological features and surgical treatment of gastric cancer in South Korea: the results of 2009 nationwide survey on surgically treated gastric cancer patients. *J Gastric Cancer* 2011; **11**: 69-77 [PMID: 22076206 DOI: 10.5230/jgc.2011.11.2.69]

5 **Nashimoto A**, Akazawa K, Isobe Y, Miyashiro I, Katai H, Kodera Y, Tsujitani S, Seto Y, Furukawa H, Oda I, Ono H, Tanabe S, Kaminishi M. Gastric cancer treated in 2002 in Japan: 2009 annual report of the JGCA nationwide registry. *Gastric Cancer* 2013; **16**: 1-27 [PMID: 22729699 DOI: 10.1007/s10120-012-0163-4]

6 **Siewert JR**, Böttcher K, Roder JD, Busch R, Hermanek P, Meyer HJ. Prognostic relevance of systematic lymph node dissection in gastric carcinoma. German Gastric Carcinoma Study Group. *Br J Surg* 1993; **80**: 1015-1018 [PMID: 8402053 DOI: 10.1002/bjs.1800800829]

7 **Gee DW**, Rattner DW. Management of gastroesophageal tumors. *Oncologist* 2007; **12**: 175-185 [PMID: 17296813 DOI: 10.1634/theoncologist.12-2-175]

8 **Frei E 3rd**. Clinical cancer research: an embattled species. *Cancer* 1982; **50**: 1979-1992 [PMID: 7127245 DOI: 10.1002/1097-0142(19821115)50:10<1979::aid-cncr2820501002>3.0.co;2-d]

9 **Plukker JT**, Mulder NH, Sleijfer DT, Grond J, Verschueren RC. Chemotherapy and surgery for locally advanced cancer of the cardia and fundus: phase II study with methotrexate and 5-fluorouracil. *Br J Surg* 1991; **78**: 955-958 [PMID: 1913116 DOI: 10.1002/bjs.1800780820]

10 **Wilke H**, Preusser P, Fink U, Gunzer U, Meyer HJ, Meyer J, Siewert JR, Achterrath W, Lenaz L, Knipp H. Preoperative chemotherapy in locally advanced and nonresectable gastric cancer: a phase II study with etoposide, doxorubicin, and cisplatin. *J Clin Oncol* 1989; **7**: 1318-1326 [PMID: 2769330 DOI: 10.1200/JCO.1989.7.9.1318]

11 **Mai M**, Takahashi Y, Fujimoto T, Omote K. [Neoadjuvant chemotherapy for far-advanced gastric carcinoma]. *Gan To Kagaku Ryoho* 1994; **21**: 431-439 [PMID: 8129383]

12 **Plukker JT**, Sleijfer DT, Verschueren RC, Van der Graaf WT, Mulder NH. Neo-adjuvant chemotherapy with carboplatin, 4-epiadriamycin and teniposide (CET) in locally advanced cancer of the cardia and the lower oesophagus: a phase II study. *Anticancer Res* 1995; **15**: 2357-2361 [PMID: 8572652]

13 **Melcher AA**, Mort D, Maughan TS. Epirubicin, cisplatin and continuous infusion 5-fluorouracil (ECF) as neoadjuvant chemotherapy in gastro-oesophageal cancer. *Br J Cancer* 1996; **74**: 1651-1654 [PMID: 8932350 DOI: 10.1038/bjc.1996.604]

14 **Cunningham D**, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, Scarffe JH, Lofts FJ, Falk SJ, Iveson TJ, Smith DB, Langley RE, Verma M, Weeden S, Chua YJ, MAGIC Trial Participants. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; **355**: 11-20 [PMID: 16822992 DOI: 10.1056/NEJMoa055531]

15 **Schuhmacher C**, Gretschel S, Lordick F, Reichardt P, Hohenberger W, Eisenberger CF, Haag C, Mauer ME, Hasan B, Welch J, Ott K, Hoelscher A, Schneider PM, Bechstein W, Wilke H, Lutz MP, Nordlinger B, Van Cutsem E, Siewert JR, Schlag PM. Neoadjuvant chemotherapy compared with surgery alone for locally advanced cancer of the stomach and cardia: European Organisation for Research and Treatment of Cancer randomized trial 40954. *J Clin Oncol* 2010; **28**: 5210-5218 [PMID: 21060024 DOI: 10.1200/JCO.2009.26.6114]

16 **Ychou M**, Boige V, Pignon JP, Conroy T, Bouché O, Lebreton G, Ducourtieux M, Bedenne L, Fabre JM, Saint-Aubert B, Genève J, Lasser P, Rougier P. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. *J Clin Oncol* 2011; **29**: 1715-1721 [PMID: 21444866 DOI: 10.1200/JCO.2010.33.0597]

17 **Al-Batran SE**, Hofheinz RD, Pauligk C, Kopp HG, Haag GM, Luley KB, Meiler J, Homann N, Lorenzen S, Schmalenberg H, Probst S, Koenigsmann M, Egger M, Prasnikar N, Caca K, Trojan J, Martens UM, Block A, Fischbach W, Mahlberg R, Clemens M, Illerhaus G, Zirlik K, Behringer DM, Schmiegel W, Pohl M, Heike M, Ronellenfitsch U, Schuler M, Bechstein WO, Königsrainer A, Gaiser T, Schirmacher P,

Hozaeel W, Reichart A, Goetze TO, Sievert M, Jäger E, Mönig S, Tannapfel A. Histopathological regression after neoadjuvant docetaxel, oxaliplatin, fluorouracil, and leucovorin versus epirubicin, cisplatin, and fluorouracil or capecitabine in patients with resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4-AIO): results from the phase 2 part of a multicentre, open-label, randomised phase 2/3 trial. *Lancet Oncol* 2016; **17**: 1697-1708 [PMID: 27776843 DOI: 10.1016/S1470-2045(16)30531-9]

18 **Al-Batran SE**, Homann N, Pauligk C, Goetze TO, Meiler J, Kasper S, Kopp HG, Mayer F, Haag GM, Luley K, Lindig U, Schmiegel W, Pohl M, Stoehlmacher J, Folprecht G, Probst S, Prasnikar N, Fischbach W, Mahlberg R, Trojan J, Koenigsmann M, Martens UM, Thuss-Patience P, Egger M, Block A, Heinemann V, Illerhaus G, Moehler M, Schenk M, Kullmann F, Behringer DM, Heike M, Pink D, Teschendorf C, Löhr C, Bernhard H, Schuch G, Rethwisch V, von Weikersthal LF, Hartmann JT, Kneba M, Daum S, Schulmann K, Weniger J, Belle S, Gaiser T, Oduncu FS, Güntner M, Hozaeel W, Reichart A, Jäger E, Kraus T, Mönig S, Bechstein WO, Schuler M, Schmalenberg H, Hofheinz RD; FLOT4-AIO Investigators. Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): a randomised, phase 2/3 trial. *Lancet* 2019; **393**: 1948-1957 [PMID: 30982686 DOI: 10.1016/S0140-6736(18)32557-1]

19 **Enzinger PC**, Burtness BA, Niedzwiecki D, Ye X, Douglas K, Ilson DH, Villaflor VM, Cohen SJ, Mayer RJ, Venook A, Benson AB, Goldberg RM. CALGB 80403 (Alliance)/E1206: A Randomized Phase II Study of Three Chemotherapy Regimens Plus Cetuximab in Metastatic Esophageal and Gastroesophageal Junction Cancers. *J Clin Oncol* 2016; **34**: 2736-2742 [PMID: 27382098 DOI: 10.1200/JCO.2015.65.5092]

20 **Kim GM**, Jeung HC, Rha SY, Kim HS, Jung I, Nam BH, Lee KH, Chung HC. A randomized phase II trial of S-1-oxaliplatin versus capecitabine-oxaliplatin in advanced gastric cancer. *Eur J Cancer* 2012; **48**: 518-526 [PMID: 22243774 DOI: 10.1016/j.ejca.2011.12.017]

21 **Al-Batran SE**, Hartmann JT, Probst S, Schmalenberg H, Hollerbach S, Hofheinz R, Rethwisch V, Seipelt G, Homann N, Wilhelm G, Schuch G, Stoehlmacher J, Derigs HG, Hegewisch-Becker S, Grossmann J, Pauligk C, Atmaca A, Bokemeyer C, Knuth A, Jäger E; Arbeitsgemeinschaft Internistische Onkologie. Phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil, leucovorin plus either oxaliplatin or cisplatin: a study of the Arbeitsgemeinschaft Internistische Onkologie. *J Clin Oncol* 2008; **26**: 1435-1442 [PMID: 18349393 DOI: 10.1200/JCO.2007.13.9378]

22 **Bedenne L**, Michel P, Bouché O, Milan C, Mariette C, Conroy T, Pezet D, Roullet B, Seitz JF, Herr JP, Paillot B, Arveux P, Bonnetain F, Binquet C. Chemoradiation followed by surgery compared with chemoradiation alone in squamous cancer of the esophagus: FFCD 9102. *J Clin Oncol* 2007; **25**: 1160-1168 [PMID: 17401004 DOI: 10.1200/JCO.2005.04.7118]

23 **Yin S**, Wang P, Xu X, Tan Y, Huang J, Xu H. The optimal strategy of multimodality therapies for resectable gastric cancer: evidence from a network meta-analysis. *J Cancer* 2019; **10**: 3094-3101 [PMID: 31289579 DOI: 10.7150/jca.30456]

24 **Macdonald JS**, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN, Haller DG, Ajani JA, Gunderson LL, Jessup JM, Martenson JA. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Engl J Med* 2001; **345**: 725-730 [PMID: 11547741 DOI: 10.1056/NEJMoa010187]

25 **Ajani JA**, Winter K, Okawara GS, Donohue JH, Pisters PW, Crane CH, Greskovich JF, Anne PR, Bradley JD, Willett C, Rich TA. Phase II trial of preoperative chemoradiation in patients with localized gastric adenocarcinoma (RTOG 9904): quality of combined modality therapy and pathologic response. *J Clin Oncol* 2006; **24**: 3953-3958 [PMID: 16921048 DOI: 10.1200/JCO.2006.06.4840]

26 **Tepper J**, Krasna MJ, Niedzwiecki D, Hollis D, Reed CE, Goldberg R, Kiel K, Willett C, Sugarbaker D, Mayer R. Phase III trial of trimodality therapy with cisplatin, fluorouracil, radiotherapy, and surgery compared with surgery alone for esophageal cancer: CALGB 9781. *J Clin Oncol* 2008; **26**: 1086-1092 [PMID: 18309943 DOI: 10.1200/JCO.2007.12.9593]

27 **van Hagen P**, Hulshof MC, van Lanschot JJ, Steyerberg EW, van Berge Henegouwen MI, Wijnhoven BP, Richel DJ, Nieuwenhuijzen GA, Hospers GA, Bonenkamp JJ, Cuesta MA, Blaisse RJ, Busch OR, ten Kate FJ, Creemers GJ, Punt CJ, Plukker JT, Verheul HM, Spillenaar Bilgen EJ, van Dekken H, van der Sangen MJ, Rozema T, Biermann K, Beukema JC, Piet AH, van Rij CM, Reinders JG, Tilanus HW, van der Gaast A; CROSS Group. Preoperative chemoradiotherapy for esophageal or junctional cancer. *N Engl J Med* 2012; **366**: 2074-2084 [PMID: 22646630 DOI: 10.1056/NEJMoa1112088]

28 **Stahl M**, Walz MK, Riera-Knorrenschild J, Stuschke M, Sandermann A, Bitzer M, Wilke H, Budach W. Preoperative chemotherapy versus chemoradiotherapy in locally advanced adenocarcinomas of the oesophagogastric junction (POET): Long-term results of a controlled randomised trial. *Eur J Cancer* 2017; **81**: 183-190 [PMID: 28628843 DOI: 10.1016/j.ejca.2017.04.027]

29 **Van Cutsem E**, Sagaert X, Topal B, Haustermans K, Prenen H. Gastric cancer. *Lancet* 2016; **388**: 2654-2664 [PMID: 27156933 DOI: 10.1016/S0140-6736(16)30354-3]

30 **Lowy AM**, Feig BW, Janjan N, Rich TA, Pisters PW, Ajani JA, Mansfield PF. A pilot study of preoperative chemoradiotherapy for resectable gastric cancer. *Ann Surg Oncol* 2001; **8**: 519-524 [PMID: 11456051]

31 **Ajani JA**, Mansfield PF, Janjan N, Morris J, Pisters PW, Lynch PM, Feig B, Myerson R, Nivers R, Cohen DS, Gunderson LL. Multi-institutional trial of preoperative chemoradiotherapy in patients with potentially resectable gastric carcinoma. *J Clin Oncol* 2004; **22**: 2774-2780 [PMID: 15254045 DOI: 10.1200/JCO.2004.01.015]

32 **Ajani JA**, Mansfield PF, Crane CH, Wu TT, Lunagomez S, Lynch PM, Janjan N, Feig B, Faust J, Yao JC, Nivers R, Morris J, Pisters PW. Paclitaxel-based chemoradiotherapy in localized gastric carcinoma: degree of pathologic response and not clinical parameters dictated patient outcome. *J Clin Oncol* 2005; **23**: 1237-1244 [PMID: 15718321 DOI: 10.1200/JCO.2005.01.305]

33 **Rivera F**, Galán M, Tabernero J, Cervantes A, Vega-Villegas ME, Gallego J, Laquente B, Rodríguez E, Carrato A, Escudero P, Massutí B, Alonso-Orduña V, Cardenal A, Sáenz A, Giralt J, Yuste AL, Antón A, Aranda E; Spanish Cooperative Group for Digestive Tumor Therapy. Phase II trial of preoperative irinotecan-cisplatin followed by concurrent irinotecan-cisplatin and radiotherapy for resectable locally advanced gastric and esophagogastric junction adenocarcinoma. *Int J Radiat Oncol Biol Phys* 2009; **75**: 1430-1436 [PMID: 19540072 DOI: 10.1016/j.ijrobp.2008.12.087]

34 **Conroy T**, Galais MP, Raoul JL, Bouché O, Gourgou-Bourgade S, Douillard JY, Etienne PL, Boige V, Martel-Lafay I, Michel P, Llacer-Moscardo C, François E, Créhange G, Abdelghani MB, Juzyna B, Bedenne L, Adenis A; Fédération Francophone de Cancérologie Digestive and UNICANCER-GI Group.

Definitive chemoradiotherapy with FOLFOX versus fluorouracil and cisplatin in patients with oesophageal cancer (PRODIGE5/ACCORD17): final results of a randomised, phase 2/3 trial. *Lancet Oncol* 2014; **15**: 305-314 [PMID: 24556041 DOI: 10.1016/S1470-2045(14)70028-2]

35 **Khushalani NI**, Leichman CG, Proulx G, Nava H, Bodnar L, Klippenstein D, Litwin A, Smith J, Nava E, Pendyala L, Smith P, Greco W, Berdzik J, Douglass H, Leichman L. Oxaliplatin in combination with protracted-infusion fluorouracil and radiation: report of a clinical trial for patients with esophageal cancer. *J Clin Oncol* 2002; **20**: 2844-2850 [PMID: 12065561 DOI: 10.1200/JCO.2002.12.032]

36 **Norton L**. A Gompertzian model of human breast cancer growth. *Cancer Res* 1988; **48**: 7067-7071 [PMID: 3191483]

37 **Hartgrink HH**, van de Velde CJ, Putter H, Songun I, Tesselaar ME, Kranenbarg EK, de Vries JE, Wils JA, van der Bijl J, van Krieken JH; Cooperating Investigators of The Dutch Gastric Cancer Group. Neo-adjuvant chemotherapy for operable gastric cancer: long term results of the Dutch randomised FAMTX trial. *Eur J Surg Oncol* 2004; **30**: 643-649 [PMID: 15256239 DOI: 10.1016/j.ejso.2004.04.013]

38 **Webb A**, Cunningham D, Scarffe JH, Harper P, Norman A, Joffe JK, Hughes M, Mansi J, Findlay M, Hill A, Oates J, Nicolson M, Hickish T, O'Brien M, Iveson T, Watson M, Underhill C, Wardley A, Meehan M. Randomized trial comparing epirubicin, cisplatin, and fluorouracil versus fluorouracil, doxorubicin, and methotrexate in advanced esophagogastric cancer. *J Clin Oncol* 1997; **15**: 261-267 [PMID: 8996151 DOI: 10.1200/JCO.1997.15.1.261]

39 **Cunningham D**, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR; Upper Gastrointestinal Clinical Studies Group of the National Cancer Research Institute of the United Kingdom. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008; **358**: 36-46 [PMID: 18172173 DOI: 10.1056/NEJMoa073149]

40 **Sumpter K**, Harper-Wynne C, Cunningham D, Rao S, Tebbutt N, Norman AR, Ward C, Iveson T, Nicolson M, Hickish T, Hill M, Oates J. Report of two protocol planned interim analyses in a randomised multicentre phase III study comparing capecitabine with fluorouracil and oxaliplatin with cisplatin in patients with advanced oesophagogastric cancer receiving ECF. *Br J Cancer* 2005; **92**: 1976-1983 [PMID: 15928658 DOI: 10.1038/sj.bjc.6602572]

41 **Rougier P**, Mahjoubi M, Lasser P, Ducreux M, Oliveira J, Ychou M, Pignon JP, Elias D, Bellefqih S, Bognel C. Neoadjuvant chemotherapy in locally advanced gastric carcinoma--a phase II trial with combined continuous intravenous 5-fluorouracil and bolus cisplatinum. *Eur J Cancer* 1994; **30A**: 1269-1275 [PMID: 7999411 DOI: 10.1016/0959-8049(94)90171-6]

42 **Van Cutsem E**, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E, Risse ML, Ajani JA; V325 Study Group. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol* 2006; **24**: 4991-4997 [PMID: 17075117 DOI: 10.1200/JCO.2006.06.8429]

43 **Al-Batran SE**, Hartmann JT, Hofheinz R, Homann N, Rethwisch V, Probst S, Stoehlmacher J, Clemens MR, Mahlberg R, Fritz M, Seipelt G, Sievert M, Pauligk C, Atmaca A, Jäger E. Biweekly fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT) for patients with metastatic adenocarcinoma of the stomach or esophagogastric junction: a phase II trial of the Arbeitsgemeinschaft Internistische Onkologie. *Ann Oncol* 2008; **19**: 1882-1887 [PMID: 18669868 DOI: 10.1093/annonc/mdn403]

44 **Al-Batran SE**, Pauligk C, Homann N, Hartmann JT, Moehler M, Probst S, Rethwisch V, Stoehlmacher-Williams J, Prasnikar N, Hollerbach S, Bokemeyer C, Mahlberg R, Hofheinz RD, Luley K, Kullmann F, Jäger E. The feasibility of triple-drug chemotherapy combination in older adult patients with oesophagogastric cancer: a randomised trial of the Arbeitsgemeinschaft Internistische Onkologie (FLOT65+). *Eur J Cancer* 2013; **49**: 835-842 [PMID: 23063354 DOI: 10.1016/j.ejca.2012.09.025]

45 **Reece-Smith AM**, Saha S, Cunnell ML, Hameed K, Bessell EM, Duffy JP, Madhusudan S, Parsons SL. MAGIC in practice: experience of peri-operative ECF/X chemotherapy in gastro-esophageal adenocarcinomas. *J Surg Oncol* 2012; **106**: 748-752 [PMID: 22674046 DOI: 10.1002/jso.23187]

46 **Achilli P**, De Martini P, Ceresoli M, Mari GM, Costanzi A, Maggioni D, Pugliese R, Ferrari G. Tumor response evaluation after neoadjuvant chemotherapy in locally advanced gastric adenocarcinoma: a prospective, multi-center cohort study. *J Gastrointest Oncol* 2017; **8**: 1018-1025 [PMID: 29299362 DOI: 10.21037/jgo.2017.08.13]

47 **Al-Batran SE**, Homann N, Pauligk C, Illerhaus G, Martens UM, Stoehlmacher J, Schmalenberg H, Luley KB, Prasnikar N, Egger M, Probst S, Messmann H, Moehler M, Fischbach W, Hartmann JT, Mayer F, Höffkes HG, Koenigsmann M, Arnold D, Kraus TW, Grimm K, Berkhoff S, Post S, Jäger E, Bechstein W, Ronellenfitsch U, Mönig S, Hofheinz RD. Effect of Neoadjuvant Chemotherapy Followed by Surgical Resection on Survival in Patients With Limited Metastatic Gastric or Gastroesophageal Junction Cancer: The AIO-FLOT3 Trial. *JAMA Oncol* 2017; **3**: 1237-1244 [PMID: 28448662 DOI: 10.1001/jamaoncol.2017.0515]

48 **Favi F**, Bollschweiler E, Berlth F, Plum P, Hescheler DA, Alakus H, Semrau R, Celik E, Mönig SP, Drebber U, Hölscher AH. Neoadjuvant chemotherapy or chemoradiation for patients with advanced adenocarcinoma of the oesophagus? A propensity score-matched study. *Eur J Surg Oncol* 2017; **43**: 1572-1580 [PMID: 28666624 DOI: 10.1016/j.ejso.2017.06.003]

49 **Lorenzen S**, Pauligk C, Homann N, Schmalenberg H, Jäger E, Al-Batran SE. Feasibility of perioperative chemotherapy with infusional 5-FU, leucovorin, and oxaliplatin with (FLOT) or without (FLO) docetaxel in elderly patients with locally advanced esophagogastric cancer. *Br J Cancer* 2013; **108**: 519-526 [PMID: 23322206 DOI: 10.1038/bjc.2012.588]

50 **Schulz C**, Kullmann F, Kunzmann V, Fuchs M, Geissler M, Vehling-Kaiser U, Stauder H, Wein A, Al-Batran SE, Kubin T, Schäfer C, Stintzing S, Giessen C, Modest DP, Ridwelski K, Heinemann V. NeoFLOT: Multicenter phase II study of perioperative chemotherapy in resectable adenocarcinoma of the gastroesophageal junction or gastric adenocarcinoma-Very good response predominantly in patients with intestinal type tumors. *Int J Cancer* 2015; **137**: 678-685 [PMID: 25530271 DOI: 10.1002/ijc.29403]

51 **Li ZY**, Koh CE, Bu ZD, Wu AW, Zhang LH, Wu XJ, Wu Q, Zong XL, Ren H, Tang L, Zhang XP, Li JY, Hu Y, Shen L, Ji JF. Neoadjuvant chemotherapy with FOLFOX: improved outcomes in Chinese patients with locally advanced gastric cancer. *J Surg Oncol* 2012; **105**: 793-799 [PMID: 22189752 DOI: 10.1002/jso.23009]

52 **Yoshikawa T**, Morita S, Tanabe K, Nishikawa K, Ito Y, Matsui T, Fujitani K, Kimura Y, Fujita J, Aoyama T, Hayashi T, Cho H, Tsuburaya A, Miyashita Y, Sakamoto J. Survival results of a randomised two-by-two factorial phase II trial comparing neoadjuvant chemotherapy with two and four courses of S-1 plus cisplatin (SC) and paclitaxel plus cisplatin (PC) followed by D2 gastrectomy for resectable advanced

gastric cancer. *Eur J Cancer* 2016; **62**: 103-111 [PMID: 27244537 DOI: 10.1016/j.ejca.2016.04.012]
53 **Ott K**, Sendler A, Becker K, Dittler HJ, Helmberger H, Busch R, Kollmannsberger C, Siewert JR, Fink U. Neoadjuvant chemotherapy with cisplatin, 5-FU, and leucovorin (PLF) in locally advanced gastric cancer: a prospective phase II study. *Gastric Cancer* 2003; **6**: 159-167 [PMID: 14520529 DOI: 10.1007/s10120-003-0245-4]
54 **Kochi M**, Fujii M, Kanamori N, Mihara Y, Funada T, Tamegai H, Watanabe M, Takayama Y, Suda H, Takayama T. Phase II Study of Neoadjuvant Chemotherapy With S-1 and CDDP in Patients With Lymph Node Metastatic Stage II or III Gastric Cancer. *Am J Clin Oncol* 2017; **40**: 17-21 [PMID: 24662266 DOI: 10.1097/COC.0000000000000058]
55 **Tsuburaya A**, Mizusawa J, Tanaka Y, Fukushima N, Nashimoto A, Sasako M; Stomach Cancer Study Group of the Japan Clinical Oncology Group. Neoadjuvant chemotherapy with S-1 and cisplatin followed by D2 gastrectomy with para-aortic lymph node dissection for gastric cancer with extensive lymph node metastasis. *Br J Surg* 2014; **101**: 653-660 [PMID: 24668391 DOI: 10.1002/bjs.9484]
56 **Feng D**, Leong M, Li T, Chen L, Li T. Surgical outcomes in patients with locally advanced gastric cancer treated with S-1 and oxaliplatin as neoadjuvant chemotherapy. *World J Surg Oncol* 2015; **13**: 11 [PMID: 25634099 DOI: 10.1186/s12957-015-0444-6]
57 **Yu Y**, Fang Y, Shen Z, Wang Y, Yan M, Cao H, Liu Y, Wang X, Cui Y, Liu F, Chen W, Li W, Li Q, Jiang H, Sun Y, Liu T. Oxaliplatin plus Capecitabine in the Perioperative Treatment of Locally Advanced Gastric Adenocarcinoma in Combination with D2 Gastrectomy: NEO-CLASSIC Study. *Oncologist* 2019 [PMID: 31239311 DOI: 10.1634/theoncologist.2019-0416]
58 **Mingol F**, Gallego J, Orduña A, Martinez-Blasco A, Sola-Vera J, Moya P, Morcillo MA, Ruiz JA, Calpena R, Lacueva FJ. Tumor regression and survival after perioperative MAGIC-style chemotherapy in carcinoma of the stomach and gastroesophageal junction. *BMC Surg* 2015; **15**: 66 [PMID: 25997454 DOI: 10.1186/s12893-015-0054-9]
59 **Bichev D**, Treese C, von Winterfeld M, Breithaupt K, Dogan Y, Schmidt SC, Daum S, Thuss-Patience PC. High Impact of Histopathological Remission for Prognosis after Perioperative Chemotherapy with ECF and ECF-Like Regimens for Gastric and Gastroesophageal Adenocarcinoma. *Oncology* 2015; **89**: 95-102 [PMID: 25823985 DOI: 10.1159/000376550]
60 **Mongan AM**, Kalachand R, King S, O'Farrell NJ, Power D, Ravi N, Muldoon C, O'Byrne K, Reynolds JV. Outcomes in gastric and junctional cancer using neoadjuvant and adjuvant chemotherapy (epirubicin, oxaliplatin, and capecitabine) and radical surgery. *Ir J Med Sci* 2015; **184**: 417-423 [PMID: 24879337 DOI: 10.1007/s11845-014-1135-y]
61 **Xue K**, Ying X, Bu Z, Wu A, Li Z, Tang L, Zhang L, Zhang Y, Li Z, Ji J. Oxaliplatin plus S-1 or capecitabine as neoadjuvant or adjuvant chemotherapy for locally advanced gastric cancer with D2 lymphadenectomy: 5-year follow-up results of a phase II-III randomized trial. *Chin J Cancer Res* 2018; **30**: 516-525 [PMID: 30510363 DOI: 10.21147/j.issn.1000-9604.2018.05.05]
62 **Yoshikawa T**, Tanabe K, Nishikawa K, Ito Y, Matsui T, Kimura Y, Hasegawa S, Aoyama T, Hayashi T, Morita S, Miyashita Y, Tsuburaya A, Sakamoto J. Accuracy of CT staging of locally advanced gastric cancer after neoadjuvant chemotherapy: cohort evaluation within a randomized phase II study. *Ann Surg Oncol* 2014; **21** Suppl 3: S385-S389 [PMID: 24595801 DOI: 10.1245/s10434-014-3615-8]
63 **Fujikawa H**, Yoshikawa T, Hasegawa S, Hayashi T, Aoyama T, Ogata T, Cho H, Oshima T, Rino Y, Morita S, Masuda M. Diagnostic value of computed tomography for staging of clinical T1 gastric cancer. *Ann Surg Oncol* 2014; **21**: 3002-3007 [PMID: 24687153 DOI: 10.1245/s10434-014-3667-9]
64 **Hwang SW**, Lee DH, Lee SH, Park YS, Hwang JH, Kim JW, Jung SH, Kim NY, Kim YH, Lee KH, Kim HH, Park DJ, Lee HS, Jung HC, Song IS. Preoperative staging of gastric cancer by endoscopic ultrasonography and multidetector-row computed tomography. *J Gastroenterol Hepatol* 2010; **25**: 512-518 [PMID: 20370729 DOI: 10.1111/j.1440-1746.2009.06106.x]
65 **Blackham AU**, Greenleaf E, Yamamoto M, Hollenbeak C, Gusani N, Coppola D, Pimiento JM, Wong J. Tumor regression grade in gastric cancer: Predictors and impact on outcome. *J Surg Oncol* 2016; **114**: 434-439 [PMID: 27199217 DOI: 10.1002/jso.24307]
66 **Becker K**, Langer R, Reim D, Novotny A, Meyer zum Buschenfelde C, Engel J, Friess H, Hofler H. Significance of histopathological tumor regression after neoadjuvant chemotherapy in gastric adenocarcinomas: a summary of 480 cases. *Ann Surg* 2011; **253**: 934-939 [PMID: 21490451 DOI: 10.1097/SLA.0b013e318216f449]
67 **Mokadem I**, Dijksterhuis WPM, van Putten M, Heuthorst L, de Vos-Geelen JM, Haj Mohammad N, Nieuwenhuijzen GAP, van Laarhoven HWM, Verhoeven RHA. Recurrence after preoperative chemotherapy and surgery for gastric adenocarcinoma: a multicenter study. *Gastric Cancer* 2019 [PMID: 30949777 DOI: 10.1007/s10120-019-00956-6]
68 **Smyth EC**, Fassan M, Cunningham D, Allum WH, Okines AF, Lampis A, Hahne JC, Rugge M, Peckitt C, Nankivell M, Langley R, Ghidini M, Braconi C, Wotherspoon A, Grabsch HI, Valeri N. Effect of Pathologic Tumor Response and Nodal Status on Survival in the Medical Research Council Adjuvant Gastric Infusional Chemotherapy Trial. *J Clin Oncol* 2016; **34**: 2721-2727 [PMID: 27298411 DOI: 10.1200/JCO.2015.65.7692]
69 **Wang FH**, Shen L, Li J, Zhou ZW, Liang H, Zhang XT, Tang L, Xin Y, Jin J, Zhang YJ, Yuan XL, Liu TS, Li GX, Wu Q, Xu HM, Ji JF, Li YF, Wang X, Yu S, Liu H, Guan WL, Xu RH. The Chinese Society of Clinical Oncology (CSCO): clinical guidelines for the diagnosis and treatment of gastric cancer. *Cancer Commun (Lond)* 2019; **39**: 10 [PMID: 30885279 DOI: 10.1186/s40880-019-0349-9]
70 **Japanese Gastric Cancer Association**. Japanese Gastric Cancer Treatment Guidelines. 5th ed. Japan: Jinyuan Press 2018;
71 Guideline Committee of the Korean Gastric Cancer Association (KGCA), Development Working Group and Review Panel. Korean Practice Guideline for Gastric Cancer 2018: An Evidence-based, Multi-disciplinary Approach. *J Gastric Cancer* 2019; **19**: 1-48 [PMID: 30944757 DOI: 10.5230/jgc.2019.19.e8]
72 **Katayama H**, Ito S, Sano T, Takahari D, Mizusawa J, Boku N, Tsuburaya A, Terashima M, Sasako M; Stomach Cancer Study Group of the Japan Clinical Oncology Group. A Phase II study of systemic chemotherapy with docetaxel, cisplatin, and S-1 (DCS) followed by surgery in gastric cancer patients with extensive lymph node metastasis: Japan Clinical Oncology Group study JCOG1002. *Jpn J Clin Oncol* 2012; **42**: 556-559 [PMID: 22525210 DOI: 10.1093/jjco/hys054]
73 **Kodera Y**. Neoadjuvant chemotherapy for gastric adenocarcinoma in Japan. *Surg Today* 2017; **47**: 899-907 [PMID: 28247105 DOI: 10.1007/s00595-017-1473-2]
74 **Hecht JR**, Bang YJ, Qin SK, Chung HC, Xu JM, Park JO, Jeziorski K, Shparyk Y, Hoff PM, Sobrero A, Salman P, Li J, Protsenko SA, Wainberg ZA, Buyse M, Afenjar K, Houé V, Garcia A, Kaneko T, Huang

Y, Khan-Wasti S, Santillana S, Press MF, Slamon D. Lapatinib in Combination With Capecitabine Plus Oxaliplatin in Human Epidermal Growth Factor Receptor 2-Positive Advanced or Metastatic Gastric, Esophageal, or Gastroesophageal Adenocarcinoma: TRIO-013/LOGiC--A Randomized Phase III Trial. *J Clin Oncol* 2016; **34**: 443-451 [PMID: 26628478 DOI: 10.1200/JCO.2015.62.6598]

75 **Satoh T**, Xu RH, Chung HC, Sun GP, Doi T, Xu JM, Tsuji A, Omuro Y, Li J, Wang JW, Miwa H, Qin SK, Chung IJ, Yeh KH, Feng JF, Mukaiyama A, Kobayashi M, Ohtsu A, Bang YJ. Lapatinib plus paclitaxel versus paclitaxel alone in the second-line treatment of HER2-amplified advanced gastric cancer in Asian populations: TyTAN--a randomized, phase III study. *J Clin Oncol* 2014; **32**: 2039-2049 [PMID: 24868024 DOI: 10.1200/JCO.2013.53.6136]

76 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]

77 **Lordick F**, Kang YK, Chung HC, Salman P, Oh SC, Bodoky G, Kurteva G, Volovat C, Moiseyenko VM, Gorbunova V, Park JO, Sawaki A, Celik I, Götte H, Melezínková H, Moehler M; Arbeitsgemeinschaft Internistische Onkologie and EXPAND Investigators. Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 490-499 [PMID: 23594786 DOI: 10.1016/S1470-2045(13)70102-5]

78 **Waddell T**, Chau I, Cunningham D, Gonzalez D, Okines AF, Okines C, Wotherspoon A, Saffery C, Middleton G, Wadsley J, Ferry D, Mansoor W, Crosby T, Coxon F, Smith D, Waters J, Iveson T, Falk S, Slater S, Peckitt C, Barbachano Y. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 481-489 [PMID: 23594787 DOI: 10.1016/S1470-2045(13)70096-2]

79 **Ohtsu A**, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, Lim HY, Yamada Y, Wu J, Langer B, Starnawski M, Kang YK. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol* 2011; **29**: 3968-3976 [PMID: 21844504 DOI: 10.1200/JCO.2011.36.2236]

80 **Shen L**, Li J, Xu J, Pan H, Dai G, Qin S, Wang L, Wang J, Yang Z, Shu Y, Xu R, Chen L, Liu Y, Yu S, Bu L, Piao Y. Bevacizumab plus capecitabine and cisplatin in Chinese patients with inoperable locally advanced or metastatic gastric or gastroesophageal junction cancer: randomized, double-blind, phase III study (AVATAR study). *Gastric Cancer* 2015; **18**: 168-176 [PMID: 24557418 DOI: 10.1007/s10120-014-0351-5]

81 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, Dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J; REGARD Trial Investigators. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768 DOI: 10.1016/S0140-6736(13)61719-5]

82 **Wilke H**, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD, Ohtsu A; RAINBOW Study Group. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1224-1235 [PMID: 25240821 DOI: 10.1016/S1470-2045(14)70420-6]

83 **Ohtsu A**, Ajani JA, Bai YX, Bang YJ, Chung HC, Pan HM, Sahmoud T, Shen L, Yeh KH, Chin K, Muro K, Kim YH, Ferry D, Tebbutt NC, Al-Batran SE, Smith H, Costantini C, Rizvi S, Lebwohl D, Van Cutsem E. Everolimus for previously treated advanced gastric cancer: results of the randomized, double-blind, phase III GRANITE-1 study. *J Clin Oncol* 2013; **31**: 3935-3943 [PMID: 24043745 DOI: 10.1200/JCO.2012.48.3552]

84 **Catenacci DVT**, Tebbutt NC, Davidenko I, Murad AM, Al-Batran SE, Ilson DH, Tjulandin S, Gotovkin E, Karaszewska B, Bondarenko I, Tejani MA, Udrea AA, Tehfe M, De Vita F, Turkington C, Tang R, Ang A, Zhang Y, Hoang T, Sidhu R, Cunningham D. Rilotumumab plus epirubicin, cisplatin, and capecitabine as first-line therapy in advanced MET-positive gastric or gastro-oesophageal junction cancer (RILOMET-1): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2017; **18**: 1467-1482 [PMID: 28958504 DOI: 10.1016/S1470-2045(17)30566-1]

85 **Shah MA**, Bang YJ, Lordick F, Alsina M, Chen M, Hack SP, Bruey JM, Smith D, McCaffery I, Shames DS, Phan S, Cunningham D. Effect of Fluorouracil, Leucovorin, and Oxaliplatin With or Without Onartuzumab in HER2-Negative, MET-Positive Gastroesophageal Adenocarcinoma: The METGastric Randomized Clinical Trial. *JAMA Oncol* 2017; **3**: 620-627 [PMID: 27918764 DOI: 10.1001/jamaoncol.2016.5580]

86 **Matsueda S**, Graham DY. Immunotherapy in gastric cancer. *World J Gastroenterol* 2014; **20**: 1657-1666 [PMID: 24587645 DOI: 10.3748/wjg.v20.i7.1657]

87 **Kono K**, Takahashi A, Ichihara F, Amemiya H, Iizuka H, Fujii H, Sekikawa T, Matsumoto Y. Prognostic significance of adoptive immunotherapy with tumor-associated lymphocytes in patients with advanced gastric cancer: a randomized trial. *Clin Cancer Res* 2002; **8**: 1767-1771 [PMID: 12060615]

88 **Jiang J**, Xu N, Wu C, Deng H, Lu M, Li M, Xu B, Wu J, Wang R, Xu J, Nilsson-Ehle P. Treatment of advanced gastric cancer by chemotherapy combined with autologous cytokine-induced killer cells. *Anticancer Res* 2006; **26**: 2237-2242 [PMID: 16821594]

89 **Jiang JT**, Shen YP, Wu CP, Zhu YB, Wei WX, Chen LJ, Zheng X, Sun J, Lu BF, Zhang XG. Increasing the frequency of CIK cells adoptive immunotherapy may decrease risk of death in gastric cancer patients. *World J Gastroenterol* 2010; **16**: 6155-6162 [PMID: 21182234 DOI: 10.3748/wjg.v16.i48.6155]

90 **Ralph C**, Elkord E, Burt DJ, O'Dwyer JF, Austin EB, Stern PL, Hawkins RE, Thistlethwaite FC. Modulation of lymphocyte regulation for cancer therapy: a phase II trial of tremelimumab in advanced gastric and esophageal adenocarcinoma. *Clin Cancer Res* 2010; **16**: 1662-1672 [PMID: 20179239 DOI: 10.1158/1078-0432.CCR-09-2870]

91 **Janjigian YY**, Bendell J, Calvo E, Kim JW, Ascierto PA, Sharma P, Ott PA, Peltola K, Jaeger D, Evans J, de Braud F, Chau I, Harbison CT, Dorange C, Tschaika M, Le DT. CheckMate-032 Study: Efficacy and

Safety of Nivolumab and Nivolumab Plus Ipilimumab in Patients With Metastatic Esophagogastric Cancer. *J Clin Oncol* 2018; **36**: 2836-2844 [PMID: 30110194 DOI: 10.1200/JCO.2017.76.6212]

92 **Muro K**, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtness B, McRee AJ, Lin CC, Pathiraja K, Lunceford J, Emancipator K, Juco J, Koshiji M, Bang YJ. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 2016; **17**: 717-726 [PMID: 27157491 DOI: 10.1016/S1470-2045(16)00175-3]

93 **Maher JC**, Krishan A, Lampidis TJ. Greater cell cycle inhibition and cytotoxicity induced by 2-deoxy-D-glucose in tumor cells treated under hypoxic vs aerobic conditions. *Cancer Chemother Pharmacol* 2004; **53**: 116-122 [PMID: 14605866 DOI: 10.1007/s00280-003-0724-7]

94 **Cai Z**, Zhao JS, Li JJ, Peng DN, Wang XY, Chen TL, Qiu YP, Chen PP, Li WJ, Xu LY, Li EM, Tam JP, Qi RZ, Jia W, Xie D. A combined proteomics and metabolomics profiling of gastric cardia cancer reveals characteristic dysregulations in glucose metabolism. *Mol Cell Proteomics* 2010; **9**: 2617-2628 [PMID: 20699381 DOI: 10.1074/mcp.M110.000661]

95 **Wiggins T**, Kumar S, Markar SR, Antonowicz S, Hanna GB. Tyrosine, phenylalanine, and tryptophan in gastroesophageal malignancy: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2015; **24**: 32-38 [PMID: 25344892 DOI: 10.1158/1055-9965.EPI-14-0980]

96 **Duan J**, Sun L, Huang H, Wu Z, Wang L, Liao W. Overexpression of fatty acid synthase predicts a poor prognosis for human gastric cancer. *Mol Med Rep* 2016; **13**: 3027-3035 [PMID: 26936091 DOI: 10.3892/mmr.2016.4902]

97 **Martin-Romano P**, Solans BP, Cano D, Subtil JC, Chopitea A, Arbea L, Lozano MD, Castanon E, Baraibar I, Salas D, Hernandez-Lizoain JL, Trocóniz IF, Rodriguez J. Neoadjuvant therapy for locally advanced gastric cancer patients. A population pharmacodynamic modeling. *PLoS One* 2019; **14**: e0215970 [PMID: 31071108 DOI: 10.1371/journal.pone.0215970]

98 **Liu Y**, Zhang KC, Huang XH, Xi HQ, Gao YH, Liang WQ, Wang XX, Chen L. Timing of surgery after neoadjuvant chemotherapy for gastric cancer: Impact on outcomes. *World J Gastroenterol* 2018; **24**: 257-265 [PMID: 29375211 DOI: 10.3748/wjg.v24.i2.257]

99 **Aichler M**, Luber B, Lordick F, Walch A. Proteomic and metabolic prediction of response to therapy in gastric cancer. *World J Gastroenterol* 2014; **20**: 13648-13657 [PMID: 25320503 DOI: 10.3748/wjg.v20.i38.13648]

100 **Biondi A**, Agnes A, Del Coco F, Pozzo C, Strippoli A, D'Ugo D, Persiani R. Preoperative therapy and long-term survival in gastric cancer: One size does not fit all. *Surg Oncol* 2018; **27**: 575-583 [PMID: 30217321 DOI: 10.1016/j.suronc.2018.07.006]

101 **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]

102 **Ajani JA**, Rodriguez W, Bodoky G, Moiseyenko V, Lichinitser M, Gorbunova V, Vynnychenko I, Garin A, Lang I, Falcon S. Multicenter phase III comparison of cisplatin/S-1 with cisplatin/infusional fluorouracil in advanced gastric or gastroesophageal adenocarcinoma study: the FLAGS trial. *J Clin Oncol* 2010; **28**: 1547-1553 [PMID: 20159816 DOI: 10.1200/JCO.2009.25.4706]

103 **Goekkurt E**, Al-Batran SE, Hartmann JT, Mogck U, Schuch G, Kramer M, Jaeger E, Bokemeyer C, Ehninger G, Stoehlmacher J. Pharmacogenetic analyses of a phase III trial in metastatic gastroesophageal adenocarcinoma with fluorouracil and leucovorin plus either oxaliplatin or cisplatin: a study of the arbeitsgemeinschaft internistische onkologie. *J Clin Oncol* 2009; **27**: 2863-2873 [PMID: 19332728 DOI: 10.1200/JCO.2008.19.1718]

104 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]

105 **Cristescu R**, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med* 2015; **21**: 449-456 [PMID: 25894828 DOI: 10.1038/nm.3850]

106 **LAUREN P**. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675 DOI: 10.1111/apm.1965.64.1.31]

107 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington MK, Carneiro F, Cree IA. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2019 [PMID: 31433515 DOI: 10.1111/his.13975]

108 **Japanese Gastric Cancer Association**. Japanese classification of gastric carcinoma: 3rd English edition. *Gastric Cancer* 2011; **14**: 101-112 [PMID: 21573743 DOI: 10.1007/s10120-011-0041-5]

109 **Qiu MZ**, Li Q, Wang ZQ, Liu TS, Liu Q, Wei XL, Jin Y, Wang DS, Ren C, Bai L, Zhang DS, Wang FH, Li YH, Xu RH. HER2-positive patients receiving trastuzumab treatment have a comparable prognosis with HER2-negative advanced gastric cancer patients: a prospective cohort observation. *Int J Cancer* 2014; **134**: 2468-2477 [PMID: 24155030 DOI: 10.1002/ijc.28559]

110 **Van Cutsem E**, de Haas S, Kang YK, Ohtsu A, Tebbutt NC, Ming Xu J, Peng Yong W, Langer B, Delmar P, Scherer SJ, Shah MA. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a biomarker evaluation from the AVAGAST randomized phase III trial. *J Clin Oncol* 2012; **30**: 2119-2127 [PMID: 22565005 DOI: 10.1200/JCO.2011.39.9824]

111 **Okines AF**, Langley RE, Thompson LC, Stenning SP, Stevenson L, Falk S, Seymour M, Coxon F, Middleton GW, Smith D, Evans L, Slater S, Waters J, Ford D, Hall M, Iveson TJ, Petty RD, Plummer C, Allum WH, Blazeby JM, Griffin M, Cunningham D. Bevacizumab with peri-operative epirubicin, cisplatin and capecitabine (ECX) in localised gastro-oesophageal adenocarcinoma: a safety report. *Ann Oncol* 2013; **24**: 702-709 [PMID: 23108952 DOI: 10.1093/annonc/mds533]

112 **Yoshikawa T**, Sasako M, Yamamoto S, Sano T, Imamura H, Fujitani K, Oshita H, Ito S, Kawashima Y, Fukushima N. Phase II study of neoadjuvant chemotherapy and extended surgery for locally advanced gastric cancer. *Br J Surg* 2009; **96**: 1015-1022 [PMID: 19644974 DOI: 10.1002/bjs.6665]

113 **Inoue T**, Yachida S, Usuki H, Kimura T, Hagiike M, Okano K, Suzuki Y. Pilot feasibility study of neoadjuvant chemoradiotherapy with S-1 in patients with locally advanced gastric cancer featuring adjacent tissue invasion or JGCA bulky N2 lymph node metastases. *Ann Surg Oncol* 2012; **19**: 2937-2945 [PMID: 22466666 DOI: 10.1245/s10434-012-2332-4]

114 **Sakuramoto S**, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, Furukawa H, Nakajima T, Ohashi Y, Imamura H, Higashino M, Yamamura Y, Kurita A, Arai K; ACTS-GC Group. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. *N Engl J Med* 2007; **357**: 1810-1820 [PMID: 17978289 DOI: 10.1056/NEJMoa072252]

115 **Mandard AM**, Dalibard F, Mandard JC, Marnay J, Henry-Amar M, Petiot JF, Roussel A, Jacob JH, Segol P, Samama G. Pathologic assessment of tumor regression after preoperative chemoradiotherapy of esophageal carcinoma. Clinicopathologic correlations. *Cancer* 1994; **73**: 2680-2686 [PMID: 8194005 DOI: 10.1002/1097-0142(19940601)73:11<2680::aid-cncr2820731105>3.0.co;2-c]

116 **Becker K**, Mueller JD, Schulmacher C, Ott K, Fink U, Busch R, Böttcher K, Siewert JR, Höfler H. Histomorphology and grading of regression in gastric carcinoma treated with neoadjuvant chemotherapy. *Cancer* 2003; **98**: 1521-1530 [PMID: 14508841 DOI: 10.1002/cncr.11660]

117 **Kajitani T**. The general rules for the gastric cancer study in surgery and pathology. Part I. Clinical classification. *Jpn J Surg* 1981; **11**: 127-139 [PMID: 7300058]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 54-65

DOI: 10.4251/wjgo.v12.i1.54

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Identification of candidate biomarkers correlated with pathogenesis of postoperative peritoneal adhesion by using microarray analysis

Yao-Yao Bian, Li-Li Yang, Yan Yan, Min Zhao, Yan-Qi Chen, Ya-Qi Zhou, Zi-Xin Wang, Wen-Lin Li, Li Zeng

ORCID number: Yao-Yao Bian (0000-0002-9968-2501); Li-Li Yang (0000-0001-5220-3094); Yan Yan (0000-0002-2702-3654); Min Zhao (0000-0003-3691-1650); Yan-Qi Chen (0000-0003-1532-1192); Ya-Qi Zhou (0000-0003-4969-9126); Zi-Xin Wang (0000-0002-1292-2454); Wen-Lin Li (0000-0002-7105-2743); Li Zeng (0000-0002-9095-9439).

Author contributions: Bian YY, Yang LL, and Yan Y contributed equally to this study; Bian YY conceived and designed the study, made the data acquisition, and prepared the manuscript with Yang LL; Yan Y and Zhao M conducted the data analysis; Chen YQ, Zhou YQ, and Wang ZX contributed to the animal experiment; Li WL and Zeng L provided several suggestions for manuscript revision.

Supported by the National Natural Science Foundation of China, No. 81704084, No. 81603529, and No. 81673982; the Science and Technology Projects of Jiangsu Provincial Bureau of Traditional Chinese Medicine, No. YB2017002 and No. YB2015002; the Natural Science Foundation of the Jiangsu Higher Education Institutions, No. 16KJB360002; the Postgraduate Research and Practice Innovation Program of Jiangsu Province, No. KYCX18_1541; the Qing Lan Project; and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), the Open Projects of the Discipline of Chinese Medicine of Nanjing University of Chinese Medicine (ZYX03KF63), Jiangsu Government Scholarship for

Yao-Yao Bian, Ya-Qi Zhou, Zi-Xin Wang, School of Nursing, Nanjing University of Chinese Medicine, Nanjing 210023, Jiangsu Province, China

Li-Li Yang, Min Zhao, Yan-Qi Chen, Li Zeng, School of First Clinical Medicine, Nanjing University of Chinese Medicine, Nanjing 210023, Jiangsu Province, China

Li-Li Yang, Wen-Lin Li, Li Zeng, Jingwen Library, Nanjing University of Chinese Medicine, Nanjing 210023, Jiangsu Province, China

Yan Yan, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China

Corresponding author: Li Zeng, PhD, Professor, School of First Clinical Medicine and Jingwen Library, Nanjing University of Chinese Medicine, 138 Xianlin Road, Nanjing 210023, Jiangsu Province, China. zengli@njucm.edu.cn

Abstract

BACKGROUND

Postoperative peritoneal adhesion (PPA), characterized by abdominal pain, female infertility, and even bowel obstruction after surgery, has always been a major concern. The occurrence and formation of adhesion are from complex biological processes. However, the molecular mechanisms underlying the basis of microarray data profile, followed by peritoneal adhesion formation, are largely unknown.

AIM

To reveal the underlying pathogenesis of PPA at the molecular level.

METHODS

The gene expression profile was retrieved from the Gene Expression Omnibus database for our analysis. We identified a panel of key genes and related pathways involved in adhesion formation using bioinformatics analysis methods. We performed quantitative PCR and western blotting *in vivo* to validate the results preliminarily.

RESULTS

In total, 446 expressed genes were altered in peritoneal adhesion. We found that several hub genes (*e.g.*, tumor necrosis factor, interleukin 1 beta, interleukin 6, C-X-C motif chemokine ligand 1, C-X-C motif chemokine ligand 2) were marked as significant biomarkers. Functional analysis suggested that these genes were

Overseas Studies and China Scholarship Council.

Institutional animal care and use committee statement: The protocol was approved by the Laboratory Animal Management Committee of the Nanjing University of Chinese Medicine.

Conflict-of-interest statement: The authors have no conflicts of interest to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Received: June 4, 2019
Peer-review started: June 4, 2019
First decision: July 31, 2019
Revised: August 5, 2019
Accepted: September 12, 2019
Article in press: September 12, 2019
Published online: January 15, 2020

P-Reviewer: Shu X
S-Editor: Ma YJ
L-Editor: Filipodia
E-Editor: Qi LL

enriched in the Toll-like receptor signaling pathway. According to the Kyoto Encyclopedia of Genes and Genomes pathway and published studies, TLR4, myeloid differentiation primary response protein 88 (MyD88), and nuclear factor kappa B (NF-κB) played essential roles in Toll-like signaling transduction. Here, we obtained a regulatory evidence chain of TLR4/MyD88/NF-κB/inflammatory cytokines/peritoneal adhesion involved in the pathogenesis of postoperative adhesion. The results of the microarray analysis were verified by the animal experiments. These findings may extend our understanding of the molecular mechanisms of PPA.

CONCLUSION
The regulatory evidence chain of TLR4/MyD88/NF-κB/inflammatory cytokines/peritoneal adhesion may play key roles in the pathogenesis of PPA. Future studies are required to validate our findings.

Key words: Postoperative peritoneal adhesion; Candidate biomarkers; Molecular pathogenesis; Bioinformatics analysis

Core tip: Postoperative peritoneal adhesion remains an urgent clinical concern due to increasing abdominal surgery. The occurrence and formation of adhesion are from complex biological processes. However, the molecular mechanisms underlying the basis of microarray data profile, followed by peritoneal adhesion formation, are largely unknown. In this study, we uncovered the underlying pathogenesis of postoperative peritoneal adhesion at the molecular level using bioinformatics analysis methods. The results were further validated using animal experiments. It showed that the regulatory evidence chain of TLR4/MyD88/NF-κB/inflammatory cytokines/peritoneal adhesion played key roles in the pathogenesis of postoperative adhesion. Our findings may provide new insights into peritoneal adhesion formation.

Citation: Bian YY, Yang LL, Yan Y, Zhao M, Chen YQ, Zhou YQ, Wang ZX, Li WL, Zeng L. Identification of candidate biomarkers correlated with pathogenesis of postoperative peritoneal adhesion by using microarray analysis. *World J Gastrointest Oncol* 2020; 12(1): 54-65
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/54.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.54

INTRODUCTION

Postoperative peritoneal adhesion (PPA) is an indefinite adhesion or fibrous cord formed among abdominal organs or tissues. It remains an urgent clinical concern due to the increasing occurrence of abdominal surgery. PPA can lead to acute or chronic complications including persistent abdominal pain and bloating, female infertility, bowel obstruction, and intestinal necrosis[1]. A previous review showed that even minimally invasive surgical techniques that are widely used to minimize surgery lesions of peritoneal trauma cannot prevent adhesion formation[2]. PPA not only increases the reoperation rate and extends hospital stay but also results in a considerable disease burden and heavy financial responsibility for individuals, families, and society[3]. PPA has an incidence rate ranging from 90% to 95%[4]. Approximately 117 per 100000 people in the United States have been hospitalized for adhesion-related problems, and the direct annual hospitalization cost for adhesion-related complications and surgery increased from US$1.3 billion in 1994 to US$50 billion in 2010 in United States[5,6].

The occurrence and development of peritoneal adhesion are complex biological processes, during which many genes and pathways are involved in the pathogenesis of adhesion formation. However, the potential biological function of adhesion formation remains limited. Therefore, there is a need to further identify the differentially expressed transcripts and related pathways associated with peritoneal adhesion formation.

In this study, we retrieved a dataset of mRNA expression microarrays from Gene

Expression Omnibus (commonly known as GEO) and identified a panel of altered genes and related pathways involved in the developmental progression of peritoneal adhesion by using bioinformatics methods. To validate our findings, we also carried out preliminary verification by using molecular biology techniques *in vivo*. The study aimed to identify candidate biomarkers to uncover the underlying pathogenesis of PPA at the molecular level.

MATERIALS AND METHODS

Data acquisition and identification of differentially expressed genes

The microarray expression dataset was downloaded from the GEO database by searching the key words: "RNA," "peritoneal adhesion," or "abdominal adhesion" and "Mus musculus" (organism). After screening, the accession number GSE123413 was obtained for analysis. The platform was GPL 21163, Agilent-074809 SurePrint G3 Mouse GE v2 8x60K Microarray. This dataset consisted of the transcriptional profiling of cecum tissues harvested at three time points (*i.e.* 3 h, 12 h, and 3 d) after the models were prepared by cauterization. The raw data were preprocessed and normalized using the affy package in R (version 3.3.4). Then the differentially expressed genes (DEGs) between sham (SH) and PPA groups at three different time points were screened separately using the limma package. $|\log_2 FC| > 1$ and $P < 0.05$ were considered statistically significant for DEGs. The overlapping DEGs of the three different time points were generated using the online tool Venny (http://bioinfogp.cnb.csic.es/tools/venny). The heat map of DEGs was obtained with the online tool Morpheus (https://software.broadinstitute.org/morpheus/).

Function and pathway enrichment analysis

To understand the underlying biological phenomena, gene ontology (GO) terms were used to determine gene annotation. Kyoto Encyclopedia of Genes and Genomes (commonly known as KEGG) enrichment was performed to locate the significant enrichment pathway. Both analyses were implemented on the Database for Annotation, Visualization and Integrated Discovery (commonly known as DAVID; http://david.abcc.ncifcrf.gov/)[7].

Protein–protein interaction (PPI) network construction and modules analysis

To further predict the interaction of peritoneal adhesion-associated protein pairs, the Search Tool for the Retrieval of Interacting Genes (commonly known as STRING)[8] was performed with a confidence score > 0.7 defined as significant. Then PPI integrated networks were mapped by Cytoscape 3.4.0 software[9]. Finally, the plug-in Molecular Complex Detection (commonly known as MCODE) was used to screen the modules of hub genes from the PPI network when node degree > 30 was considered as cut-off criteria.

Animal experiments

Twenty male Sprague-Dawley rats (8 wk old, weighing 280 ± 20 g) were purchased from the Qinglongshan Experimental Animal Breeding Farm (Nanjing, China). The rats were randomly divided into two groups: SH (n = 10) and PPA (n = 10). Both groups were housed in a standard condition of 12 h light-dark cycle (light on at 07:00 a.m.) under a controlled temperature of 22 ± 2°C. All animals were provided plenty of food and water and allowed to acclimatize to this condition 3 d before use. All experiments in this study followed the Guidelines of Accommodation and Care for Animals formulated by the Chinese Convention for the protection of vertebrate animals used for experimental and other scientific purposes and were authorized by the Laboratory Animal Management Committee of the Nanjing University of Chinese Medicine.

Surgical procedures and adhesion quality

The cecum cauterization model was established by a previous study[10]. After preoperative fasting for 12 h, the rats were placed under anesthesia with 1.0%–1.5% isoflurane. A 1.5 cm midline incision was made in the abdominal wall after traditional skin preservation and sterilization under aseptic conditions. The cecum was isolated and then cauterized using bipolar forceps to inflict a coagulation function for 1 s. Finally, the cecum was restored into the abdominal cavity, and the abdominal wall was sutured. After 3 d, the rats were sacrificed. Two independent investigators who were blinded to both groups evaluated the adhesion quality on the basis of a five-stage grading score system[11,12] shown in Table 1[13].

Masson staining

Table 1 Peritoneal adhesion scoring system

Grade	Score	Adhesion area	Description
0	0	None	None
I	1	0%–25%	Thin, avascular, transparent
II	2	25%–50%	Thick, avascular, opaque
III	3	50%–75%	Thick, capillaries, opaque, sharp dissection required
IV	4	75%–100%	Thick, opaque, large vessels, sharp dissection required

After fixation in 10% neutral formalin for 48 h, the cecum tissues were embedded in paraffin, and cut into sections. Masson staining was performed to estimate the collagen deposition. Represented views were visualized under a microscope (DM2500; Leica, Germany).

RNA extraction and quantitative PCR

Total RNA was extracted from the damaged cecum using TRIzol reagent (Invitrogen, United States). The complementary DNA (cDNA) was generated *via* reverse transcription by using the First Stand cDNA Synthesis Kit (Thermo Fisher Scientific, United States). UltraSYBR One Step RT-qPCR Kit (Cwbio Technology, China) was used according to the manufacturer's protocol. Based on GAPDH as standardization, the expression levels were conducted by using the $2^{-\Delta\Delta CT}$ analysis method. The primer sequences are shown in Table 2.

Western blot analysis

Tissue proteins were extracted by the RIPA Protein Extraction Kit (Beyotime Biotechnology, China) according to routine protocols. The extracted proteins were added to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to PVDF membranes. Then the membranes were incubated with primary antibody at 4°C overnight against Toll-like receptor 4 (TLR4, 1:200), myeloid differentiation primary response protein 88 (MyD88, 1:200), nuclear factor kappa B (NF-κB, 1:200), and β-actin (1:2000) (all from Santa Cruz Biotechnology, United States). After incubation with secondary antibody for 1 h at 37°C, protein expression was viewed by the Chemiluminescence Imaging system (Bio-Rad, United States).

Statistical analysis

Statistical analyses were conducted using SPSS 19.0 software. All data are presented as the mean ± SD. Statistical comparisons within the two groups were made by the unpaired Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

RESULTS

DEG identification

The GSE123413 expression profile dataset consisted of the expression data matrix of 56743 gene probes. The raw data were processed and normalized with R software, as presented in Figure 1A. We identified the DEGs of three time points and found 457 overlapping genes. Of these genes, 446 expressed genes were altered, among which 183 were upregulated and 263 were downregulated. The expression levels of dysregulated genes are shown in Figure 1B.

Functional and pathway enrichment analyses

Analyses of the above-obtained 446 genes were performed using DAVID for biological process annotation and KEGG pathway enrichment. GO term assignment analysis suggested that the most enriched GOs were enriched in inflammatory response (GO: 0006954), neutrophil chemotaxis (GO: 0030593), cytokine activity (GO: 0005125), cellular response to interleukin-1 (IL-1) (GO: 0071347), immune response (GO: 0006955), and response to lipopolysaccharide (GO: 0032496), as presented in Figure 2A. According to pathway enrichment analysis, the enriched altered genes were involved in PPA-related pathways such as the cytokine-cytokine receptor interaction and tumor necrosis factor (TNF) signaling pathway (Figure 2B).

PPI network construction and modules analysis

The 743 pairs involving 376 proteins were mapped in the PPI network through the

Table 2 Primers used for qPCR

Gene	Primer sequence, 5'-3'
TNF-α-forward	TCATTCCTGCTCGTGGCGGG
TNF-α-reverse	CGGCTGACGGTGGGGTGAG
IL1β-forward	TCGGCCAAGACAGGTCGCTCA
IL1β-reverse	TGGTTGCCCATCAGAGGCAAGG
IL6-forward	CCACTGCCTTCCCTACTTCA
IL6-reverse	ACAGTGCATCATCGCTGTTC
CXCL1-forward	GGCAGGGATTCACTTCAAGA
CXCL1-reverse	GCCATCGGTGCAATCTATCT
CXCL2-forward	ATCCAGAGCTTGACGGTGAC
CXCL2-reverse	AGGTACGATCCAGGCTTCCT
GAPDH-forward	GCTACACTGAGGACCAGGTT
GAPDH-reverse	CCCAGCATCAAAGGTGGAAG

STRING website. The genes with relatively high degrees were considered hub genes related to peritoneal adhesion such as TNF (degree = 43), IL-6 (degree = 42), C-X-C motif chemokine ligand 2 (CXCL2; degree = 39), CXCL1 (degree = 38), and IL-1β (degree = 34). These genes may play pivotal roles in PPA progression. To further depict the complex relationship, the obtained interaction pairs were constructed using Cytoscape software (Figure 3A). After analysis using the plug-in MCODE, the top two significant modules were identified and enriched in the inflammatory response by DAVID. Module 1 involved 18 genes and 153 connections, and module 2 implicated 30 genes and 176 connections that were markedly enriched in the TLR signaling pathway, as shown in Figure 3B and Table 3.

Macroscopic evaluation of adhesions

The incisions of all rats were primarily healed, without infection or other complications. The adhesion grades and scores among groups are shown in Table 4. In the SH group, the adhesion grade ranged from 0 to 1, which indicated the absence of adhesion or minor adhesion. However, the adhesion grade ranged from 2 to 4, and the score was approximately 3.4, which suggested that dense connective tissues were present in the PPA groups. The magnitude of peritoneal adhesions is presented in Figure 4.

Masson staining of adhesive tissue

Microscopically, collagen fibers appeared blue and muscle fibers were red in color. The model group displayed a deep and extensive range of blue, which indicated increased collagen fiber accumulation. However, the SH group displayed a light and narrow range of blue color, which suggested decreased collagen fiber accumulation (Figure 5).

Quantitative PCR validation of hub gene expression

To verify the bioinformatics analysis, the expression levels of the hub genes (TNF, IL-1β, IL6, CXCL1, and CXCL2) of the cecum tissues were quantified by quantitative PCR (qPCR) between the adhesion models and SH controls. The results indicated that the mRNA expression levels significantly increased ($P < 0.05$, Figure 6) in PPA groups compared with the SH groups.

Western blot validation of protein expression

In our study, functional enrichment of modules analysis suggested that the significant hub genes were markedly enriched in the TLR signaling pathway. Increasing evidence[14-16] suggests that TLR4, MyD88, and NF-κB play essential roles in the inflammatory pathway such as Toll-like signaling. On the basis of this evidence, we measured the expression levels of the abovementioned proteins by western blotting. The protein expression levels of TLR4, MyD88, and NF-κB in the PPA groups were significantly upregulated compared with those in the SH group (Figure 7).

DISCUSSION

PPA is triggered immediately after surgery, and starts from the inflammation

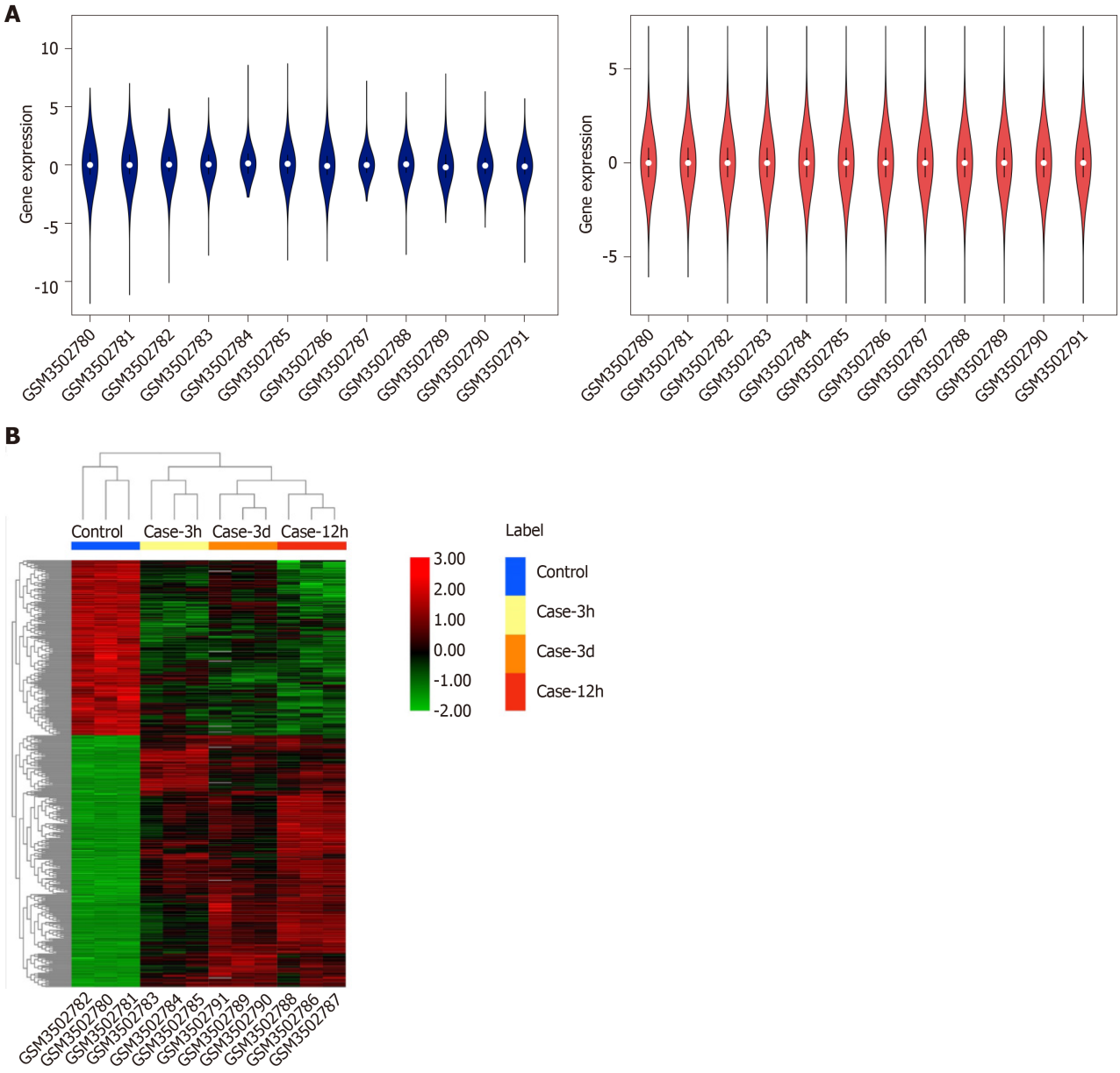

Figure 1 Box plots of data normalization and hierarchical cluster heatmap. A: Box plots of data normalization. The blue box plot represents the data before normalization, whereas the red box plot represents the normalized data; B: Heatmap of the obtained DEGs.

response, which aims to repair injured lesion. Adhesive formation is a strictly timed process. After peritoneal injury, acute inflammation occurs within minutes. Then numerous macrophages and neutrophils migrate onto the damaged lesions within hours, which can trigger a series of cascade reactions. The proliferation and migration of fibroblasts and mesothelium cell further enhance fibrinolysis activity, and the epithelial tissue is repaired within 3 d. If the repair is delayed, adhesion formation occurs from the third day. Hence, it is urgent to identify the potential biomarkers and associated pathways related to adhesion formation within 3 d at the molecular level.

To achieve this goal, we provided a bioinformatics analysis of DEGs and the associated pathways related to adhesion formation on the basis of the available GEO dataset. Consequently, 446 DEGs at three time points were identified. Based on the top 20 GO terms, the altered genes displayed a variety of functionalities in inflammatory and immune response. The results were supported by a previous study[17], thereby indicating that the importance of the inflammatory immune response in the abdominal microenvironment is involved in adhesion formation. Given the implication of gene function enrichment, the DEGs were enriched in the cytokine–cytokine receptor interaction and TNF signaling pathway. Thus, the importance of the biological process and associated inflammatory pathways may be related to adhesion formation.

The pathophysiological process of adhesion formation is rapid, cascaded, and

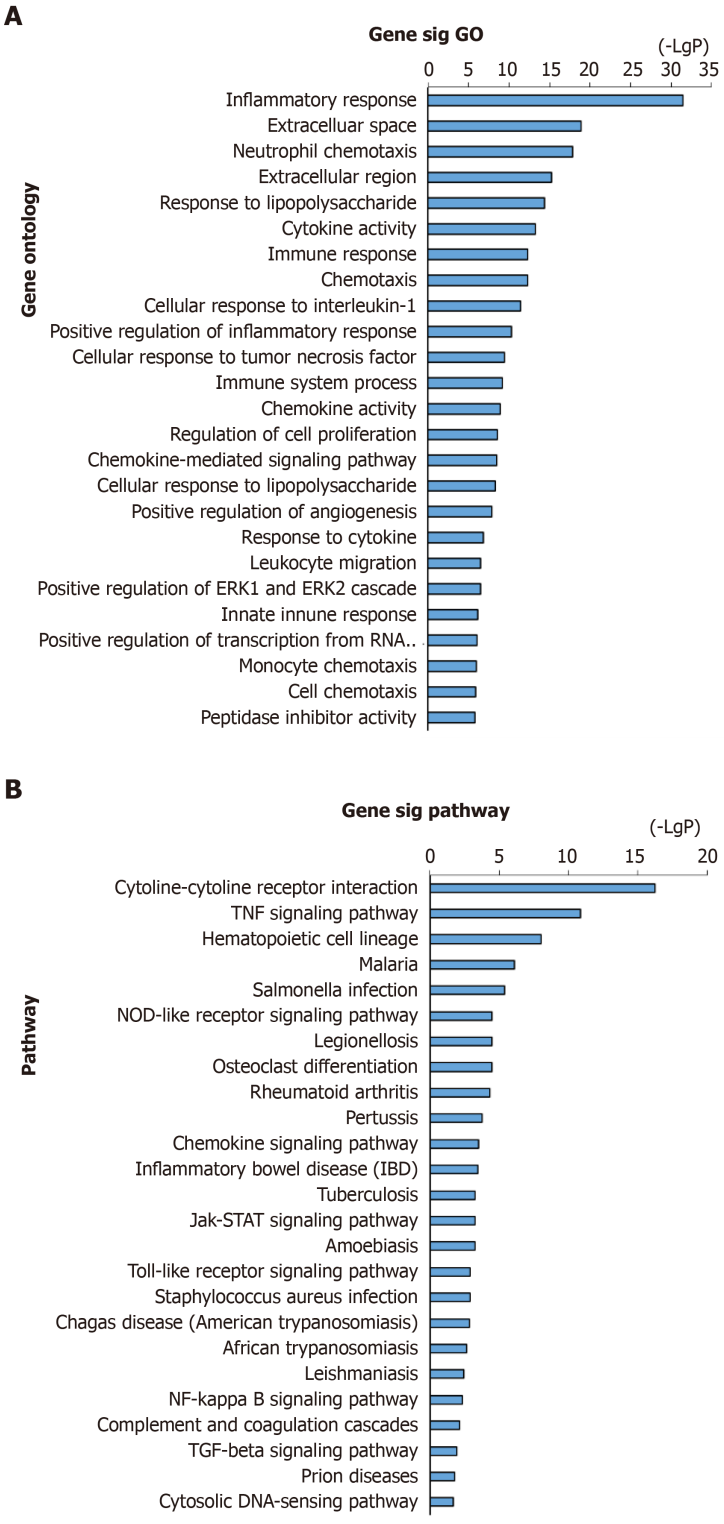

Figure 2 GO annotation and pathway enrichment of DEGs in adhesion formation. A: GO analysis of DEGs (top 20); B: KEGG enrichment of DEGs (top 20). GO: Gene ontology; DEGs: Differentially expressed genes; KEGG: Kyoto Encyclopedia of Genes and Genomes.

complex, during which many genetic and epigenetic modifications of driving genes occur. Among the hub genes, TNF, IL-1β, IL-6, CXCL1, and CXCL2, which may act as important regulators of wound inflammatory, were marked with a high degree. Pro-inflammatory and anti-inflammatory cytokines expressed by these genes had double effects on the balance of the microenvironment. Once the imbalance is broken, adhesion occurs. We primarily validated the crucial roles of these mRNAs in the progression of adhesion formation by using qPCR analysis in the animal experiments. The results were consistent with those of microarray analysis. These signatures that

Table 3 KEGG analysis of two clusters (top five)

Term	Definition	Genes	*P* value
Module 1			
mmu04062	Chemokine signaling pathway	*CXCL1, GNGT1, PPBP, CXCL5, CXCL3, CXCL2, CCL9, PF4, CXCR2, CCL4*	3.70E-11
mmu04060	Cytokine–cytokine receptor interaction	*CXCL1, PPBP, CXCL5, CXCL2, CCL9, PF4, CXCR2, CCL4*	2.51E-07
mmu04080	Neuroactive ligand–receptor interaction	*C5AR1, CHRM2, GALR2, FPR1, ADRA2C, FPR2*	2.10E-04
mmu05132	*Salmonella* infection	*CXCL1, CXCL3, CXCL2, CCL4*	5.11E-04
mmu05150	*Staphylococcus aureus* infection	*C5AR1, FPR1, FPR2*	5.11E-04
Module 2			
mmu04640	Hematopoietic cell lineage	*CSF3, IL6, CD55, TNF, CD44, IL1B*	4.19E-10
mmu04620	Malaria	*CSF3, IL6, TNF, IL1B, THBS1*	1.14E-08
mmu05323	Rheumatoid arthritis	*IL6, IL17A, CCL3, TNF, IL1B*	3.58E-07
mmu04620	TLR signaling pathway	*IL6, CCL3, TNF, IL1B*	9.63E-07
mmu05142	Chagas disease	*IL6, CCL3, TNF, SERPINE1, IL1B*	3.80E-05

KEGG: Kyoto Encyclopedia of Genes and Genomes.

are associated with peritoneal adhesion have also been extensively reported[18-20]. For example, IL-1β is used as an important short-term medium and can be regarded as a reliable biomarker in peritoneal adhesion formation. IL-6 contributes to epithelial cell proliferation and the accumulation of inflammatory cells and fibers into injury sites, following the promotion of the pathological process of adhesion, thereby inducing adhesion formation. The preoperative application of anti-IL-1β and anti-IL-6 antibodies can effectively reduce the occurrence of postoperative abdominal adhesion[19,21]. TNF-α, as an immune regulator factor, combined with TNFR2 can activate the NF-κB signaling pathway in either a classical or non-classical manner, which further mediates the imbalance of inflammatory adhesion and eventually leads to adhesion formation. TNF-α is a pro-fibrogenic cytokine that can promote fibroblast proliferation and stimulate the peritoneal mesothelium cells to increase PAI-1 synthesis, which can further inhibit plasminogen activation and promote fiber accumulation, thereby resulting in adhesion formation. Adhesion-associated CXCL1 and CXCL2 also recruits circulating leukocytes to the injury sites that are involved in the inflammatory response[22]. Thus, these genes might act as key biomarkers in adhesion formation.

Based on the KEGG pathway, these hub genes were markedly enriched in TLR signaling pathway. TLRs are cellular transmembrane receptors and pattern recognition receptors that recognize pathogen-associated molecular patterns in congenital immune system. Gaining function of TLRs, TLR2, TLR3, and TLR4 have vital roles during acute wound repair[23]. Among these receptors, TLR4 is the most studied in tissue healing. The endogenous ligand MyD88 produced during the inflammatory process was identified by TLR4, which can further induce NF-κB activation and downstream regulator (*i.e.* IL-1, IL-6, TNF, CXCL1, and CXCL2) expression[24]. To further elucidate the critical roles of TLR4, MyD88, and NF-κB in the pathogenesis of adhesion formation, western blotting was applied to measure the protein expression in the two groups. The results showed that the proteins mentioned above were highly expressed in the PPA group. Hence, we initially speculated that the regulatory chain of "TLR4/MyD88/NF-κB/inflammatory cytokines" may serve key functions on postoperative adhesion formation. Our findings may provide preliminary evidence about TLR4/MyD88/NF-κB signal transduction in the molecular mechanism of postoperative adhesion formation. To the best of our knowledge, only one study reported that the TLR4/MyD88/NF-κB signaling pathway is a potential pathway in preventing peritoneal inflammation in peritoneal dialysis[25]. Further functional and gene knockout studies are warranted to elucidate the exact effects on the transcriptional expression of genes regulated by NF-κB axis activation.

Taken together, according to bioinformatics analysis, a series of adhesion-related hub genes and a regulatory pathway were identified. To further verify the underlying molecular mechanism in adhesion formation, experiment validation was conducted *in vivo*. The regulatory evidence chain of TLR4/MyD88/NF-κB/inflammatory cytokines/peritoneal adhesion was involved in the pathogenesis of PPA. These findings may provide initial sights into the underlying mechanisms of peritoneal adhesion formation.

Table 4 Grading scores of peritoneal adhesions, *n* = 10

Group	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Scores, mean ± SD
SH group	7	3	0	0	0	0.30 ± 0.483
PPA group	0	0	1	4	5	3.40 ± 0.699[b]

[b]$P < 0.01$, *vs* SH group. PPA: Postoperative peritoneal adhesion; SH: Sham.

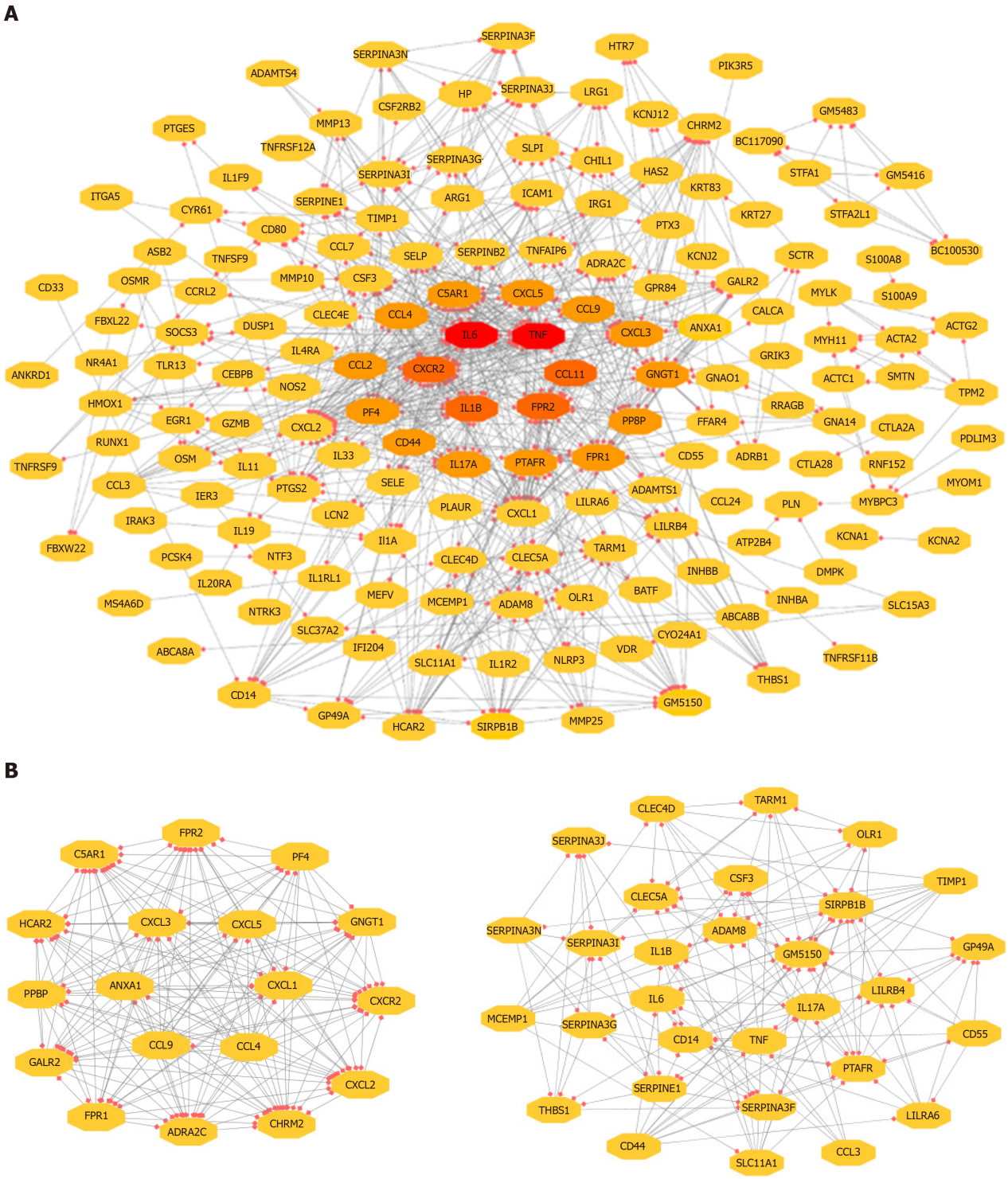

Figure 3 Visualization of the PPI network of identified DEGs. A: Network of the adhesion formation-associated genes; B: Two significant modules selected from the PPI network. Red: Greater degree; Yellow: Lesser degree. DEG: Differentially expressed genes; PPI: Protein–protein interaction.

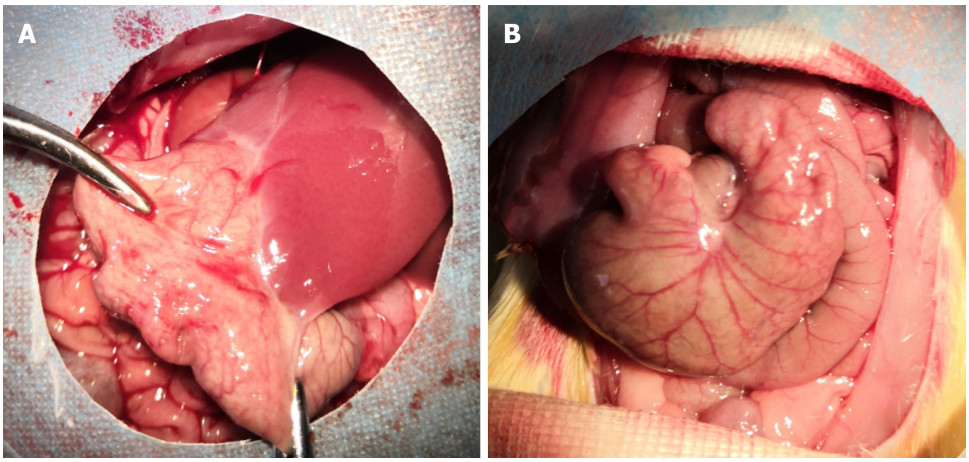

Figure 4 Representative images of PPA in each group. A: PPA group; B: Sham group.

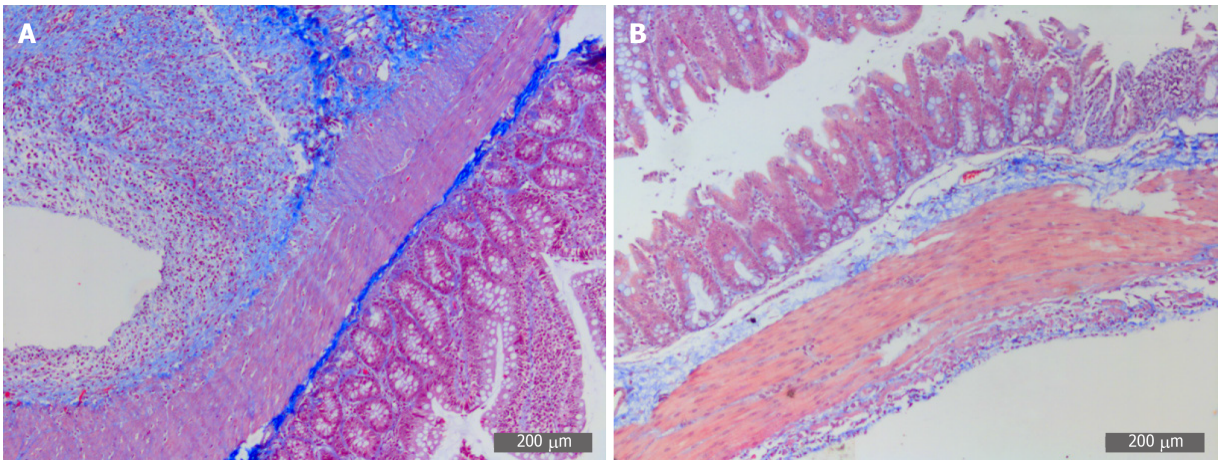

Figure 5 Representative images of Masson staining of adhesive tissues (200×). A: Masson staining in the PPA group; B: Masson staining in the SH group. PPA: Postoperative peritoneal adhesion; SH: Sham.

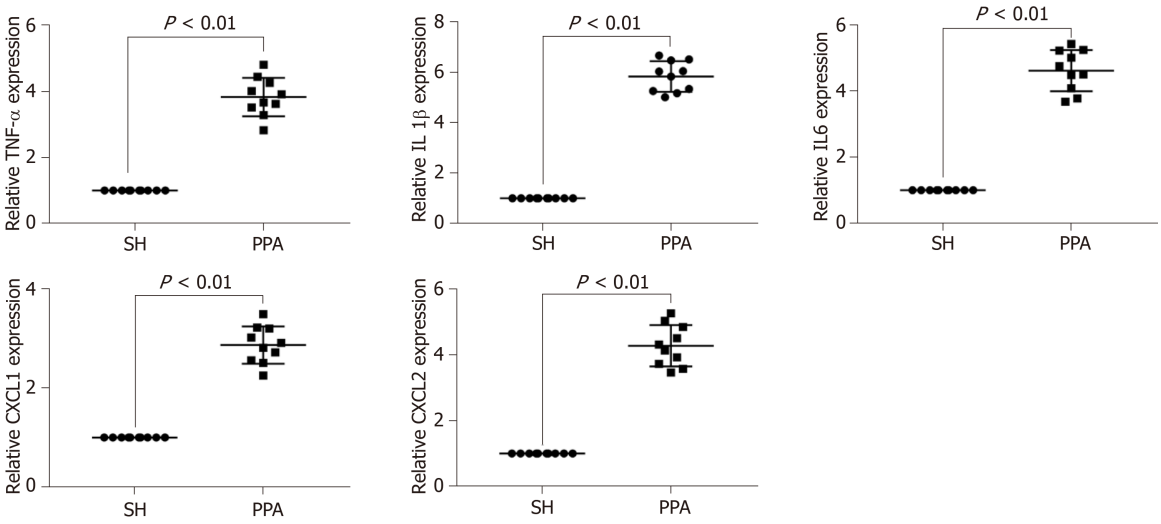

Figure 6 Representative mRNA expression level by qPCR. Expression levels of the mRNAs (TNF-α, IL1β, IL6, CXCL1, and CXCL2) were performed using qPCR, results are expressed as mean ± SD. PPA: Postoperative peritoneal adhesion; SH: Sham.

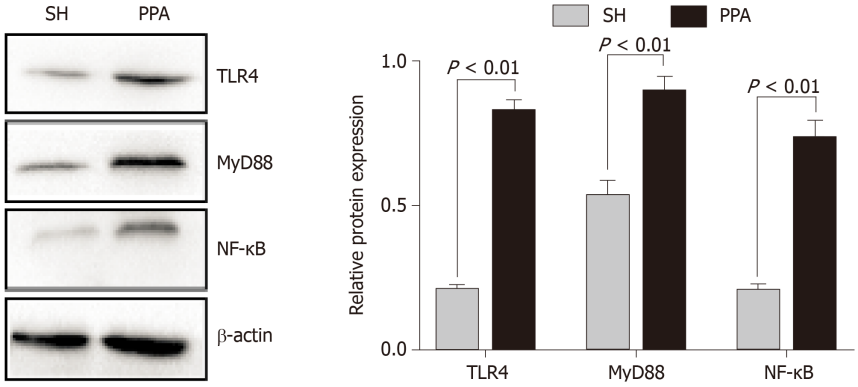

Figure 7 Relative protein levels by western blot analysis. The expression levels of TLR4, MyD88, and NF-κB were obtained using western blot analysis. The results are expressed as mean ± SD. PPA: Postoperative peritoneal adhesion; SH: Sham.

ARTICLE HIGHLIGHTS

Research background

Postoperative peritoneal adhesion (PPA), characterized with abdominal pain, female infertility, and even bowel obstruction after surgery, has always been a major concern. The occurrence and formation of adhesion are from complex biological processes. However, the molecular mechanisms of the basis of microarray data profile, followed by PPA formation, are largely unknown.

Research motivation

The occurrence and development of PPA is a complex biological process, during which many genes and pathways are involved in the pathogenesis of adhesion formation. As such, we developed microarray analysis combined with experimental methods to understand the underlying mechanisms of PPA at the transcriptomic and molecular levels.

Research objectives

The aim of this study was to uncover the molecular mechanisms of PPA formation after surgery using bioinformatics analysis, and to validate the results using rodent adhesion models.

Research methods

The gene expression profile was retrieved from the Gene Expression Omnibus database for our analysis. A panel of key altered genes and related pathways involved in adhesion formation were identified using bioinformatics analysis methods. And the microarray results were verified by performing quantitative PCR and western blotting *in vivo* preliminarily.

Research results

In total, 446 expressed genes were altered in peritoneal adhesion. We found that several hub genes (*e.g.*, TNF, IL-1β, IL-6, CXCL1, CXCL2) were marked as significant biomarkers associated with PPA. Functional analysis suggested that these genes were enriched in the Toll-like receptor signaling pathway. According to the Kyoto Encyclopedia of Genes and Genomes pathway and published studies, TLR4, MyD88, and NF-κB played essential roles in Toll-like signaling transduction. Here, we gained a regulatory evidence chain of TLR4/MyD88/NF-κB/inflammatory cytokines/peritoneal adhesion involved in the pathogenesis of PPA. The results of the microarray analysis were consistent with the animal experiments.

Research conclusions

Our findings provide initial evidence about the regulatory evidence chain of TLR4/MyD88/NF-κB/inflammatory cytokines/peritoneal adhesion in the pathogenesis of PPA. Future studies are required to validate the results.

Research perspectives

These findings may extend our understanding of the molecular mechanisms of PPA. Further functional and gene knockout studies are warranted to elucidate the exact effects on the transcriptional expression of genes regulated by NF-κB axis activation.

REFERENCES

1 **Makarchian HR**, Kasraianfard A, Ghaderzadeh P, Javadi SM, Ghorbanpoor M. The effectiveness of heparin, platelet-rich plasma (PRP), and silver nanoparticles on prevention of postoperative peritoneal adhesion formation in rats. *Acta Cir Bras* 2017; **32**: 22-27 [PMID: 28225914 DOI: 10.1590/s0102-865020170103]

2 **Ten Broek RPG**, Stommel MWJ, Strik C, van Laarhoven CJHM, Keus F, van Goor H. Benefits and harms of adhesion barriers for abdominal surgery: a systematic review and meta-analysis. *Lancet* 2014; **383**: 48-59 [PMID: 24075279 DOI: 10.1016/S0140-6736(13)61687-6]

3 **Brochhausen C**, Schmitt VH, Mamilos A, Schmitt C, Planck CN, Rajab TK, Hierlemann H, Kirkpatrick CJ. Expression of CD68 positive macrophages in the use of different barrier materials to prevent peritoneal adhesions-an animal study. *J Mater Sci Mater Med* 2017; **28**: 15 [PMID: 27995493 DOI: 10.1007/s10856-016-5821-3]

4 **ten Broek RP**, Issa Y, van Santbrink EJ, Bouvy ND, Kruitwagen RF, Jeekel J, Bakkum EA, Rovers MM, van Goor H. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. *BMJ* 2013; **347**: f5588 [PMID: 24092941 DOI: 10.1136/bmj.f5588]

5 **Ray NF**, Denton WG, Thamer M, Henderson SC, Perry S. Abdominal adhesiolysis: inpatient care and expenditures in the United States in 1994. *J Am Coll Surg* 1998; **186**: 1-9 [PMID: 9449594 DOI: 10.1016/S1072-7515(97)00127-0]

6 **Parker MC**, Wilson MS, Menzies D, Sunderland G, Clark DN, Knight AD, Crowe AM; Surgical and Clinical Adhesions Research (SCAR) Group. The SCAR-3 study: 5-year adhesion-related readmission risk following lower abdominal surgical procedures. *Colorectal Dis* 2005; **7**: 551-558 [PMID: 16232234 DOI: 10.1111/j.1463-1318.2005.00857.x]

7 **Huang DW**, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA. The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol* 2007; **8**: R183 [PMID: 17784955 DOI: 10.1186/gb-2007-8-9-r183]

8 **von Mering C**, Huynen M, Jaeggi D, Schmidt S, Bork P, Snel B. STRING: a database of predicted functional associations between proteins. *Nucleic Acids Res* 2003; **31**: 258-261 [PMID: 12519996 DOI: 10.1093/nar/gkg034]

9 **Shannon P**, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003; **13**: 2498-2504 [PMID: 14597658 DOI: 10.1101/gr.1239303]

10 **Kosaka H**, Yoshimoto T, Yoshimoto T, Fujimoto J, Nakanishi K. Interferon-gamma is a therapeutic target molecule for prevention of postoperative adhesion formation. *Nat Med* 2008; **14**: 437-441 [PMID: 18345012 DOI: 10.1038/nm1733]

11 **Koçak I**, Unlü C, Akçan Y, Yakin K. Reduction of adhesion formation with cross-linked hyaluronic acid after peritoneal surgery in rats. *Fertil Steril* 1999; **72**: 873-878 [PMID: 10560992 DOI: 10.1016/s0015-0282(99)00368-4]

12 **Kennedy R**, Costain DJ, McAlister VC, Lee TD. Prevention of experimental postoperative peritoneal adhesions by N,O-carboxymethyl chitosan. *Surgery* 1996; **120**: 866-870 [PMID: 8909523 DOI: 10.1016/s0039-6060(96)80096-1]

13 **Cai X**, Hu S, Yu B, Cai Y, Yang J, Li F, Zheng Y, Shi X. Transglutaminase-catalyzed preparation of crosslinked carboxymethyl chitosan/carboxymethyl cellulose/collagen composite membrane for postsurgical peritoneal adhesion prevention. *Carbohydr Polym* 2018; **201**: 201-210 [PMID: 30241812 DOI: 10.1016/j.carbpol.2018.08.065]

14 **Gay NJ**. Role of self-organising myddosome oligomers in inflammatory signalling by Toll-like receptors. *BMC Biol* 2019; **17**: 15 [PMID: 30786893 DOI: 10.1186/s12915-019-0637-5]

15 **Li J**, Csakai A, Jin J, Zhang F, Yin H. Therapeutic Developments Targeting Toll-like Receptor-4-Mediated Neuroinflammation. *ChemMedChem* 2016; **11**: 154-165 [PMID: 26136385 DOI: 10.1002/cmdc.201500188]

16 **Yang Y**, Lv J, Jiang S, Ma Z, Wang D, Hu W, Deng C, Fan C, Di S, Sun Y, Yi W. The emerging role of Toll-like receptor 4 in myocardial inflammation. *Cell Death Dis* 2016; **7**: e2234 [PMID: 27228349 DOI: 10.1038/cddis.2016.140]

17 **Koninckx PR**, Gomel V, Ussia A, Adamyan L. Role of the peritoneal cavity in the prevention of postoperative adhesions, pain, and fatigue. *Fertil Steril* 2016; **106**: 998-1010 [PMID: 27523299 DOI: 10.1016/j.fertnstert.2016.08.012]

18 **Saba AA**, Godziachvili V, Mavani AK, Silva YJ. Serum levels of interleukin 1 and tumor necrosis factor alpha correlate with peritoneal adhesion grades in humans after major abdominal surgery. *Am Surg* 1998; **64**: 734-6; discussion 737 [PMID: 9697902]

19 **Saba AA**, Kaidi AA, Godziachvili V, Dombi GW, Dawe EJ, Libcke JH, Silva YJ. Effects of interleukin-6 and its neutralizing antibodies on peritoneal adhesion formation and wound healing. *Am Surg* 1996; **62**: 569-572 [PMID: 8651553 DOI: 10.1097/00000478-199607000-00019]

20 **Kaidi AA**, Gurchumelidze T, Nazzal M, Figert P, Vanterpool C, Silva Y. Tumor necrosis factor-alpha: a marker for peritoneal adhesion formation. *J Surg Res* 1995; **58**: 516-518 [PMID: 7745964 DOI: 10.1006/jsre.1995.1081]

21 **Saed GM**, Kruger M, Diamond MP. Expression of transforming growth factor-beta and extracellular matrix by human peritoneal mesothelial cells and by fibroblasts from normal peritoneum and adhesions: effect of Tisseel. *Wound Repair Regen* 2004; **12**: 557-564 [PMID: 15453838 DOI: 10.1111/j.1067-1927.2004.012508.x]

22 **Cassidy MR**, Sheldon HK, Gainsbury ML, Gillespie E, Kosaka H, Heydrick S, Stucchi AF. The neurokinin 1 receptor regulates peritoneal fibrinolytic activity and postoperative adhesion formation. *J Surg Res* 2014; **191**: 12-18 [PMID: 24836694 DOI: 10.1016/j.jss.2014.04.030]

23 **Chen L**, DiPietro LA. Toll-Like Receptor Function in Acute Wounds. *Adv Wound Care (New Rochelle)* 2017; **6**: 344-355 [PMID: 29062591 DOI: 10.1089/wound.2017.0734]

24 **Jiang D**, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, Homer RJ, Goldstein DR, Bucala R, Lee PJ, Medzhitov R, Noble PW. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 2005; **11**: 1173-1179 [PMID: 16244651 DOI: 10.1038/nm1315]

25 **Choi SY**, Ryu HM, Choi JY, Cho JH, Kim CD, Kim YL, Park SH. The role of Toll-like receptor 4 in high-glucose-induced inflammatory and fibrosis markers in human peritoneal mesothelial cells. *Int Urol Nephrol* 2017; **49**: 171-181 [PMID: 27722989 DOI: 10.1007/s11255-016-1430-9]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 66-76

DOI: 10.4251/wjgo.v12.i1.66

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Abnormal CD44 activation of hepatocytes with nonalcoholic fatty accumulation in rat hepatocarcinogenesis

Miao Fang, Min Yao, Jie Yang, Wen-Jie Zheng, Li Wang, Deng-Fu Yao

ORCID number: Miao Fang (0000-0002-3211-6230); Min Yao (0000-0002-5473-0186); Jie Yang (0000-0001-8518-4512); Wen-Jie Zheng (0000-0002-3073-4596); Li Wang (0000-0003-2838-9807); Deng-Fu Yao (0000-0002-3448-7756).

Author contributions: Fang M, Yao M, and Yang J contributed equally to this work and wrote the first draft; Fang M and Yang J conducted the animal model study; Zheng WJ and Wang L analyzed the data; Yao M and Yao DF revised the manuscript; All authors approved the final version of the manuscript.

Supported by the Projects of the Ministry of S. and T. National Key Research and Development Program, No. 2018YFC0116902; the National Natural Science Foundation of China, No. 31872738; the National Natural Science Foundation of China, No. 81673241; the National Natural Science Foundation of China, No. 81702419; the National Natural Science Foundation of China, No. 81873915; and the Jiangsu Medical Science of China, No. BE2016698.

Institutional animal care and use committee statement: The study protocol was approved by the Animal Medical Ethics Committee of Nantong University.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: No additional unpublished data are available.

Miao Fang, Min Yao, Jie Yang, Medical School of Nantong University, Nantong 226001, Jiangsu Province, China

Wen-Jie Zheng, Deng-Fu Yao, Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China

Li Wang, Department of Medical Informatics, Medical School of Nantong University, Nantong 226001, Jiangsu Province, China

Corresponding author: Deng-Fu Yao, MD, PhD, Professor, Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, 20 West Temple Road, Nantong 226001, Jiangsu Province, China. yaodf@ahnmc.com

Abstract

BACKGROUND

Prevalence of nonalcoholic fatty liver disease (NAFLD) is rapidly increasing, and NAFLD has become one of the most common chronic liver diseases worldwide. With abnormal CD44 activation, the severe form of NAFLD can progress to liver cirrhosis and hepatocellular carcinoma (HCC). Thus, the molecular mechanism of CD44 in NAFLD needs to be identified.

AIM

To investigate the relationship between CD44 activation and malignant transformation of rat hepatocytes under nonalcoholic lipid accumulation.

METHODS

Sprague-Dawley rats were fed a high-fat (HF) for 12 wk to entice NAFLD and then with HF plus 2-fluorenylacetamide (0.05%) to induce HCC. Rats were sacrificed every 2 wk, and subsequently divided into the groups based on liver pathological examination (hematoxylin and eosin staining): NAFLD, denaturation, precancerosis, HCC, and control. Liver CD44 mRNA was detected by OneArray. Liver fat as assessed by Oil red O staining or CD44 by immunohistochemical assay was compared with their integral optic density. Serum CD44, alanine aminotransferase, aspartate aminotransferase, triglyceride, total cholesterol, and AFP levels were quantitatively tested.

RESULTS

Elevated CD44 was first reported in hepatocarcinogenesis, with increasing expression from NAFLD to HCC at the protein or mRNA level. The CD44 integral optic density values were significantly different between the control

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Manuscript source: Unsolicited manuscript

Received: April 15, 2019
Peer-review started: April 15, 2019
First decision: May 16, 2019
Revised: July 26, 2019
Accepted: October 1, 2019
Article in press: October 1, 2019
Published online: January 15, 2020

P-Reviewer: Gassler N, Ho HK, Ozaki I, Perse M
S-Editor: Tang JZ
L-Editor: Filipodia
E-Editor: Qi LL

group and the NAFLD (t = 25.433, P < 0.001), denaturation (t = 48.822, P < 0.001), precancerosis (t = 27.751, P < 0.001), and HCC (t = 16.239, P < 0.001) groups, respectively. Hepatic CD44 can be secreted into the blood, and serum CD44 levels in HCC or precancerous rats were significantly higher (P < 0.001) than those in any of the other rats. Positive correlations were found between liver CD44 and CD44 mRNA (rs = 0.373, P = 0.043) and serum CD44 (rs = 0.541, P = 0.002) and between liver CD44 mRNA and serum CD44 (rs = 0.507, P = 0.004). Moreover, significant correlations were found between liver CD44 and liver AFP (rs = 0.572, P = 0.001), between serum CD44 and serum AFP (rs = 0.608, P < 0.001), and between CD44 mRNA and AFP mRNA (rs = 0.370, P = 0.044).

CONCLUSION
The data suggested that increasing CD44 expression is associated with the malignant transformation of hepatocytes in NAFLD.

Key words: Hepatocarcinogenesis; CD44; Nonalcoholic fatty liver disease; Animal model; Dynamic expressions

Core tip: CD44, which belongs to a family of adhesion molecules, is a marker of cancer stem cells and is related to the transformation of nonalcoholic fatty liver disease to nonalcoholic steatohepatitis and hepatocellular carcinoma. Dynamic expression of CD44 in livers or blood at protein or mRNA level was first investigated at different stages of the progression of fat accumulating fatty liver. Increasing CD44 expression could be one of the most important progenitors and was associated with the malignant transformation of hepatocytes with lipid accumulation.

Citation: Fang M, Yao M, Yang J, Zheng WJ, Wang L, Yao DF. Abnormal CD44 activation of hepatocytes with nonalcoholic fatty accumulation in rat hepatocarcinogenesis. *World J Gastrointest Oncol* 2020; 12(1): 66-76
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/66.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.66

INTRODUCTION

Hepatocellular carcinoma (HCC) is the main form of primary liver cancer characterized by high malignancy, easy recurrence and metastasis, and geographical diversity, and both its incidence and mortality are increasing in the world[1,2]. Despite improved treatment modalities, the prognosis of HCC patients is still rather poor because of frequent metastasis and recurrence[3,4]. Major risk factors, except for infection with hepatitis B virus or hepatitis C virus, are nonalcoholic fatty liver disease (NAFLD) and metabolic-related disorders[5-7]. The incidence of NAFLD has significantly increased, and the proportion of HCC due to malignant transformation of NAFLD shows an increasing trend. Lipid accumulation is strictly linked to chronic hepatocyte damage, resulting in the generation of an inflammation microenvironment and creation of a pro-oncogenic milieu, thus promoting malignant transformation of hepatocytes with no mitochondrial carnitine palmitoyl transferase-II activity[8]. Recently, accumulating evidence supports that HCC contains a small subpopulation of cancer stem-like cells (CSC)[9] with potential biomarkers (CD44, CD133, and aldehyde dehydrogenase 1) that might be important factors in HCC occurrence[10], of which CD44 could be a key player in non-alcoholic steatohepatitis[11].

Transmembrane glycoprotein CD44 is closely associated with aggressive behavior and poor prognosis in a variety of human malignancies[12,13]. It can bind to hyaluronic acid (the most important ligand), collagen, fibrin, and laminin, mediate specific adhesion between cells as well as between cells and the extracellular matrix, and be involved in many biological processes such as transmitting intracellular signals and regulating the growth, invasion, and metastasis of HCC[14,15]. CD44 is one of the most frequently reported CSC markers in NAFLD, and CD44 positive cells have CSC properties, such as self-renewal and tumorigenicity. Recently, high CD44 expression has been closely linked to NAFLD progression to HCC[16]. However, the relationship

between CD44 expression and hepatocarcinogenesis is still controversial, with unclear particular mechanisms. The objective of this study was to highlight correlations between the alterations of CD44 expression and malignant transformation of lipid-accumulating hepatocytes.

MATERIALS AND METHODS

Fatty-accumulated HCC model

In total, 78 4-wk-old male Sprague-Dawley rats, weighing 100-120 g, were randomly divided into either a control group (n = 12) or an NAFLD model group (n = 66). All animals were raised at 22 ± 2 °C, with a light/dark period of 12 h, and a humidity of 55%. According to a previous method[8], the rats of the control group were fed a routine diet, whereas those of the NAFLD model group were fed a high fat diet (10% egg yolk powder, 10% lard, 4% cholesterol, 1% cholic acid, and 75% common feed) for 2 wk. Then, the NAFLD rats (n = 42) were given a high fat diet plus 0.05% of 2-fluorenylacetamide (2-FAA, Sigma, St Louis, MO, United States) to induce HCC formation. Two control rats, four NAFLD rats, and one HCC rat were sacrificed by ether anesthetization every 2 wk. Blood samples were collected from the heart and stored at -20 °C, and liver tissues were taken after operation, frozen quickly in liquid nitrogen, and stored at -80 °C. Liver tissues were used for Oil red O, hematoxylin and eosin, and immunohistochemical (IHC) staining. All *in vivo* procedures were performed in accordance with the guidelines of the Animal Care and Use Committee of Nantong University, China.

Histopathological analysis

Dried paraffin-embedded sections were deparaffinized in xylene, rehydrated with a graded series of ethanol, and stained with hematoxylin for 5 min. Subsequently, the sections were immerged in hydrochloric acid and ammonia for seconds, rinsed for 1 h, placed in distilled water for a moment, decolorized with 70% and 90% alcohol for 10 min each, and stained with eosin for 3 min. After dyeing, the sections were dehydrated with 100% alcohol, cleared with xylene, and sealed with resin. Based on the alterations of histopathological characteristics under a microscope, the livers were divided into control, NAFLD, denaturation, precancerosis, and HCC groups.

Oil red O staining

We prepared the application fluid and filtered it according to the kit manufacturer's instructions. The frozen slices stored in the refrigerator at -80 °C in advance were placed at room temperature for 10 min, then stained with reagent one for 15 min and washed with distilled water at 37 °C for 20 s. After that, they were stained again with reagent two for 3-5 min and washed with distilled water at 37 °C for 30 s. Subsequently, we added the water-based sealant to the surface before drying. The slices were observed and photographed under microscope and analyzed by Image-Pro Plus v6.0 software with integral optic density (IOD) value[17]. For measuring IOD, the image system comprised a Leica CCD camera DFC420 connected to a Leica DM IRE2 microscope (Leica Microsystems Imaging Solutions Ltd, Cambridge, United Kingdom). Photographs of representative fields were captured under high-power magnification (× 200) with Leica QWin Plus v3 software. The IOD value of each image was measured with Image-Pro Plus v6.0 software (Media Cybernetics Inc, Bethesda, MD, United States).

Biochemical analysis

Serum total cholesterol (Tch) and triglyceride (TG) levels were measured with a kit from Nanjing Jiancheng Biotechnology Company (Nanjing, China). Briefly, the blank, calibration, and sample wells were set up. We added 10 μL distilled water as well as standards and samples with 1000 μL working liquid into corresponding wells. After being incubated at 37 °C for 10 min, absorbance of each well was read on a spectrophotometer, with 510 nm as the primary wavelength, and the average concentration was calculated according to the formula.

IHC staining

The liver sections were put in 80 °C drying box for 2 h. Then, after being dewaxed, dehydrated, and washed by flowing water, the slices were soaked in the citrate antigen recovery buffer and heated in the microwave oven until boiling for 5 min. Each slice was exposed to 100 μL 3% H_2O_2, incubated, and washed with phosphate buffered saline with primary rabbit anti-human CD44 antibody (ab157107, Abcam, Cambridge, United Kingdom) at 1:100 dilution. After that, polymer reinforcements and horseradish peroxidase-conjugated goat anti-rabbit IgG (ab97051, Abcam) at 1:500

dilutions were followed and repeatedly washed. Finally, the slices were added with 3,3'-diaminobenzidine dye liquor, counterstained with Hematoxylin, and soaked in 0.1% HCl. After rinsing, blueness, dehydration in ethanol, clearness with xylene, and sealing with neutral balsam were observed by optical microscope (MX53 Olympus, Tokyo, Japan) and analyzed with the Image Pro plus v6.0 software with IOD value[17].

Analysis of alpha-fetoprotein (AFP) and CD44 transcription

According to the protocol, every 100 mg tissue was homogenized in a glass grinder with 1 mL TRI reagent and then transferred into Eppendorf (EP) tube, in which the reagent was mixed up and down 10 times, and rested for 5 min at room temperature. Next, 0.2 mL chloroform was added, mixed, rested, and then centrifuged (12000 rpm, 4 °C, 15 min). The upper water was transferred into a new aseptic EP tube, mixed with 0.5 mL isopropanol, and centrifuged (12000 rpm, 4 °C, 10 min). Afterwards, the supernatant was removed, sRNA precipitation was hacked and washed with 80% ethanol, and the centrifugation (7500 rpm, 4 °C, 5 min) was repeated. Finally, the supernatant was carefully poured out, the precipitation was dried (30 min, until RNA precipitation became transparent, not completely dry), and then the pellet dissolved with 30 μL of DEPC water. The quantity and quality of the RNA samples were determined with the use of the NanoDrop ND-1000 spectrophotometer.

The strand of cDNA and antisense RNA was synthesized by using OneArray plus RNA amplification kit developed by the Hualian Company (Beijing, China). In the process, aa-UTP and NHS-CyeDye were added to make aRNA become CyeDye-aRNA to complete calibration. After purification, we made the hybridization between the product and Phalanx OneArray™ and, furthermore, entered the analysis process after cleaning and signal detection. The scanner was the Agilent Microarray Scanner (G2505C, Santa Clara, CA, United States). Finally, transcriptional levels of AFP and CD44 in five groups of rats were.

Liver tissues

Five groups of rats were created based on pathological hematoxylin and eosin (H and E) staining. Liver tissue (20 mg) was mixed with 200 μL of mixed radioimmunoprecipitation assay buffer (UNOCI Biological Company, WB020) in 1.5 mL EP tubes and homogenized. The tissue was preserved with ice for 4 h and centrifuged for 5 min at 12000 *g*. The supernatant was divided into two parts: one was stored at -80 °C after measuring the concentration and the other was denatured in boiling water with 5 × protein loading buffer for 5 min and stored at -80 °C.

Serum samples

About 5 mL of blood was taken from the rat heart and incubated at 4 °C overnight. After centrifugation (2000 rpm, 20 min), we removed sera into the EP tube. Based on H and E staining, we divided all sera into five groups and stored them at -80 °C and avoided repeated freeze-thaw cycles.

Enzyme linked immunosorbent assay (ELISA)

The concentration of AFP and CD44 in the liver homogenate and in the sera of rats was detected according to the manufacturer's instructions of the ELISA kit (Cloud-Clone Corp, Katy, TX, United States). We set the blank and added 100 μL standards, liver homogenates, and serum to the microplate, where the reagents were incubated 1 h at 37 °C. Then, we removed the liquid, added prepared biotinylated labeled detector antibody, and incubated the samples at 37 °C. Subsequently, we aspirated and washed each tube, added prepared streptavidin-horseradish peroxidase mixture to each tube, incubated the mixture again, aspirated and washed each well, added the TMB solution to each well until color developed, and then added the Stop solution. The optical density values were measured at 450 nm on a microplate reader (Biotek Synergy, Winooski, VT, United States), and the corresponding protein concentration for each sample was obtained by a standard curve.

Statistical analysis

Image pro plus 6.0, GraphPad prism 5.0 (La Jolla, CA, United States), and Photoshop software were used to analyze data and generate figures. Microsoft Excel and IBM SPSS statistics 23 software (Armonk, NY, United States) were applied to analyze data and calculate the mean ± SD. The Student's t test was used to compare CD44 and AFP levels in liver homogenates and sera of rats. A $P < 0.05$ was considered significant.

RESULTS

NAFLD models with lipid accumulation

Rat livers with lipid accumulation and circulating lipid levels are shown in Figure 1. Compared with the normal control (Figure 1A and A1), the rat NAFLD models have been successfully made with lipid accumulation (Figure 1B and B1). After the rats were fed with a high fat plus 2-FAA diet, the rat livers were collected at the early (Figure 1C and C1), middle (Figure 1D and D1), and last (Figure 1E and E1) stage. The corresponding liver sections by the Oil red O staining were confirmed with over fatty accumulation in hepatocytes, except for control rats, whose levels of hepatic lipid were relatively quantified by the IOD (Figure 1F). Compared with the control group, hepatocyte lipid contents were significantly higher in the NAFLD (t = 12.461, P < 0.001), hepatocytes denaturation (t = 6.541, P = 0.02), precancerosis (t = 14.133, P = 0.005), and HCC (t = 9.797, P = 0.009) groups, respectively. Furthermore, the circulating total cholesterol (Figure 1G) levels with 2-3 times or triglycerides (Figure 1H) levels with 1.50-4.53 times in any group of all rats with high fat diet were significantly higher (P < 0.05) than those in the control rats.

CD44 alteration in hepatocarcinogenesis

The alterations of liver histopathological examination and the IHC analysis of liver CD44 expression in rat hepatocarcinogenesis are shown in Figure 2. According to pathological results with H and E staining, the rat livers were divided into five groups: the controls (n = 12, Figure 2A) with normal diet only, the NAFLD formation (n = 24, Figure 2B) with high fat diet, the hepatocytes damage (denaturation, n = 17, Figure 2C) at early stage, the precancerosis (n = 15, Figure 2D) at middle stage, and the HCC formation (n = 10, Figure 2E) at last stage after high fat diet plus 2-FAA. The hepatic CD44 levels of the corresponding sections were analyzed by immunohistochemistry with anti-rat CD44 antibodies. Liver CD44 was overexpressed in rat hepatocytes (Figure 2B1, C1, D1 and E1) except for normal controls (Figure 2A1). The IOD values of CD44 expression (Figure 2F) were significantly different between the control group and the NAFLD (t = 25.433, P < 0.001), hepatocytes denaturation (t = 48.822, P < 0.001), precancerosis (t = 27.751, P < 0.001), and HCC (t = 16.239, P < 0.001) groups, respectively. Also, the liver damage with abnormal liver alanine aminotransferase (Figure 2G) or aspartate aminotransferase (Figure 2H) activity was higher in any group of the rats with high fat diet (P < 0.05) than in the control rats during malignant transformation of NAFLD.

Quantitative analysis of CD44 in hepatocarcinogenesis

The dynamic alterations of liver or circulating CD44 expression at protein level and comparative analysis with AFP expression in rat hepatocarcinogenesis are shown in Table 1. In the rat liver tissues, CD44 expression was lower in the control group and was significantly increasing in the NAFLD group; no significant difference of liver AFP was found between the control and NAFLD groups. After the NAFLD rats were fed with 2-FAA in hepatocarcinogenesis, the increasing liver CD44 expression was significantly higher in the precancerosis and HCC groups than in the control, NAFLD, and denaturation groups; the increased liver AFP expression was significantly higher in the denaturation, precancerosis, and HCC groups than in the control or NAFLD group. In the circulating blood of rats, CD44 expression was lower in the control group. No significant difference of serum CD44 or AFP was found between the control group and the NAFLD group. However, the serum CD44 or AFP level in the denaturation, precancerosis, or HCC group of the NAFLD rats with 2-FAA in hepatocarcinogenesis was significantly higher than that in the control or NAFLD group. Significantly close correlations were found between liver CD44 and serum CD44 (rs = 0.541, P = 0.002) and liver AFP (rs = 0.572, P = 0.001) and between serum CD44 and serum AFP (rs = 0.608, P < 0.001).

Expression of CD44 mRNA in hepatocarcinogenesis

The dynamic expression of liver CD44 mRNA and the comparative analysis with AFP mRNA in rat hepatocarcinogenesis are shown in Table 2. The level of liver CD44 mRNA or AFP mRNA expression in the control group was low. Moreover, a similar observation was found in the NAFLD group, and there was no significant difference of liver CD44 mRNA or AFP mRNA found between the control and NAFLD groups. After the NAFLD rats were fed with 2-FAA in hepatocarcinogenesis, the expression of liver CD44 mRNA in the denaturation, precancerosis, or HCC group was significantly higher than that in the control or NAFLD group; liver AFP mRNA expression in the precancerosis or HCC group was significantly higher than that in any of the control, NAFLD, or denaturation group. Significantly close correlations were found between liver CD44 (Table 1) and CD44 mRNA (rs = 0.373, P = 0.043) and between liver CD44 mRNA and serum CD44 (Table 1, rs = 0.507, P = 0.004) or AFP mRNA (rs = 0.370, P = 0.044).

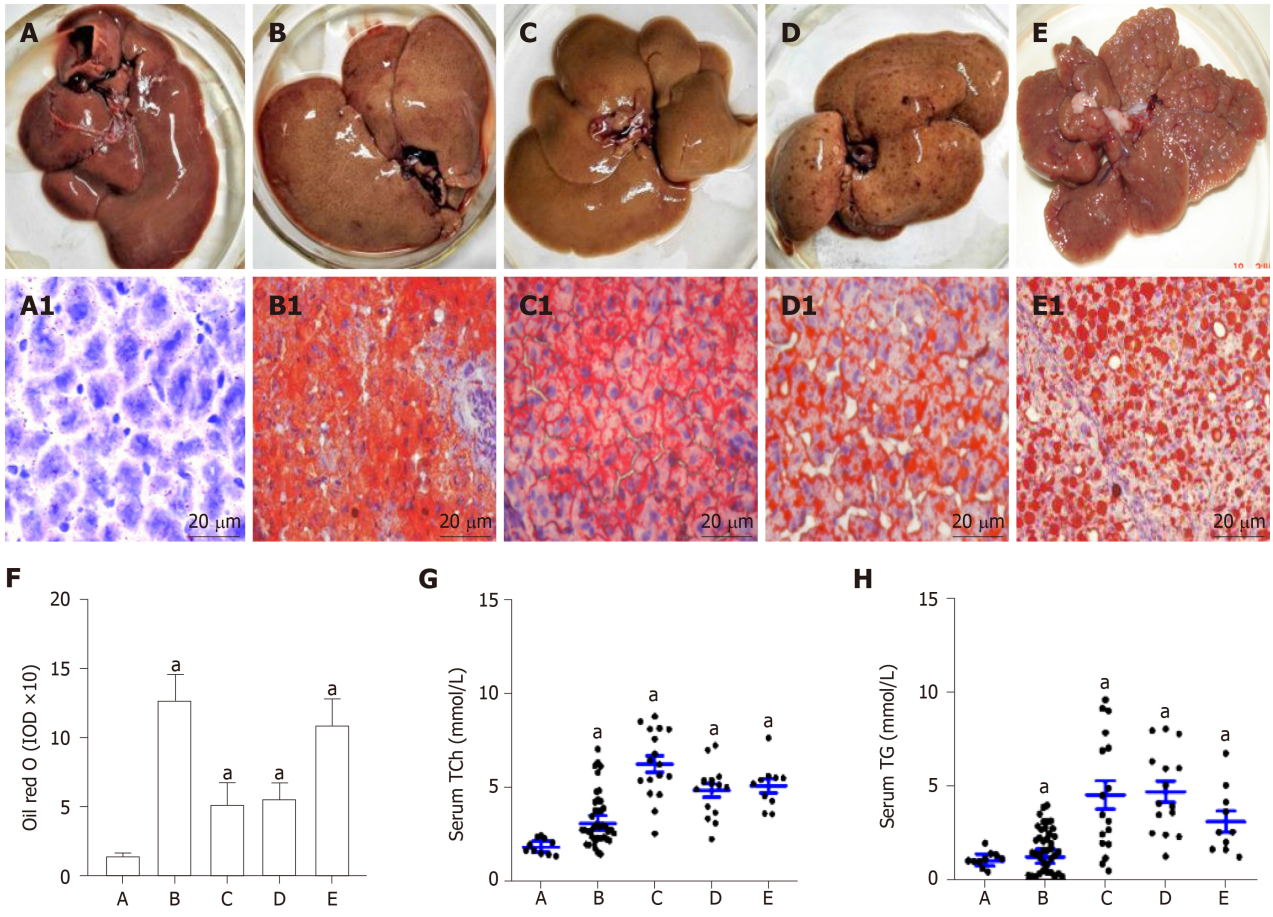

Figure 1 Rat livers with lipid accumulation and circulating lipid levels. A: The livers of control rats with normal diet; B: The livers of the rats with high fat diet; C: The livers of the rats with high fat plus 2-fluorenylacetamide (2-FAA) diet at the early stage; D: The livers of the rats with high fat plus 2-FAA diet at the middle early stage; and E: The livers of the rats with high fat plus 2-FAA diet at the last stage; A1: Normal controls; B1-E1: The sections of the corresponding to above livers were stained with the Oil red O assay, and over lipid accumulation in rat hepatocytes; F: The integral optic density values represented hepatic lipid levels of the corresponding to above livers; G: The alterations of serum total cholesterol level; and H: The alterations of serum triglycerides level. Original magnification of liver sections (× 400) from Figure 1A1 to Figure 1E1. $^{a}P < 0.05$ *vs* control group. IOD: Integral optic density; Tch: Total cholesterol; TG: Triglycerides.

DISCUSSION

Alterations of hepatic metabolism are critical to the malignant transformation of hepatocytes[18,19]. The incidence of NAFLD among healthy populations is increasing and has become one of the most common causes of HCC worldwide[20-22]. An accumulation of ectopic fat, including visceral obesity and fatty liver, leads to dysfunction of the adipose tissue, with impaired production of adipocytokines and inactivity of mitochondrial inner membrane (carnitine palmitoyl transferase-II)[8,23]; abnormal CD44 expressions in NAFLD lead to the emergence of a microenvironment favorable to HCC development. Human HCC follows a pattern of pathologic evolution involving multistep processes, starting from hepatocyte injury and cirrhosis to low-grade dysplastic nodules, high-grade dysplastic nodules, early liver cancer, and progressed HCC[24,25]. However, the correlation between CD44 and hepatocarcinogenesis is still controversial. In this study, the increasing features of CD44 activation at different stages were first investigated in the cascade of NAFLD to HCC progression.

Hepatocarcinogenesis is of fundamental importance to analyze the dynamic alteration of HCC-related biomarkers and to understand the molecular mechanisms of cancer development[26-28]. NAFLD models with lipid accumulation were confirmed with Oil red O staining, and then the malignant transformation of rat hepatocytes induced with 2-FAA was identified by histopathological H and E examination. The lipid IOD value of the rat liver sections in the NAFLD group was significantly higher than that in the control group, with increasing serum triglyceride or total cholesterol levels and higher hepatic enzymatic alanine aminotransferase or aspartate aminotransferase activity. After the NAFLD rats were fed 2-FAA, rat hepatocytes were malignantly transformed from normal liver cells to denaturation at the early-, to precancerosis at the middle-, and to HCC formation at last-stage. The data indicated

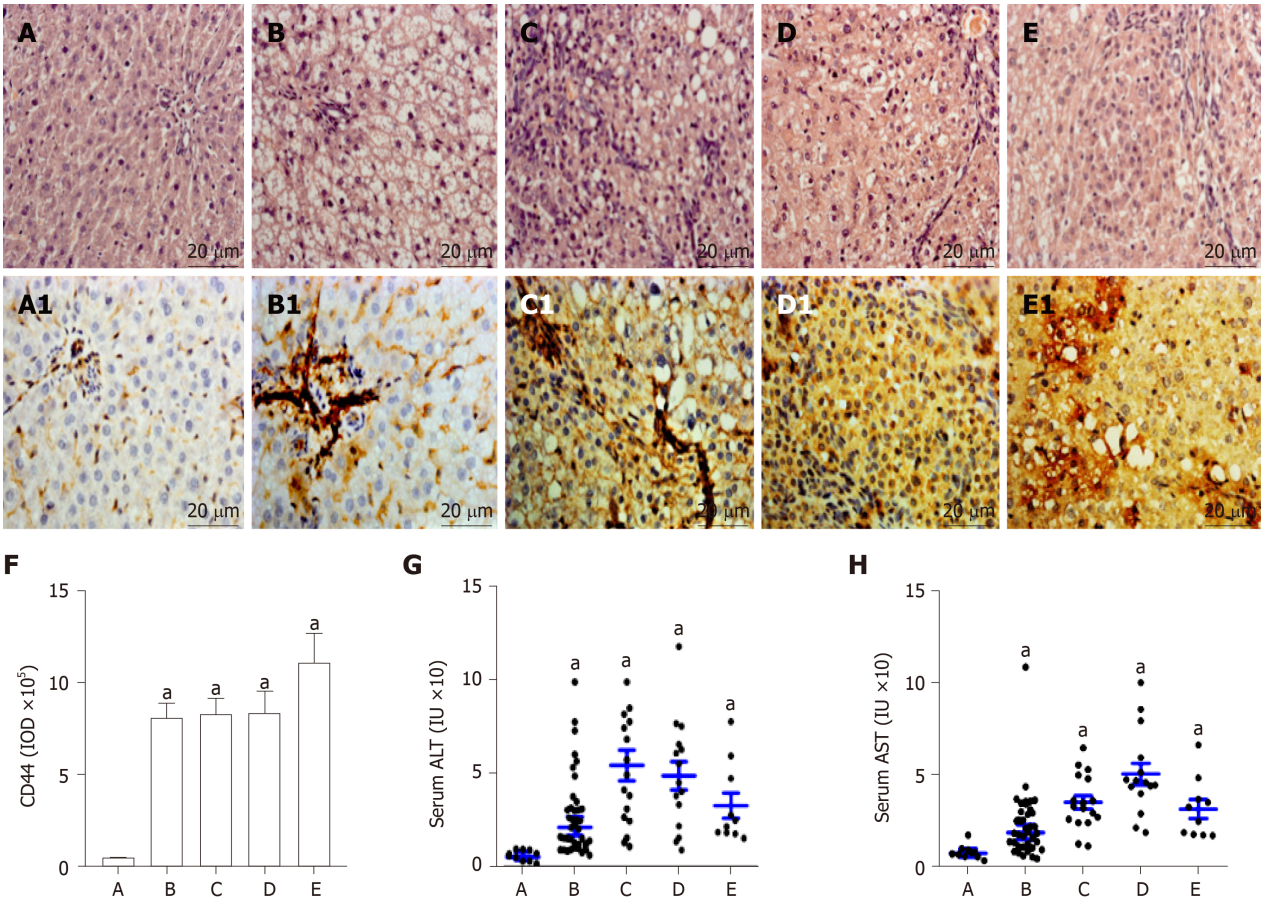

Figure 2 Pathohistology and hepatic CD44 in rat hepatocarcinogenesis. According to pathohistological examination with H and E staining, the rat livers were divided into five groups. A: The normal controls (A1); B: The nonalcoholic fatty liver disease formation (B1); C: The hepatocytes damage (denaturation, C1); D: The precancerosis (D1); and E: The HCC formation (E1); A1: Normal controls; B1-E1: The sections of the corresponding to above livers were analyzed by CD44 immunohistochemistry with anti-rat CD44 antibody, and the overexpression of CD44 in rat hepatocytes; F: The IOD values represented hepatic CD44 expression levels; G: The alterations of serum alanine aminotransferase (ALT) activity; and H: The alterations of serum AST activity. Original magnification of liver sections (× 400) from Figure 2A1 to Figure 2E1. [a]*P* < 0.05 *vs* control group. IOD: Integral optic density.

that the rat models with lipid accumulation were suitable to observe the CD44 activation from NAFLD involving inflammatory with abnormal metabolism to HCC progression[29,30].

The fastest growing cause of cancer-related death is HCC, which is at least partly attributable to the rising incidence of NAFLD that encompasses a broad spectrum of conditions, ranging from non-progressive bland steatosis to hepatocarcinogenesis[31,32]. In line with these clinical risk factors, high-fat administration over a prolonged period results in spontaneous HCC development. Liver CD44 was overexpressed in all rat livers except for normal controls. Significant difference of the CD44 IOD values was found between control rats and NAFLD, denaturation, precancerosis, or HCC rats, suggesting that elevated CD44 level could contribute to malignant transformation of hepatocytes and HCC development[33,34]. As a hyaluronic acid receptor, CD44, whose expression could be rapidly induced in a STAT3-dependent manner, potentiates AKT activation to escape p53-induced death and responds to proliferative signals that become HCC progenitors[13].

CD44 as a major adhesion molecule of the extracellular matrix has been implicated in a wide range of biological processes, such as transmitting intracellular signals and regulating the growth, invasion, and metastasis of tumors[12,35]. The binding of CD44 with active hyaluronic acid in rat nonalcoholic steatohepatitis (NASH) could induce the accumulation of leukocytes around hepatic sinusoid, and its deficiency could not completely prevent inflammation. CD44 expression in NASH patients is significantly decreased while a fatty disappears after the liver operation. Both CD44 gene knockout and wild type mice with methionine and choline deficient diet were fed to induce NAFLD[36]. In this study, abnormal CD44 expression had a relationship between NAFLD with liver ballooning and malignant transformation of hepatocytes. Although the complex molecular mechanisms of CD44 in rat hepatocarcinogenesis needs to be explored further, the molecular profiling of NAFLD related to increasing CD44

Table 1 Dynamic alterations of liver or serum CD44 and alpha-fetoprotein at protein level in rat hepatocarcinogenesis

Group	*n*	Liver CD44, ng/per mg liver	Serum CD44, ng/mL	Liver AFP, ng/per mg liver	Serum AFP, ng/mL
Control	12	1.465 ± 0.341	9.193 ± 1.176	1.757 ± 0.452	0.881 ± 0.092
NAFLD	24	1.920 ± 0.311[a]	10.432 ± 2.288	2.185 ± 0.553	0.958 ± 0.131
Denaturation	17	1.830 ± 0.460[a]	19.913 ± 7.277[a]	3.023 ± 0.797[a]	1.460 ± 0.394[a]
Precancerosis	15	2.203 ± 0.303[a]	20.628 ± 2.756[a]	3.282 ± 0.683[a]	1.622 ± 0.418[a]
HCC	10	2.577 ± 0.425[a]	29.597 ± 6.907[a]	3.877 ± 0.625[a]	1.830 ± 0.537[a]

[a]$P < 0.05$ *vs* control group. NAFLD: Nonalcoholic fatty liver disease; AFP: Alpha-fetoprotein; HCC: Hepatocellular carcinoma.

expression during HCC development holds great translational potential for individualized surveillance, prevention, and therapy[16].

Recent evidence indicated that HCC contains a small subpopulation of cells called CSCs that were key drivers of HCC formation and progression, especially relating to invasion and metastasis[33,37]. Among potential CSCs markers, such as CD44, CD133, and aldehyde dehydrogenase 1, several studies similarly utilized CD44 positivity to isolate cells with stem cell-like and cancer-initiating properties from other cancer cells. Interestingly, some CSCs biomarkers have been used to identify by immunohistochemistry CSCs in HCC[10,38]. In this study, both CD44 and AFP were involved in HCC progression, with abnormal expression at the protein or mRNA level and provided a concise overview on the molecular pathogenesis of the NAFLD-NASH-HCC sequence, suggesting that CD44 as a hepatic progenitor might be an important factor in hepatocyte malignant transformation.

In conclusion, to the best of our knowledge, this is the first report to investigate the relationship between increasing CD44 activation and malignant transformation of hepatocytes. The findings are promising, and the initial evidence confirmed that hepatic CD44 was one of the early molecules from NAFLD to HCC progression. However, the investigation of liver histology had not analyzed the relationship between CD44 level and liver fibrosis. Future studies should evaluate liver tissues concerning the degree of fibrosis and CD44 activation, clarify the molecular mechanisms or HCC-related signal pathways of the upregulation of CD44 expression, and elucidate the role of CD44 as a hepatic progenitor in hepatocyte malignant transformation[39,40].

Table 2 Dynamic alterations of liver CD44 mRNA and AFP mRNA expression in rat hepatocarcinogenesis

Group	*n*	Liver CD44 mRNA, × 10^7/per mg tissues	Liver AFP mRNA, × 10^8/per mg tissues
Control	12	1.844 ± 0.305	4.859 ± 0.636
NAFLD	24	2.234 ± 0.441	4.150 ± 0.439
Denaturation	17	3.008 ± 0.436[a]	5.575 ± 1.672
Precancerosis	15	2.942 ± 0.530[a]	6.749 ± 0.949[a]
HCC	10	3.593 ±1.554[a]	5.731 ± 0.404[a]

[a]$P < 0.05$ *vs* control group. AFP: Alpha-fetoprotein; HCC: Hepatocellular carcinoma; NAFLD: Nonalcoholic fatty liver disease.

ARTICLE HIGHLIGHTS

Research background

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, and its prevalence is rapidly increasing worldwide. The severe form of NAFLD can progress to liver cirrhosis and hepatocellular carcinoma (HCC). Recently, several related papers expounded that CD44 played an important role in NAFLD and that there was rather little known knowledge about CD44 expression in different stages of hepatocyte malignant transformation correlated with fatty accumulation.

Research motivation

Although CD44 is initially regarded as an adhesion molecule, which has a close relationship with tumor growth, invasion, and metastasis of HCC, the abnormal activation of CD44 in NAFLD has yet to be discovered, and the fact that CD44 is overexpressed in hepatocytes with fatty accumulation needs to be investigated.

Research objectives

CD44 is a non-kinase transmembrane glycoprotein, and its expression is high in malignant tumors and low in benign and low-metastatic tumors. This new mechanism of CD44 expression with fatty metabolism was worthy to be explored. The objective of this study was to initiate the investigation of the relationship between CD44 activation and hepatocyte malignant transformation under nonalcoholic lipid accumulation

Research methods

In order to clarify the mechanism of CD44 high expression and NAFLD, the models with lipid accumulation were constructed and then the malignant transformation of rat hepatocytes was induced with 2-fluorenylacetamide. Histopathological alterations were identified from normal liver cells to denaturation at the early-, to precancerosis at the middle-, and to HCC formation at last-stage by hematoxylin and eosin examination, with increasing CD44 activation from NAFLD involving inflammation with abnormal metabolism to HCC progression.

Research results

CD44 in hepatocarcinogenesis of rat liver cells was increased from NAFLD to HCC at the protein or mRNA level. Significant difference of CD44 was found between the control group and the NAFLD, denaturation, precancerosis, or HCC group, respectively. Serum CD44 levels in HCC or precancerous rats were significantly higher than those in any of the other rats. Positive correlations were found between liver CD44 mRNA and circulating CD44 or alpha-fetoprotein.

Research conclusions

To the best of our knowledge, this is the first report to investigate the relationship between increasing CD44 activation and malignant transformation of hepatocytes. Hepatic CD44 mRNA and circulating CD44 expression are early molecules contributing to the progression from NAFLD to HCC. The new findings are promising, and the initial evidence confirmed that hepatic CD44 is one of the early molecules leading to the progression from NAFLD to HCC.

Research perspectives

CD44 represents a continuous increasing expression during the entire process of hepatocyte malignant transformation associated with fatty accumulation. Targeting CD44 might prevent NAFLD from turning into HCC and might become a potential therapeutic strategy for HCC. Moreover, further experiments should be conducted to collect the data of CD44 in normal people and of NAFLD, hepatitis, cirrhosis, and HCC and to clarify the molecule mechanism of high expression and carcinogenesis of CD44.

ACKNOWLEDGEMENTS

The authors thank Dr. FitzGibbon T for comments on earlier drafts of the manuscript.

REFERENCES

1 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]
2 **Lee WY**, Bachtiar M, Choo CCS, Lee CG. Comprehensive review of Hepatitis B Virus-associated hepatocellular carcinoma research through text mining and big data analytics. *Biol Rev Camb Philos Soc* 2019; **94**: 353-367 [PMID: 30105774 DOI: 10.1111/brv.12457]
3 **Calandri M**, Mauri G, Yevich S, Gazzera C, Basile D, Gatti M, Veltri A, Fonio P. Fusion Imaging and Virtual Navigation to Guide Percutaneous Thermal Ablation of Hepatocellular Carcinoma: A Review of the Literature. *Cardiovasc Intervent Radiol* 2019; **42**: 639-647 [PMID: 30809699 DOI: 10.1007/s00270-019-02167-z]
4 **Fetzer DT**, Rodgers SK, Seow JH, Dawkins AA, Joshi G, Gabriel H, Kamaya A. Ultrasound Evaluation in Patients at Risk for Hepatocellular Carcinoma. *Radiol Clin North Am* 2019; **57**: 563-583 [PMID: 30928078 DOI: 10.1016/j.rcl.2019.01.004]
5 **Athuluri-Divakar SK**, Hoshida Y. Generic chemoprevention of hepatocellular carcinoma. *Ann N Y Acad Sci* 2019; **1440**: 23-35 [PMID: 30221358 DOI: 10.1111/nyas.13971]
6 **Wong CR**, Nguyen MH, Lim JK. Hepatocellular carcinoma in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2016; **22**: 8294-8303 [PMID: 27729736 DOI: 10.3748/wjg.v22.i37.8294]
7 **Sumida Y**, Seko Y, Yoneda M; Japan Study Group of NAFLD (JSG-NAFLD). Novel antidiabetic medications for non-alcoholic fatty liver disease with type 2 diabetes mellitus. *Hepatol Res* 2017; **47**: 266-280 [PMID: 28019064 DOI: 10.1111/hepr.12856]
8 **Gu JJ**, Yao M, Yang J, Cai Y, Zheng WJ, Wang L, Yao DB, Yao DF. Mitochondrial carnitine palmitoyl transferase-II inactivity aggravates lipid accumulation in rat hepatocarcinogenesis. *World J Gastroenterol* 2017; **23**: 256-264 [PMID: 28127199 DOI: 10.3748/wjg.v23.i2.256]
9 **Castelli G**, Pelosi E, Testa U. Liver Cancer: Molecular Characterization, Clonal Evolution and Cancer Stem Cells. *Cancers (Basel)* 2017; **9** [PMID: 28930164 DOI: 10.3390/cancers9090127]
10 **Zoller H**, Tilg H. Nonalcoholic fatty liver disease and hepatocellular carcinoma. *Metabolism* 2016; **65**: 1151-1160 [PMID: 26907206 DOI: 10.1016/j.metabol.2016.01.010]
11 **Patouraux S**, Rousseau D, Bonnafous S, Lebeaupin C, Luci C, Canivet CM, Schneck AS, Bertola A, Saint-Paul MC, Iannelli A, Gugenheim J, Anty R, Tran A, Bailly-Maitre B, Gual P. CD44 is a key player in non-alcoholic steatohepatitis. *J Hepatol* 2017; **67**: 328-338 [PMID: 28323124 DOI: 10.1016/j.jhep.2017.03.003]
12 **Iqbal J**, Sarkar-Dutta M, McRae S, Ramachandran A, Kumar B, Waris G. Osteopontin Regulates Hepatitis C Virus (HCV) Replication and Assembly by Interacting with HCV Proteins and Lipid Droplets and by Binding to Receptors αVβ3 and CD44. *J Virol* 2018; **92** [PMID: 29669827 DOI: 10.1128/JVI.02116-17]
13 **Dhar D**, Antonucci L, Nakagawa H, Kim JY, Glitzner E, Caruso S, Shalapour S, Yang L, Valasek MA, Lee S, Minnich K, Seki E, Tuckermann J, Sibilia M, Zucman-Rossi J, Karin M. Liver Cancer Initiation Requires p53 Inhibition by CD44-Enhanced Growth Factor Signaling. *Cancer Cell* 2018; **33**: 1061-1077.e6 [PMID: 29894692 DOI: 10.1016/j.ccell.2018.05.003]
14 **Luo Y**, Tan Y. Prognostic value of CD44 expression in patients with hepatocellular carcinoma: meta-analysis. *Cancer Cell Int* 2016; **16**: 47 [PMID: 27330410 DOI: 10.1186/s12935-016-0325-2]
15 **Park NR**, Cha JH, Jang JW, Bae SH, Jang B, Kim JH, Hur W, Choi JY, Yoon SK. Synergistic effects of CD44 and TGF-β1 through AKT/GSK-3β/β-catenin signaling during epithelial-mesenchymal transition in liver cancer cells. *Biochem Biophys Res Commun* 2016; **477**: 568-574 [PMID: 27320862 DOI: 10.1016/j.bbrc.2016.06.077]
16 **Gu J**, Yao M, Yao D, Wang L, Yang X, Yao D. Nonalcoholic Lipid Accumulation and Hepatocyte Malignant Transformation. *J Clin Transl Hepatol* 2016; **4**: 123-130 [PMID: 27350942 DOI: 10.14218/JCTH.2016.00010]
17 **Chen KJ**, Jin RM, Shi CC, Ge RL, Hu L, Zou QF, Cai QY, Jin GZ, Wang K. The prognostic value of Niemann-Pick C1-like protein 1 and Niemann-Pick disease type C2 in hepatocellular carcinoma. *J Cancer* 2018; **9**: 556-563 [PMID: 29483961 DOI: 10.7150/jca.19996]
18 **Brar G**, Tsukamoto H. Alcoholic and non-alcoholic steatohepatitis: global perspective and emerging science. *J Gastroenterol* 2019; **54**: 218-225 [PMID: 30643981 DOI: 10.1007/s00535-018-01542-w]
19 **Safaei A**, Arefi Oskouie A, Mohebbi SR, Rezaei-Tavirani M, Mahboubi M, Peyvandi M, Okhovatian F, Zamanian-Azodi M. Metabolomic analysis of human cirrhosis, hepatocellular carcinoma, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis diseases. *Gastroenterol Hepatol Bed Bench* 2016; **9**: 158-173 [PMID: 27458508]
20 **Marengo A**, Rosso C, Bugianesi E. Liver Cancer: Connections with Obesity, Fatty Liver, and Cirrhosis. *Annu Rev Med* 2016; **67**: 103-117 [PMID: 26473416 DOI: 10.1146/annurev-med-090514-013832]
21 **Younossi Z**, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, Eguchi Y, Wong VW, Negro F, Yilmaz Y, Romero-Gomez M, George J, Ahmed A, Wong R, Younossi I, Ziayee M, Afendy A; Global Nonalcoholic Steatohepatitis Council. Nonalcoholic Steatohepatitis Is the Fastest Growing Cause of Hepatocellular Carcinoma in Liver Transplant Candidates. *Clin Gastroenterol Hepatol* 2019; **17**: 748-755.e3 [PMID: 29908364 DOI: 10.1016/j.cgh.2018.05.057]
22 **Livadariu R**, Timofte D, Danilă R, Ionescu L, Diaconu C, Soroceanu P, Sângeap AM, Drug VL, Trifan A. Nonalcoholic fatty liver disease and its complications--assessing the population at risk. a small series report and literature review. *Rev Med Chir Soc Med Nat Iasi* 2015; **119**: 346-352 [PMID: 26204635]
23 **Ye Q**, Qian BX, Yin WL, Wang FM, Han T. Association between the HFE C282Y, H63D Polymorphisms and the Risks of Non-Alcoholic Fatty Liver Disease, Liver Cirrhosis and Hepatocellular Carcinoma: An Updated Systematic Review and Meta-Analysis of 5,758 Cases and 14,741 Controls. *PLoS One* 2016; **11**: e0163423 [PMID: 27657935 DOI: 10.1371/journal.pone.0163423]
24 **Piccinin E**, Villani G, Moschetta A. Metabolic aspects in NAFLD, NASH and hepatocellular carcinoma: the role of PGC1 coactivators. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 160-174 [PMID: 30518830 DOI: 10.1038/s41575-018-0089-3]
25 **Calzadilla Bertot L**, Adams LA. The Natural Course of Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 2016; **17** [PMID: 27213358 DOI: 10.3390/ijms17050774]
26 **Chen K**, Ma J, Jia X, Ai W, Ma Z, Pan Q. Advancing the understanding of NAFLD to hepatocellular carcinoma development: From experimental models to humans. *Biochim Biophys Acta Rev Cancer* 2019; **1871**: 117-125 [PMID: 30528647 DOI: 10.1016/j.bbcan.2018.11.005]
27 **Lau JKC**, Zhang X, Yu J. Animal Models of Non-alcoholic Fatty Liver Diseases and Its Associated Liver

Cancer. *Adv Exp Med Biol* 2018; **1061**: 139-147 [PMID: 29956212 DOI: 10.1007/978-981-10-8684-7_11]
28 **Estes C**, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2018; **67**: 123-133 [PMID: 28802062 DOI: 10.1002/hep.29466]
29 **Wu J**. Utilization of animal models to investigate nonalcoholic steatohepatitis -associated hepatocellular carcinoma. *Oncotarget* 2016; **7**: 42762-42776 [PMID: 27072576 DOI: 10.18632/oncotarget.8641]
30 **Jacobs A**, Warda AS, Verbeek J, Cassiman D, Spincemaille P. An Overview of Mouse Models of Nonalcoholic Steatohepatitis: From Past to Present. *Curr Protoc Mouse Biol* 2016; **6**: 185-200 [PMID: 27248434 DOI: 10.1002/cpmo.3]
31 **Sun LM**, Lin MC, Lin CL, Liang JA, Jeng LB, Kao CH, Lu CY. Nonalcoholic Cirrhosis Increased Risk of Digestive Tract Malignancies: A Population-Based Cohort Study. *Medicine (Baltimore)* 2015; **94**: e2080 [PMID: 26656334 DOI: 10.1097/MD.0000000000002080]
32 **Reid DT**, Eksteen B. Murine models provide insight to the development of non-alcoholic fatty liver disease. *Nutr Res Rev* 2015; **28**: 133-142 [PMID: 26494024 DOI: 10.1017/S0954422415000128]
33 **Asai R**, Tsuchiya H, Amisaki M, Makimoto K, Takenaga A, Sakabe T, Hoi S, Koyama S, Shiota G. CD44 standard isoform is involved in maintenance of cancer stem cells of a hepatocellular carcinoma cell line. *Cancer Med* 2019; **8**: 773-782 [PMID: 30636370 DOI: 10.1002/cam4.1968]
34 **Fan Z**, Xia H, Xu H, Ma J, Zhou S, Hou W, Tang Q, Gong Q, Nie Y, Bi F. Standard CD44 modulates YAP1 through a positive feedback loop in hepato- cellular carcinoma. *Biomed Pharmacother* 2018; **103**: 147-156 [PMID: 29649630 DOI: 10.1016/j.biopha.2018.03.042]
35 **Yang Z**, Qin W, Chen Y, Yuan B, Song X, Wang B, Shen F, Fu J, Wang H. Cholesterol inhibits hepatocellular carcinoma invasion and metastasis by promoting CD44 localization in lipid rafts. *Cancer Lett* 2018; **429**: 66-77 [PMID: 29746928 DOI: 10.1016/j.canlet.2018.04.038]
36 **Gao Y**, Ruan B, Liu W, Wang J, Yang X, Zhang Z, Li X, Duan J, Zhang F, Ding R, Tao K, Dou K. Knockdown of CD44 inhibits the invasion and metastasis of hepatocellular carcinoma both *in vitro* and *in vivo* by reversing epithelial- mesenchymal transition. *Oncotarget* 2015; **6**: 7828-7837 [PMID: 25797261 DOI: 10.18632/oncotarget.3488]
37 **Gu Y**, Wei X, Sun Y, Gao H, Zheng X, Wong LL, Jin L, Liu N, Hernandez B, Peplowska K, Zhao X, Zhan QM, Feng XH, Tang ZY, Ji J. miR-192-5p Silencing by Genetic Aberrations Is a Key Event in Hepatocellular Carcinomas with Cancer Stem Cell Features. *Cancer Res* 2019; **79**: 941-953 [PMID: 30530815 DOI: 10.1158/0008-5472.CAN-18-1675]
38 **Kim BH**, Park JW, Kim JS, Lee SK, Hong EK. Stem Cell Markers Predict the Response to Sorafenib in Patients with Hepatocellular Carcinoma. *Gut Liver* 2019; **13**: 342-348 [PMID: 30600675 DOI: 10.5009/gnl18345]
39 **Lee YB**, Ha Y, Chon YE, Kim MN, Lee JH, Park H, Kim KI, Kim SH, Rim KS, Hwang SG. Association between hepatic steatosis and the development of hepatocellular carcinoma in patients with chronic hepatitis B. *Clin Mol Hepatol* 2019; **25**: 52-64 [PMID: 30360031 DOI: 10.3350/cmh.2018.0040]
40 **Tian Y**, Mok MT, Yang P, Cheng AS. Epigenetic Activation of Wnt/β-Catenin Signaling in NAFLD-Associated Hepatocarcinogenesis. *Cancers (Basel)* 2016; **8** [PMID: 27556491 DOI: 10.3390/cancers8080076]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 77-82

DOI: 10.4251/wjgo.v12.i1.77

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Case Control Study

Laparoscopic dissection of the hepatic node: The trans lesser omentum approach

Offir Ben-Ishay

ORCID number: Offir Ben-Ishay (0000-0003-3603-3843).

Author contributions: Ben-Ishay O designed, drafted, and critically reviewed the manuscript.

Institutional review board statement: The study was approved by the institutional review board of the Rambam Health Care Campus, Haifa, Israel.

Informed consent statement: Informed consent was exempt by the institutional review board due to the retrospective nature of the study and no impact on the clinical outcome of the study.

Conflict-of-interest statement: The author has no conflict of interest.

STROBE statement: The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

Offir Ben-Ishay, Department of General Surgery, Rambam Health Care Campus, Haifa 35254, Israel

Corresponding author: Offir Ben-Ishay, MD, Director, Surgical Oncology, Department of General Surgery, Division of Surgery, Rambam Health Care Campus, 8 Ha'Aliyah St., Haifa 35254, Israel. o_ben-ishay@rambam.health.gov.il

Abstract

BACKGROUND

Diagnosis of lympho-proliferative diseases is sometimes challenging. Excisional lymph node biopsy is the standard of care. Five percent of the patients will present with abdominal or retroperitoneal lymphadenopathy alone. Advancements in endoscopic techniques allow for access to fine needle biopsy in complicated areas, but this often does not meet the standard guidelines for diagnosis.

AIM

To investigate the results of laparoscopic excisional biopsy of the hepatic node (LEBHN) through a trans lesser omentum approach.

METHODS

Data of all patients undergoing LEBHN were collected retrospectively from patients' electronic charts over a period of 1 year. Data collected included age, gender, suspected disease, number of previous biopsies and biopsy method, surgical approach, intraoperative complications, operative time, post-operative complications, mortality, and final diagnosis.

RESULTS

Six patients were operated in this technique during the time frame of the study, 66.6% (n = 4) were females, and median age was 55 years (range: 25-72 years). We present no conversions from laparoscopy to laparotomy, and mean operating time was 51.2 min. Mean length of hospital stay was 1 d, and morbidity and mortality were nil. Most importantly, this technique offered definite diagnosis and appropriate treatment in all patients. Final diagnosis included two patients with lymphoma (Hodgkin and Follicular), two patients with sarcoidosis, and two patients with reactive lymph nodes with no evidence of malignancy.

CONCLUSION

In conclusion, this technique seems to be feasible and safe and may offer a simple

Manuscript source: Unsolicited manuscript

Received: July 2, 2019
Peer-review started: July 3, 2019
First decision: July 31, 2019
Revised: August 24, 2019
Accepted: September 26, 2019
Article in press: September 26, 2019
Published online: January 15, 2020

P-Reviewer: Isik A, Sergi CM
S-Editor: Ma YJ
L-Editor: Filipodia
E-Editor: Qi LL

approach for a definite diagnosis for what seems to be a complicated anatomical area.

Key words: Lymph node; Diagnosis; Lymphoma; Laparoscopy; Biopsy; Retroperitoneum

Core tip: Diagnosis of lympho-proliferative diseases is sometimes challenging, and laparoscopy is an essential tool. Laparoscopic excisional biopsy of the hepatic node seems to be feasible and safe and may offer a simple approach for a definite diagnosis for what seems to be a complicated anatomical area.

Citation: Ben-Ishay O. Laparoscopic dissection of the hepatic node: The trans lesser omentum approach. *World J Gastrointest Oncol* 2020; 12(1): 77-82
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/77.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.77

INTRODUCTION

Diagnosis of lympho-proliferative diseases is sometimes challenging. Based upon the current international guidelines[1,2], a surgically excised tissue biopsy is widely accepted as the gold standard for the diagnosis of lymphoma. An excisional biopsy of a lymph node (LN) allows assessment of the micro-architecture and provides adequate material for immunocytochemistry, flow cytometry, fluorescence *in situ* hybridization studies, and extraction of DNA and RNA for molecular diagnostics. The major disadvantages of surgical biopsies are the probable need for general anesthesia and deferrals due to the need of a surgical consult and operating room time. These issues are addressed through percutaneous and endoscopic core needle biopsies or fine needle aspiration with cellblock techniques.

When possible, a superficial LN, most often from the groin or axilla, should be excised, and this can be done simply under local anesthesia in day care setup. Often though this is not possible, and an intra-abdominal LN must be obtained.

The intra-abdominal lymphatic system is complex, and lymphadenopathy is often retroperitoneal, along the celiac axis and in the pelvis. Traditionally, laparoscopic biopsies of retroperitoneal or celiac nodes are considered complicated. Lymph nodes that are distributed along the celiac trunk include the root of the left gastric (station 7), the proper hepatic artery (station 8), and the splenic artery (station 9).

The hepatic node is the lymph node lying on the proper hepatic artery right above the neck of the pancreas. This node is quite often enlarged and has an abnormal fluorodeoxyglucose uptake on positron emission tomography (Figure 1).

Approach to the supra-pancreatic area is complex; the current study presents our experience with six patients who underwent laparoscopic excision of the hepatic node (LEBHN) through a trans lesser omentum approach.

MATERIALS AND METHODS

Surgical technique

After general anesthesia was induced, the patient was placed in a lithotomy position, and the operating table was maintained in 20° into the reverse Trendelenburg position. The surgeon was located between the legs of the patient, while the first assistant and the camera operator were placed to the left and the right side of the patient respectively.

After insufflation of the abdomen with CO_2 through a veres needle, a 10 mm trocar was placed above the umbilicus. The camera was than inserted, and an exploration of the abdomen was performed. A second 10 mm trocar was placed in mid left abdomen, and three 5 mm trocars were placed sub-xifoide, mid right quadrant, and left upper quadrant.

The left lobe of the liver was retracted upwards towards the diaphragm, and the lesser omentum with its pars flaccida was exposed. The pars flaccida was excised, the supra-pancreatic area was exposed, and the hepatic node was then observed (Figure

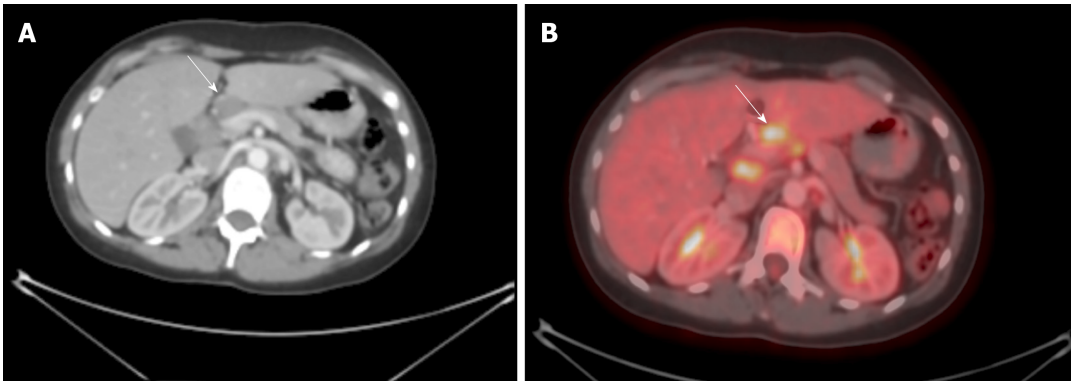

Figure 1 Abnormal FDG uptake on positron emission tomography. A: Computed tomography scan of the abdomen showing enlarged hepatic LN; B: Positron emission tomography showing abnormal FDG uptake in the same LN. FDG: Fluorodeoxyglucose; LN: Lymph node.

2A). The peritoneal layer above the hepatic node was carefully dissected with an electric cautery, and the node was than dissected carefully with bipolar energy (Figure 2B).

Data collection

Data of all patients undergoing LEBHN over a period of 1 year were collected retrospectively within the array of the surgical oncology service of the Department of general surgery at the Rambam Health Care Campus. Data included demographics, preoperative data including number of previous biopsies, previous biopsy methods used, and operative and post-operative data. Operative and postoperative complications were recorded.

RESULTS

Six patients were operated in this technique during the time frame of the study, 66.6% (n = 4) were females, and the median age was 55 years (25-72). Two patients had a suspected recurrence of a previously treated lymphoma. Three patients had one previous attempt for tissue diagnosis, and one had three of them. One patient had percutaneous attempt for biopsy, four had endoscopic biopsies trough endoscopic ultrasound, and one patient had a mediastinoscopy with mediastinal biopsy. Table 1 depicts the demographic operative and post-operative characteristics of the entire cohort. All patients were operated in a laparoscopic approach with no conversions to laparotomy. All patients had an uneventful operation with no operative or post-operative complications. Mean length of hospital stay was 1 d, and morbidity and mortality were nil.

DISCUSSION

Retroperitoneal lymphadenopathy occurs in various hematologic and granulomatous diseases. Approximately 5% of patients will have abdominal lymphadenopathy without enlargement of LN in superficial areas[4]. Advancements in percutaneous and endoscopic techniques allow diagnosis through minor ambulatory procedures. Laparoscopic abdominal and retroperitoneal lymph node biopsy was previously proven to be feasible and safe in the diagnosis of lympho-proliferative diseases[3-13]. Biopsy of celiac nodes or hepatic nodes though has been described occasionally in the literature. The current study describes six patients that underwent a laparoscopic celiac node biopsy in a trans lesser omentum approach. The advantages of this technique over other endoscopic approaches are the high chances for a definitive diagnosis, a better diagnostic yield, and early instigation of treatment.

The approach to the supra-pancreatic area is possible through dissection of the gastro-colic ligament and entrance to the lesser sac, this technique allows full exposure of the pancreas, splenic artery, and hepatic artery. Pisano *et al*[4] used this approach in four cases. This technique carried some disadvantages as mobilization of the large curvature of the stomach increases the rate of complications, such as intraoperative hemorrhage and injury to the stomach, and significant increases the operating time.

In our small case series, there were no conversions to laparotomy. The outcomes of

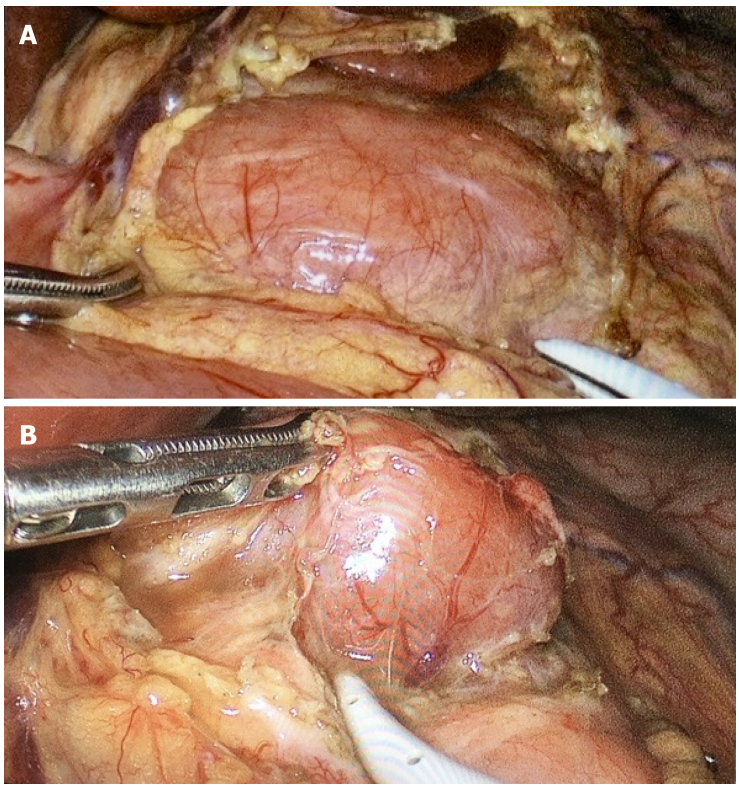

Figure 2 The left lobe of the liver is retracted upwards towards the diaphragm and the lesser omentum with its pars flaccida is exposed. A: Exposure of the hepatic node excising the lesser omentum; B: Dissection of the hepatic node.

operative time, blood loss, and complications were acceptable. All patients received an accurate diagnosis without any false-negative results.

Although the results of this current patient series are good, the small sample size does not permit firm conclusions.

In conclusion, LEBHN seems to be feasible and safe, and surgeons may use this simple approach to a fairly complicated anatomical area to patients who do not have access to superficial lymph nodes.

Table 1 Patients' demographic and operative characteristics

Age	Gender	No of Biopsies	FNA	FNB	ORT	LOS	Complications	Diagnosis
72	F	1	Yes	No	71	1	No	Follicular LY
39	F	1	No	No	53	1	No	Hodgkin LY
25	M	0	No	No	49	1	No	Sarcoidosis
60	F	0	No	No	65	1	No	Reactive LN
69	F	3	Yes	Yes	47	1	No	Sarcoidosis
51	M	1	No	No	22	1	No	Reactive LN

F: Female; M: Male; FNA: Fine needle aspiration; FNB: Fine needle biopsy; ORT: Operating time; LOS: Length of hospital stay; LY: Lymphoma.

ARTICLE HIGHLIGHTS

Research background

Diagnosis of lympho-proliferative diseases is challenging. Although an excisional biopsy of a complete lymph node is the gold standard for diagnosis, endoscopic or percutaneous techniques are often used due to the surgical challenge the location of the lymph node imposes.

Research motivation

The current study describes a small case series of laparoscopic dissection of the hepatic node through a trans lesser omentum approach. This approach is rarely discussed in the English literature.

Research objectives

The study describes the clinical and surgical results of this novel technique.

Research methods

A single center, retrospective evaluation of patients undergoing laparoscopic dissection and excisional biopsy of the hepatic node.

Research results

During the time frame of the study, six patients were operated using this novel technique, with no conversions to laparotomy, no intra and post-operative complications and acceptable operating time. Most importantly, surgery yielded a definite diagnosis in all patients, and there was no need for further investigation.

Research conclusions

Laparoscopic dissection of the hepatic node seems to be feasible and safe, and surgeons may use this simple approach to a fairly complicated anatomical area in highly selected patients who do not have access to superficial lymph nodes.

Research perspectives

This study suggests that approach to the hepatic node and the celiac axis is easily and safely performed through a trans lesser omentum approach. This may facilitate future discussion on how to achieve the diagnosis of lympho-proliferative disease in patients who do not have enlarged and pathological superficial lymph nodes.

REFERENCES

1 **Cheson BD**, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, Lister TA; Alliance, Australasian Leukaemia and Lymphoma Group; Eastern Cooperative Oncology Group; European Mantle Cell Lymphoma Consortium; Italian Lymphoma Foundation; European Organisation for Research; Treatment of Cancer/Dutch Hemato-Oncology Group; Grupo Español de Médula Ósea; German High-Grade Lymphoma Study Group; German Hodgkin's Study Group; Japanese Lymphorra Study Group; Lymphoma Study Association; NCIC Clinical Trials Group; Nordic Lymphoma Study Group; Southwest Oncology Group; United Kingdom National Cancer Research Institute. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol* 2014; **32**: 3059-3068 [PMID: 25113753 DOI: 10.1200/JCO.2013.54.8800]

2 **Tilly H**, Gomes da Silva M, Vitolo U, Jack A, Meignan M, Lopez-Guillermo A, Walewski J, André M, Johnson PW, Pfreundschuh M, Ladetto M; ESMO Guidelines Committee. Diffuse large B-cell lymphoma (DLBCL): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015; **26** Suppl 5: v116-v125 [PMID: 26314773 DOI: 10.1093/annonc/mdv304]

3 **Durai R**, Mir N, Ng PC. Laparoscopic retroperitoneal/mesenteric lymph node sampling: a safe and effective technique. *Singapore Med J* 2011; **52**: 758-762 [PMID: 22009398 DOI: 10.1007/s00508-011-0065-1]

4 **Pisano G**, Calò PG, Piras S, Sanna S, Manca A, Tatti A, Nicolosi A. Laparoscopic lymph node biopsy in the diagnosis of lymphoma. Indications and results. *Ann Ital Chir* 2012; **83**: 469-476 [PMID: 23082720

DOI: 10.2319/012413-76.1]
5 **Cowles RA**, Yahanda AM. Laparoscopic biopsy of abdominal retroperitoneal lymphadenopathy for the diagnosis of lymphoma. *J Am Coll Surg* 2000; **191**: 108-113 [PMID: 10898191 DOI: 10.1016/S1072-7515(00)00279-9]
6 **Gossot D**, de Kerviler E, Brice P, Mariette X, Meignin V, Cazals-Hatem D, Frija J, Célérier M. Surgical endoscopic techniques in the diagnosis and follow-up of patients with lymphoma. *Br J Surg* 1998; **85**: 1107-1110 [PMID: 9718007 DOI: 10.1046/j.1365-2168.1998.00774.x]
7 **Strickler JG**, Donohue JH, Porter LE, Habermann TM. Laparoscopic biopsy for suspected abdominal lymphoma. *Mod Pathol* 1998; **11**: 831-836 [PMID: 9758362]
8 **Walsh RM**, Heniford BT. Role of laparoscopy for Hodgkin's and non-Hodgkin's lymphoma. *Semin Surg Oncol* 1999; **16**: 284-292 [PMID: 10332774 DOI: 10.1002/(SICI)1098-2388(199906)16:43.0.CO;2-F]
9 **Silecchia G**, Fantini A, Raparelli L, De Leo A, Vitolo D, Monarca B, Bezzi M, Rosato P, Basso N. Management of abdominal lymphoproliferative diseases in the era of laparoscopy. *Am J Surg* 1999; **177**: 325-330 [PMID: 10326853 DOI: 10.1016/S0002-9610(99)00056-2]
10 **Silecchia G**, Raparelli L, Perrotta N, Fantini A, Fabiano P, Monarca B, Basso N. Accuracy of laparoscopy in the diagnosis and staging of lymphoproliferative diseases. *World J Surg* 2003; **27**: 653-658 [PMID: 12734679 DOI: 10.1007/s00268-003-6692-6]
11 **Asoglu O**, Porter L, Donohue JH, Cha SS. Laparoscopy for the definitive diagnosis of intra-abdominal lymphoma. *Mayo Clin Proc* 2005; **80**: 625-631 [PMID: 15887430 DOI: 10.4065/80.5.625]
12 **Casaccia M**, Torelli P, Cavaliere D, Panaro F, Nardi I, Rossi E, Spriano M, Bacigalupo A, Gentile R, Valente U. Laparoscopic lymph node biopsy in intra-abdominal lymphoma: high diagnostic accuracy achieved with a minimally invasive procedure. *Surg Laparosc Endosc Percutan Tech* 2007; **17**: 175-178 [PMID: 17581460 DOI: 10.1097/SLE.0b013e31804b41c9]
13 **Daly SC**, Klairmont M, Arslan B, Vigneswaran Y, Roggin KF, Ujiki MB, Denham W, Millikan KW, Luu MB, Deziel DJ, Myers JA. Laparoscopy has a superior diagnostic yield than percutaneous image-guided biopsy for suspected intra-abdominal lymphoma. *Surg Endosc* 2015; **29**: 2496-2499 [PMID: 25492451 DOI: 10.1007/s00464-014-4004-x]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 83-91

DOI: 10.4251/wjgo.v12.i1.83

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Multi-institutional retrospective analysis of FOLFIRI in patients with advanced biliary tract cancers

Jonathan D Mizrahi, Valerie Gunchick, Kabir Mody, Lianchun Xiao, Phanikeerthi Surapaneni, Rachna T Shroff, Vaibhav Sahai

ORCID number: Jonathan D Mizrahi (0000-0002-3710-9742); Valerie Gunchick (0000-0002-9392-0644); Kabir Mody (0000-0003-3254-2990); Lianchun Xiao (0000-0003-0972-2766); Phani Keerthi Surapaneni (0000-0003-2712-7709); Rachna T Shroff (0000-0003-0066-2672); Vaibhav Sahai (0000-0003-1892-1548).

Author contributions: Sahai V, Shroff RT and Mody K designed the research study; Mizrahi JD, Gunchick V, Mody K, Surapaneni KP, Shroff RT and Sahai V collected the data; Xiao L, Mizrahi JD, Gunchick V and Sahai V analyzed and interpreted the data; Mizrahi JD, Gunchick V and Sahai V wrote the manuscript; All authors have read and approve the final manuscript.

Institutional review board statement: The study was reviewed and approved by the individual Institutional Review Boards at MD Anderson Cancer Center, University of Michigan and Mayo Clinic.

Informed consent statement: Informed consent was waived as patient data were collected and de-identified for analysis.

Conflict-of-interest statement: The authors report no conflicts of interest relevant to this article.

Open-Access: This article is an open-access article which was selected by an in-house editor and

Jonathan D Mizrahi, Lianchun Xiao, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77005, United States

Valerie Gunchick, Vaibhav Sahai, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109, United States

Kabir Mody, Phanikeerthi Surapaneni, Division of Medical Oncology, Mayo Clinic Cancer Center, Jacksonville, FL 32224, United States

Rachna T Shroff, University of Arizona Cancer Center, University of Arizona, Tucson, AZ 85724, United States

Corresponding author: Jonathan D Mizrahi, MD, Academic Fellow, Division of Cancer Medicine, the University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd Unit 463, Houston, TX 77030, United States. jdmizrahi@mdanderson.org

Abstract

BACKGROUND

Gemcitabine plus platinum is the standard of care first-line treatment for advanced biliary tract cancers (BTC). There is no established second-line therapy, and retrospective reviews report median progression-free survival (PFS) less than 3 mo on second-line therapy. 5-Fluorouracil plus irinotecan (FOLFIRI) is a commonly used regimen in patients with BTC who have progressed on gemcitabine plus platinum, though there is a paucity of data regarding its efficacy in this population.

AIM

To assess the efficacy of FOLFIRI in patients with biliary tract cancers.

METHODS

We retrospectively identified patients with advanced BTC who were treated with FOLFIRI at MD Anderson, University of Michigan and Mayo Clinic in Jacksonville. Data were collected on patient demographics, BTC subtype, response per RECIST v1.1, progression and survival.

RESULTS

Ninety-eight patients were included of which 74 (75%) had metastatic and 24 (25%) had locally advanced disease at the time of treatment with FOLFIRI. The median age was 60 (range, 22-86) years. The number of patients with extrahepatic

Manuscript source: Unsolicited manuscript

Received: May 8, 2019
Peer-review started: May 10, 2019
First decision: July 31, 2019
Revised: August 9, 2019
Accepted: September 12, 2019
Article in press: September 12, 2019
Published online: January 15, 2020

P-Reviewer: Cao ZF, Frena A
S-Editor: Ma YJ
L-Editor: Filipodia
E-Editor: Liu MY

cholangiocarcinoma, gall bladder cancer and intrahepatic cholangiocarcinoma were 10, 17 and 71, respectively. FOLFIRI was used as 1st, 2nd, 3rd or 4th – Nth lines in 8, 50, 36 and 4 patients, respectively. Median duration on FOLFIRI in the entire cohort was 2.2 (range, 0.5-8.4) mo. The median PFS and overall survival were 2.4 (95% confidence interval (CI): 1.7-3.1) and 6.6 (95%CI: 4.7-8.4) mo, respectively. Median PFS for patients treated with FOLFIRI in 1st, 2nd, 3rd or 4th – Nth lines were 3.1, 2.5, 2.3 and 1.5 mo, respectively. Eighteen patients received concurrent bevacizumab (n = 13) or EGFR-targeted therapy (n = 5) with FOLFIRI, with a median PFS of 2.7 mo (95%CI: 1.7-5.1).

CONCLUSION
In this largest multi-institution retrospective review of 98 patients with BTC treated with FOLFIRI, efficacy appears to be modest with outcomes similar to other cytotoxic chemotherapy regimens.

Key words: Biliary tract neoplasms; Fluorouracil; Irinotecan; Cholangiocarcinoma; Retrospective studies

Core tip: We retrospectively analyzed patients with advanced biliary tract cancers treated with 5-fluorouracil plus irinotecan at three institutions, MD Anderson, University of Michigan and Mayo Clinic in Jacksonville. We identified 98 patients with a median age of 60 years, most (72%) of whom had intrahepatic cholangiocarcinoma. Fifty and 36 patients were treated in the second and third-line settings, respectively. The median progression-free survival and overall survival were 2.4 (95%CI: 1.7-3.1) and 6.6 (95%CI: 4.7-8.4) mo, respectively.

Citation: Mizrahi JD, Gunchick V, Mody K, Xiao L, Surapaneni P, Shroff RT, Sahai V. Multi-institutional retrospective analysis of FOLFIRI in patients with advanced biliary tract cancers. *World J Gastrointest Oncol* 2020; 12(1): 83-91
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/83.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.83

INTRODUCTION

Biliary tract cancers (BTCs) are rare but aggressive malignancies that arise from epithelial cells in the bile ducts or gallbladder. BTCs are anatomically classified as intrahepatic and extrahepatic (perihilar and distal) cholangiocarcinoma (CCA), and gallbladder carcinoma (GBCA)[1-3].

In the United States alone, more than 12000 people are estimated to be diagnosed with BTC in 2019[4]. Advanced BTCs are considered aggressive cancers with a reported median overall survival (OS) of approximately 12 mo. Over 85000 people lost their lives to BTC between 1999 and 2014[5], and mortality rates continue to rise[5,6]. It is clear that more effective management strategies are needed to reverse these rising rates, particularly for patients with advanced BTCs. Standard of care first-line therapy for these patients involves multi-agent chemotherapy with gemcitabine and cisplatin[7-9]. In the phase 3 ABC-02 trial published in 2010, gemcitabine and cisplatin was demonstrated to improve median OS to 11.7 mo from 8.1 mo with gemcitabine alone. However, durable response rates are infrequent, and a substantial number of patients progress quickly. Additional strategies and subsequent lines of therapy remain largely investigational with no clear standard at present, although FOLinic acid and Fluorouracil in combination with either OXaliplatin (FOLFOX) or IRInotecan (FOLFIRI) are often used[10-12].

The efficacy of FOLFIRI as a first or second-line treatment has been previously assessed in small retrospective studies. In a single institution review of 17 patients with advanced BTC treated with FOLFIRI as first-line therapy, the authors noted a median progression free survival (PFS) and OS of 2.6 and 6.5 mo, respectively[13]. In another retrospective analysis of 64 patients with advanced BTC treated with either FOLFIRI or XELIRI as second-line therapy, Brieau *et al*[10] observed a similar median PFS and OS of 2.6 and 6.2 mo, respectively. Additionally, a smaller retrospective

analysis of five BTC patients treated with either FOLFIRI or FOLFOX as second-line therapy reported a median PFS and OS of 4.4 and 6.1 mo, respectively[14].

The primary objective of this retrospective analysis was to identify the efficacy of irinotecan-based regimens in the management of patients with advanced BTC in a larger multi-institutional cohort.

MATERIALS AND METHODS

Study cohort

The study was individually approved by the institutional review boards at University of Michigan, University of Texas MD Anderson Cancer Center and Mayo Clinic Cancer Center at Jacksonville. The informed consent was waived for this HIPAA compliant retrospective study. The eligibility criteria included patients aged 18 years or older with pathologic confirmation of BTC and advanced unresectable or metastatic disease on imaging. Eligible subjects must have received irinotecan-based systemic chemotherapy. Patients with ICD9 and ICD10 diagnosis codes for BTC were retrospectively identified at each institution with at least one encounter between January 2007 and October 2017. Data were collected on patient demographics, subtype of BTC, response per RECIST v1.1, progression and survival. In addition, genomic analysis data were also collected, when available.

Statistical analysis

Descriptive statistics such as mean, standard deviation, median and range, frequency and percentage were used to summarize patient characteristics. The Kaplan-Meier method was applied to estimate survival outcomes (Figure 1), *i.e.*, OS and PFS, and the log rank test was used for comparison of these outcomes between subgroups of patients. The OS time was calculated as the time period from the date of the treatment start to the date of death or to the date of the last follow-up for patients alive, and patients alive were censored for the analysis of OS. The PFS time was calculated as the time period from the date of start of treatment to the date of progression or death, whichever occurred first; and patients alive and without progression were censored to the date of the last follow-up. SAS software v9.4 (SAS institute Inc., Cary, NC, United States) and Splus software v8.2 (TIBCO Software Inc., Palo Alto, CA, Unites States).

RESULTS

A total of 98 consecutive patients who met the eligibility criteria were included in the analysis. The median age was 60 years (range, 22-86 years), and 46 (46.9%) subjects were women. Sixty-one patients were identified at MD Anderson, 26 at University of Michigan, and 11 at Mayo Clinic Cancer Center in Jacksonville. Seventy-four (75%) patients had distant metastases at the time of treatment with FOLFIRI, while 24 (25%) had locally advanced disease. The majority of patients had intrahepatic CCA (n = 71), compared with 17 with GBCA and 10 with extrahepatic CCA. The patient baseline characteristics are detailed in Table 1.

The median duration on FOLFIRI, or FOLFIRI-containing regimens, was 2.2 mo (range, 0.5 to 8.4), and the median PFS was 2.4 mo (95% confidence interval (CI): 1.7-3.1) for the entire cohort. The median PFS for patients treated with FOLFIRI as 1st, 2nd, 3rd or 4th – Nth line therapy was 3.1 (95%CI: 1.4-4.8), 2.4 (95%CI: 1.8-3.7), 2.3 (95%CI: 1.5-3.1) and 1.5 (95%CI: 0.9-2.0) mo, respectively. The median OS for patients treated with FOLFIRI as 1st, 2nd, 3rd or 4th – Nth line therapy was 12.3 (95%CI: 5.6-23.4), 7.7 (95%CI: 4.9-10.5), 5.0 (95%CI: 3.6-7.3) and 7.5 (95%CI: 5.2-9.8) mo, respectively. The median OS for the cohort was 6.6 mo (95%CI: 4.7-8.4) from start of therapy. The best overall response rate was 9.8% per RECIST v1.1 with a disease control rate of 45.1%.

Thirteen patients received vascular endothelial growth factor targeted therapy with bevacizumab, and five patients received anti-epidermal growth factor receptor therapy with erlotinib (n = 4) and panitumumab (n = 1) concurrently with FOLFIRI. Patients in both of these groups of patients exhibited a median PFS of 2.7 mo.

There was no statistically significant difference in median PFS for patients with locally advanced disease when compared to those with distant metastases (3.2 *vs* 2.1 mo, P = 0.16) at the time of FOLFIRI treatment. There was a trend towards prolonged median OS for patients with locally advanced cancer compared to those with distant metastases (9.3 *vs* 5.6 mo, P = 0.08) (Table 2).

Thirty-four (35%) of the patients included in the study had genomic profiling of their BTC completed, including five with extrahepatic CCA, 27 with intrahepatic CCA and two with GBCA. The genomic profiling results are summarized in Table 3. The

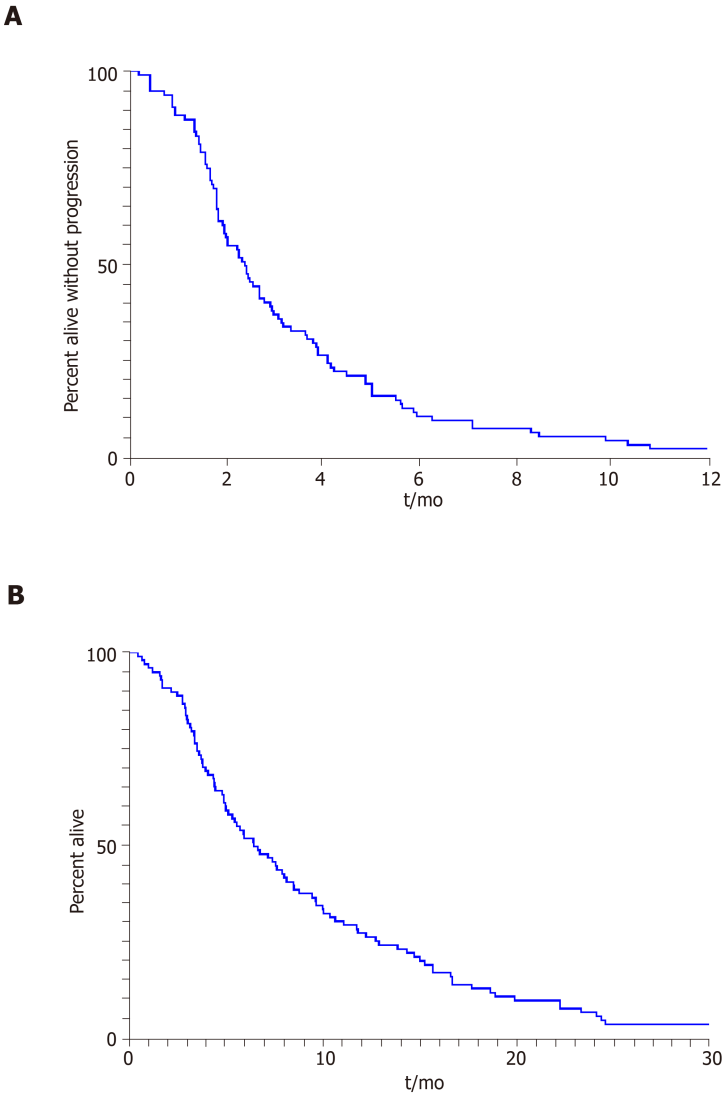

Figure 1 The Kaplan-Meier method was applied to estimate survival outcomes. A: Progression-free survival; B: Overall survival.

most frequent alterations identified included mutations in *TP53* (35.3%), *IDH1* and *IDH2* (29.4%) and *KRAS* (20.6%) genes. *FGFR*2 fusions were identified in four patients with intrahepatic CCA and two patients with extrahepatic CCA, however, the *IDH1* and *IDH2* mutations were restricted to the intrahepatic subtype.

DISCUSSION

In this multi-institution retrospective study, FOLFIRI, or FOLFIRI-containing regimens had modest efficacy with a median PFS of 2.4 mo and OS of 6.6 mo in patients with advanced unresectable or metastatic BTC. To our knowledge, this is the largest analysis of outcomes with FOLFIRI in BTCs. Expectedly, patients treated with FOLFIRI earlier in the course of their therapy tended to have longer PFS (P = 0.53), likely due to use of other 5-fluorouracil (5-FU) containing regimens prior to FOLFIRI and development of multi-drug resistance. Additionally, patients with locally advanced stage may have longer PFS (3.2 *vs* 2.1 mo; P = 0.16) compared to those with distant metastasis.

The majority of the patients included in our analysis had intrahepatic CCA (72%). This subtype of BTC may be associated with better outcomes compared to extrahepatic CCA and GBCA[15], which could potentially bias our results. However, a difference in survival was not seen in our patients based on subtype of BTC, though the sample size of patients with extrahepatic CCA and GBCA was small.

The survival outcomes we describe are comparable to those reported by Brieau *et*

Table 1 Patient baseline characteristics

Characteristic	Value
Total, *n*	98
Age in yr, median (range)	60 (22-86)
Gender, *n (%)*	
Female	46 (47)
Male	52 (53)
Institution, *n (%)*	
MD Anderson Cancer Center	61 (62)
University of Michigan	26 (27)
Mayo Clinic Cancer Center	11 (11)
Stage at Treatment with FOLFIRI, *n (%)*	
Locally advanced	24 (25)
Metastatic	74 (75)
Subtype of BTC, *n (%)*	
Extrahepatic cholangiocarcinoma	10 (10)
Intrahepatic cholangiocarcinoma	71 (72)
Gallbladder carcinoma	17 (17)
Line of Therapy, *n (%)*	
First	8 (8)
Second	50 (51)
Third	36 (37)
Fourth or greater	4 (4)
Irinotecan-based regimen, *n (%)*	
FOLFIRI	77 (79)
FOLFIRI + bevacizumab	13 (13)
FOLRIRI + anti-EGFR	5 (5)
FOLFIRINOX	2 (2)
FOLFIRI + nab-paclitaxel	1 (1)
ECOG performance status, *n (%)*	
0	11 (11)
1	48 (49)
2	3 (3)
3	2 (2)
Not documented	34 (35)

BTC: Biliary tract cancer; ECOG: Eastern Cooperative Group; EGFR: Epidermal growth factor receptor; FOLFIRI: Folinic acid, 5-fluorouracil and irinotecan; FOLFIRINOX: Folinic acid, 5-fluorouracil, irinotecan and oxaliplatin.

al[10] and Moretto *et al*[13] utilizing FOLFIRI and similar to published data regarding other chemotherapy regimens, such as FOLFOX in this patient population[16,17]. FOLFOX has been evaluated in multiple prospective studies as second-line therapy with a reported time to progression of 3.1 mo in a 37 patient phase II trial from China[18] and a PFS of 3.9 mo in a 66 patient observational study from Japan[19]. A retrospective analysis of 144 patients with BTCs treated with second-line chemotherapy (70% regimens 5-FU based) at a single institution in Germany found an overall response rate of 9.7% with a disease control rate of 33.6% and median OS of 9.9 mo[20]. An additional retrospective study of 18 patients from an institution in Chile showed a median PFS and OS of 3.2 and 4.6 mo, respectively[17]. There are several other clinical trials accruing patients for second-line therapy, including the phase Ib/II trial evaluating the combination of 5-FU, folinic acid and nanoliposomal irinotecan in conjunction with an anti-PD1 antibody nivolumab (BilT-03)[21].

In the subgroup of 18 patients who were treated concurrently with either anti-EGFR or anti-vascular endothelial growth factor targeted therapy, there did not appear to be a clinical benefit of the additional drug, though this was a small cohort. This is in contrast to a single institution analysis from France of 13 patients with metastatic intrahepatic CCA treated with FOLFIRI with bevacizumab as second-line

Table 2 Subgroup analysis of median progression-free survival

Variable	Median PFS (95%CI)	*P* value	Median OS (95%CI)	*P* value
Gender				
Female	2.4 (1.6–3.6)	0.65	6.6 (4.5–9.9)	0.65
Male	2.3 (1.5–3.4)		6.9 (4.6–10.3)	
Subtype of BTC				
Extrahepatic cholangiocarcinoma	3.7 (1.5–18.9)	0.14	8.0 (1.8–22.3)	0.61
Intrahepatic cholangiocarcinoma	2.3 (1.9-2.8)		6.5 (4.5–9.7)	
Gallbladder carcinoma	2.1 (1.8–3.7)		6.5 (5.2–10.1)	
Stage at treatment with FOLFIRI				
Locally Advanced	3.2 (2.0–5.2)	0.16	9.3 (5.9–14.7)	0.08
Metastatic	2.1 (1.3–3.3)		5.6 (3.5–8.8)	
Line of therapy				
First	3.1 (1.4–4.8)	0.24	12.3 (5.6–23.4)	0.08
Second	2.4 (1.8–3.7)		7.7 (4.9–10.5)	
Third	2.3 (1.5–3.1)		5.0 (3.6–7.3)	
Fourth or greater	1.5 (0.9–2.0)		7.5 (5.2–9.8)	
ECOG performance status				
0 or 1	2.5 (2.0–3.1)	0.44	7.7 (5.6–11.9)	0.03
2 or greater	1.5 (0.8–4.9)		2.9 (1.7–8.6)	
Undocumented	2.1 (1.6–3.4)		5.3 (3.8–8.2)	
Genomic analysis				
KRAS				
Wildtype	2.4 (1.1–5.1)	0.12	11.8 (5.5–25.4)	0.06
Mutant	3.7 (1.7–8.0)		7.5 (3.5–16.1)	
FGFR				
Wildtype	2.5 (1.0–6.0)	0.29	8.0 (3.3–19.4)	0.56
Fusion	4.3 (1.8–10.5)		13.4 (5.5–32.2)	
IDH1				
Wildtype	2.7 (1.2–6.0)	0.02	10.8 (4.9–23.9)	0.14
Mutant	2.1 (0.9–4.6)		4.1 (2.3–11.1)	

BTC: Biliary tract cancer; ECOG: Eastern Cooperative Group; FOLFIRI: FOLINIC acid, 5-fluorouracil, irinotecan; OS: Overall survival; PFS: Progression-free survival.

therapy that reported a best overall response rate of 38.4%, median PFS of 8 mo and OS of 20 mo[22].

We identified no significant correlation between specific somatic mutations and patient outcomes or response to FOLFIRI, though this conclusion is limited by the small number of patients in our study. Recently, the most promising therapeutic advances in BTC have resulted from the identification and targeting of actionable driver somatic mutations. Multiple phase II clinical trials have yielded encouraging results by taking advantage of driver mutations such as *FGFR*, *IDH1*, *IDH2* and *BRAF*[23-26]. However, most patients with BTCs do not harbor mutations that are currently targetable, limiting the benefits of these recent advances to only a select cohort.

Given the lack of other standard therapies for patients with BTCs who have progressed on first-line therapy, our results indicate that FOLFIRI may indeed have a role in these patients. The results of our study further emphasize the need for more effective treatment options for patients with advanced unresectable or metastatic BTCs after failure of first-line systemic chemotherapy, especially in absence of actionable driver mutations.

Table 3 Genomic profiling by tumor type

Total patients profiled, *n* = 34	Cholangiocarcinoma		Gallbladder carcinoma, *n* = 2 (6%)
	Extrahepatic, *n* = 5 (15%)	Intrahepatic, *n* = 27 (79%)	
Mutation	***n* (% of profiled)**	***n* (% of profiled)**	***n* (% of profiled)**
TP53	-	10 (37)	2 (100)
IDH1	-	8 (30)	-
KRAS	1 (20)	6 (22)	-
FGFR2	2 (40)	4 (15)	-
IDH2	-	2 (7)	-
PBRM1	-	2 (7)	-
BAP1	-	2 (7)	-
NF1	-	1 (4)	1 (50)
ARID1A	-	1 (4)	1 (50)
CDKN2A	-	-	1 (50)
MET	-	1 (4)	-
CCND1	-	1 (4)	-
PBX1	-	1 (4)	-
MYC	-	1 (4)	-
RB1	-	1 (4)	-
MAP3K1	-	1 (4)	-
S76	-	1 (4)	-
SPTA1	-	1 (4)	-
RET	-	1 (4)	-
ALK	-	1 (4)	-
ATM	-	1 (4)	-
CCNE1	-	1 (4)	-
GNAS	-	1 (4)	-
SMAD4	-	1 (4)	-
PIK3CA	-	1 (4)	-
PIK3CB	-	1 (4)	-
PTEN	-	1 (4)	-
PALB2	-	1 (4)	-
ARID2	-	1 (4)	-
BRCA2	-	1 (4)	-
BRAF	-	1 (4)	-
MDM2	1 (20)	-	-
FRS2	1 (20)	-	-

ARTICLE HIGHLIGHTS

Research background

Advanced biliary tract cancers (BTC) are aggressive malignancies without an established standard of care after progression on first-line combination chemotherapy with gemcitabine plus cisplatin. Fluoropyrimidine-based therapies, such as 5-fluorouracil plus either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) are commonly used in this setting. There is limited data on the efficacy of such regimens in patients with BTCs, particularly in the patients who have progressed on first-line therapy.

Research motivation

There is a significant need for evidence-based treatment of patients with advanced BTCs who have previously progressed of first-line systemic chemotherapy. Only small, primarily single-institution analyses have been published about the role of FOLFIRI in this population. We sought to combine the experiences of multiple institutions to provide the largest dataset with this regimen.

Research objectives

Our study assessed the efficacy of FOLFIRI in patients with BTC by measuring progression-free survival and overall survival.

Research methods
We retrospectively identified patients with advanced, unresectable BTC who were treated with FOLFIRI at three institutions: MD Anderson, University of Michigan and Mayo Clinic in Jacksonville. We collected data on survival, response per RECIST v1.1, patient demographics and tumor characteristics.

Research results
Ninety-eight patients were included in our analysis, most of whom were treated in the second and third-line setting. Median duration on FOLFIRI was 2.2 mo. Median progression-free survival was 2.4 mo (95%CI: 1.7-3.1), and median overall survival was 6.6 mo (95%CI: 4.7-8.4).

Research conclusions
The efficacy of FOLFIRI for patients with BTCs appears to be modest with survival outcomes that are similar to historical controls of other retrospectively examined second-line cytotoxic therapy options.

Research perspectives
Based on this multi-institutional analysis, FOLFIRI seems to have a limited role in the treatment of patients with BTCs, though there are no prospective studies that have assessed this regimen in this patient population. The recently reported results of the randomized phase III ABC-06 trial demonstrating an increase in OS with modified FOLFOX plus active symptom control compared to active symptom control alone likely makes this a more appealing treatment option for most patients who have progressed on gemcitabine plus cisplatin.

REFERENCES

1 **Bridgewater J**, Galle PR, Khan SA, Llovet JM, Park JW, Patel T, Pawlik TM, Gores GJ. Guidelines for the diagnosis and management of intrahepatic cholangiocarcinoma. *J Hepatol* 2014; **60**: 1268-1289 [PMID: 24681130 DOI: 10.1016/j.jhep.2014.01.021]

2 **Bridgewater JA**, Goodman KA, Kalyan A, Mulcahy MF. Biliary Tract Cancer: Epidemiology, Radiotherapy, and Molecular Profiling. *Am Soc Clin Oncol Educ Book* 2016; **35**: e194-e203 [PMID: 27249723 DOI: 10.14694/EDBK_160831]

3 **Wistuba II**, Gazdar AF. Gallbladder cancer: lessons from a rare tumour. *Nat Rev Cancer* 2004; **4**: 695-706 [PMID: 15343276 DOI: 10.1038/nrc1429]

4 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 7-34 [PMID: 30620402 DOI: 10.3322/caac.21551]

5 **Yao KJ**, Jabbour S, Parekh N, Lin Y, Moss RA. Increasing mortality in the United States from cholangiocarcinoma: an analysis of the National Center for Health Statistics Database. *BMC Gastroenterol* 2016; **16**: 117 [PMID: 27655244 DOI: 10.1186/s12876-016-0527-z]

6 **Khan SA**, Toledano MB, Taylor-Robinson SD. Epidemiology, risk factors, and pathogenesis of cholangiocarcinoma. *HPB (Oxford)* 2008; **10**: 77-82 [PMID: 18773060 DOI: 10.1080/13651820801992641]

7 **Valle J**, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP, Roughton M, Bridgewater J; ABC-02 Trial Investigators. Cisplatin plus gemcitabine *vs* gemcitabine for biliary tract cancer. *N Engl J Med* 2010; **362**: 1273-1281 [PMID: 20375404 DOI: 10.1056/NEJMoa0908721]

8 **Okusaka T**, Nakachi K, Fukutomi A, Mizuno N, Ohkawa S, Funakoshi A, Nagino M, Kondo S, Nagaoka S, Funai J, Koshiji M, Nambu Y, Furuse J, Miyazaki M, Nimura Y. Gemcitabine alone or in combination with cisplatin in patients with biliary tract cancer: a comparative multicentre study in Japan. *Br J Cancer* 2010; **103**: 469-474 [PMID: 20628385 DOI: 10.1038/sj.bjc.6605779]

9 **Park JO**, Oh DY, Hsu C, Chen JS, Chen LT, Orlando M, Kim JS, Lim HY. Gemcitabine Plus Cisplatin for Advanced Biliary Tract Cancer: A Systematic Review. *Cancer Res Treat* 2015; **47**: 343-361 [PMID: 25989801 DOI: 10.4143/crt.2014.308]

10 **Brieau B**, Dahan L, De Rycke Y, Boussaha T, Vasseur P, Tougeron D, Lecomte T, Coriat R, Bachet JB, Claudez P, Zaanan A, Soibinet P, Desrame J, Thirot-Bidault A, Trouilloud I, Mary F, Marthey L, Taieb J, Cacheux W, Lièvre A. Second-line chemotherapy for advanced biliary tract cancer after failure of the gemcitabine-platinum combination: A large multicenter study by the Association des Gastro-Entérologues Oncologues. *Cancer* 2015; **121**: 3290-3297 [PMID: 26052689 DOI: 10.1002/cncr.29471]

11 **Chun YS**, Javle M. Systemic and Adjuvant Therapies for Intrahepatic Cholangiocarcinoma. *Cancer Control* 2017; **24**: 1073274817729241 [PMID: 28975832 DOI: 10.1177/1073274817729241]

12 **Lamarca A**, Palmer DH, Wasan HS, Ross PJ, Ma YT, Arora A, Falk S, Gillmore R, Wadsley J, Patel K, Anthoney A, Maraveyas A, Waters JS, Hobbs C, Barber S, Ryder D, Ramage J, Davies LM, Bridgewater JA, Valle JW, on behalf of the Advanced Biliary Cancer (ABC) Working Group. ABC-06 | A randomised phase III, multi-centre, open-label study of active symptom control (ASC) alone or ASC with oxaliplatin / 5-FU chemotherapy (ASC+mFOLFOX) for patients (pts) with locally advanced / metastatic biliary tract cancers (ABC) previously-treated with cisplatin/gemcitabine (CisGem) chemotherapy. *J Clin Oncol* 2019; 4003 [DOI: 10.1200/JCO.2019.37.15_suppl.4003]

13 **Moretto R**, Raimondo L, De Stefano A, Cella CA, Matano E, De Placido S, Carlomagno C. FOLFIRI in patients with locally advanced or metastatic pancreatic or biliary tract carcinoma: a monoinstitutional experience. *Anticancer Drugs* 2013; **24**: 980-985 [PMID: 23928570 DOI: 10.1097/CAD.0b013e328364e66b]

14 **Fiteni F**, Jary M, Monnien F, Nguyen T, Beohou E, Demarchi M, Dobi E, Fein F, Cleau D, Fratté S, Nerich V, Bonnetain F, Pivot X, Borg C, Kim S. Advanced biliary tract carcinomas: a retrospective multicenter analysis of first and second-line chemotherapy. *BMC Gastroenterol* 2014; **14**: 143 [PMID:

25117717 DOI: 10.1186/1471-230X-14-143]
15 **Lamarca A**, Ross P, Wasan HS, Hubner RA, McNamara MG, Lopes A, Manoharan P, Palmer D, Bridgewater J, Valle JW. Advanced intrahepatic cholangiocarcinoma: post-hoc analysis of the ABC-01, -02 and -03 clinical trials. *J Natl Cancer Inst* 2019 [PMID: 31077311 DOI: 10.1093/jnci/djz071]
16 **Rogers JE**, Law L, Nguyen VD, Qiao W, Javle MM, Kaseb A, Shroff RT. Second-line systemic treatment for advanced cholangiocarcinoma. *J Gastrointest Oncol* 2014; **5**: 408-413 [PMID: 25436118 DOI: 10.3978/j.issn.2078-6891.2014.072]
17 **Leal JL**, Roa JC, Jarufe N, Madrid J, Ibanez C, Herrera ME, Garrido M, Nervi B. Second-line FOLFOX chemotherapy in patients with metastatic gallbladder cancer and cholangiocarcinoma. *J Clin Oncol* 2014; **32**: 322-322 [DOI: 10.1200/jco.2014.32.3_suppl.322]
18 **He S**, Shen J, Sun X, Liu L, Dong J. A phase II FOLFOX-4 regimen as second-line treatment in advanced biliary tract cancer refractory to gemcitabine/cisplatin. *J Chemother* 2014; **26**: 243-247 [PMID: 24070164 DOI: 10.1179/1973947813Y.0000000133]
19 **Dodagoudar C**, Doval DC, Mahanta A, Goel V, Upadhyay A, Goyal P, Talwar V, Singh S, John MC, Tiwari S, Patnaik N. FOLFOX-4 as second-line therapy after failure of gemcitabine and platinum combination in advanced gall bladder cancer patients. *Jpn J Clin Oncol* 2016; **46**: 57-62 [PMID: 26603355 DOI: 10.1093/jjco/hyv148]
20 **Schweitzer N**, Kirstein MM, Kratzel AM, Mederacke YS, Fischer M, Manns MP, Vogel A. Second-line chemotherapy in biliary tract cancer: Outcome and prognostic factors. *Liver Int* 2019; **39**: 914-923 [PMID: 30716200 DOI: 10.1111/liv.14063]
21 **University of Michigan Rogel Cancer Center**. Phase Ib/II trial of Nal-irinotecan and nivolumab as second-line treatment in patients with advanced biliary tract cancer. [accessed 2019 Apr 25]. In: ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: https://ClinicalTrials.gov/show/NCT03785873 ClinicalTrials.gov Identifier: NCT03785873
22 **Guion-Dusserre JF**, Lorgis V, Vincent J, Bengrine L, Ghiringhelli F. FOLFIRI plus bevacizumab as a second-line therapy for metastatic intrahepatic cholangiocarcinoma. *World J Gastroenterol* 2015; **21**: 2096-2101 [PMID: 25717243 DOI: 10.3748/wjg.v21.i7.2096]
23 **Lowery MA**, Abou-Alfa GK, Burris HA, Janku F, Shroff RT, Cleary JM, Azad NS, Goyal L, Maher EA, Gore L, Hollebecque A, Beeram M, Trent JC, Jiang L, Ishii Y, Auer J, Gliser C, Agresta SV, Pandya SS, Zhu AX. Phase I study of AG-120, an IDH1 mutant enzyme inhibitor: Results from the cholangiocarcinoma dose escalation and expansion cohorts. *J Clin Oncol* 2017; **35**: 4015-4015 [DOI: 10.1200/JCO.2017.35.15_suppl.4015]
24 **Javle M**, Lowery M, Shroff RT, Weiss KH, Springfeld C, Borad MJ, Ramanathan RK, Goyal L, Sadeghi S, Macarulla T, El-Khoueiry A, Kelley RK, Borbath I, Choo SP, Oh DY, Philip PA, Chen LT, Reungwetwattana T, Van Cutsem E, Yeh KH, Ciombor K, Finn RS, Patel A, Sen S, Porter D, Isaacs R, Zhu AX, Abou-Alfa GK, Bekaii-Saab T. Phase II Study of BGJ398 in Patients With FGFR-Altered Advanced Cholangiocarcinoma. *J Clin Oncol* 2018; **36**: 276-282 [PMID: 29182496 DOI: 10.1200/jco.2017.75.5009]
25 **Hollebecque A**, Lihou C, Zhen H, Abou-Alfa GK, Borad M, Sahai V, Catenacci DVT, Murphy A, Vaccaro G, Paulson A, Oh D-Y, Féliz L. Interim results of fight-202, a phase II, open-label, multicenter study of INCB054828 in patients (pts) with previously treated advanced/metastatic or surgically unresectable cholangiocarcinoma (CCA) with/without fibroblast growth factor (FGF)/FGF receptor (FGFR) genetic alterations. *Ann Oncol* 2018; 29 [DOI: 10.1093/annonc/mdy282.139]
26 **Wainberg ZA**, Lassen UN, Elez E, Italiano A, Curigliano G, Braud FGD, Prager G, Greil R, Stein A, Fasolo A, Schellens JHM, Wen PY, Boran AD, Burgess P, Gasal E, Ilankumaran P, Subbiah V. Efficacy and safety of dabrafenib (D) and trametinib (T) in patients (pts) with BRAF V600E–mutated biliary tract cancer (BTC): A cohort of the ROAR basket trial. *J Clin Oncol* 2019; **37**: 187-187 [DOI: 10.1200/JCO.2019.37.4_suppl.187]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 92-100

DOI: 10.4251/wjgo.v12.i1.92

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Simultaneous transarterial chemoembolization and radiofrequency ablation for large hepatocellular carcinoma

Feng Duan, Yan-Hua Bai, Li Cui, Xiao-Hui Li, Jie-Yu Yan, Mao-Qiang Wang

ORCID number: Feng Duan (0000-0001-8432-590X); Yan-Hua Bai (0000-0002-7331-7194); Li Cui (0000-0001-6161-6958); Xiao-Hui Li (0000-0002-1172-5707); Jie-Yu Yan (0000-0003-1142-7248); Mao-Qiang Wang (0000-0002-0299-5289).

Author contributions: Duan F and Wang MQ designed research; Duan F, Li XH, Yan JY, Wang MQ performed clinical research; Bai YH and Cui L analyzed data; Duan F and Cui L wrote the paper, all authors read and approved the final manuscript.

Institutional review board statement: This study was reviewed and approved by the General Hospital of People's Liberation Army Institutional Review Board.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous data.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest related to this article.

Data sharing statement: No additional unpublished data are available.

Feng Duan, Yan-Hua Bai, Li Cui, Xiao-Hui Li, Jie-Yu Yan, Mao-Qiang Wang, Department of Interventional Radiology, General Hospital of Chinese People's Liberation Army, Beijing 100853, China

Corresponding author: Mao-Qiang Wang, MD, PhD, Professor, Department of Interventional Radiology, General Hospital of Chinese People's Liberation Army, No. 28 Fuxing Road, Haidian District, Beijing 100853, China. wangmq@vip.sina.com

Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) is a common cancer and a leading cause of tumor-related death. Patients with large HCC (≥ 8 cm) are at an advanced stage and have poor prognosis, and hepatic resection may not be suitable, and the incidence of postoperative recurrence is high.

AIM

To evaluate recurrence and mid-term survival of patients with large HCC treated by transcatheter arterial chemoembolization (TACE) and radiofrequency ablation (RFA).

METHODS

This was a retrospective study. From 2010 to 2013, 46 consecutive patients with large HCC were treated with simultaneous TACE and RFA. Thirty-five of 46 patients had a single tumor. Progression-free survival (PFS) and overall survival (OS) were analyzed at 2 years and 3 years, respectively.

RESULTS

Forty-six patients treated by simultaneous TACE and RFA had no significant complications and treatment was successful. After 3 years, median PFS and OS were 10.21 ± 1.58 mo and 26.44 ± 2.26 mo, retrospectively. The survival rate was 67.5% after 2 years and 55.67% after 3 years.

CONCLUSION

These preliminary data show that simultaneous TACE and RFA are safe and effective for large HCC.

Key words: Chemoembolization; Radiofrequency ablation; Hepatocellular carcinoma; Simultaneous treatment; Transcatheter arterial chemoembolization; Radiofrequency ablation

Manuscript source: Unsolicited manuscript

Received: September 5, 2019
Peer-review started: September 5, 2019
First decision: October 18, 2019
Revised: October 25, 2019
Accepted: December 6, 2019
Article in press: December 6, 2019
Published online: January 15, 2020

P-Reviewer: Alsina A, Mathur A, Ko E
S-Editor: Wang JL
L-Editor: A
E-Editor: Qi LL

Core tip: Hepatocellular carcinoma (HCC) is a common cancer and a leading cause of tumor-related death. Patients who have large HCC (≥ 8 cm) are at advanced stages and have poor prognosis. Interventional treatment including transcatheter arterial chemoembolization (TACE) and radiofrequency ablation (RFA) are commonly used for HCC. However, for patients with large HCC, the use of TACE alone and RFA alone can only lead to partial tumor necrosis with poor local control. Our study showed that simultaneous combination of TACE and RFA may improve therapeutic efficacy and survival for patients with large HCC.

Citation: Duan F, Bai YH, Cui L, Li XH, Yan JY, Wang MQ. Simultaneous transarterial chemoembolization and radiofrequency ablation for large hepatocellular carcinoma. *World J Gastrointest Oncol* 2020; 12(1): 92-100
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/92.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.92

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common cancer and a leading cause of tumor-related death[1,2]. Patients who have large HCC (≥ 8 cm) are generally at advanced stages and have poor prognosis[3,4]. Hepatectomy may not suitable for patients who have large HCC or dysfunction of liver reserve and few patients are suitable for surgery. Besides, the postoperative recurrence is high[5].

Transcatheter arterial chemoembolization (TACE) and radiofrequency ablation (RFA) is commonly used for liver cancer. For TACE, the best candidates are patients with no symptoms and well-preserved liver function, as well as multifocal tumors with no vascular invasion or extrahepatic spread. However, TACE alone only leads to partial tumor necrosis. For small liver cancers (< 3 cm), RFA and surgery are comparable when it comes to therapeutic efficacy[6,7], but for tumors > 3 cm, RFA has poor local tumor control[8,9]. Therefore, combination of TACE and RFA may improve therapeutic efficacy and extend survival time.

In the present study, we evaluated the efficacy of combined TACE and RFA for large HCC. We retrospectively followed up 46 patients who received the combination treatment from March 2010 to November 2013 and assessed mid-term efficacy of the combination treatment modality as a novel strategy.

MATERIALS AND METHODS

Patient data

A total of 46 consecutively identified patients with large HCCs (at least one lesion diameter ≥ 8 cm) were enrolled. The baseline characteristics of these patients were as follows: (1) 42 men and four women; (2) Median age: 53.5 years (range 36–70 years); and (3) According to the Barcelona Clinic Liver Cancer (BCLC) staging classification, advanced HCC was classified as B/C (42/4); liver function: Child–Pugh class A (n = 45) and class B (n = 1). The mean tumor size was 8.17 cm (range 8.0–14.0 cm) (Tables 1 and 2). Sex, age, tumor stage, tumor size, number of tumors, Child–Pugh score, vascular invasion (tumor thrombus in the first branch or trunk of the portal vein) and pseudocapsule were taken into consideration as factors for subgroup analysis. This study was approved by the Ethics Committee of the Chinese People's Liberation Army General Hospital, and patients' informed consent was obtained. The diagnosis of HCC was based on imaging findings and/or α-fetoprotein (AFP) levels. Tumor stage was classified according to the BCLC classification system. The patients were surgically unsuitable, and without arteriovenous fistula or ascites.

Treatment protocol

After routine preoperative preparation, TACE was performed first, under sterile conditions and general anesthesia[10]. The right femoral artery was cannulated by a 4F vascular sheath (Radifocus Introducer II; Terumo Corp., Japan) and Seldinger's technique. Selective celiac artery and superior mesenteric artery angiography was performed by 4F hepatic artery catheter (HA; Terumo), which was through the

Table 1 Baseline characteristics of patients before treatment

Characteristics	Before treatment
Sex, M/F	42/4
Age, yr, median (range)	53.5 (36-70)
BCLC stage B/C [1]	42/4
Child–Pugh class A/B/C	45/1/0
ECOG performance status 0/1[2]	42/4/0
Laboratory values, median (range)	
WBC count, 10^9/L	4.98 (2.23-10.09)
Platelet count, 10^9/L	158 (49-371)
Hemoglobin, g/dL	135 (76-159)
Serum AST, IU/L	18.85 (16.20-101.60)
Serum ALT, IU/L	28.7 (9.6-178.8)
Serum total bilirubin, mg/dL	13.2 (5.0-41.4)
Serum albumin, g/dL	38.45 (28.8-45.1)
INR	1.10 (0.92-1.33)
Serum creatinine, mg/dL	65.05 (40.30-106.20)
Serum AFP, ng/mL, baseline < 20	7.86 (1.28-24200.00) (n = 29)
Serum AFP, ng/mL, baseline > 20	170.80 (20.02-24200.00) (n = 17)
Tumor burden and distribution	
Unifocal/multifocal	35/11
Unilobar/bilobar	44/2
Lesion diameter (cm)	
Largest lesion diameter (mean, range)	14.0 (9.47, 8.2-14.0)

[1]Barcelona Clinic Liver Cancer staging system.
[2]Eastern Cooperative Oncology Group performance status. BCLC: Barcelona Clinic Liver Cancer; ECOG: Eastern Cooperative Oncology Group; WBC: White blood cell; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International Normalized Ratio; AFP: α-fetoprotein.

vascular sheath. Maximum catheter selectivity of the hepatic artery was achieved using a microcatheter (Progreat; Terumo), with administration of an embolic agent into the tumor feeding arteries. Drug dose varied from 15 to 20 mL lipiodol (Guerbet Corp., France) each procedure, 30–50 mg doxorubicin (Pfizer Pharmaceuticals Ltd., United States), 100–150 mg oxaliplatin (Sanofi Pharmaceuticals Co. Ltd., France), depending on the tumor size, patient's weight and laboratory results. Lipiodol chemotherapeutic agents were injected until stasis to minimize reflux into nontarget vessels. Administration of agents continued until quiescence, and was observed in the arteries that directly fed the tumor (*i.e.*, the control column was fully cleared in five heart beats). After administration of 20 mL lipiodol, gelatin sponge, which served as a supplement, was injected if stasis was not achieved. If the inferior phrenic, internal thoracic artery branches and omental branches fed the tumor, these collateral arteries were embolized accordingly.

Percutaneous RFA was immediately performed after TACE. It was under general anesthesia and with the guidance of digital subtraction angiography (DSA) combined with cone-beam computed tomography (CBCT)[11]. One multipolar RF probe (RITA Co., Crystal Lake, IL, USA) with 5–7 cm maximum ablation diameter and 10–15 cm length was used during RFA. Guided by fluoroscopy, the RF probe was inserted into the center of the tumor. During puncture, both the lateral and postural views were obtained. CBCT was then performed to confirm the position of the RF probe (Figure 1). Ablation began when the target position was reached. The operation parameters were power, 150–200 W; and ablation time, 15 min when temperature rose until 105 °C. According to tumor size and maximum ablation diameter, RFA was performed 2–5 times. Puncture tract ablation was carried out to avoid bleeding and tumor seeding.

Patient follow-up and clinical data collection

Enhanced magnetic resonance imaging was used for follow-up every 1–2 mo during the first year, and every 2–4 months afterwards. Tumor recurrence or metastasis was recognized as disease progression. Comprehensive treatment including TACE, RFA,

Table 2 Baseline characteristics of patients after treatment

Characteristics	3 d after treatment
Laboratory values, median (range)	
WBC count, 10^9/L	4.985 (2.300-9.050)
Platelet count, 10^9/L	141 (44-259)
Hemoglobin, g/dL	136.5 (98.0-165.0)
Serum AST, IU/L	28.2 (15.0-103.7)
Serum ALT, IU/L	32.15 (8.40-74.30)
Serum total bilirubin, mg/dL	14.45 (5.40-44.80)
Serum albumin, g/dL	37.75 (24.30-46.30)
INR	1.08 (0.95-1.41)
Serum creatinine, mg/dL	65.7 (45.4-134.6)
Serum AFP, ng/mL, baseline < 20	5.60 (1.12-24200.00) (n = 29)
Serum AFP, ng/mL, baseline > 20	916.2 (21.1-24200.0) (n = 17)

WBC: White blood cell; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International Normalized Ratio; AFP: α-fetoprotein.

radiotherapy, and sorafenib was performed on patients with disease progression. Two independent authors followed up all the clinical data and follow-up outcomes.

Statistical analysis

SPSS for Windows (SPSS Inc., Chicago, IL, United States) was used for analyzing data. The estimated local tumor progression and overall survival (OS) rates were compared by the Kaplan–Meier method. Cox proportional hazards model was used to fit survival time for each variable. $P < 0.05$ was considered to be a significant difference.

RESULTS

Treatment response

Figure 2 shows a representative condition after TACE and RFA combination treatment. As shown in Tables 1 and 2, there were no significant differences between laboratory results before and 3 d after treatment. After 2 years, OS was 18.43 ± 1.34 mo and progression-free survival (PFS) was 9.40 ± 1.31 mo; however, after 3 years, OS was 26.44 ± 2.26 mo and PFS was 10.21 ± 1.58 mo. Figure 3 shows the OS and PFS results.

Subset analysis showed similar OS and PFS (Table 3). Among these subsets, four groups showed different results, which were the vascular invasion group, non-vascular invasion group, male group, and female group (marked as A, B, C and D, respectively). OS in the A and D groups was shorter than in the B and C groups. The P values for B and C were 0.019 and 0.031, respectively.

Adverse effects and complications

Clinical adverse events included fever, pain, nausea, fatigue, transient reduction in blood counts and transient elevations of aspartate aminotransferase and alanine aminotransferase levels, but were mostly limited to grade 1 and 2 (Table 2). No severe complications associated with our treatment protocol were noted.

DISCUSSION

HCC is a leading cause of liver-disease-related mortality. Although rapid progress in treatment for large liver cancer has been made in the past few years, neither the prognosis nor postoperative outcomes are satisfactory.

According to a previous report, the combination of TACE and RFA has a synergistic effect on HCC inactivation[12]. The combination improves treatment efficacy, prolongs survival, and reduces recurrence rate. Thus, the combined treatment is superior to TACE or RFA alone[5,13]. So far, treatments are generally launched separately in practice. The time interval between the two modalities was 1 d to 4 wk. Because of the possible collateral formation and elimination of lipiodol chemotherapeutic agents after embolism, the effects of TACE or RFA alone are not

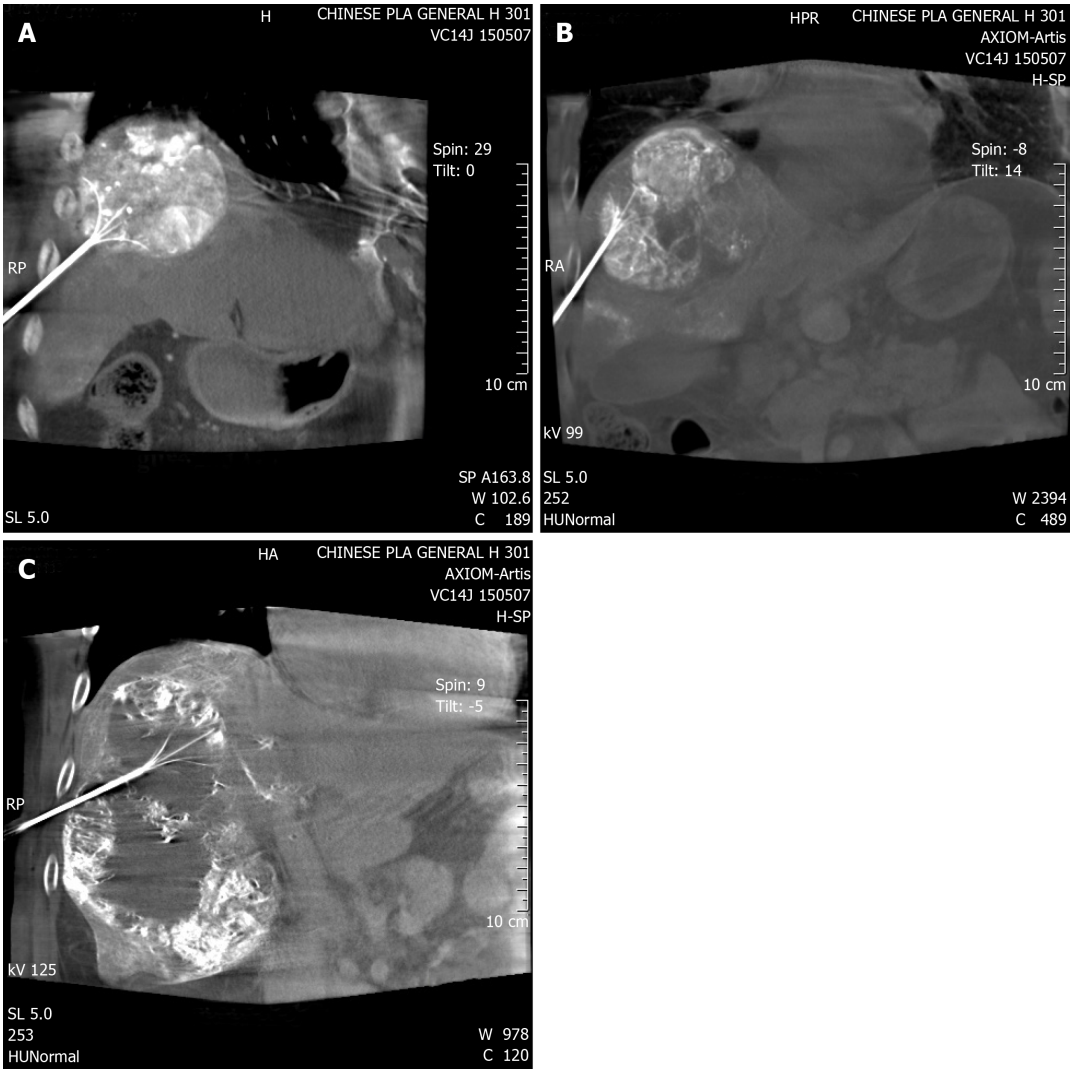

Figure 1 Cone-beam computed tomography image confirmed the position of the radiofrequency probe. A-C: Radiofrequency probe inserted at an angle to avoid lung damage.

synergistic[6,14,15]. Therefore, evaluating the effect of the combined treatment is necessary.

The present study involved 3-year follow-up of the efficacy of simultaneous TACE and RFA in patients with large HCC. This combination treatment may have the following advantages. First, DSA or CBCT can clearly show blood vessels. Both imaging modalities allow successful puncture of the liver and can verify treatment efficacy during the treatment in real time[16,17]. Second, during combination treatment, iodine oil precipitates around the lesions. Thus, it can be used as a heat-transmitting medium to improve ablation efficiency and make the surrounding HCC microenvironment inactive[18]. This can reduce tumor recurrence by improving ablation efficacy. Third, TACE can block blood flow into the tumor, thereby reducing heat loss during RFA[19]. Fourth, after TACE, ablation can be performed immediately, which may also localize damage such as liquefaction necrosis as well as coagulation sclerosis. Moreover, the immediate combination procedure is considered to reduce the side effects of TACE[20]. Finally, in one session of treatment, combination of TACE and RFA can be performed, which may reduce financial burden for the patient. In general, TACE with simultaneous RFA leads to synergistic effects of thermal ablation and chemotherapy. No significant adverse effects were observed in our study. In short, for the efficacy and survival of patients with large HCCs, TACE with simultaneous RFA may be a useful and novel tactic.

During our follow-up, the incidence of intrahepatic and extrahepatic metastases was higher in patients with vascular invasion than in those without vascular invasion. This indicates that tumor thrombus exhibits poor response to treatment. Multivariate Cox proportional hazard analysis also demonstrated that vascular invasion was an important prognostic factor. In addition, the male and female patients showed

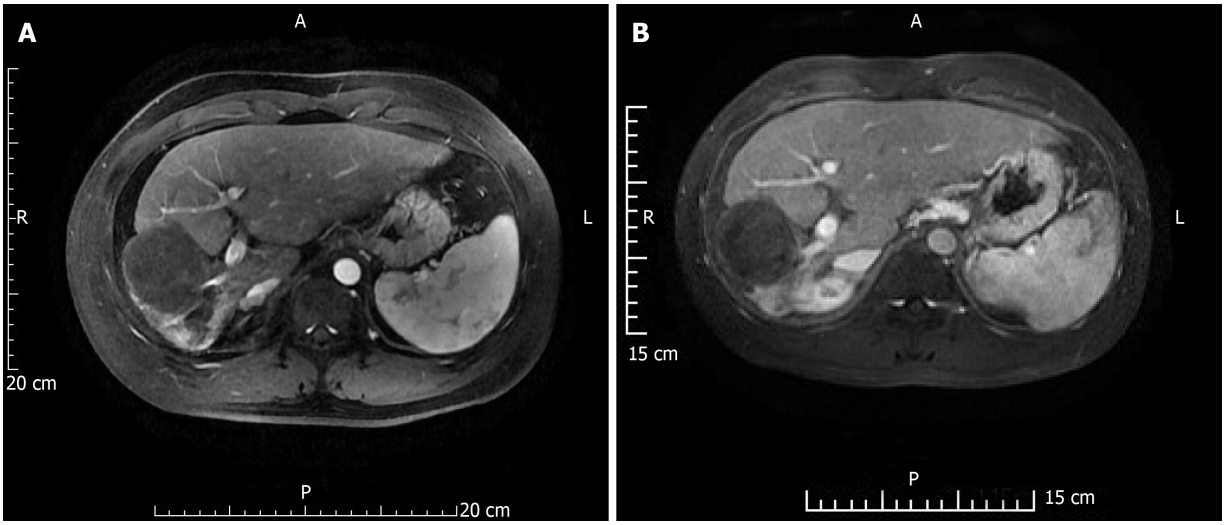

Figure 2 A 41-year-old male patient re-examined 2 and 4 years after combination therapy. A: Magnetic resonance imaging (MRI) at 2 years; B: MRI at 4 years. Hepatocellular carcinoma lesions showed pyknosis and necrosis.

significant differences in OS and PFS. The cause of the false-positive result may be the small size of the female group. The pseudocapsule group showed better treatment efficacy mainly because the pseudocapsule may enhance the thermal aggregation effect of ablation, resulting in greater tumor inactivation. However, it was not significant, possibly because of the small sample size. The other subgroups did not show significant differences.

The main limitation of our study was that it was retrospective. Thus, a multicenter prospective study, with a large sample size should be conducted to evaluate further the outcome of TACE and RFA combination treatment in patients with large HCCs.

In conclusion, these preliminary data show that the simultaneous TACE and RFA is a safe, effective and valuable strategy for large HCC, as it improves efficacy and prognosis.

Table 3 Multivariate analysis using Cox proportional hazard model, *n* (%)

Characteristics	*n*	Median OS (mo)	1-yr survival	2-yr survival	3-yr survival	*P* value
Sex						0.031
Female	4	10.5	2 (50.0)	1 (25.0)	0	
Male	42	34.5	31 (73.8)	27 (64.3)	23 (54.5)	
Age (yr)						0.264
≤ 60	37	34	26 (70.3)	22 (59.5)	20 (54.1)	
> 60	9	35	7 (77.8)	6 (66.7)	4 (44.4)	
BCLC staging						0.657
B	10	37.5	8 (80.0)	8 (80.0)	7 (70.0)	
C	36	30	25 (69.4)	20 (55.6)	17 (47.2)	
Size of tumor (cm)						0.300
8-10	23	34	18 (78.3)	14 (60.9)	12 (51.4)	
> 10	23	26	15 (65.2)	14 (60.9)	12 (52.2)	
No. of tumors						0.087
Single	35	34	26 (74.3)	23 (65.7)	20 (56.7)	
Multiple	11	19	7 (63.6)	5 (54.5)	4 (36.4)	
Child–Pugh class						0.640
A	45	34	32 (77.8)	28 (62.2)	24 (53.3)	
B	1	19	1 (100)	0	0	
Pseudocapsule						0.289
Yes	11	44	10 (90.9)	9 (81.8)	7 (63.6)	
No	35	30	23 (65.7)	19 (54.3)	17 (48.2)	
Vascular invasion						0.019
Yes	4	8	1 (25.0)	1 (25.0)	0	
No	42	34.5	32 (76.2)	27 (64.3)	24 (56.8)	
AFP positive[1]						0.051
Yes	17	14.5	9 (52.9)	6 (35.3)	6 (35.3)	
No	29	35	24 (79.3)	22 (75.9)	18 (61.4)	

[1]AFP ≥ 200 ng/mL. BCLC: Barcelona Clinic Liver Cancer; OS: Overall survival; AFP: α-fetoprotein.

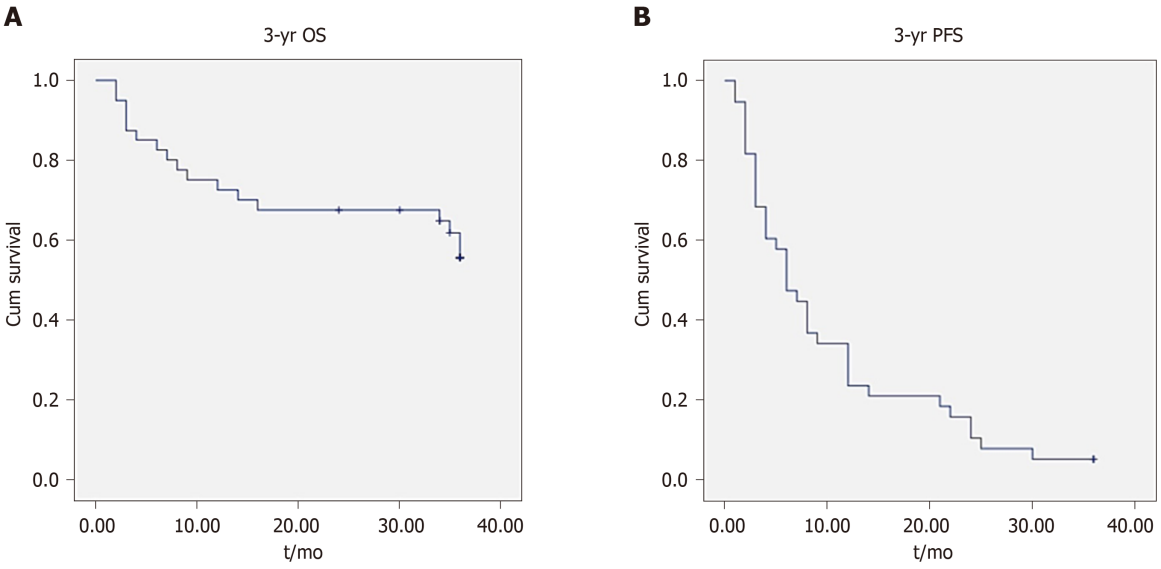

Figure 3 Kaplan–Meier analysis of overall survival and progression-free survival. Kaplan–Meier survival curves shown for patients with large hepatocellular carcinomas treated with combination therapy. A: 3-year overall survival; B: 3-year progression-free survival. OS: Overall survival; PFS: Progression-free survival.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is a common cancer and a leading cause of tumor-related death. Patients who have large HCC (≥ 8 cm) are at advanced stages and have poor prognosis. Transcatheter arterial chemoembolization (TACE) and radiofrequency ablation (RFA) is commonly used for patients with large HCC, however, both treatments has their own limitation. Recently study showed that combination of TACE and RFA may improve therapeutic efficacy, but how to combine these two treatment modalities is still a controversial topic.

Research motivation

The combination of TACE and RFA has a synergistic effect on HCC inactivation; however, most treatments are generally launched separately in practice, the effects of TACE or RFA alone are not synergistic very well. Therefore, evaluating the effect of the simultaneous combined treatment is necessary.

Research objectives

In the present study, we evaluated the efficacy and safety of simultaneous combined TACE and RFA for large HCC, to figure out how to combine these two treatment modalities.

Research methods

A retrospective study was conducted. From 2010 to 2013, 46 consecutive patients with large HCC were treated with simultaneous TACE and RFA. Thirty-five of 46 patients had a single tumor. Progression-free survival (PFS) and overall survival (OS) were analyzed at 2 years and 3 years, respectively.

Research results

Forty-six patients treated by simultaneous TACE and RFA had no significant complications and treatment was successful. After 3 years, median PFS and OS were 10.21 ± 1.58 and 26.44 ± 2.26 mo, retrospectively. The survival rate was 67.5% after 2 years and 55.67% after 3 years.

Research conclusions

These preliminary data show that simultaneous TACE and RFA are safe and effective for large HCC.

Research perspectives

With the simultaneous combination of TACE and RFA, it is expected to bring us a better treatment for large HCC.

REFERENCES

1 **European Association For The Study Of The Liver**. European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

2 **Wang FS**, Fan JG, Zhang Z, Gao B, Wang HY. The global burden of liver disease: the major impact of China. *Hepatology* 2014; **60**: 2099-2108 [PMID: 25164003 DOI: 10.1002/hep.27406]

3 **Alnaggar M**, Niu L, Li J, Yao F, Wang Y, Zeng J, Ye J, Chen J, Mu F, Xu K. Cryoprotective therapy for huge hepatocellular carcinoma: a study of 14 patients with a single lesion. *Cryobiology* 2014; **69**: 457-461 [PMID: 25445461 DOI: 10.1016/j.cryobiol.2014.10.004]

4 **Xue TC**, Jia QA, Ge NL, Chen Y, Zhang BH, Ye SL. Imbalance in systemic inflammation and immune response following transarterial chemoembolization potentially increases metastatic risk in huge hepatocellular carcinoma. *Tumour Biol* 2015; **36**: 8797-8803 [PMID: 26058874 DOI: 10.1007/s13277-015-3632-7]

5 **Kim JW**, Shin SS, Kim JK, Choi SK, Heo SH, Lim HS, Hur YH, Cho CK, Jeong YY, Kang HK. Radiofrequency ablation combined with transcatheter arterial chemoembolization for the treatment of single hepatocellular carcinoma of 2 to 5 cm in diameter: comparison with surgical resection. *Korean J Radiol* 2013; **14**: 626-635 [PMID: 23901320 DOI: 10.3348/kjr.2013.14.4.626]

6 **Min JH**, Lee MW, Cha DI, Jeon YH, Shin SW, Cho SK, Rhim H, Lim HK. Radiofrequency ablation combined with chemoembolization for intermediate-sized (3-5 cm) hepatocellular carcinomas under dual guidance of biplane fluoroscopy and ultrasonography. *Korean J Radiol* 2013; **14**: 248-258 [PMID: 23483753 DOI: 10.3348/kjr.2013.14.2.248]

7 **Song MJ**, Bae SH, Lee JS, Lee SW, Song DS, You CR, Choi JY, Yoon SK. Combination transarterial chemoembolization and radiofrequency ablation therapy for early hepatocellular carcinoma. *Korean J Intern Med* 2016; **31**: 242-252 [PMID: 26874512 DOI: 10.3904/kjim.2015.112]

8 **Guo W**, He X, Li Z, Li Y. Combination of Transarterial Chemoembolization (TACE) and Radiofrequency Ablation (RFA) vs. Surgical Resection (SR) on Survival Outcome of Early Hepatocellular Carcinoma: A Meta-Analysis. *Hepatogastroenterology* 2015; **62**: 710-714 [PMID: 26897959]

9 **Dai WC**, Cheung TT, Chok KS, Chan AC, Sharr WW, Tsang SH, Yuen WK, Chan SC, Fan ST, Lo CM, Poon RT. Radiofrequency ablation versus transarterial chemoembolization for unresectable solitary hepatocellular carcinomas sized 5-8 cm. *HPB (Oxford)* 2015; **17**: 226-231 [PMID: 25284590 DOI: 10.1111/hpb.12324]

10 **Duan F**, Yu W, Wang Y, Liu FY, Song P, Wang ZJ, Yan JY, Yuan K, Wang MQ. Trans-arterial chemoembolization and external beam radiation therapy for treatment of hepatocellular carcinoma with a tumor thrombus in the inferior vena cava and right atrium. *Cancer Imaging* 2015; **15**: 7 [PMID: 26007646 DOI: 10.1186/s40644-015-0043-3]

11 **Wang ZJ**, Wang MQ, Duan F, Song P, Liu FY, Chang ZF, Wang Y, Yan JY, Li K. Transcatheter arterial chemoembolization followed by immediate radiofrequency ablation for large solitary hepatocellular carcinomas. *World J Gastroenterol* 2013; **19**: 4192-4199 [PMID: 23864783 DOI: 10.3748/wjg.v19.i26.4192]

12 **Ginsburg M**, Zivin SP, Wroblewski K, Doshi T, Vasnani RJ, Van Ha TG. Comparison of combination therapies in the management of hepatocellular carcinoma: transarterial chemoembolization with radiofrequency ablation versus microwave ablation. *J Vasc Interv Radiol* 2015; **26**: 330-341 [PMID: 25534635 DOI: 10.1016/j.jvir.2014.10.047]

13 **Hou YF**, Wei YG, Yang JY, Wen TF, Xu MQ, Yan LN, Li B. Combined hepatectomy and radiofrequency ablation versus TACE in improving survival of patients with unresectable BCLC stage B HCC. *Hepatobiliary Pancreat Dis Int* 2016; **15**: 378-385 [PMID: 27498577 DOI: 10.1016/S1499-3872(16)60089-9]

14 **Choe WH**, Kim YJ, Park HS, Park SW, Kim JH, Kwon SY. Short-term interval combined chemoembolization and radiofrequency ablation for hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 12588-12594 [PMID: 25253962 DOI: 10.3748/wjg.v20.i35.12588]

15 **Gadaleta C**, Catino A, Ranieri G, Fazio V, Gadaleta-Caldarola G, Cramarossa A, Armenise F, Canniello E, Vinciarelli G, Laricchia G, Mattioli V. Single-step therapy -- feasibility and safety of simultaneous transarterial chemoembolization and radiofrequency ablation for hepatic malignancies. *In Vivo* 2009; **23**: 813-820 [PMID: 19779117]

16 **Shibata T**, Shibata T, Maetani Y, Kubo T, Itoh K, Togashi K, Hiraoka M. Transthoracic percutaneous radiofrequency ablation for liver tumors in the hepatic dome. *J Vasc Interv Radiol* 2004; **15**: 1323-1327 [PMID: 15525754 DOI: 10.1097/01.RVI.0000132297.97113.C4]

17 **Kato T**, Yamagami T, Hirota T, Matsumoto T, Yoshimatsu R, Nishimura T. Transpulmonary radiofrequency ablation for hepatocellular carcinoma under real-time computed tomography-fluoroscopic guidance. *Hepatogastroenterology* 2008; **55**: 1450-1453 [PMID: 18795709]

18 **Tamai T**, Oshige A, Tabu K, Tabu E, Ijyuin S, Sakae H, Onishi H, Muromachi K, Saisyoji A, Oda K, Kumagai K, Mawatari S, Moriuchi A, Sakurai K, Hori T, Ido A. Utility of percutaneous radiofrequency ablation alone or combined with transarterial chemoembolization for early hepatocellular carcinoma. *Oncol Lett* 2017; **14**: 3199-3206 [PMID: 28927066 DOI: 10.3892/ol.2017.6476]

19 **Bholee AK**, Peng K, Zhou Z, Chen J, Xu L, Zhang Y, Chen M. Radiofrequency ablation combined with transarterial chemoembolization versus hepatectomy for patients with hepatocellular carcinoma within Milan criteria: a retrospective case-control study. *Clin Transl Oncol* 2017; **19**: 844-852 [PMID: 28070766 DOI: 10.1007/s12094-016-1611-0]

20 **Chen L**, Sun J, Yang X. Radiofrequency ablation-combined multimodel therapies for hepatocellular carcinoma: Current status. *Cancer Lett* 2016; **370**: 78-84 [PMID: 26472630 DOI: 10.1016/j.canlet.2015.09.020]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 101-112

DOI: 10.4251/wjgo.v12.i1.101

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Adenosquamous carcinoma may have an inferior prognosis to signet ring cell carcinoma in patients with stages I and II gastric cancer

Yu-Xin Chu, Hong-Yun Gong, Qin-Yong Hu, Qi-Bin Song

ORCID number: Yu-Xin Chu (0000-0001-5526-997X); Hong-Yun Gong (0000-0001-7465-7820); Qin-Yong Hu (0000-0002-0764-3792); Qi-Bin Song (0000-0003-2387-6438).

Author contributions: Chu YX drafted the manuscript; Gong HY performed data extraction; Hu QY performed statistical analysis; and Song QB supervised the study.

Supported by the National Natural Science Foundation of China, No. 81670123 and No. 81670144.

Institutional review board statement: This study was approved by the Institutional Review Board of Wuhan University Renmin Hospital.

Informed consent statement: The patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: We have no financial relationships to disclose.

Yu-Xin Chu, Hong-Yun Gong, Qin-Yong Hu, Qi-Bin Song, Department of Oncology (Division I), Cancer Center, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China

Corresponding author: Qinyong Hu, MD, Academic Research, Department of Oncology I, Cancer Center, Renmin Hospital of Wuhan University, Jiefang Road No. 238, Wuhan 430060, Hubei Province, China. rm001223@whu.edu.cn

Abstract

BACKGROUND

Primary gastric adenosquamous carcinoma (ASC) is an exceedingly rare histological subtype. Gastric signet ring cell carcinoma (SRC) is a unique subtype with distinct tumor biology and clinical features. The prognosis of gastric ASC *vs* SRC has not been well established to date. We hypothesized that further knowledge about these distinct cancers would improve the clinical management of such patients.

AIM

To investigate the clinicopathological characteristics and prognosis of gastric ASC *vs* SRC.

METHODS

A cohort of gastric cancer patients was retrospectively collected from the Surveillance, epidemiology, and end results program database. The 1:4 propensity score matching was performed among this cohort. The clinicopathological features and prognosis of gastric ASC were compared with gastric SRC by descriptive statistics. Kaplan-Meier method was utilized to calculate the median survival of the two groups of patients. Cox proportional hazard regression models were used to identify prognostic factors.

RESULTS

Totally 6063 patients with gastric ASC or SRC were identified. A cohort of 465 patients was recruited to the matched population, including 370 patients with SRC and 95 patients with ASC. Gastric ASC showed an inferior prognosis to SRC after propensity score matching. In the post-matching cohort, the median cancer specific survival was 13.0 (9.7-16.3) mo in the ASC group *vs* 20.0 (15.7-24.3) mo in the SRC group, and the median overall survival had a similar trend ($P < 0.05$). ASC and higher tumor-node-metastasis stage were independently associated

Received: August 30, 2019
Peer-review started: August 30, 2019
First decision: October 14, 2019
Revised: October 25, 2019
Accepted: October 31, 2019
Article in press: October 31, 2019
Published online: January 15, 2020

P-Reviewer: Imaeda H, Koch TR
S-Editor: Zhang L
L-Editor: Wang TQ
E-Editor: Qi LL

with a poor survival, while radiotherapy and surgery were independent protective factors for improved prognosis. Subgroup survival analysis revealed that the prognosis of ASC was inferior to SRC only in stages I and II patients.

CONCLUSION
ASC may have an inferior prognosis to SRC in patients with stages I and II gastric cancer. Our study supports radiotherapy and surgery for the future management of this clinically rare entity.

Key words: Adenosquamous carcinoma; Signet ring cell carcinoma; Surveillance, Epidemiology, and End results; Propensity score matching; Prognosis; Survival

Core tip: The prognosis of gastric adenosquamous carcinoma (ASC) *vs* signet ring cell carcinoma (SRC) has not been well established to date. Our study used both propensity score matching method and multivariate Cox regression analysis to adjust the potential bias caused by the imbalanced distribution of confounding factors. We found that ASC may have an inferior prognosis to SRC in patients with stages I and II gastric cancer. Radiotherapy and surgery were proved to be independent protective factors for improving their prognosis.

Citation: Chu YX, Gong HY, Hu QY, Song QB. Adenosquamous carcinoma may have an inferior prognosis to signet ring cell carcinoma in patients with stages I and II gastric cancer. *World J Gastrointest Oncol* 2020; 12(1): 101-112
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/101.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.101

INTRODUCTION

Gastric cancer (GC) is still the second leading cause of cancer-related mortality worldwide[1]. It is also the fifth most frequently diagnosed cancer and was responsible for over 1000000 new cases in 2018 and an estimated 783000 deaths globally[2]. GC has increasingly been recognized as a heterogeneous disease, each histologic subtype of GC differs in its biology, especially in its metabolic profiles[3], so histology is very important in individualized evaluation of patients with GC. Among the various histologic types of GC, signet ring cell carcinoma (SRC) is a unique subtype with distinct tumor biology and clinical features, so it should be analyzed separately[4]. By contrast, adenosquamous carcinoma (ASC) in GC is relatively rare. ASC accounts for only 0.2%-0.4% of all gastric carcinomas[5]. According to the World Health Organization international histological classification of tumors, SRC is defined as a tumor with only intracellular mucin pools[6]. Comparatively, the diagnosis of ASC requires coexistence of both adenocarcinoma and squamous cell carcinoma in the primary tumor, and squamous component should exceed 25% of the primary tumor[7].

Previous studies revealed that primary gastric ASC exhibited early tumor progression and a worse prognosis than some typical gastric carcinomas[8]. There have been two major proposed mechanisms to explain the poor prognosis of ASC in GC. First, this rare subtype may have more extensive tumor depth and higher frequencies of lymphatic and vascular permeations of the carcinoma cells[9]. Second, adenocarcinoma predominate histology may be associated with a higher risk of metastatic disease compared to squamous carcinoma predominate histology[10]. Due to the rare incidence, most of the literature about gastric ASC is described in case reports. The study on gastric ASC with large series is still lacking. The prognosis of ASC *vs* SRC has not been well established to date. Actually, a variety of issues about gastric ASC are still unresolved.

In this study, we utilized the Surveillance, Epidemiology, and End Results (SEER) database to extract a large cohort of patients to investigate the survival differences between ASC and SRC. Cancer-specific survival (CSS) and overall survival (OS) were comprehensively compared between the two groups of patients. We sought to clarify the clinicopathological characteristics and prognosis of gastric ASC *vs* SRC based on a large population analysis. Our study may intensify the current knowledge about these

tumors and provide additional guidance for their management.

MATERIALS AND METHODS

Patient selection

All the data in this study were extracted from SEER 18 registries Custom Data (with additional treatment fields). The SEER database comprises 18 cancer registries and covers approximately 30% of the United States population. The patients were selected using SEER Stat version 8.3.5 software directly. The patient information in SEER database is completely de-identified and publicly available, so this study was exempt from ethical approval from human study subcommittee. We initiated the following inclusion criteria to select eligible patients: (1) All patients were diagnosed from 2004 to 2015; (2) Primary site was the stomach; (3) Behavior recode for analysis was malignant; (4) Primary gastric cancer was the first or only cancer diagnosis; (5) Histological types were confined only to SRC (ICD-03, 8490/3) and ASC (ICD-03, 8560/3); and (6) The follow-up data were complete. The diagnosis was not gained from any death certificate or autopsy. Those patients with unknown information about table variables were excluded.

Data collection

The following variables were extracted for each patient: Age at diagnosis, gender, race, marital status, tumor size, tumor-node-metastasis (TNM) stage, tumor depth, LN metastasis, distant metastasis, radiation, surgery, histological type, survival months, CSS, and OS. CSS was defined as the time from the date of diagnosis to the date of death caused by gastric cancer. OS was defined as the duration from diagnosis to death from any cause. In our study, CSS was the primary endpoint, and OS was the secondary endpoint.

Statistical analysis

The patients were divided into patients with gastric SRC *vs* those with ASC. Given that the two cohorts dichotomized above were not randomized, unbalanced variables might engender selection bias, so we utilized a 1:4 propensity-score matching (PSM) method to control the non-random assignment of patients. A logistic regression model that predicts the likelihood of being assigned to ASC was constructed and set as the propensity score. The propensity scores were calculated according to unbalanced covariates. The PSM adopted nearest-neighbor matching algorithm. The caliper width was 0.01. No replacement was allowed, and all patients were matched only once. The baseline characteristics were compared in both matched and unmatched cohorts by chi-square tests. The survival curves of each histologic group were compared by Kaplan-Meier plots with log-rank test. Univariable and multivariable Cox proportional hazard regression models were used to identify prognostic factors in the post-matching cohort. Variables with $P < 0.05$ in univariate analysis were further adjusted through multivariate analysis. PSM was conducted with R version 3.5.3. Statistical analyses were completed with SPSS statistical software, version 25.0 (SPSS, Chicago, IL, United States). A two-tailed P value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics before PSM

Preliminarily, 10646 patients with gastric ASC or SRC were collected, but 4583 cases were excluded because of any missing data or unknown of table variables. Finally, a total of 6063 eligible patients were included in this study. Among the unmatched cohort, 5968 (98.4%) patients had SRC and 95 (1.6%) patients had ASC. The distributions of age, gender, race, marital status, LN metastasis, and radiation were significantly different between the two groups ($P < 0.05$). Compared with those SRC patients, the ASC patients were more likely to have age > 60 years old (66.3% *vs* 52.7%), be male (74.7% *vs* 52.7%) while less female (25.3% *vs* 47.3%), had a relatively higher proportion of white population (77.9% *vs* 69.5%), and be married (77.9% *vs* 61.7%). As for LN metastasis, the ASC patients showed more N1 (48.4% *vs* 34.9%) and N2 (16.8% *vs* 15.5%). With respect to radiation, more ASC patients received radiotherapy (35.8% *vs* 23.7%). No differences were observed in terms of tumor size, TNM stage, tumor depth, distant metastasis, or surgery ($P > 0.05$). The patients' characteristics before PSM are summarized in Table 1.

Table 1 Patient characteristics dichotomized by histological type before propensity score matching, *n* (%)

Characteristic	SRC *n* = 5968 (98.4)	ASC *n* = 95 (1.6)	Total *n* = 6063 (100)	*P* value
Age (yr)				0.008
≤ 60	2823 (47.3)	32 (33.7)	2855 (47.1)	
> 60	3145 (52.7)	63 (66.3)	3208 (52.9)	
Gender				< 0.001
Male	3146 (52.7)	71 (74.7)	3217 (53.1)	
Female	2822 (47.3)	24 (25.3)	2846 (46.9)	
Race				0.045
White	4147 (69.5)	74 (77.9)	4221 (69.6)	
Black	725 (12.1)	13 (13.7)	738 (12.2)	
Others	1096 (18.4)	8 (8.4)	1104 (18.2)	
Marital status				0.001
Not married	2287 (38.3)	21 (22.1)	2308 (38.1)	
Married	3681 (61.7)	74 (77.9)	3755 (61.9)	
Tumor size (mm)				0.220
≤ 50	2271 (38.1)	42 (44.2)	2313 (38.1)	
> 50	3697 (61.9)	53 (55.8)	3750 (61.9)	
TNM Stage				0.299
I	1595 (26.7)	21 (22.1)	1616 (26.7)	
II	888 (14.9)	20 (21.1)	908 (15.0)	
III	1129 (18.9)	15 (15.8)	1144 (18.9)	
IV	2356 (39.5)	39 (41.1)	2395 (39.5)	
Tumor depth				0.139
T1	1398 (23.4)	16 (16.8)	1414 (23.3)	
T2	2177 (36.5)	45 (47.4)	2222 (36.6)	
T3	1384 (23.2)	18 (18.9)	1402 (23.1)	
T4	1009 (16.9)	16 (16.8)	1025 (16.9)	
LN metastasis				0.011
N0	2472 (41.4)	31 (32.6)	2503 (41.3)	
N1	2080 (34.9)	46 (48.4)	2126 (35.1)	
N2	926 (15.5)	16 (16.8)	942 (15.5)	
N3	490 (8.2)	2 (2.1)	492 (8.1)	
Distant metastasis				0.966
No	4197 (70.3)	67 (70.5)	4264 (70.3)	
Yes	1771 (29.7)	28 (29.5)	1799 (29.7)	
Radiotherapy				0.006
No	4553 (76.3)	61 (64.2)	4614 (76.1)	
Yes	1415 (23.7)	34 (35.8)	1449 (23.9)	
Surgery				0.231
No	2047 (34.3)	27 (28.4)	2074 (34.2)	
Yes	3921 (65.7)	68 (71.6)	3989 (65.8)	

SRC: Signet ring cell carcinoma; ASC: Adenosquamous carcinoma; TNM: Tumor-node-metastasis; LN: Lymph node.

Patient characteristics after PSM

A 1:4 PSM was initiated. The logit of propensity score for histological type was derived from other covariates. Totally 465 patients were matched, including 95 ASC patients and 370 SRC patients. After the PSM, all covariates were well balanced with no significant differences between the two groups ($P > 0.05$). The patients' characteristics categorized by histology after PSM are displayed in Table 2.

Table 2 Patient characteristics dichotomized by histological type after propensity score matching, *n* (%)

Characteristic	SRC	ASC	Total	*P* value
	n = 370 (79.6)	*n* = 95 (20.4)	*n* = 465 (100)	
Age (yr)				0.754
≤ 60	131 (35.4)	32 (33.7)	163 (35.1)	
> 60	239 (64.6)	63 (66.3)	302 (64.9)	
Gender				0.809
Male	272 (73.5)	71 (74.7)	343 (73.8)	
Female	98 (26.5)	24 (25.3)	122 (26.2)	
Race				0.845
White	290 (78.4)	74 (77.9)	364 (78.3)	
Black	44 (11.9)	13 (13.7)	57 (12.3)	
Others	36 (9.7)	8 (8.4)	44 (9.5)	
Marital status				0.901
Not married	84 (22.7)	21 (22.1)	105 (22.6)	
Married	286 (77.3)	74 (77.9)	360 (77.4)	
Tumor size (mm)				0.828
≤ 50	159 (43.0)	42 (44.2)	201 (43.2)	
> 50	211 (57.0)	53 (55.8)	264 (56.8)	
Stage				0.365
I	94 (25.4)	21 (22.1)	115 (24.7)	
II	55 (14.9)	20 (21.1)	75 (16.1)	
III	77 (20.8)	15 (15.8)	92 (19.8)	
IV	144 (38.9)	39 (41.1)	183 (39.4)	
Tumor depth				0.598
T1	77 (20.8)	16 (16.8)	93 (20.0)	
T2	156 (42.2)	45 (47.4)	201 (43.2)	
T3	84 (22.7)	18 (18.9)	102 (21.9)	
T4	53 (14.3)	16 (16.8)	69 (14.8)	
LN metastasis				0.151
N0	151 (40.8)	31 (32.6)	182 (39.1)	
N1	134 (36.2)	46 (48.4)	180 (38.7)	
N2	69 (18.6)	16 (16.8)	85 (18.3)	
N3	16 (4.3)	2 (2.1)	18 (3.9)	
Distant metastasis				0.840
No	257 (69.5)	67 (70.5)	324 (69.7)	
Yes	113 (30.5)	28 (29.5)	141 (30.3)	
Radiotherapy				0.786
No	232 (62.7)	61 (64.2)	293 (63.0)	
Yes	138 (37.3)	34 (35.8)	172 (37.0)	
Surgery				0.883
No	108 (29.2)	27 (28.4)	135 (29.0)	
Yes	262 (70.8)	68 (71.6)	330 (71.0)	

SRC: Signet ring cell carcinoma; ASC: Adenosquamous carcinoma; TNM: Tumor-node-metastasis; LN: Lymph node.

Comparison of the prognosis between gastric SRC and ASC before PSM

As for the 6063 patients finally enrolled, 4560 patients were dead at the end of the last follow-up. Moreover, 4160 patients were dead from gastric cancer specifically. The prognosis of gastric SRC *vs* ASC before PSM was compared. The Kaplan-Meier plots showed that the prognosis of SRC was comparable to that of ASC in both CSS and OS curves (Figure 1, $P > 0.05$). The median CSS of the SRC group was 16.0 (15.2-16.8) mo, while that of the ASC group was 13.0 (9.7-16.3) mo ($P = 0.101$; Table 3). Similarly, the

median OS of the SRC group was not significantly different from that of the ASC group (P = 0.084; Table 3). Hence, the results indicated the prognosis was not statistically different between gastric SRC and ASC before PSM.

Comparison of the prognosis in matched groups

We initiated a 1:4 (ASC:SRC) matched case-control analysis by PSM, in order to adjust the baseline characteristic differences between the two groups. The PSM analysis resulted in a balanced cohort including the ASC group (n = 95) and the SRC group (n = 370). As for the cohort after PSM, statistically significant differences appeared in both CSS and OS, dejecting the ASC group compared with the SRC group (P < 0.05 for both endpoints; Figure 2). Furthermore, the median CSS was 13.0 (9.7-16.3) mo in ASC *vs* 20.0 (15.7-24.3) mo in SRC group (P = 0.027; Table 3). In parallel, the median OS of the ASC group was also inferior to that of the SRC group (Table 3, P = 0.017). The survival curves of CSS and OS after PSM are exhibited in Figure 2. Obviously, the ASC patients had an inferior prognosis to SRC patients in matched groups.

Identify predictors of survival

The Cox proportional hazard models were constructed to evaluate the impact of clinicopathological factors on CSS of the post-matching cohort (Table 4). In univariate analysis, the variables significantly associated with CSS were histological type, marital status, tumor size, TNM stage, tumor depth, distant metastasis, radiation, and surgery (P < 0.05). ASC was found to be a risk factor for poor prognosis [hazard ratio (HR) = 1.343, 95%CI = 1.029-1.752, P < 0.05]. All the significant variables mentioned above were subsequently included to the multivariate Cox regression analysis. Multivariable analysis confirmed some of the prognostic factors identified in univariate analysis. After adjusting for other confounding predictors, histological type and TNM stage were proved to be independent risk factors for poor survival (HR > 1, P < 0.05), while radiotherapy and surgery were independent protective factors for favorable prognosis (HR < 1, P < 0.05). Anyway, ASC was still associated with an inferior prognosis to SRC (HR = 1.316, 95%CI = 1.004-1.726, P < 0.05). The detailed results are available in Table 4.

Subgroup survival analysis

Given that TNM stage is also independently associated with the patients' survival after PSM, we performed a subgroup analysis to highlight the impact of histological type on the prognosis of patients. The Kaplan-Meier plots revealed that the CSS of gastric ASC was worse than that of gastric SRC in both stages I (P < 0.001) and II (P < 0.05) patients. However, no significant survival difference was found for ASC *vs* SRC in either stage III or IV (P > 0.05). Thus, the prognosis of ASC was inferior to SRC only in stages I and II patients. The survival curves of CSS stratified by TNM stage are illustrated in Figure 3.

DISCUSSION

Primary gastric ASC is an extremely rare subtype[11]. The clinicopathological characteristics and prognosis of gastric ASC are still poorly understood. Based on a large cohort from the SEER database, we utilized PSM analysis to evaluate the prognosis of ASC *vs* SRC for patients with gastric cancer. Moreover, we also used Cox proportional hazard regression models to identify prognostic factors for the post-matching population. The overall results suggest that ASC had an inferior survival to SRC in patients with gastric cancer. ASC and higher TNM stages were independently associated with a poor prognosis.

The clinicopathological features and prognosis of gastric ASC have been reviewed by several previous studies. Based on the National Cancer Database analysis, a recent original research has reported the clinical features and outcomes of gastric squamous cell carcinoma (SCC) and ASC. They collected 61215 patients with primary gastric cancer. ASC only accounted for 0.5%. The median OS was 9.9 mo in ASC *vs* 13.2 mo in adenocarcinoma. On multivariate analysis, ASC histology was still associated with a worse survival compared to adenocarcinoma[12]. Furthermore, another study reported the clinical features and outcomes of 167 gastric ASC cases. Only 109 cases with R0 resection were recruited in survival analysis. Their results revealed that the median OS time was 17 mo for patients with gastric ASC receiving R0 resection. They also found that the prognosis of gastric ASC was significantly poorer than that of gastric adenocarcinoma[13]. Quan *et al*[14] also reported that the median OS of gastric ASC was 12 mo, and 87.5% of the patients survived for less than 24 mo after diagnosis. In our present study, we compared the survival outcomes of gastric ASC with SRC. As for our matched cohort, the median OS was 12.0 (9.5-14.5) mo in ASC *vs* 19.0 (14.9-23.1)

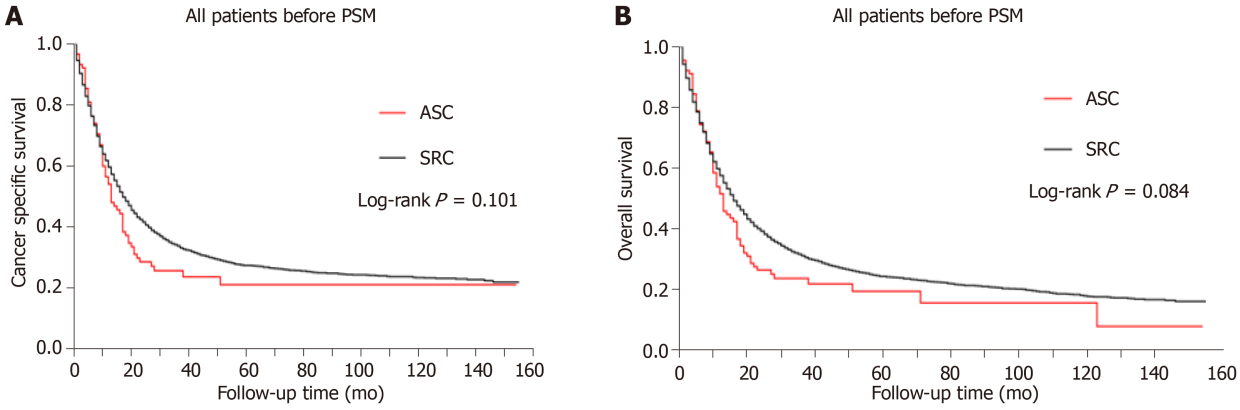

Figure 1 Kaplan-Meier survival curves by histology before propensity score matching. A: Cancer-specific Survival ($P > 0.05$); B: Overall Survival ($P > 0.05$). ASC: Adenosquamous carcinoma; SRC: Signet ring cell carcinoma.

mo in SRC group. In parallel, the median CSS of ASC was also significantly worse than that of SRC. Consistently, the prognosis of ASC was inferior to that of SRC after PSM analysis.

When it comes to the prognostic factors for gastric ASC, we found that the histological type ASC and higher TNM stage were independent risk factors for poor survival (HR > 1, $P < 0.05$), while radiotherapy (HR = 0.587; 95%CI: 0.444-0.776, $P < 0.001$) and surgery were independent protective factors for favorable prognosis (HR < 1, $P < 0.05$). So far, surgery remains the optimal treatment for gastric cancer without distant metastasis[15]. So the survival advantage of gastrectomy has been further confirmed by our study. Additionally, radiotherapy has been reported to be an effective adjuvant treatment for improving the OS in patients with gastric cancer after resection[16]. Considering that squamous cell carcinoma is generally sensitive to radiation therapy, the squamous component of gastric ASC may specifically benefit from radiotherapy[17]. Therefore, our study has provided evidence to support radiotherapy for patients with gastric ASC.

In addition to histological type, other confounders such as tumor TNM stage may also account for the potentially important survival differences. In order to further adjust the confounding factors, we performed subgroup survival analysis by TNM stage. Our results revealed that the CSS of gastric ASC was significantly worse than that of SRC in stages I and II patients, whereas no significant survival difference was found for stages III and IV patients. A recent study revealed that half of gastric ASC cases were diagnosed at advanced stages, and most patients had lymph node metastasis[18]. These results suggest that gastric ASC has an aggressive clinical course compared with conventional gastric cancer. The prognosis of stages I and II ASC patients should be concerned.

In terms of the prognosis for patients with gastric SRC, a recent review has indicated that early SRC had a better clinical outcome, but advanced SRC was generally considered to have a worse prognosis. Therapeutic strategies are still controversial for these patients[19]. Consistently, our study also revealed that stages I and II SRC patients had better survival curves than ASC patients. Their median CSS was 20.0 (15.7-24.3) mo, and median OS was 19.0 (14.9-23.1) mo. Our Cox proportional hazard regression models identified radiotherapy and surgery as independent protective factors for improving their prognosis (HR < 1, $P < 0.05$). Hence, our results may improve the therapeutic recommendations for these patients.

There are several limitations in our study. First, the retrospective nature of the current study could not exclude the possibility of selection bias. Although we could balance known covariates by PSM analysis, there may be unmeasured confounders not addressed in propensity matching. Hence, the results of our study should be interpreted cautiously. Second, the constituent ratio of adenocarcinoma and SCC components varied among different primary tumors. The prognostic value of constituent ratio on the survival of gastric ASC could not be evaluated. Third, there were limited data about cancer recurrence and subsequent involved sites in SEER database, so the patterns of recurrence and corresponding impact on the prognosis of patients remain unclear. In spite of the limitations stated above, SEER registry data usually have high completeness and are representative of the real-world patient population. Thus, the results of our study are still considerably convincing.

The major strength of our study is that we used both PSM method and multivariate Cox regression analysis to adjust the potential bias caused by the imbalanced

Table 3 Comparison of median survival of the patients before and after propensity score matching

	Patients, *n*	Median CSS 95%CI, mo	Median OS 95%CI, mo
Before PSM			
SRC	5968	16.0 (15.2-16.8)	15.0 (14.3-15.7)
ASC	95	13.0 (9.7-16.3)	12.0 (9.5-14.5)
P value		0.101	0.084
After PSM			
SRC	370	20.0 (15.7-24.3)	19.0 (14.9-23.1)
ASC	95	13.0 (9.7-16.3)	12.0 (9.5-14.5)
P value		0.027	0.017

CSS: Cancer-specific survival; OS: Overall survival; PSM: Propensity-score matching; SRC: Signet ring cell carcinoma; ASC: Adenosquamous carcinoma.

distribution of confounding factors. This doubly robust estimation combines two approaches to evaluate the causal effect of exposures on outcomes, which will encourage researchers to more fully interpret their findings on both scales[20].

In summary, gastric ASC differs significantly from gastric SRC in terms of clinicopathological characteristics. ASC may have an inferior prognosis to SRC in patients with stages I and II gastric cancer, so greater attention should be paid to these patients. Histological type ASC and higher TNM stage are associated a poor survival, but radiotherapy and surgery are independent protective factors for improving their prognosis. Our study supports radiotherapy and surgery for the future management of this clinically rare entity.

Table 4 Cox regression analysis of cancer-specific survival (*n* = 465)

Characteristic	Univariate Cox		Multivariate Cox	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Histological type				
SRC	Reference		Reference	
ASC	1.343 (1.029-1.752)	0.030	1.316 (1.004-1.726)	0.047
Age (yr)				
≤ 60	Reference		NI	
> 60	1.027 (0.819-1.288)	0.815		
Gender				
Male	Reference		NI	
Female	0.952 (0.740-1.223)	0.698		
Race				
White	Reference		NI	
Black	1.199 (0.870-1.652)	0.268		
Others	0.883 (0.597-1.304)	0.531		
Marital status				
Not married	Reference		Reference	
Married	0.768 (0.593-0.994)	0.045	0.709 (0.540-0.932)	0.014
Tumor size (mm)				
≤ 50	Reference		Reference	
> 50	1.994 (1.587-2.503)	<0.001	1.217 (0.947-1.564)	0.125
Stage				
I	Reference		Reference	
II	1.482 (0.993-2.212)	0.054	1.564 (1.021-2.394)	0.040
III	2.472 (1.714-3.564)	< 0.001	2.460 (1.601-3.780)	< 0.001
IV	5.179 (3.739-7.175)	< 0.001	2.884 (1.665-4.997)	< 0.001
Tumor depth				
T1	Reference		Reference	
T2	0.962(0.705-1.312)	0.805	1.296 (0.923-1.821)	0.135
T3	1.478(1.051-2.077)	0.025	1.482 (0.986-2.228)	0.058
T4	1.801 (1.243-2.609)	0.002	1.070 (0.699-1.638)	0.757
LN metastasis				
N0	Reference		NI	
N1	0.978(0.760-1.258)	0.863		
N2	1.218 (0.901-1.647)	0.200		
N3	1.642 (0.985-2.737)	0.057		
Distant metastasis				
No	Reference		Reference	
Yes	4.303 (3.397-5.451)	< 0.001	1.278 (0.778-2.100)	0.333
Radiotherapy				
No	Reference		Reference	
Yes	0.484(0.383-0.612)	< 0.001	0.587 (0.444-0.776)	< 0.001
Surgery				
No	Reference		Reference	
Yes	0.244(0.192-0.311)	< 0.001	0.450 (0.319-0.635)	< 0.001

HR: Hazard ratio; NI: Not included; LN: Lymph node; SRC: Signet ring cell carcinoma; ASC: Adenosquamous carcinoma; TNM: Tumor-node-metastasis.

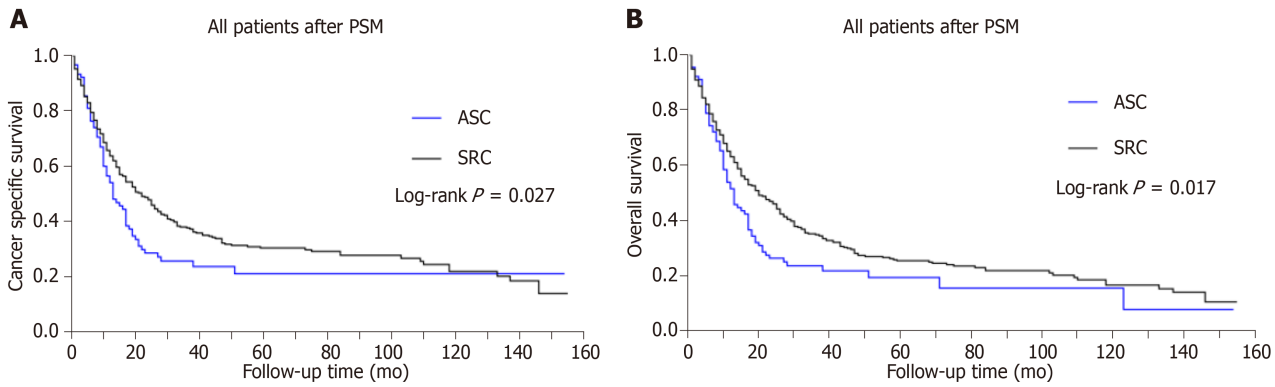

Figure 2 Kaplan-Meier survival curves by histology after propensity score matching. A: Cancer-specific Survival ($P < 0.05$); B: Overall Survival ($P < 0.05$). ASC: Adenosquamous carcinoma; SRC: Signet ring cell carcinoma.

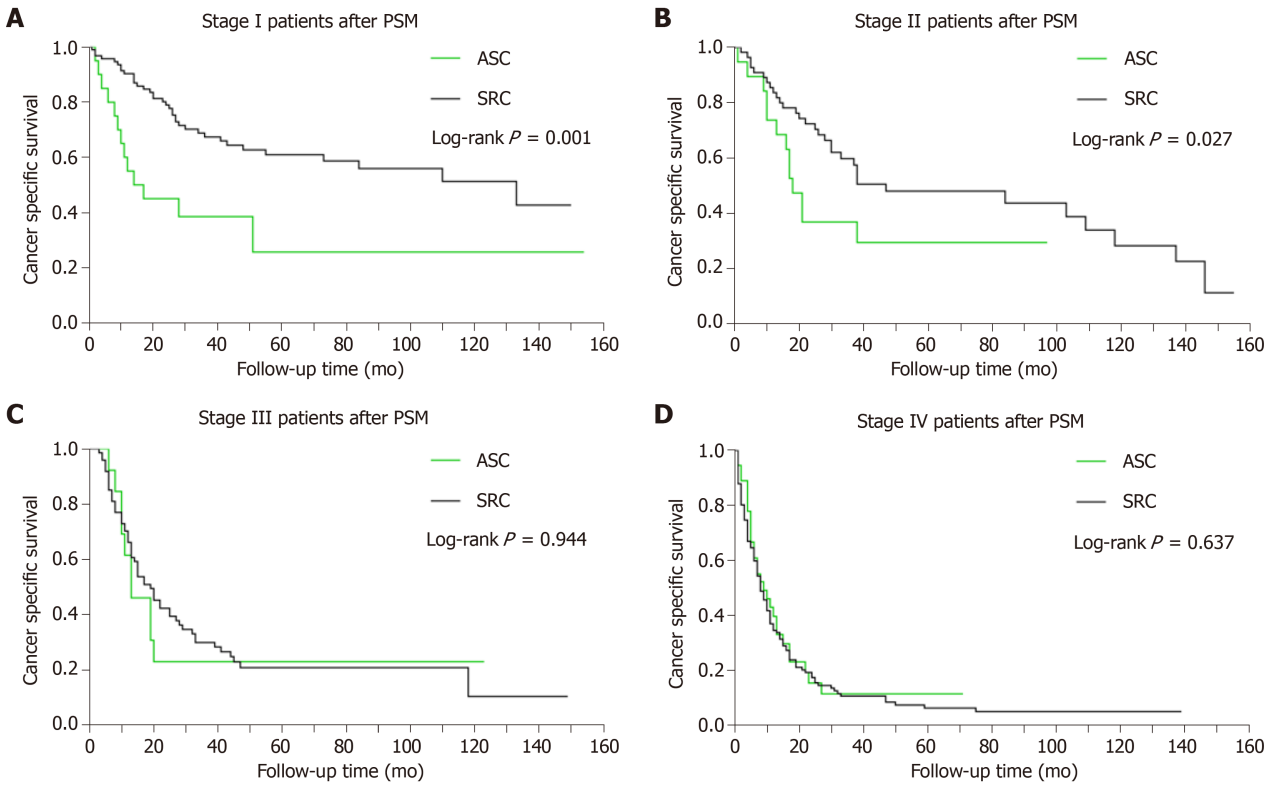

Figure 3 Kaplan-Meier plots of adenosquamous carcinoma *vs* signet ring cell carcinoma stratified by TNM stage. A: Stage I ($P < 0.05$); B: Stage II ($P < 0.05$); C: Stage III ($P > 0.05$); D: Stage IV ($P > 0.05$). ASC: Adenosquamous carcinoma; SRC: Signet ring cell carcinoma.

ARTICLE HIGHLIGHTS

Research background

Adenosquamous carcinoma (ASC) is a rare entity in gastric cancer, which exhibits early tumor progression and a poorer prognosis than other typical gastric adenocarcinoma. Gastric signet ring cell carcinoma (SRC) is a unique subtype with distinct tumor biology and clinical features. We hypothesized that further knowledge about these distinct cancers would improve the clinical management of such patients.

Research motivation

Given the relative rarity of these two subtypes in gastric cancer, the study on gastric ASC with large series is still lacking. The clinicopathological characteristics and prognosis of ASC *vs* SRC has not been well established to date. The current study adopted a large cohort of such patients from the Surveillance, Epidemiology, and End Results (SEER) database. Study on the clinicopathological features, treatment, and prognosis of such patients may bring deeper knowledge on these tumors and provide additional assistance for their treatment.

Research objectives
The goal of our study was to evaluate the clinicopathological characteristics and prognosis of ASC *vs* SRC based on a large cohort from the SEER database. Achieving this objective may provide additional assistance for their management.

Research methods
We conducted a retrospective study using a large cohort from the SEER database. The clinicopathological features of patients with ASC *vs* SRC were comprehensively compared by chi-square tests. We used both propensity-score matching (PSM) method and multivariate Cox regression analysis to adjust the potential bias caused by the imbalanced distribution of confounding factors. Clinical outcomes including cancer-specific survival (CSS) and overall survival (OS) were also compared by the Kaplan-Meier method. The prognostic factors were identified.

Research results
A total of 6063 eligible patients were collected. After PSM, 370 patients with SRC and 95 patients with ASC were analyzed. In the post-matching cohort, gastric ASC showed an inferior prognosis to SRC in both CSS and OS. ASC and higher TNM stage were independently associated with a poor survival (HR > 1, $P < 0.05$), while radiotherapy (HR = 0.587; 95%CI: 0.444-0.776, $P < 0.001$) and surgery were independent protective factors for favorable prognosis (HR < 1, $P < 0.05$). Subgroup survival analysis revealed that the inferior prognosis was most significant in stages I and II patients.

Research conclusions
ASC may have an inferior prognosis to SRC in patients with stages I and II gastric cancer, so greater attention should be paid to these patients. Our study supports radiotherapy and surgery for the future management of this clinically rare entity. Improved clinical and biological understanding of ASC *vs* SRC may lead to more individualized therapy for such patients.

Research perspectives
Our study shows that gastric ASC has an inferior prognosis to SRC in stages I and II patients. Precautions should be taken to such patients. Radiotherapy and surgery have the potential to improve their clinical outcomes. Future long-term prospective studies are warranted to validate our findings.

REFERENCES

1 **Li Q**, Zou J, Jia M, Li P, Zhang R, Han J, Huang K, Qiao Y, Xu T, Peng R, Song Q, Fu Z. Palliative Gastrectomy and Survival in Patients With Metastatic Gastric Cancer: A Propensity Score-Matched Analysis of a Large Population-Based Study. *Clin Transl Gastroenterol* 2019; **10**: 1-8 [PMID: 31116140 DOI: 10.14309/ctg.0000000000000048]

2 **Di Sibio A**, Romano L, Giuliani A, Varrassi M, De Donato MC, Iacopino A, Perri M, Schietroma M, Carlei F, Di Cesare E, Masciocchi C. Nerve root metastasis of gastric adenocarcinoma: A case report and review of the literature. *Int J Surg Case Rep* 2019; **61**: 9-13 [PMID: 31302320 DOI: 10.1016/j.ijscr.2019.07.001]

3 **Yuan LW**, Yamashita H, Seto Y. Glucose metabolism in gastric cancer: The cutting-edge. *World J Gastroenterol* 2016; **22**: 2046-2059 [PMID: 26877609 DOI: 10.3748/wjg.v22.i6.2046]

4 **Chon HJ**, Kim C, Cho A, Kim YM, Jang SJ, Kim BO, Park CH, Hyung WJ, Ahn JB, Noh SH, Yun M, Rha SY. The clinical implications of FDG-PET/CT differ according to histology in advanced gastric cancer. *Gastric Cancer* 2019; **22**: 113-122 [PMID: 29948387 DOI: 10.1007/s10120-018-0847-5]

5 **Ebi M**, Shimura T, Yamada S, Hirata Y, Tsukamoto H, Okamoto Y, Mizoshita T, Tanida S, Kataoka H, Kamiya T, Inagaki H, Joh T. A patient with gastric adenosquamous carcinoma with intraperitoneal free cancer cells who remained recurrence-free with postoperative S-1 chemotherapy. *Intern Med* 2012; **51**: 3125-3129 [PMID: 23154717 DOI: 10.2169/internalmedicine.51.8402]

6 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington MK, Carneiro F, Cree IA. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2019 [PMID: 31433515 DOI: 10.1111/his.13975]

7 **Shirahige A**, Suzuki H, Oda I, Sekiguchi M, Mori G, Abe S, Nonaka S, Yoshinaga S, Sekine S, Kushima R, Saito Y, Fukagawa T, Katai H. Fatal submucosal invasive gastric adenosquamous carcinoma detected at surveillance after gastric endoscopic submucosal dissection. *World J Gastroenterol* 2015; **21**: 4385-4390 [PMID: 25892891 DOI: 10.3748/wjg.v21.i14.4385]

8 **Faria GR**, Eloy C, Preto JR, Costa EL, Almeida T, Barbosa J, Paiva ME, Sousa-Rodrigues J, Pimenta A. Primary gastric adenosquamous carcinoma in a Caucasian woman: a case report. *J Med Case Rep* 2010; **4**: 351 [PMID: 21034475 DOI: 10.1186/1752-1947-4-351]

9 **Mori M**, Iwashita A, Enjoji M. Adenosquamous carcinoma of the stomach. A clinicopathologic analysis of 28 cases. *Cancer* 1986; **57**: 333-339 [PMID: 3942965 DOI: 10.1002/1097-0142(19860115)57:2<333::aid-cncr2820570224>3.0.co;2-u]

10 **Chen YY**, Li AF, Huang KH, Lan YT, Chen MH, Chao Y, Lo SS, Wu CW, Shyr YM, Fang WL. Adenosquamous carcinoma of the stomach and review of the literature. *Pathol Oncol Res* 2015; **21**: 547-551 [PMID: 25567665 DOI: 10.1007/s12253-014-9890-7]

11 **Bae HI**, Seo AN. Early Gastric Adenosquamous Carcinoma Resected Using Endoscopic Submucosal Dissection. *Case Rep Gastroenterol* 2019; **13**: 165-172 [PMID: 31123442 DOI: 10.1159/000499447]

12 **Akce M**, Jiang R, Alese OB, Shaib WL, Wu C, Behera M, El-Rayes BF. Gastric squamous cell carcinoma and gastric adenosquamous carcinoma, clinical features and outcomes of rare clinical entities: a National Cancer Database (NCDB) analysis. *J Gastrointest Oncol* 2019; **10**: 85-94 [PMID: 30788163 DOI: 10.21037/jgo.2018.10.06]

13 **Feng F**, Zheng G, Qi J, Xu G, Wang F, Wang Q, Guo M, Lian X, Zhang H. Clinicopathological features and prognosis of gastric adenosquamous carcinoma. *Sci Rep* 2017; **7**: 4597 [PMID: 28676632 DOI: 10.1038/s41598-017-04563-2]

14 **Quan J**, Zhang R, Liang H, Li F, Liu H. The clinicopathologic and prognostic analysis of adenosquamous and squamous cell carcinoma of the stomach. *Am Surg* 2013; **79**: E206-E208 [PMID: 23635572]

15 **Alsina M**, Miquel JM, Diez M, Castro S, Tabernero J. How I treat gastric adenocarcinoma. *ESMO Open* 2019; **4**: e000521 [PMID: 31354966 DOI: 10.1136/esmoopen-2019-000521]

16 **Stumpf PK**, Amini A, Jones BL, Koshy M, Sher DJ, Lieu CH, Schefter TE, Goodman KA, Rusthoven CG. Adjuvant radiotherapy improves overall survival in patients with resected gastric adenocarcinoma: A National Cancer Data Base analysis. *Cancer* 2017; **123**: 3402-3409 [PMID: 28513823 DOI: 10.1002/cncr.30748]

17 **Moro K**, Nagahashi M, Naito T, Nagai Y, Katada T, Minagawa M, Hasegawa J, Tani T, Shimakage N, Usuda H, Gabriel E, Kawaguchi T, Takabe K, Wakai T. Gastric adenosquamous carcinoma producing granulocyte-colony stimulating factor: a case of a rare malignancy. *Surg Case Rep* 2017; **3**: 67 [PMID: 28493097 DOI: 10.1186/s40792-017-0338-7]

18 **Miyake H**, Miyasaka C, Ishida M, Miki H, Inoue K, Tsuta K. Simultaneous gastric adenosquamous carcinoma and gastric carcinoma with lymphoid stroma: A case report. *Mol Clin Oncol* 2019; **11**: 77-80 [PMID: 31289682 DOI: 10.3892/mco.2019.1860]

19 **Pernot S**, Voron T, Perkins G, Lagorce-Pages C, Berger A, Taieb J. Signet-ring cell carcinoma of the stomach: Impact on prognosis and specific therapeutic challenge. *World J Gastroenterol* 2015; **21**: 11428-11438 [PMID: 26523107 DOI: 10.3748/wjg.v21.i40.11428]

20 **Antonelli J**, Cefalu M, Palmer N, Agniel D. Doubly robust matching estimators for high dimensional confounding adjustment. *Biometrics* 2018; **74**: 1171-1179 [PMID: 29750844 DOI: 10.1111/biom.12887]

World Journal of Gastrointestinal Oncology

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

World J Gastrointest Oncol 2020 January 15; 12(1): 113-123

DOI: 10.4251/wjgo.v12.i1.113

ISSN 1948-5204 (online)

EVIDENCE-BASED MEDICINE

Validity of studies suggesting preoperative chemotherapy for resectable thoracic esophageal cancer: A critical appraisal of randomized trials

Giulia Manzini, Ursula Klotz, Doris Henne-Bruns, Michael Kremer

ORCID number: Giulia Manzini (0000-0002-8032-8043); Ursula Klotz (0000-0003-2725-1187); Doris Henne-Bruns (0000-0002-5699-9031); Michael Kremer (0000-0001-9364-9420).

Author contributions: Manzini G, Klotz U and Henne-Bruns DH contributed substantially to the conception and design of the study; Manzini G and Klotz U contributed to the analysis and interpretation of the data. Manzini G performed the meta-analysis. Manzini G and Kremer M wrote the manuscript. All authors gave their final approval of the version to be published.

Conflict-of-interest statement: The authors report no relevant conflicts of interest.

Manuscript source: Invited manuscript

Giulia Manzini, Ursula Klotz, Doris Henne-Bruns, Michael Kremer, Department of General and Visceral Surgery, University of Ulm, Ulm 89081, Germany

Michael Kremer, Department of General and Visceral Surgery, Hospital of Aarau, Aarau 5000, Switzerland

Corresponding author: Doris Henne-Bruns, MD, Professor, Department of General and Visceral Surgery, University Hospital of Ulm, Albert-Einstein-Allee 23, Ulm 89081, Germany. doris.henne-bruns@uniklinik-ulm.de

Abstract

BACKGROUND

In 2015, Kidane published a Cochrane review and meta-analysis to summarise the impact of preoperative chemotherapy versus surgery alone on survival for resectable thoracic esophageal cancer. The authors concluded that preoperative chemotherapy improved overall survival (OS).

AIM

The aim of this article was to assess the validity of the three most powerful studies included in the Cochrane review and the meta-analysis supporting the advantage of preoperative chemotherapy and to investigate the impact of an exclusion of these three studies on the result of the meta-analysis.

METHODS

OS was selected as the endpoint of interest. Among the ten included papers which analysed this endpoint, we identified the three publications with the highest weights influencing the final result. The validity of these papers was analysed using the CONSORT checklist for randomized controlled trials. We performed a new meta-analysis without the three studies to assess their impact on the general result of the original meta-analysis.

RESULTS

The three analysed studies revealed several inconsistencies. Inappropriate answers were found in up to one third of the items of the CONSORT checklist. Missing information about sample-size calculation and power, unclear or inadequate randomisation, and missing blinded set-up were the most common findings. When the three criticized studies were excluded in the meta-analysis, preoperative chemotherapy showed no benefit in OS.

Received: March 22, 2019
Peer-review started: March 22, 2019
First decision: July 31, 2019
Revised: September 20, 2019
Accepted: October 14, 2019
Article in press: October 14, 2019
Published online: January 15, 2020

P-Reviewer: Okamoto H
S-Editor: Zhang L
L-Editor: A
E-Editor: Liu MY

CONCLUSION
The three most powerful publications in the Cochrane review show substantial deficits. After the exclusion of these studies from the meta-analysis, preoperative chemotherapy does not seem to result in an advantage in survival. We suggest a more critical appraisal regarding the validity of single studies.

Key words: Esophageal cancer; Assessment of validity; Meta-analysis; CONSORT; Overall survival

Core tip: The quality of single studies is crucial in order to perform valid meta-analyses that are often used as basis for guideline recommendations. We critically analysed a recent Cochrane meta-analysis that supports the use of preoperative chemotherapy for resectable thoracic esophageal cancer in order to improve overall survival. The most powerful included studies showed several inconsistencies according to the requirements of the Consort checklist for randomized controlled trials. After the exclusion of these studies from the meta-analysis, preoperative chemotherapy does not seem to result in an advantage in survival. We suggest a more critical appraisal regarding the validity of single studies.

Citation: Manzini G, Klotz U, Henne-Bruns D, Kremer M. Validity of studies suggesting preoperative chemotherapy for resectable thoracic esophageal cancer: A critical appraisal of randomized trials. *World J Gastrointest Oncol* 2020; 12(1): 113-123
URL: https://www.wjgnet.com/1948-5204/full/v12/i1/113.htm
DOI: https://dx.doi.org/10.4251/wjgo.v12.i1.113

INTRODUCTION

Esophageal cancer is the eighth most common cancer in the world and the sixth most common cause of death from cancer with an overall ratio of mortality of 0.88[1]. Although it accounts for only 3.2% of all cancers, the incidence of esophageal cancer is increasing with an incidence of 572/100000 new cases/year in 2018[2].

Surgery is the treatment of choice for localized esophageal cancer[3-4] with a potential to provide loco-regional control, as well as long-term survival[5]. Curative resection is possible in only 15% to 39% of the cases[6-9]. Surgery is the only curative treatment, but it alone often fails to overcome the natural history of the disease owing to the presence of occult micrometastases, and fatal distant and loco-regional disease relapse is common. Median survival after esophagectomy with curative intention is 15 to 18 mo with a 5-year survival rate of 20% to 25%[5]. Therefore, clinicians are now inclined to use some form of multidisciplinary treatment including surgery as a standard of care for locally advanced esophageal cancer, which is defined as disease restricted to the esophagus or resectable periesophageal tissue (T2-T4) and/or lymph-node involvement (N1-N3) in the absence of distant metastasis[10].

The optimal multimodality treatment is still controversial. Potential contentious issues exist regarding the (1) ideal preoperative, perioperative or postoperative approach and (2) ideal combination of radiotherapy (RTx), chemotherapy (CTx) or concurrent chemoradiation. Various randomized and non-randomized trials and several meta-analyses have been conducted to address this topic, but established standard guidelines still vary considerably or even fail to propose a specific treatment regime[11]. Preoperative (radio-)chemotherapy aims to exterminate micro-metastases, enhance resectability by down-staging the tumour, improve loco-regional control and provide relief of dysphagia[11,12].

Several studies have investigated whether preoperative CTx leads to improved cure rates, but reports remain conflicting. The initial Cochrane review of preoperative CTx for resectable esophageal cancer[13] concluded that no survival advantage was associated with CTx. The same result was found by Urschel *et al*[14] after inclusion of 11 randomized trials in a meta-analysis. Ychou *et al*[15], Boonstra *et al*[16] and MRC Allum *et al*[17] subsequently reported a survival benefit for patients receiving neoadjuvant CTx. After inclusion of these last three studies, the updated Cochrane Review and meta-analysis by Kidane *et al*[18] on the same topic found an improvement in overall survival

(OS) [hazard ratio (HR): 0.88; 95% confidence interval (CI): 0.80 to 0.96] for patients receiving preoperative CTx. A total of ten randomized controlled studies with OS as the primary endpoint were included in this meta-analysis.

The aim of our study was to assess the validity of the studies by Ychou *et al*[15], Boonstra *et al*[16] and MRC Allum *et al*[17] included in the updated Cochrane Review and the meta-analysis by Kidane *et al*[18], which confirmed the benefit of preoperative CTx on survival for resectable thoracic esophageal cancer with the intention to invite everyone to critically interpret not only the results, but also the methodology by which the results were achieved. We performed a variety of meta-analyses excluding or including studies depending on their validity and attributed power and discuss those findings in regard to current recommendations of esophageal carcinoma guidelines.

MATERIALS AND METHODS

The meta-analysis by Kidane *et al*[18] included a total of ten studies. Four (40%) (Ychou *et al*[15], Boonstra *et al*[16], MRC Allum *et al*[17], and Law *et al*[19]) found a statistically significant advantage in survival in patients after preoperative CTx for resectable thoracic esophageal cancer (HR: < 1 with a significant 95%CI). All the other six included studies (60%) were not statistically significant[20-25].

In the first part of the results section we assessed the validity of the three most powerful studies included in the Cochrane review by Kidane *et al*[18], which found a statistically significant advantage in survival in patients receiving preoperative CTx before resection for thoracic esophageal cancer. These studies were those of Ychou *et al*[15], Boonstra *et al*[16] and MRC Allum *et al*[17].

In the second part of the results section, we performed a new meta-analysis without these aforementioned three studies. Among the three analysed studies, Boonstra *et al*[16] had the higher validity, so we performed another meta-analysis assuming that this study is valid enough to be included in the meta-analysis.

Finally, we present the results of the meta-analysis excluding the four statistically significant studies confirming the survival advantage for patients treated with preoperative CTx. In this last case, only statistically non-significant studies were included in the meta-analysis.

Selection of the studies and assessment of their validity

We used the same methodology as described in our previous publication[26] to analyse the validity of the Cochrane review. From the several endpoints investigated in the Cochrane review by Kidane *et al*[18], we identified OS as a major endpoint of interest. Among the ten studies identified by the authors of the Cochrane review investigating OS, we selected the three most powerful studies as weighted by the review's authors which support the advantage of preoperative CTx: Ychou *et al*[15], Boonstra *et al*[16] and MRC Allum *et al*[17]. The weights assigned to these three studies by the authors of the systematic review according to their sample size, precision of the estimates and width of the confidence intervals were 24.5%, 24.1% and 20.5%, respectively. We then assessed the validity of these studies using the CONSORT checklist 2010[27], which is a validated instrument for the evaluation of randomized controlled trials (RCTs) and contains a total of 37 items. The checklist with all items and their precise description is available in the Appendix of our previous publication[26]. We then asked whether the positive result in the Cochrane review is supported by sufficient validity. Figure 1 illustrates our methodology. Two independent review authors (UK and GM) assessed the validity of each of the three publications.

Meta-analysis

We repeated the meta-analysis without the three analysed studies (n = 7) and compared the result with the original meta-analysis comprising ten studies. Since Boonstra *et al*[16] has the higher validity among the analysed studies, we then conducted a second meta-analysis only excluding Ychou *et al*[15] and MRC Allum *et al*[17]. In a next step, we assumed that all single studies with a statistically significant benefit of preoperative CTx for thoracic resectable esophageal cancer (n = 4) were not valid enough and performed a second meta-analysis with the remaining six studies. The results were compared with the original meta-analysis (n = 10 studies). The meta-analyses were performed with R, version 3.2.0, with the package "meta" (http://www.r-project.org/foundation).

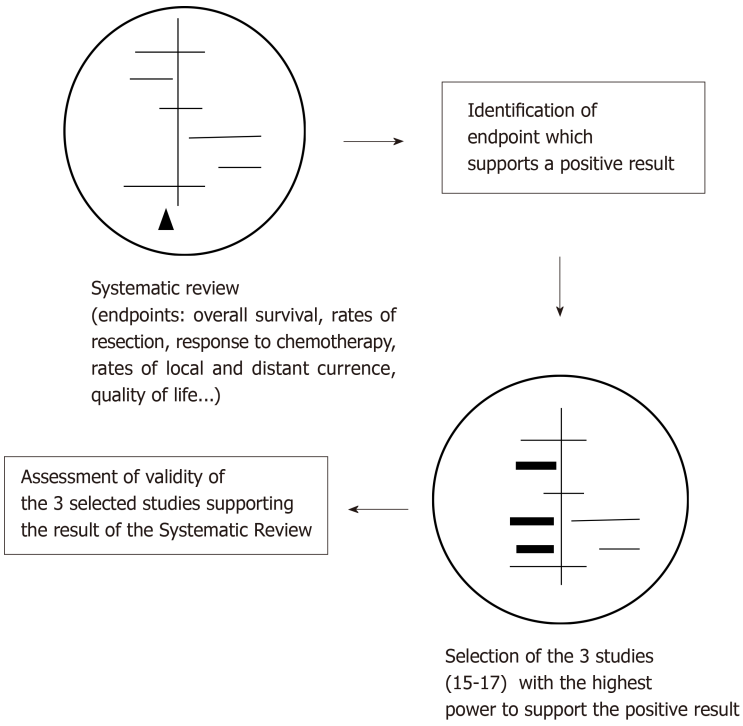

Figure 1 Four steps to the analysis of validity of a systematic review according to our previous work[26]. We identified the endpoint of interest (overall survival) and selected the three most powerful studies addressing this endpoint on the basis of the assigned weights by the authors of the systematic review, as these studies contributed essentially to the positive result of the systematic review. We finally assessed the validity of these studies by using the CONSORT checklist.

RESULTS

Assessment of the validity of the studies

Table 1 presents a summary of the three analysed papers. The results are reported for each of the three included studies. Table 2 summarizes all the items present in the CONSORT checklist showing how the studies dealt with them. In this section, we describe the problems of each study. Eleven of the 34 validity criteria (32.4%) were not met in the study by Ychou *et al*[15]. Three items were not applicable. The randomisation occurred by phone call through a centralized randomisation system, and then the assignment was stratified according to centre, performance status, and tumour site using the minimisation procedure. Due to the use of the minimisation method, allocation concealment was not maintained. Blinding was not possible in this study, as the control group did not receive any preoperative treatment. Inclusion of untreated controls limits the interpretation of the study. Specifically, the difference between the intervention and control group may be caused by a non-specific effect, such as a placebo effect. Moreover, 50% of the patients in the intervention group also received postoperative CTx. Regarding sample size, in the methods section the authors described that 250 patients (178 deaths) were required to achieve the needed power. The trial was closed earlier due to difficulties in patient recruitment. At the closure time, a total of 224 patients (156 deaths) had been included, raising the question of whether the power was sufficient. Moreover, patients with stomach adenocarcinoma were also included in the study at a later time after changing the inclusion criteria. Taken together, these issues lead to insufficient validity of the report; therefore the described effect cannot be considered as clinically relevant.

In Boonstra *et al*[16], we identified poor validity in 8 of the 33 validity criteria (24.2%). Four items were not applicable. Again, as in the previous study, the use of untreated controls limits the interpretation of the study. Blinding was not possible in this work either, as the control group did not receive any preoperative treatment. Central randomisation was performed, but the process is not clearly described. Therefore, it is not possible to ascertain whether allocation concealment was maintained or not. Additionally, random assignment was stratified by age, gender, weight loss and tumour length. As also pointed out by the authors of the Cochrane review, it is unclear whether an intention-to-treat (ITT) analysis had been performed, as information on withdrawals was missing or unclear.

Table 1 Summary of the three analysed studies

Study (year)	Boonstra *et al*[16]	MRC Allum *et al*[17]	Ychou *et al*[15]
Number of included patients (intervention *vs* control)	85 *vs* 84	400 *vs* 402	113 *vs* 111
Inclusion criteria	100% squamous-cell cancer of thoracic oesophagus (upper, middle and lower third), T1-3, any N, M0 (M1a eligible if distal oesophageal cancer and suspected celiac nodes) < 80 yr of age, Karnofsky > 70	Squamous-cell cancer, adenocarcinoma, undifferentiated, upper, middle and lower thirds of oesophagus, as well as the gastric cardia	Resectable adenocarcinoma of the lower third of the oesophagus or gastro-oesophageal junction or stomach 18-75 years of age, WHO performance status 0 or 1, adequate renal (Cr < 120 mol/L) and hematologic functions
Intervention group	Preop. CTx[a]: Cisplatin, Etoposid *iv*. po. + surgery	Preop.CTx: Cisplatin, 5-FU + preop.radiotherapy + surgery	Preop.CTx: 5-FU, Cisplatin + surgery
Control group	Surgery	Preop. radiotherapy + surgery	Surgery
Outcome (intervention *vs* control)	Median overall survival 16 mo *vs* 12 mo, $P = 0.03$, by the log-rank test, HR[b]: 0.71; (95%CI[c]: 0.51-0.98)	Overall survival is significantly greater in CS group (HR: 0.84, 95%CI: 0.72-0.98, $P = 0.03$)	Overall survival significantly higher in CS group (HR for death 0.69, 95%CI: 0.50-0.95, $P = 0.02$) 5-year survival: 38% (95%CI: 29%-47%) in the CS group *vs* 24% (95%CI: 26%-44%) in the S group
Weight assigned in the Cochrane review (%)	24.1	20.5	24.5

[a]CTx: chemotherapy,
[b]HR: hazard ratio,
[c]CI: Confidence interval. HR: Hazard ratio; CS: Chemotherapy + surgery; WHO: World Health Organization.

As the validity of the report is not sufficient, the described effect cannot be considered as clinically relevant.

MRC Allum *et al*[17] described the long-term results of a previously published study by the same group in 2002. If information was not found in the last studies, we checked if the needed information was available in the first publication[28]. Taking this into consideration, 11 of the 33 validity criteria were not met (33.3%) by MRC Allum *et al*[17]. Four items were not applicable. As in the previous study, the use of untreated controls limits the interpretation of the study. Blinding was also not possible because the control group did not receive any preoperative treatment. Due to the use of the minimisation method, allocation concealment is not maintained. A power calculation is missing.

In the previous publication by the same group in 2002[28], the sponsor appointed the writing committee, which interpreted data, wrote the report and submitted it for publication. The risk profiles of the two groups are slightly different with a certain probability of unbalanced risk distribution in favour of the intervention group regarding age and degree of dysphagia.

As the validity of the report is not sufficient, the described effect cannot be considered as clinically relevant.

Meta-analyses

Figure 2 shows the result of the meta-analysis when the three analysed studies were excluded. A total of seven studies were included. One study (Law *et al*[19]) showed a positive and statistically significant result in favour of the use of preoperative CTx before resection of thoracic esophageal cancer. Six of the included studies were not statistically significant by themselves. The new meta-analysis estimate had a HR of 0.94 with a 95%CI (0.81; 1.09) under assumption of a fixed-effect model and a HR of 0.92 with a 95%CI (0.75; 1.13) under assumption of a mixed-effect model. Regardless of the assumed model, the new estimate does not confirm the advantage of preoperative CTx for resectable thoracic esophageal cancer. The estimate of the original meta-analysis was 0.88 with a 95%CI (0.80; 0.96). The exclusion of the three studies completely changed the result of the meta-analysis. In Boonstra *et al*[16], only 24.2% of the items on the CONSORT checklist were inappropriately answered, so we assumed that the validity of this study was enough to be included in the meta-analysis. We performed a new meta-analysis excluding only Ychou *et al*[15] and MRC Allum *et al*[17] (Figure 3). We found a HR of 0.90 with a 95%CI (0.81; 1.00) under assumption of a fixed-effect model and a HR of 0.90 with a 95%CI (0.78; 1.05) under assumption of a mixed-effect model. Again, regardless of the assumed model, the new estimate does not confirm the advantage of preoperative CTx for resectable thoracic esophageal cancer.

Table 2 Assessment of validity of the three analysed studies according to the CONSORT checklist (REF)

Section/Topic	Item number	Boonstra *et al*[16]	MRC Allum *et al*[17]	Ychou *et al*[15]
Title and Abstract	1a	Yes	Yes	No
	1b	Yes	Yes	Yes
Introduction				
Background and objectives	2a	Yes	Yes	Yes
	2b	Yes	Yes	Yes
Methods				
Trial design	3a	Yes	Yes	Yes
	3b	Not applicable	Not applicable	Yes
Participants	4a	Yes	Yes	Yes
	4b	Yes	No	No
Interventions	5	Yes	No	Yes
Outcomes	6a	Yes	Yes	Yes
	6b	Not applicable	Not applicable	Not applicable
Sample size	7a	Yes	No	Yes
	7b	Not applicable	Not applicable	Yes
Randomisation				
-Sequence generation	8a	No	Yes	Yes
	8b	No	No	No
-Allocation concealment mechanism	9	No	No	No
- Implementation	10	No	No	No
Blinding	11a	No	Yes	No
	11b	Yes	No	No
Statistical methods	12a	Yes	Yes	Yes
	12b	Yes	Yes	Not applicable
Results				
Participant flow	13a	Yes	Yes	Yes
	13b	Yes	Yes	Yes
Recruitment	14a	Yes	Yes	Yes
	14b	Not applicable	Not applicable	Nes
Baseline data	15	Yes	Yes	Yes
Numbers analysed	16	Yes	Yes	Yes
Outcomes and estimation	17a	Yes	Yes	Yes
	17b	Yes	Yes	Yes
Ancillary analysis	18	Yes	Yes	Not applicable
Harms	19	Yes	No	Yes
Discussion				
Limitations	20	Yes	Yes	Yes
Generalisability	21	No	No	No
Interpretation	22	Yes	Yes	Yes
Other information				
Registration	23	No	No	No
Protocol	24	No	No	No
Funding	25	Yes	Yes	No

Finally, we performed a second meta-analysis (Figure 4) also excluding Law *et al*[19], which found a positive and statistically significant result as well. After the exclusion of all four studies with positive and statistically significant results, the new meta-analysis consisted of only six statistically non-significant studies. The new meta-analysis estimate was HR 1.04 with a 95%CI (0.88; 1.22), confirming the lack of a survival advantage for patients undergoing preoperative CTx before resection of the thoracic esophageal cancer.

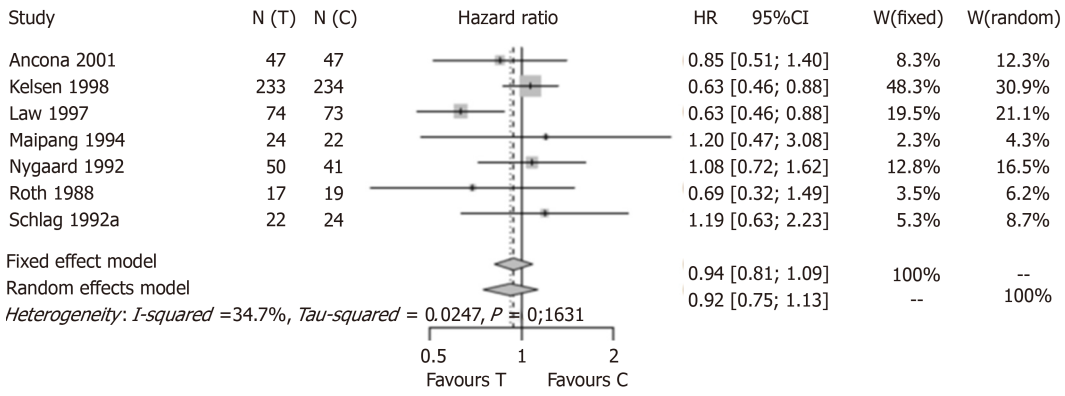

Figure 2 Meta-analysis of seven studies after excluding the three analysed studies. HR: Hazard ratio; N(T): Number of patients in the experimental group; N(C): Number of patients in the control group; W(fixed): Weight assigned to the study by using a fixed-effect model; W(random): Weight assigned to the study by using a random-effect model.

DISCUSSION

In the present manuscript, we assessed the validity of three studies included in the meta-analysis by Kidane *et al*[18], which supports the results of improved survival in patients treated with preoperative CTx for resectable thoracic esophageal cancer. It is important to identify possible bias in the three studies which support the result of the meta-analysis because bias jeopardizes validity. We demonstrated that these three studies are not valid enough to be included in a Cochrane review. When excluded from the meta-analysis, the overall result of the meta-analysis is no longer significant.

We will first illustrate the problems we discovered in the three mathematically most influential studies supporting the conclusions and, in a second step, discuss our findings after performing the new meta-analyses.

Common problems in all studies

The lack of a placebo-controlled and blinded study affects the validity of the three studies and, consequently, the validity of the review. As discussed in our previous work[26], without a placebo control, it is impossible to differentiate between specific pharmacological and placebo effects. A placebo effect is defined as the "…response of a subject to a substance or any procedure known to be without specific therapeutic effect for the condition being treated[29]." Several studies demonstrated that perceptual characteristics of drugs[30], the route of administration[31], laboratory tests[32], diagnosis[33] and the doctor-patient relationship play an important role in the outcome of an illness[34-37]. Information regarding treatment or no treatment alone is sufficient to elicit a placebo effect[38]. Moreover, patients' and doctors' preferences could also have influenced the results in an open study[39]. Patients assigned to the control group feel disadvantaged because they expect to be treated. Furthermore, when there is no concealment of treatment allocation, the randomisation procedure is compromised because of conscious or subconscious bias[40]. It is important to perform an ITT analysis to maintain the balance distribution of risk factors between groups achieved by a randomisation procedure. A correct ITT analysis was only conducted in the studies by MRC Allum *et al*[17] and Ychou *et al*[15]. These aspects collectively affect the validity of the reports and, therefore, the described effects cannot be considered as clinically relevant.

Specific problems of the study by Ychou et al[15]

In the study by Ychou *et al*[15], a minimisation method is used. Minimisation[41-44], a type of dynamic allocation, is gaining popularity especially in clinical cancer trials. In this design, the new subject's treatment assignment is determined by evaluating the potential covariate imbalance that would result if he or she were assigned to the treatment or to the control group[45]. Minimisation aims at achieving balance over a large number of prespecified prognostic factors simultaneously. We raise concerns over this design, as it compromises adequate generation of an allocation sequence and concealment in this study. Investigators using minimisation can actually determine the group to which a prospective subject would be allocated and then decide whether this is positive or negative in terms of creating an imbalance in some key predictor of outcome not considered in the imbalance function. Despite adding randomisation, so that the treatment that minimises the imbalance function for a given patient is not necessarily allocated to that patient, there is a high probability of this being the case[46].

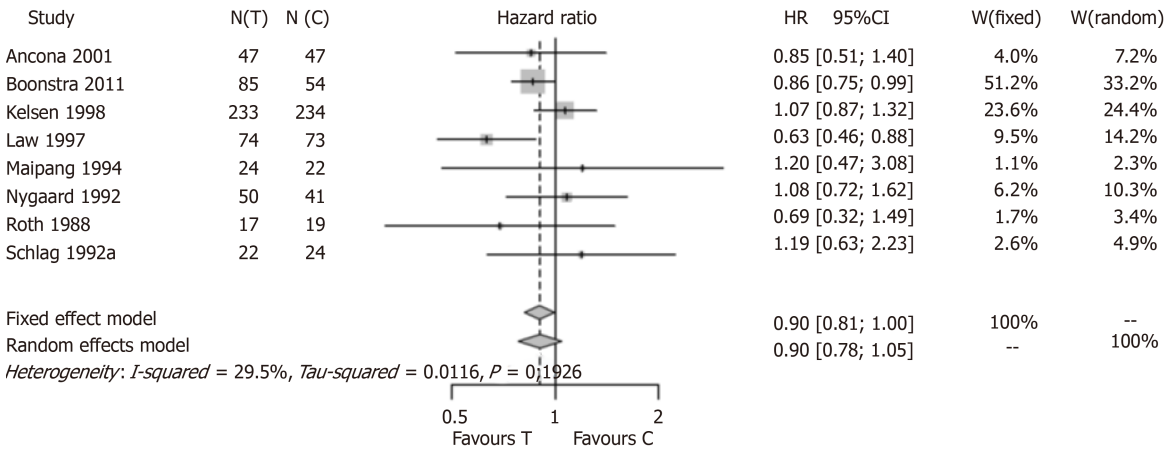

Figure 3 Meta-analysis of eight studies after excluding the studies by Ychou and MRC Allum. HR: Hazard ratio; N(T): Number of patients in the experimental group; N(C): Number of patients in the control group; W(fixed): Weight assigned to the study by using a fixed-effect model; W(random): Weight assigned to the study by using a random-effect model.

The European Medicines Agency's Committee[47] states that "dynamic allocation is strongly discouraged".

Specific problems of the study by Boonstra et al[16]

In this study, as in the study by Ychou *et al*[15], the randomisation process is not exhaustively described; they only mentioned that a central randomisation took place. A description of the randomisation process is completely lacking. Aside from this problem, which is extremely relevant, we find that this study was conducted well in comparison to the other two.

Specific problems of the study by MRC Allum et al[17]

This study reports long follow-up results of a previously published study by the same authors (2002)[28]. As in the study by Ychou *et al*[15], minimisation was used, raising the same concerns as previously described. A power calculation is completely missing. Finally, a sponsor-related conflict of interest was identified by our analysis.

As recently shown by Shnier *et al*[48], financial conflicts of interest and relationships between guideline authors and drug companies are common and represent a source of bias in studies. As authoritative value is assigned to guidelines, it is important to develop formal policies to limit the potential influence of any conflict of interest on guideline recommendations. This is the only way to improve the quality of medical publications. Only valid studies are reliable studies. For an expert pool aiming to publish guidelines, it is necessary to scrutinise the validity of single studies and of meta-analyses as well, as low-quality studies can lead to a distortion of the summary-effect estimate[49].

In the second part of our analysis we performed the meta-analysis first without the three analysed studies and showed that the result of the meta-analysis is no longer significant. This result coincides with previous big studies and the original meta-analysis by Malthaner *et al*[13]. Moreover, as we find that the study by Boonstra *et al*[16] was quite well done in comparison to the other two, we performed a new meta-analysis excluding only the studies of Ychou *et al*[15] and MRC Allum *et al*[17]. The estimate also showed no benefit of preoperative CTx before surgical resection. As expected, when all studies with positive results are eliminated from the meta-analysis, the estimate is not significant.

Implications for practice

According to the results of the Cochrane review, preoperative CTx should be used for patients with resectable thoracic esophageal cancer. However, it is important to note that some of the included trials contain limitations so that definitive assessments of this topic should be delayed until future trials are properly developed. The three analysed studies that were chosen because of their attributed weights are not sufficiently valid to be included in a meta-analysis, which is also true for most of the other studies included.

Despite finding several inconsistencies and substantial deficits in the included high-power studies, the aim of this work is not primarily to identify the best therapeutic treatment for esophageal cancer, but to increase awareness of the quality of studies and their impact on medical treatment when used in meta-analyses or

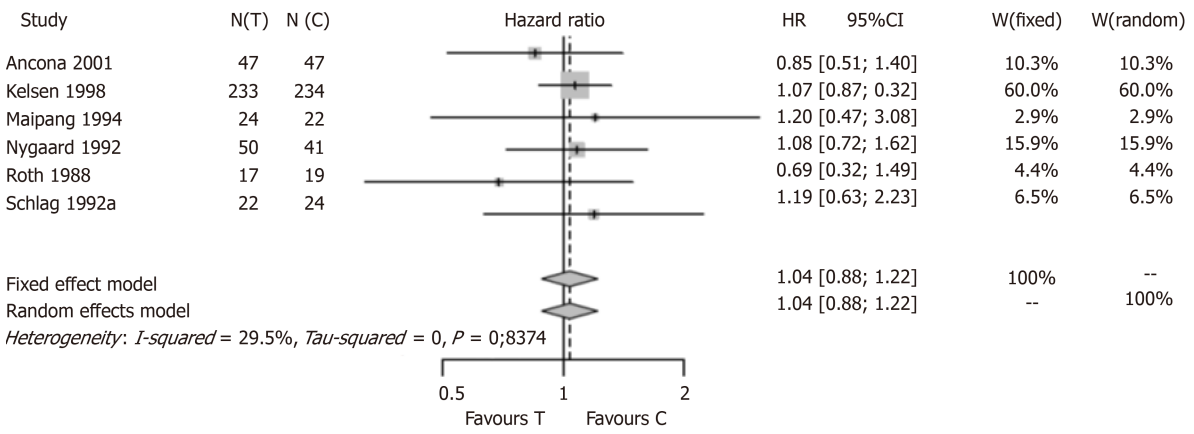

Figure 4 Meta-analysis of six studies after excluding all studies which found a statistically significant survival advantage in the experimental group. HR: hazard ratio; N(T): Number of patients in the experimental group; N(C): Number of patients in the control group; W(fixed): Weight assigned to the study by using a fixed-effect model; W(random): Weight assigned to the study by using a random-effect model.

Cochrane reviews. Especially studies that were performed before implementation of the CONSORT checklist show a variety of inconsistencies that would exclude publication according to current quality standards. High-quality RCTs decrease the risk of inherent bias and therefore receive higher attributed weight in meta-analyses. The inclusion of several low-power studies with serious deficits can overpower well conducted studies and change the outcome.

The analysed Cochrane review was published in 2015; only three included studies were performed after 2009, but seven before 2001, some even dating back to before the 1990s. At that time, no standardised reporting procedure, like the CONSORT checklist, existed. Therefore, the findings are quite heterogeneous. The three most powerful studies were the last ones published and still show a substantial lack in standardisation according to the CONSORT checklist, which was first published in 1996 and revised in 2001 and 2010.

As the incidence of esophageal carcinoma is relatively low, studies often include adenocarinoma and squamous-cell carcinoma without discrimination. Even worse, in some of the studies adjuvant treatment was not only CTx, but sometimes also RCTx for squamous-cell carcinoma. Both inherently different carcinoma types with different neoadjuvant treatment regimens were included in a single group. To analyse the role of neoadjuvant CTx in this context, two groups needed to be established: RTx alone *vs* RCTx as neoadjuvant therapy as performed by Herskovic *et al*[50]. In this paper, adenocarcinoma and squamous-cell carcinoma of the esophagus were also put into one group.

Multimodale therapy in patients with esophageal cancer is now the standard treatment in most centres today and is recommended in several national guidelines[51-52].

In Germany, S3 guidelines for esophageal carcinoma were updated in 2018[51]. Several newer publications, usually multicentric randomised controlled studies, were taken into account.

The evaluated Cochrane review by Kidane is not mentioned in the current German S3 guideline for the standardised treatment of esophageal carcinoma. However, the analysed studies by Ychou *et al*[15], Boonstra *et al*[16] and MRC Allum *et al*[17] with observed inconsistencies are mentioned and included. Thanks to the authors of the German S3 guideline, the current data is critically presented and not all study results are included in the recommendation for standardised treatment: "In squamous cell carcinoma, no consistent increase in survival after CTx alone – despite the positive study by Boonstra – could be observed by metaanalyses." (page 101 German S3 guidelines AWMF-Registernummer: 021/023OL).

In conclusion, multimodal therapy of advanced esophageal carcinoma represents the current gold standard for treatment. We observed several deficits of the analysed studies in the Cochrane review by Kidane. Interestingly, this review was not taken into account in the current german S3 guideline for treatment of esophageal carcinoma, and the analyzed single studies are there critically reviewed and set in context with similar research papers. Well performed (multicentric) randomized controlled studies are needed to be analysed together in a meta-analyse. High-quality single studies are required, as they determine the outcome of meta-analyses that can influence the recommendations of national guidelines.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Globocan 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet] Lyon, France: International Agency for Research on Cancer 2013. pp. cited 2015-06-25. Available from: http://globocan.iarc.fr.

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

3 **DeMeester TR**, Barlow AP. Surgery and current management for cancer of the esopha-gus. Current problems in Surgery. 1988; 535-605 doi:10.1016/0011-3840(88)90027-5

4 **Lerut T**. Esophageal surgery at the end of the millennium. *Journal of Thoracic and Cardiovascular Surgery* 1998; **116**: 1-20 [DOI: 10.1016/S0022-5223(98)70237-5]

5 **DeVita Jr**, Vincent T, Rosenberg, Steven A, Lawrence, Theodore S, DeVita VT, Lawrence TS, Rosenberg SA. Cancer of the Esophagus. DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology. Philadelphia: Wolters Kluwer; 2015; 574-612

6 **M Fok**, J Wong, SWK Cheng and SYK Law. A comparison of outcome after resection for squa-mous cell carcinomas and adenocarcinomas of the esophagus and cardia. *Surgery Gy-necology and Obstetrics* 1992; **175**: 107-112

7 **Lerut T**, De Leyn P, Coosemans W, Van Raemdonck D, Scheys I, LeSaffre E. Surgical strategies in esophageal carcinoma with emphasis on radical lymphadenectomy. *Ann Surg* 1992; **216**: 583-590 [PMID: 1444650 DOI: 10.1097/00000658-199211000-00010]

8 **Liebermann MD**, Shriver CD, Bleckner S, Burt M. Carcinoma of the esophagus. Prog-nostic significance of histologic type. Journal of Thoracic and Cardiovascular Surgery. 1995; 130-138 [DOI: 10.1016/S0022-5223(95)70428-0]

9 **Orringer MB**. Multimodality therapy for esophageal carcinoma--update. *Chest* 1993; **103**: 406S-409S [PMID: 8462336 DOI: 10.1378/chest.103.4_Supplement.406S]

10 **Keditsu KK**, Jiwnani S, Karimundackal G, Pramesh CS. Multimodality management of esophageal cancer. *Indian J Surg Oncol* 2013; **4**: 96-104 [PMID: 24426708 DOI: 10.1007/s13193-013-0216-0]

11 **Garg PK**, Sharma J, Jakhetiya A, Goel A, Gaur MK. Preoperative therapy in locally advanced esophageal cancer. *World J Gastroenterol* 2016; **22**: 8750-8759 [PMID: 27818590 DOI: 10.3748/wjg.v22.i39.8750]

12 **Li F**, Ding N, Zhao Y, Yuan L, Mao Y. The current optimal multimodality treatments for oesophageal squamous-cell carcinoma: A systematic review and meta-analysis. *Int J Surg* 2018; **60**: 88-100 [PMID: 30389537 DOI: 10.1016/j.ijsu.2018.10.037]

13 **Malthaner R**, Fenlon D. Preoperative CTx for resectable thoracic esophageal cancer. *Cochrane Database of Systematic Reviews* 2001; 1 [DOI 10.1002/14651858.CD001556.pub2]

14 **Urschel JD**, Vasan H, Belwett CJ. A meta-analysis of randomized controlled trials that compared neoadjuvant CTx and surgery to surgery alone for resectable esophageal cancer. *American Journal of Surgery* 2002; **183**: 274-9 [DOI: 10.1016/S0002-9610(02)00795-X]

15 **Ychou M**, Boige V, Pignon JP, Conroy T, Bouché O, Lebreton G, Ducourtieux M, Bedenne L, Fabre JM, Saint-Aubert B, Genève J, Lasser P, Rougier P. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. *J Clin Oncol* 2011; **29**: 1715-1721 [PMID: 21444866 DOI: 10.1200/JCO.2010.33.0597]

16 **Boonstra JJ**, Kok TC, Wijnhoven BP, van Heijl M, van Berge Henegouwen MI, Ten Kate FJ, Siersema PD, Dinjens WN, van Lanschot JJ, Tilanus HW, van der Gaast A. Chemotherapy followed by surgery versus surgery alone in patients with resectable oesophageal squamous cell carcinoma: long-term results of a randomized controlled trial. *BMC Cancer* 2011; **11**: 181 [PMID: 21595951 DOI: 10.1186/1471-2407-11-181]

17 **Allum WH**, Stenning SP, Bancewicz J, Clark PI, Langley RE. Long-term results of a randomized trial of surgery with or without preoperative chemotherapy in esophageal cancer. *J Clin Oncol* 2009; **27**: 5062-5067 [PMID: 19770374 DOI: 10.1200/JCO.2009.22.2083]

18 **Kidane B**, Coughlin S, Vogt K, Malthaner R. Preoperative chemotherapy for resectable thoracic esophageal cancer. *Cochrane Database Syst Rev* 2015; CD001556 [PMID: 25988291 DOI: 10.1002/14651858.CD001556.pub3]

19 **Law S**, Fok M, Chow S, Chu KM, Wong J. Preoperative CTx versus surgical therapy alone for squamous cell carcinoma of the esophagus: a prospective randomized trial. *Journal of Thoracic and Cardiovascular Surgery* 1997; **114**: 210-217 [DOI: 10.1016/S0022-5223(97)70147-8]

20 **Ancona E**, Ruol A, Santi S, Merigliano S, Sileni VC, Koussis H, Zaninotto G, Bonavina L, Peracchia A. Only pathologic complete responce to neoadjuvant chemotheraphy improves significantly the long term survival of patients with resectable esophageal squamous cell carcinoma: Final report of a randomized, controlled trial of preoperative chemotherapy versus surgery alone. *Cancer* 2001; **91**: 2165-2174

21 **Kelsen DP**, Ginsberg R, Pajak TF, Sheahan DG, Gunderson L, Mortimer J, Estes N, Haller DG, Ajani J, Kocha W, Minsky BD, Roth JA. Chemotherapy followed by surgery compared with surgery alone for localized esophageal cancer. *N Engl J Med* 1998; **339**: 1979-1984 [PMID: 9869669 DOI: 10.1056/NEJM199812313392704]

22 **Maipang T**, Vasinanukorn P, Petpichetchian C, Chamroonkul S, Geater A, Chansawwaang S, Kuapanich R, Panjapiyakul C, Watanaarepornchai S, Punperk S. Induction chemotherapy in the treatment of patients with carcinoma of the esophagus. *J Surg Oncol* 1994; **56**: 191-197 [PMID: 7518020 DOI: 10.1002/jso.2930560314]

23 **Nygaard K**, Hagen S, Hansen HS, Hatlevoll R, Hultborn R, Jakobsen A, Mäntyla M, Modig H, Munck-Wikland E, Rosengren B. Pre-operative radiotherapy prolongs survival in operable esophageal carcinoma: a randomized, multicenter study of pre-operative radiotherapy and chemotherapy. The second Scandinavian trial in esophageal cancer. *World J Surg* 1992; **16**: 1104-9; discussion 1110 [PMID: 1455880 DOI: 10.1007/BF02067069]

24 **Roth JA**, Pass HI, Flanagan MM, Graeber GM, Rosenberg JC, Steinberg S. Randomized clinical trial of preoperative and postoperative adjuvant chemotherapy with cisplatin, vindesine, and bleomycin for carcinoma of the esophagus. *Journal of Thoracic Cardiovascular Surgery* 1988; **96**: 242-8

25 **Schlag PM**. Randomized trial of preoperative chemotherapy for squamous cell cancer of the esophagus. The Chirurgische Arbeitsgemeinschaft Fuer Onkologie der Deutschen Gesellschaft Fuer Chirurgie Study Group. *Arch Surg* 1992; **127**: 1446-1450 [PMID: 1365692 DOI: 10.1001/archsurg.1992.01420120080015]

26 **Manzini G**, Henne-Bruns D, Kremer M. Validity of studies suggesting postsurgical chemotherapy for

resectable gastric cancer: critical appraisal of randomised trials. *BMJ Open Gastroenterol* 2017; **4**: e000138 [PMID: 29177062 DOI: 10.1136/bmjgast-2017-000138]
27 **Moher D**, Schulz KF, Altman DG; CONSORT GROUP (Consolidated Standards of Reporting Trials). The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *Ann Intern Med* 2001; **134**: 657-662 [PMID: 11304106 DOI: 10.7326/0003-4819-134-8-200104170-00011]
28 Medical Research Council Esophageal Cancer working Party. Surgical resection with or without preoperative chemotheraphy in esophageal cancer: a randomized controlled trial. *The Lancet* 2002; **359**: 1727-1733 [DOI: 10.1016/S0140-6736(02)08651-8]
29 **Benedetti F**, Amanzio M. The neurobiology of placebo analgesia: from endogenous opiois to cholecystokinin. *Progr Neurobiol* 1997; **52**: 109-125 [DOI: 10.1016/S0301-0082(97)00006-3]
30 **Buckalew LW**, Coffield KE. An investigation of drug expectancy as a function of capsule color and size and preparation form. *J Clin Psychopharmacol* 1982; **2**: 245-248 [DOI: 10.1097/00004714-198208000-00003]
31 **Kowalczyk M**, Wall A, Turek T, Kulej M, Scigała K, Kawecki J. Computerized tomography evaluation of cortical bone properties in the tibia. *Ortop Traumatol Rehabil* 2007; **9**: 187-197 [PMID: 17514164 DOI: 10.1002/9780470514412.ch10]
32 **Sox HC**, Margulies I, Sox CH. Psychologically mediated effects of diagnostic tests. *Ann Intern Med* 1981; **95**: 680-685 [PMID: 7305144 DOI: 10.7326/0003-4819-95-6-680]
33 **Thomas KB**. General practice consultations: is there any point in being positive? *Br Med J (Clin Res Ed)* 1987; **294**: 1200-1202 [PMID: 3109581 DOI: 10.1136/bmj.294.6581.1200]
34 **Bass MJ**, Buck C, Turner L, Dickie G, Pratt G, Robinson HC. The physician's actions and the outcome of illness in family practice. *J Fam Pract* 1986; **23**: 43-47 [PMID: 3723083]
35 **Gracely RH**, Dubner R, Deeter WR, Wolskee PJ. Clinician's expectations influence placebo analgesia. *Lancet* 1985; **1**: 1-43 [PMID: 2856960 DOI: 10.1016/s0140-6736(85)90984-5]
36 **Greenfield S**, Kaplan S, Ware JE. Expanding patient involvement in care. Effects on patient outcomes. *Ann Intern Med* 1985; **102**: 520-528 [PMID: 3977198 DOI: 10.7326/0003-4819-102-4-520]
37 **Stewart MA**. Effective physician-patient communication and health outcomes: a review. *CMAJ* 1995; **152**: 1423-1433 [PMID: 7728691]
38 **Waber RL**, Shiv B, Carmon Z, Ariely D. Commercial features of placebo and therapeutic efficacy. *JAMA* 2008; **299**: 1016-1017 [PMID: 18319411 DOI: 10.1001/jama.299.9.1016]
39 **Porzsolt F**, Eisemann M, Habs M, Wyer P. Form follows function: pragmatic controlled trials (PCTs) have to answer different questions and require different designs than randomized controlled trials (RCTs). *Z Gesundh Wiss* 2013; **21**: 307-313 [PMID: 23687408 DOI: 10.1007/s10389-012-0544-5]
40 **Altman DG**, Schulz KF. Statistics notes: Concealing treatment allocation in randomised trials. *BMJ* 2001; **323**: 446-447 [PMID: 11520850 DOI: 10.1136/bmj.323.7310.446]
41 **Pocock SJ**, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975; **31**: 103-115 [PMID: 1100130 DOI: 10.2307/2529712]
42 **Taves DR**. Minimization: a new method of assigning patients to treatment and control groups. *Clin Pharmacol Ther* 1974; **15**: 443-453 [PMID: 4597226 DOI: 10.1002/cpt1974155443]
43 **Wei L-J**. A class of designs for sequential clinical trials. *Journal of the American Statistical Association* 1977; **72**: 382-386 [DOI: 10.1080/01621459.1977.10481005]
44 **Wei L-J**. The adaptive biased coin design for sequential experiments. *The Annals of Statistics* 1978; **6**: 92-100 [DOI: 10.1214/aos/1176344068]
45 **Xu Z**, Proschan M, Lee S. Validity and power considerations on hypothesis testing under minimization. *Stat Med* 2016; **35**: 2315-2327 [PMID: 26787557 DOI: 10.1002/sim.6874]
46 **Berger VW**. Minimization, by its nature, precludes allocation concealment, and invites selection bias. *Contemp Clin Trials* 2010; **31**: 406 [PMID: 20457277 DOI: 10.1016/j.cct.2010.05.001]
47 Committee for Proprietary Medicinal Products (CPMP). Committee for Proprietary Medicinal Products (CPMP): points to consider on adjustment for baseline covariates. *Stat Med* 2004; **23**: 701-709 [PMID: 14981670 DOI: 10.1002/sim.1647]
48 **Shnier A**, Lexchin J, Romero M, Brown K. Reporting of financial conflicts of interest in clinical practice guidelines: a case study analysis of guidelines from the Canadian Medical Association Infobase. *BMC Health Serv Res* 2016; **16**: 383 [PMID: 27528247 DOI: 10.1186/s12913-016-1646-5]
49 **Stang A**. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; **25**: 603-605 [PMID: 20652370 DOI: 10.1007/s10654-010-9491-z]
50 **Herskovic A**, Martz K, al-Sarraf M, Leichman L, Brindle J, Vaitkevicius V, Cooper J, Byhardt R, Davis L, Emami B. Combined chemotherapy and radiotherapy compared with radiotherapy alone in patients with cancer of the esophagus. *N Engl J Med* 1992; **326**: 1593-1598 [PMID: 1584260 DOI: 10.1056/NEJM199206113262403]
51 S3-Leitlinie Diagnostik und Therapie der Plattenepithelkarzinome und Adenokarzinome des Esophagus. Publiziert bei: AWMF online. Leitlinien programm Onkoligie 2018. Version 2.0.
52 **Martin-Richard M**, Díaz Beveridge R, Arrazubi V, Alsina M, Galan Guzmán M, Custodio AB, Gómez C, Muñoz FL, Pazo R, Rivera F. SEOM Clinical Guideline for the diagnosis and treatment of esophageal cancer (2016). *Clin Transl Oncol* 2016; **18**: 1179-1186 [PMID: 27900538 DOI: 10.1007/s12094-016-1577-y]

Published By Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-2238242
E-mail: bpgoffice@wjgnet.com
Help Desk:https://www.f6publishing.com/helpdesk
https://www.wjgnet.com