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REVIEW

Novel biomarkers for patient stratification in colorectal cancer: A review of definitions, emerging concepts, and data

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Abstract

Colorectal cancer (CRC) treatment has become more personalised, incorporating a combination of the individual patient risk assessment, gene testing, and chemother-

apy with surgery for optimal care. The improvement of staging with high-resolution imaging has allowed more selective treatments, optimising survival outcomes. The next step is to identify biomarkers that can inform clinicians of expected prognosis and offer the most beneficial treatment, while reducing unnecessary morbidity for the patient. The search for biomarkers in CRC has been of significant interest, with questions remaining on their impact and applicability. The study of biomarkers can be broadly divided into metabolic, molecular, microRNA, epithelial-to-mesenchymal-transition (EMT), and imaging classes. Although numerous molecules have claimed to impact prognosis and treatment, their clinical application has been limited. Furthermore, routine testing of prognostic markers with no demonstrable influence on response to treatment is a questionable practice, as it increases cost and can adversely affect expectations of treatment. In this review we focus on recent developments and emerging biomarkers with potential utility for clinical translation in CRC. We examine and critically appraise novel imaging and molecular-based approaches; evaluate the promising array of microRNAs, analyze metabolic profiles, and highlight key findings for biomarker potential in the EMT pathway.

Key words: Biomarker; Colorectal cancer; Epithelial-to-mesenchymal-transition pathway; Molecular biomarker; MicroRNA; Metabolic biomarker; Imaging biomarker; Tumour regression grade

Core tip: Biomarkers are an emerging field that can potentially guide the diagnosis, prognosis, and treatment course in rectal cancer. Here, the current definitions, classifications, recent developments and emerging biomarkers with potential utility for clinical translation in colorectal cancer are reviewed by international experts for a better understanding in surgery.

Chand M, Keller DS, Mirnezami R, Bullock M, Bhangu A, Moran B, Tekkis PP, Brown G, Mirnezami A, Berho M. Novel biomarkers for patient stratification in colorectal cancer: A review of definitions, emerging concepts, and data. *World J Gastrointest Oncol* 2018; 10(7): 145-158 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i7/145.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i7.145

INTRODUCTION

Colorectal cancer (CRC) is one of the most common types of cancer and cancer related deaths worldwide, with more than a third of the incidence involving the rectum[1,2]. Historically, rectal cancer was associated with the worst oncological outcomes[3]. The choice of treatment for rectal cancer was traditionally based upon the histologic type of malignancy, stage of the disease, the tumour-node-metastasis (TNM) staging system, and circumferential resection margin (CRM) status[2,4]. These variables provide clinical utility, help determine the need for neoadjuvant chemoradiotherapy (CRT) in patients with a threatened or involved CRM, post-operative adjuvant treatment in stage Ⅲ disease, and are prognostic of oncological outcome. Nevertheless, they provide an incomplete picture, as many patients with predicted early-stage disease harbour lymph node and systemic micrometastases, which can ultimately result in local and/or distant disease recurrence. Administration of neoadjuvant CRT is also sub-optimal as this treatment modality has many side effects, some of which are fatal, while others impair quality of life (QOL). Response to CRT is also unpredictable; up to 30% of patients will have a complete pathological response (pCR = tumour regression grade 1, TRG1), and could have omitted surgery altogether[5,6]. In 10% of cases however, no reduction in tumour volume is achieved, (tumour regression grade 5, TRG5); patients get no benefit from CRT, but are exposed to its side effects and may also experience cancer progression from delay to surgery[7]. These observations underscore the limitations of current methods for accurate stratification of patients with rectal cancer, and highlight the pressing need to identify biomarkers indicative of aggressive disease and/or response to CRT, in order to avoid patient under- or over-treatment.

With the advent of the "holy plane", standards for utilising chemoradiation, the application of minimally invasive surgery, and multidisciplinary tumour boards to guide care, the diagnosis, staging and management of rectal cancer has improved significantly in the past 25 years[8-18]. However, considerable variation still exists in management and outcomes, and recurrence continues to be a problem, with 5-year survival rates stubbornly below 60% in most European countries[19]. To further improve outcomes, there is a paradigm shift in the methods of diagnosis, staging, determining the patient's prognosis, and developing a personalized therapeutic course using advances in molecular biology, genetics, biochemistry, imaging, and the individual patient's personal risk assessment, neoadjuvant chemoradiotherapy, and adjuvant chemotherapy with surgery to optimise care[20].

The routine evaluation of microsatellite instability (MSI) and KRAS/NRAS/BRAF mutational status in clinical practice, for risk stratification in stage Ⅱ CRC and to determine the utility of monoclonal antibody-based adjuvant therapy, such as panitumumab or cetuximab, in metastatic disease, provides a clear proof-of-concept that more tailored therapeutic strategies can be translated to improve patient care through identification of biomarkers with functional activity. In this review, we explore the recent developments and emerging biomarkers with potential utility for clinical translation in CRC. We examine and critically appraise both novel imaging and molecular pathology based approaches; evaluating the promising array of microRNAs with biomarker potential; examining the developing techniques

and studies analysing metabolic profiles, and highlight key findings in the biomarker potential in the epithelial-to-mesenchymal-transition (EMT) pathway.

BIOMARKERS: TERMS OF REFERENCE, CONCEPTS, AND CLASSIFICATION

From the Biomarkers Definitions Working Group, the formal definition of a biomarker is a tumour characteristic that can be objectively measured and evaluated as an indicator(s) of normal biological or pathogenic processes, or pharmacologic responses to a therapeutic intervention that identify increased or decreased risk of patient benefit or harm[21,22]. Biomarkers can take multiple forms when used to detect or confirm presence of disease or to identify affected individuals[23]. Table 1 shows the categorisation of biomarkers. Most biomarkers applicable in CRC are prognostic - providing information about the likelihood of a condition, disease recurrence or progression; or predictive - providing information about the likelihood to respond to specific treatments. A cause of confusion around biomarkers has been the loose application of their definition and application. Distinguishing between predictive and prognostic biomarkers- which may not be mutually exclusive- has been another source of confusion in patient stratification and developing treatment strategies[23]. Another source of confusion is the inconsistent terminology previously used, restricting the scope of biomarkers to describing biological molecules or monitoring the treatment response. The current definition laid out by Cancer Research United Kingdom provides a standardised vocabulary for investigators, explicitly stating, "molecular, histologic, radiographic or physiologic characteristics are examples of biomarkers"[24]. With this progression, biomarkers may be used in a variety of situations and serve a number of purposes - as a diagnostic tool; for risk-stratification and staging of disease; as an estimator of prognosis; and, for prediction of disease response. The study of such biomarkers can be broadly divided into metabolic; miRNA; EMT; and imaging biomarkers. This review describes the current status of biomarkers in CRC within this framework.

MOLECULAR MARKERS ASSOCIATED WITH CARCINOGENESIS PATHWAYS

The search for molecular markers in CRC has been of significant recent interest. Extensive research has revealed that CRC develops through three major pathways: (1) chromosomal abnormalities that lead to mutations of oncogenes and tumour suppressor genes (classic pathway), characterised by the adenoma-carcinoma progression; (2) the microsatellite instability pathway that results from defects in the DNA repair system; and (3) the methylation pathway characterized by the epigenetic (post cellular division) methylation of numerous genes (methylator pathway). Hundreds of molecules involved in the chromosomal instability pathway have been associated with prognosis, however, only 1 single marker- the epidermal growth factor receptor (EGFR) pathway-has successfully proven clinical utility to date, largely due to the complexity and redundancy of cellular pathways, as well as the lack of therapies that can target the different biomarkers.

The EGFR pathway is the most clinically relevant molecule involved in the chromosomal instability pathway, and the EGFR serves as the main target for treatment in locally advanced CRC. However, this treatment is only useful for patients with wild-type KRAS (wtKRAS)[25]. Abnormal activation of the EGFR signalling pathways in CRC is mainly associated with three mutations in the mitogen-activated protein kinase and phosphatidylinositol-3-kinase (PI3K) pathways - KRAS, NRAS, and BRAF; these three mutations are reported to occur in more than half of all CRC cases[26]. Mutation of some of the components of the EGFR pathway, specifically BRAF V600E, KRAS (exon 2, 3, 4), and NRAS mutation (exon 2, 3, 4) cause the malignant cells to become resistant to anti-EGFR therapy; thus, patients should not be treated with either cetuximab or panitumumab. As a result, all patients with metastatic CRC should have investigation of KRAS/NRAS and BRAF mutation status prior to the start of treatment. KRAS/NRAS and BRAF mutational status may be performed by a variety of techniques, detailed discussion of the different methodologies is out of the scope of this review, however it is essential to emphasize that several technical factors including tissue fixation and tumour volume amongst others may affect the accuracy of the test results leading to erroneous information with the consequent impact on the decision making process. Furthermore, any tumour molecular analysis should be performed only by a certified laboratory that can prove competency and proficiency to perform testing.

Microsatellite instability status (MSI) (high or low) is the primary molecular marker for stratification of stage Ⅱ CRC. In node negative CRC, patients that are MSI-high have better outcomes than MSI-low tumours; therefore, adjuvant chemotherapy is usually not indicated in MSI-high tumours. MSI-high tumours arise in the setting of a defective DNA repair machinery, although several proteins have been implicated in DNA repair, abnormalities in MSH2, MSH6, PMS2 and MLH1 are the most commonly described. MSI-high tumours may be the result of an inherited mutation of the DNA repair genes (Lynch syndrome) or, more commonly, the abnormal epigenetic methylation of the *MLH1* promoter gene (sporadic MSI-high CRC). Analysis of the DNA repair system may be directly investigated by the tissue expression of MSH2, MSH6, PMS2 and MLH1 by immunohistochemistry, or alternatively by determination of microsatellite status by PCR.

The CpG Island Methylator Phenotype (methylator) pathway has been associated with a constellation of clinical (elderly patients, female, right-sided colon tumours) and histological features (poorly differentiated

Table 1 Biomarker types and definitions

Biomarker type	Objective
Diagnostic biomarker	These aim to identify the type of cancer, *e.g.*, PSA, CEA. They may also be used to monitor or detect disease recurrence
Pharmacological biomarker	These are used to measure response to a specific drug treatment. They are based on accurate pharmokinetic data and measure treatment response in early drug trials, *e.g.*, drug therapy to angiogenesis
Predictive biomarker	These are used to identify individuals who will most likely show a survival benefit to a specific targeted treatment, *e.g.*, improvement in local recurrence risk following treatment for circumferential resection margin involvement
Prognostic biomarker	These indicate the progress of disease and to estimate the risk of disease recurrence for example. They are used to estimate survival outcome and are independent of treatment strategy, *e.g.*, nodal disease
Risk/predisposition biomarker	These aim to identify individuals who are at significant risk of developing tumours, *e.g.*, *MLH1* gene
Screening biomarker	These are used to identify disease at an early stage, *e.g.*, PSA
Surrogate response biomarker	These can be used as an alternative to a clinically meaningful endpoint. Therefore there must be correlation with a clinical endpoint, *e.g.*, CEA

tumours and advanced stage disease). This pattern seen in approximately 15%-20% of CRCs, and involves atypical methylation of the mismatch repair gene *MLH1*. The precursor lesions in CIMP cancers are serrated polyps, not adenomatous lesions, with the initial mutation occurring most often in the *BRAF* oncogene[27]. BRAF mutations transform normal mucosa to aberrant crypt foci, hyperplastic, or sessile serrated polyps (SSP). With promoter methylation, loss of p16 occurs, allowing cells to progress to advanced polyps[28]. Increasing activity leads to methylation of MLH1, silencing transcription. Loss of MLH1 results in MMR deficiency and the MSI-H CRC phenotype. This is clinically important for diagnosis and therapeutic planning. An estimated 85% of MMR deficiency CRC is due to methylation of the MLH1 promoter region. BRAF can be used to distinguish between MLH1 promoter methylation and Lynch syndrome as the cause of CRC. A positive BRAF mutation is associated with the methylator pathway, and indicates MLH1 down-regulation through somatic methylation of the gene's promoter region, not through a germline mutation. BRAF mutations are rare in Lynch Syndrome-related CRC. On the converse, MLH1 promotor methylation in the absence of a BRAF mutation is consistent with Lynch Syndrome. Figure 1 shows a clinical algorithm for testing MMR deficiency. Several promising new therapies aimed at demethylation of genes are being developed.

METABOLIC PROFILING APPROACHES

In recent years the majority of molecular profiling approaches applied to the study of rectal cancer have focused on macromolecules (DNA, RNA, protein). While these avenues of research continue to offer significant insights into rectal cancer development and progression[29,30], it is widely accepted that a macromolecular, "bottom up" view of system activity cannot provide all the answers to facilitate precision approaches for rectal cancer diagnosis, prognosis and therapeutic personalisation[31]. Metabonomics (metabolomics/metabolic profiling) offers a dynamic "top down" view of system activity and is defined as the systematic, time-dependent measurement of metabolic shifts occurring in response to drugs, environmental stimuli or disease[32-34]. This approach provides rich *micro*molecular data downstream of the genome and proteome, offering a genuine functional "snapshot" of system activity[33].

The basic concept of altered cancer metabolism is well described across a variety of cancer subtypes[35-38]; the Warburg effect[39] is central to our understanding of cancer metabolism and glycolytic flux forms the basis for [^{18}F]-fluorodeoxyglucose enhanced positron emission tomography (FDG-PET) solid tumour imaging[40]. Current and next-generation nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)-based profiling platforms offer a means of interrogating the cancer metabolome in unprecedented detail and moving beyond the Warburg phenomenon to identify an entirely new pool of disease-relevant biomolecular data. These profiling approaches are likely to have three main areas of application in rectal cancer phenotyping: (1) to identify novel metabolic fingerprints for accurate and ultra-fast tumour tissue diagnosis, staging and grading; (2) to develop metabolite-based models for prediction of response to chemo and/or radiotherapy; and (3) to devise novel next-generation targeted therapies designed to disrupt specific metabolic pathways implicated in rectal cancer.

NMR spectroscopy techniques are highly versatile and have been developed and applied for metabolic profiling of liquid-state and solid-state systems[41,42]. The technique of HR-MAS NMR has been introduced more recently to overcome spectral line-broadening effects seen with conventional NMR analysis of solids[41]. This approach allows acquisition of tissue-specific high-resolution spectra, which in combination with chemometric data treatment methods have the capacity to identify novel molecular signatures within rectal cancer tissue[43]. Recent work in this area has demonstrated increased abundance of taurine, glycine, lactate and scyllo-inositol in cancerous relative to healthy rectal mucosa, with a relative reduction in abundance observed for lipids and glucose[44] (Figure 2). These findings can be used to determine tissue status (cancerous or healthy) by entirely biochemical means, and have also revealed strong differences in metabolite profiles according to tumour stage[44]. From a pharmaco-

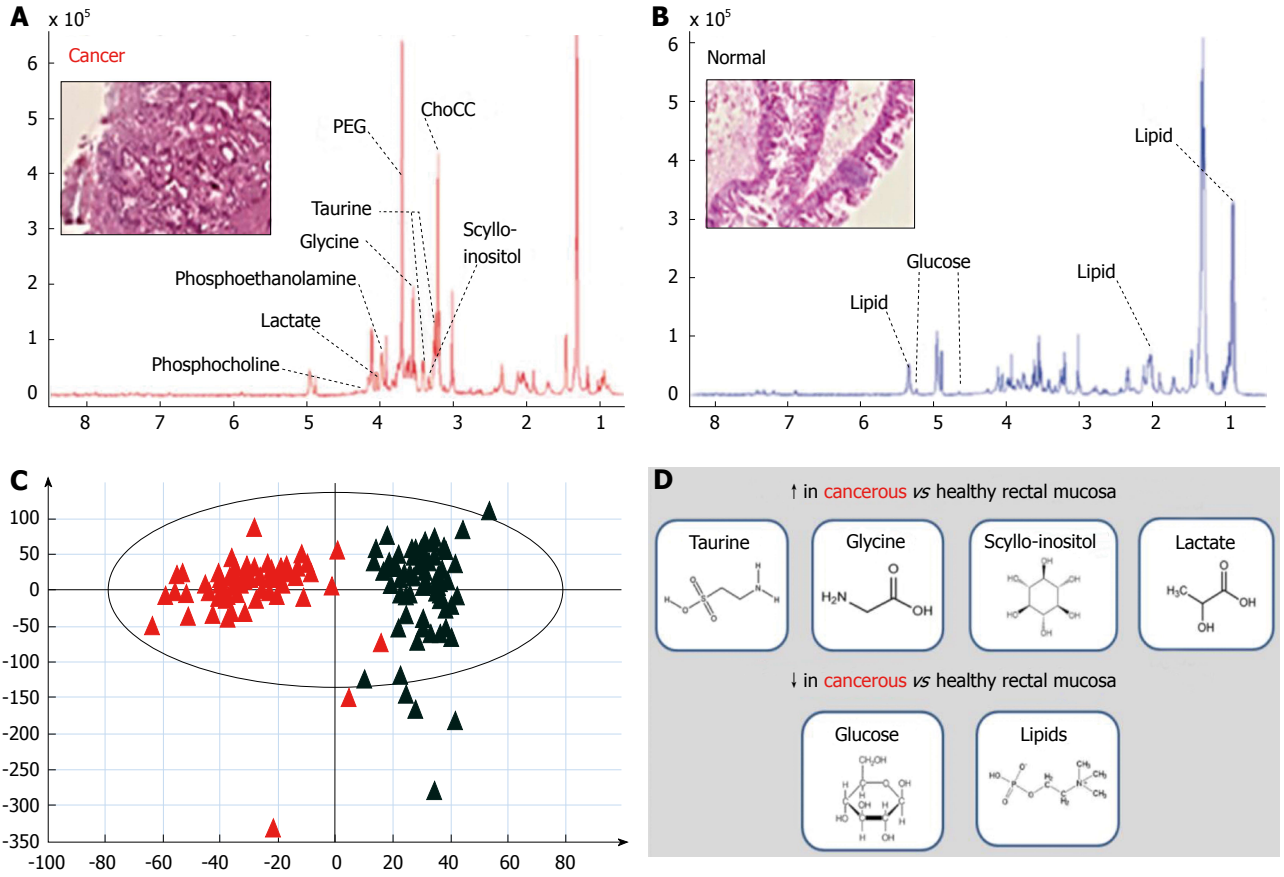

Figure 1 High-resolution magic angle spinning nuclear magnetic resonance spectroscopy of intact rectal cancer tissue biopsies. A and B: Annotated representative HR-MAS NMR spectral metabolite pattern for rectal cancer (A) and healthy rectal mucosa (B); C and D: Acquired data can then be subjected to supervised and un-supervised multivariate analysis using PCA and PLS-DA (C) to determine metabolic processes up- and down-regulated in cancerous tissue (D) (original data). NMR: Nuclear magnetic resonance; PCA: Principal component analysis; PLS-DA: Partial least squares discriminant analysis.

therapeutic perspective these discoveries offer the chance to develop novel anti-cancer agents; for example, taurine (2-aminoethane sulphonic acid), a common beta-amino acid has a known role in a number of fundamental physiological functions including cellular osmoregulation, cell-membrane stabilization and protein assembly[45]. Exploiting this finding by disrupting taurine handling within the rectal cancer microenvironment may offer a means of developing next-generation targeted agents for rectal cancer down-staging[46].

Mass spectrometry approaches have shown recent promise in the development of metabolite-based biomarker discovery for prediction of response to chemoradiotherapy. Crotti *et al*[47] described novel peptidomic methodology in an analysis of samples of serum collected pre- and post-CRT subjected to matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry. A comparison of pre-treatment serum fingerprints from responders [Mandard tumour regression grade (TRG) 1 and 2] and non-responders (Mandard TRG 3-5) identified three peptides (m/z 1082.552, m/z 1098.537 and 1104.538) that were capable of robust class separation. Kim and colleagues also used a MALDI-based approach, but specifically sought to evaluate the abundance of low-mass ions (< m/z 1000) in serum samples acquired from 73 patients with locally advanced rectal cancer, prior to CRT[48]. A panel of nine low-mass ions were found to have discriminatory capacity, with hypoxanthine (HX; m/z 137.08) and phosphoenolpyruvic acid (PEP; m/z 169.04) highlighted as the most significant. Lower levels of HX and higher levels of PEP were shown to strongly correlate with improved response to CRT (TRG 1, 2). These studies indicate the exciting potential for the development of a circulating biomarker panel to predict chemoradiosensitivity prior to commencing therapy.

MiRNA AND RESPONSE TO TREATMENT

MicroRNAs (miRNA) are highly conserved, short, non-coding nucleotide segments that regulate gene expression post-transcriptionally through repressing translation or targeting mRNAs for degradation[49]. miRNA genes account for between 2%-5% of the human genome and are commonly clustered within introns[50]. Each miRNA is estimated to interact with multiple mRNA targets and, as a consequence, thus, these sequences may regulate more than 30% of all human genes[51,52]. Oncogenes and tumour-suppressor genes are being discovered under miRNA control, with the majority of miRNA genes found within cancer-associated genomic regions[53,54]. In CRC,

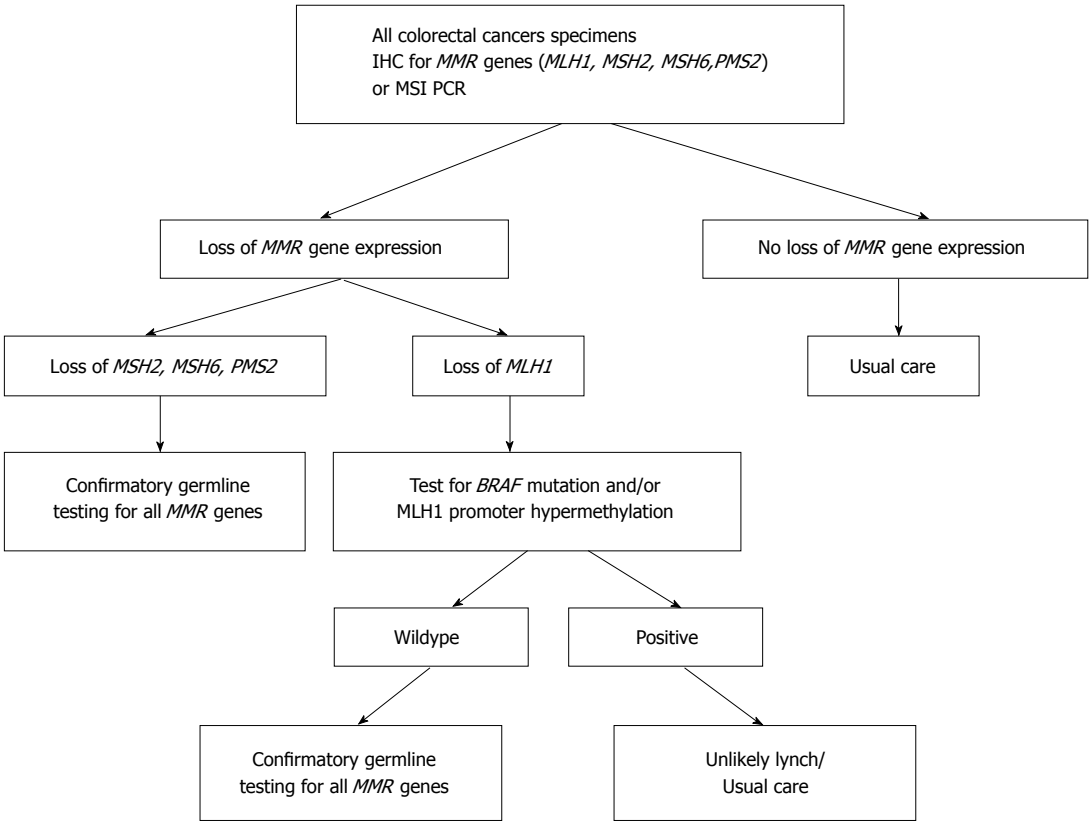

Figure 2 Algorithm for testing of mismatch repair genes in colorectal cancer for Lynch syndrome. MMR: Mismatch repair; MSI: Microsatellite instability.

abnormally expressed miRNAs disrupt cellular signal transduction and cell survival pathways, such as Wnt/β-catenin, EGFR, and p53, linking miRNA to known events in the pathway of malignant transformation[55].

Accumulating evidence suggests that miRNAs may also have powerful clinical applications. miRNA expression profiles are capable of discriminating tumours of different developmental origin[56]. Furthermore, the expression of individual miRNAs may be used to predict patient survival, tumour stage, the presence of lymph node metastases and the response to therapy in CRC[55,57,58].

Three studies have specifically examined the utility of miRNA expression signatures in predicting chemoradiotherapy response in rectal cancer[59-61]. Della Vittoria Scarpati *et al*[59] examined miRNA expression in fresh-frozen pre-treatment tumour specimens from 38 patients with locally advanced (T3/T4 Node +ve) rectal cancer and compared miRNA profiles in patients with complete (Mandard TRG 1; n = 9) and incomplete (Mandard TRG > 1; n = 29) pathological responses to a standardised neoadjuvant chemoradiotherapy regime consisting of capecitabine, oxaliplatin and 45 Gy of pelvic conformal radiotherapy. Thirteen significantly differentially expressed miRNAs were subsequently validated using high sensitivity TaqMan® qRT-PCR, of which 2; miR-622 and miR-630, were found to predict chemoradiotherapy response with 100% sensitivity and specificity[59].

A similar analysis of 20 patients undergoing combined radiotherapy and capecitabine/5-FU chemotherapy compared "responders", namely those displaying a positive response to treatment (Mandard TRG 1 and 2) with "non-responders" (Mandard TRG 3-5). TaqMan Low Density Arrays identified a miRNA signature consisting of 8 miRNAs capable of correctly classifying 90% (9/10) of responders and 90% (9/10) of non-responders[60].

A third study, which used formalin fixed rather than fresh rectal cancer specimens identified a miRNA signature consisting of just 3 miRNAs (miR-153, miR-16 and miR-590-5p), capable of distinguishing patients with complete and incomplete responses to therapy, however the value of this data is unclear as patient demographics, tumour characteristics, study end-points and the neoadjuvant treatment strategy were not clearly described[61].

As profiling methodology and the definition of tumour regression vary between these 3 studies, inter-study comparisons are of limited value; however it is important to note that no overlap is observed between the miRNA signatures described. This suggests that an miRNA based "therapy-response" prediction tool is some way from becoming a reality however; other studies have clearly established that miRNAs do play a role in regulating the tissue response to neoadjuvant therapy in CRC[62-64]. Perhaps by focusing on the contribution of miRNAs within the biological pathways that govern resistance and/or sensitivity to neo-adjuvant therapy in rectal cancer, more clinically pertinent data will emerge on the role of miRNA as a potential biomarker in cancer treatment strategies[65].

EMERGING TECHNOLOGY, LIQUID BIOPSIES

The term "liquid biopsy" in cancer arose when circulating tumor cells (CTC) were proposed as alternatives to conventional tissue biopsy in breast cancer for prognosis and evaluation of treatment responses[66]. The theory has continued to grow experimentally and has gained particular traction in CRC. The clinical applications of liquid biopsy in CRC continue to grow, including detecting premalignant and early-stage cancers, identification of aggressive phenotypes and high-risk patients, assessing tumor heterogeneity, residual, and recurrent disease, and monitoring treatment response[67]. In colon cancers, liquid biopsies may hold prognostic information beyond the nodal status for determining whether to administer adjuvant chemotherapy, while in rectal cancer, liquid biopsy may have roles for both primary disease evaluation and monitoring treatment response[68]. Possible sources of liquid biopsies include blood, urine, saliva, and stool, which contain cancer-derived subcellular components, such as circulating tumor DNA (ctDNA) and circulating miRNAs.

Tumour-tissue remains the "gold standard", but the advent of ctDNA analysis from blood samples has promise as a non-invasive biomarkers. Studies have reported a direct relationship between ctDNA levels and tumor burden, stage, vascularity, cellular turnover, and response to therapy[69-71]. It can enable efficient temporal assessment of disease status, response to intervention, and early detection of recurrence superior to current strategies, such as CEA[72]. ctDNA can monitor and recognize high-risk individuals, as the plasma tumour DNA levels are significantly higher in patients with increased advanced/stage Ⅳ disease, recurrence, or metastasis[73,74]. ctDNA may be sensitive to detect with early, presumably curable CRC from common mutations, which could have implication for diagnostic testing[75]. Meta-analysis has demonstrated high overall sensitivity and specificity for detecting the *KRAS* oncogene mutation in CRC, showing it may be a viable alternative to tissue analysis for the detection of *KRAS* mutations and subsequent therapeutic planning[75]. Further, comparative analysis between CTCs and ctDNA in metastatic CRC has shown strong concordance between ctDNA and tissue for RAS, BRAF, and ERBB2 mutations (84.6%) and greater detectability than CTCs with a smaller amount of blood sampling[76]. ctDNA may hold specific promise as a biomarker to guide therapy in post-operative locally advanced rectal cancer, but further studies are needed for validation[77]. There are limitations to ctDNA as a biomarker. Although ctDNA targets offer a high specificity, it is scarce in circulating biofluids- representing less than 1% of the total circulating free DNA and may be inadequate as clinically applicable diagnostic biomarkers. The best source of ctDNA is still uncertain and the size of the DNA released from dead cancer cells is longer than that of non-neoplastic DNA[70,78]. Large scale controlled trials are needed for validation.

miRNA is an alternate for liquid biopsy. miRNAs have features making them ideal candidates for development as disease-specific biomarkers, and may offer superior sensitivity and specificity compared with ctDNA for diagnosing CRC[79]. miRNAs are generally stable in blood and other body fluids due to their small size and their ability to escape from RNase-mediated degradation. miRNA expression levels are different in tumour compared to normal colon tissues[80]. miRNA are actively secreted from living cells, while most ctDNA is dependent on release from apoptotic or necrotic cells[81,82]. miRNA-based diagnostic markers and panels have been identified for early detection, risk of recurrence at the time of diagnosis, complement to CEA for identification of distant metastasis, and stratification of patients with poor prognosis and greater likelihood of metastasis to the lymph nodes, liver, and peritoneum[80,83-88]. These miRNAs are detailed in Table 2. While a promising tool for "precision medicine", there are limitations of circulating miRNAs as biomarkers in CRC. The existing studies use relatively small sample sizes, are retrospective in design, and utilized non-standardized sampling procedures. Larger, controlled studies are needed in order to validate the best purification method and clinical use of circulating miRNAs in CRC.

An example of a blood sample-based diagnostic biomarker that could make a clinical impact is methylated *Septin 9* (m*SEPT9*), which is validated to distinguish CRC from normal blood using real-time PCR[89]. This non-invasive, blood-based tool for CRC could improve screening and surveillance compliance over colonoscopy and other screening methods[90]. While monitoring of mSEPT9 may hold promise for CRC screening, a larger study population and more prospective studies are needed to validate mSEPT9 as a diagnostic biomarker in CRC.

ROLE OF EPITHELIAL MESENCHYMAL TRANSITION IN PRODUCING RECTAL CANCER CELLS WITH A RADIORESISTANCE PHENOTYPE

EMT is a physiological process resulting in transformation of stable epithelial cells into mobile mesenchymal cells[91]. While EMT is a normal process during human development, it has also been shown to occur in carcinogenesis[92]. In this situation, the resulting abnormal mesenchymal cells, which evade the influence of normal cellular control mechanisms, display an aggressive and invasive phenotype. These cells are increasingly linked to formation of micro-metastases, and causation of resistance to the effects of radiotherapy.

EMT cellular biology

Down-regulation of membranous E-cadherin is the classical finding of EMT. This results in loss of intercellular epithelial junctional complexes, promoting migration of

Table 2 Candidate liquid biopsy/circulating miRNA biomarkers[145]

Expression level	Diagnostic biomarker	Prognostic biomarker (malignant potential, tumor recurrence)	Predictive biomarker (chemosensitivity)
High	miR-92a, miR-141, let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, miR-23a, miR-378	miR-141, miR-320, miR-596, miR-203	miR-106a, miR-484, miR-130b
Low		miR-15a, miR-103, miR-148a, miR451	

Adapted from Tsutomu Kawaguchi *et al*. Circulating MicroRNAs: A Next-Generation Clinical Biomarker for Digestive System Cancers. *Int J Mol Sci* 2016; 17: 1459.

cells[93-95]. The microRNA-200 family has been identified as a key post-transcriptional regulator of this process, through its targeting of E-cadherin transcriptional receptors[96]. Subsequent escape from growth factor control, with uncontrolled proliferation, results from the EMT process[94,95]. An end consequence of this pathway is tumour budding, defined as the presence of single cells or small cell clusters at the invasive front of tumour growth[97]. Tumour budding is highly likely to be associated to EMT at the poorly differentiated invasive front[97-100].

Current evidence

There is increasing evidence linking EMT to chemoresistance in ovarian, pancreatic and breast cancer cell lines[101-104], and in human lung cancer specimens[105]. Emerging evidence is also relating EMT to response to chemoradiotherapy in CRC. This initially arose from testing chemoresistance in colorectal cell lines[106-108]. However newer human evidence is relating EMT as an independent biomarker of tumour budding, lymph node metastases, and radioresistance[109]. The largest of these demonstrated that, in 103 patients with advanced rectal cancer, an EMT phenotype was associated with non-response to neoadjuvant therapy and reduced cancer specific survival[110]. More evidence from human rectal cancer tissue is urgently needed to assess its potential as a biomarker.

Windows for intervention

A genetic predisposition to loss of E-cadherin and subsequent EMT may be causative, meaning that pre-treatment biopsy analysis presents a window for intervention. Radiotherapy may also be a traumatic triggering stimulus which forces some cells into an EMT phenotype, meaning other methods for patient selection may be required; overlap in causation is likely.

EMT as a prognostic and therapeutic biomarker

The biological action of metformin down-regulates the EMT transcription factors and up regulations E-cadherin[110]. Its low toxicity profile makes it a feasible option in EMT prevention attempts, with subsequent improvements in response to neoadjuvant therapies[111,112]. Additionally, cyclo-oxygenase (COX) inhibitors have shown potential to prevent EMT by reducing vimentin expression and increasing cell surface E-cadherin expression in cell line models[113]. However, due to their serious associated cardiovascular side-effects, the particular COX agent and dose require optimisation before widescale use[113,114]. The potential role of post-transcriptional microRNA-200 regulation presents a further potential therapeutic target[96].

ROLE OF IMAGING BIOMARKERS IN DETECTION AND MONITORING DISEASE

The concept of an imaging biomarker is relatively new, but one which is becoming an increasingly important component of many phase Ⅱ/Ⅲ clinical trials as a surrogate endpoint. Imaging biomarkers may allow objective assessment of the tumour response to therapy and/or non-invasively detect early disease. Currently, the imaging techniques that seek to quantify treatment response in CRC can be broadly divided into those which measure tumour size and those which measure tumour activity. Whilst size criteria are the more commonly used biomarkers to assess radiological response in clinical trials because of their association with survival outcomes, it is the functional imaging techniques which are feted as having the greatest potential in uncovering the underlying biological processes which lead to cancer.

Measuring changes in tumour size

Reduction in tumour size has been shown to be a useful biomarker[115]. This can be measured in one-, two- or three-dimensions by various routine imaging techniques such as CT and MRI[116]. However, the two commonly used criteria - WHO[117] and RECIST[118] (Table 1); have contrasting characteristics, in particular in the technique used to measure tumour size - only one dimension using RECIST criteria. Further limitations to using size measurements have been deciding on what degree of tumour bulk reduction constitutes a significant clinical response. An example of this is has been shown by Morgan *et al*[119], who investigated the effect of a VEGF receptor inhibitor on colorectal metastases, whereby significant size reduction was not met with an equally significant overall response (< 10%). However the novel MRI-based tumour regression grade (mrTRG), which stratifies response on the degree of fibrosis visualised in the tumour following chemoradiotherapy, has been shown to be a useful clinical tool[120]. The degree of fibrosis seen on MRI following CRT on a scale analogous to histopathological tumour regression grade (TRG)[121] - tumour signal that has been completely replaced by radiological evidence of fibrosis is defined as radiological

complete response (mrTRG1-2)[122]. These findings have been validated in a prospectively enrolled, multicentre study[123] and used to influence treatment decisions in particular "deferral of surgery" programs. In the above study, multivariate analysis showed mrTRG hazard ratios (HR) were independently significant for overall and disease-free survival. Using fibrosis as a radiological feature is not limited to measuring tumour size but can be used to quantify other prognostic factors such as extramural venous invasion (EMVI), for example[120]. A further study using prospectively collected data on EMVI response to neo-adjuvant chemoradiotherapy showed hazard ratio of 2.37 for DFS in tumours which had undergone more than 50% fibrosis of tumour signal in extramural vasculature[124].

Measuring tumour activity

These techniques involve analysis of images to quantify the functional activity of tumours. The most common example of this is positron emission tomograhy (PET) with Fluorodeoxyglucose (18-FDG), which relies on the principle of a differential glycolytic rate seen in tumour cells. Using the glucose analogue 18-FDG gives an assessment of tumour metabolism[125,126] by quantification of standard uptake values (SUV). However as timing of the scans from administration of the 18-FDG and subsequent clearance rates may vary between centres and patients, comparisons and standardisation of technique has been difficult. It is also important to note that until now, there has been no validation of response.

Dynamic contrast-enhanced (DCE) CT/MRI provides a detailed assessment of tumour bloodflow through acquisition of data as specific contrast material passes through the vasculature. DCE-CT has the potential to identify angiogenesis and has been shown to be able to distinguish from diverticular disease as well as detect early liver metastases[127,128]. Although reports have identified a correlation between tumour blood flow, the development of metastases, and decreased survival outcomes[129,130], this has not been translated to widespread clinical application. Vascular endothelial growth factor (VEGF) is upregulated in up to 78% of CRCs[131,132] and is a potential target for functional imaging techniques. Bevacizmab is an anti-VEGF-A monoclonal antibody and DCE-MRI has been used in rectal cancer to evaluate treatment response using conjugation with a radioclueotide[133-135]. The analysis in DCE-MRI uses two compartments of plasma and extravascular-extracellular space to compare contrast agent - K^{trans} is the constant which is used to depict the bloodflow. Several studies have validated K^{trans} with expression of growth factors, such as VEGF and immunohistochemical confirmation of vessel architecture[136-139]. Reduction in K^{trans} using Vatalanib (tyrosine kinase inhibitor which target VEGF receptor-2) for metastatic CRC with liver disease have shown promising results in the phase Ⅰ/Ⅱ setting[119,140] but not been translated to survival benefit in phase Ⅲ trials.

Diffusion weighted imaging (DWI) assesses the movement of water molecules within cells using diffusion-weighted gradients to T2 sequences. Quantitative analysis is possible by calculation of the apparent diffusion coefficients (ADC), which are inversely correlated with tumour cellularity. DWI has been effective in detecting small liver metastases and differentiation from inflammatory lesion[141-143], as well as detecting lymph node metastases[144], but application has been limited to mainly experimental work.

CONCLUSION

The interest in biomarkers relating to rectal cancer is clearly increasing. They form a new aspect of clinical and laboratory research which help translate these concepts to more meaningful applications in patient management. Much of the current literature is still in its embryonic stage, but as more results from clinical trials using biomarker endpoints and outcome measures become available, there will be a better understanding by clinicians of their potential, with possible future application to improve the predictive and prognosis of rectal cancer.

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REVIEW

HER2 inhibition in gastro-oesophageal cancer: A review drawing on lessons learned from breast cancer

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Abstract

Human epidermal growth factor receptor 2 (HER2)-inhibition is an important therapeutic strategy in HER2-amplified gastro-oesophageal cancer (GOC). A significant proportion of GOC patients display HER2 amplification, yet HER2 inhibition in these patients has not displayed the success seen in HER2 amplified breast cancer. Much of the current evidence surrounding HER2 has been obtained from studies in breast cancer, and we are only recently beginning to improve our understanding of HER2-amplified GOC. Whilst there are numerous licensed HER2 inhibitors in breast cancer, trastuzumab remains the only licensed HER2 inhibitor for HER2-amplified GOC. Clinical trials investigating lapatinib, trastuzumab emtansine, pertuzumab and MM-111 in GOC have demonstrated disappointing results and have not yet changed the treatment paradigm. Trastuzumab deruxtecan may hold promise and is currently being investigated in phase Ⅱ trials. HER2 amplified GOC differs from breast cancer due to inherent differences in the HER2 amino-truncation and mutation rate, loss of HER2 expression, alterations in HER2 signalling pathways and differences in insulin-like growth factor-1 receptor and MET expression. Epigenetic alterations involving different microRNA profiles in GOC as compared to breast cancer and intrinsic differences in the immune environment are likely to play a role. The key to effective treatment of HER2 amplified GOC lies in understanding these mechanisms and tailoring HER2 inhibition for GOC patients in order to improve clinical outcomes.

Key words: Human epidermal growth factor receptor 2; Gastro-oesophageal cancer; Trastuzumab; Resistance; Biomarkers; Breast cancer

Core tip: Human epidermal growth factor receptor 2 (HER2)-inhibition is an important therapeutic strategy

in HER2-amplified gastro-oesophageal cancer (GOC). A significant proportion of GOC patients display HER2 amplification, yet HER2 inhibition in these patients has not displayed the success seen in HER2 amplified breast cancer. We evaluate current clinical and laboratory evidence surrounding HER2 inhibition in GOC. Inherent differences in the HER2 receptor, signalling pathways, associated microRNA signature and immune environment may partly explain the disappointing clinical trial outcomes seen in GOC. Only with improved understanding of HER2 inhibition can effective treatment be provided in order to improve clinical outcomes for patients.

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INTRODUCTION

Cancer therapy is becoming increasingly personalised and molecularly targeted, using biomarkers to identify patients most likely to respond to therapy. Human epidermal growth factor receptor 2 (HER2)-amplified cancer is defined as cancer with HER2 protein overexpression ± *HER2* gene amplification[1]. It represents a molecularly-defined subgroup of malignancy and is known to exist in breast and gastro-oesophageal cancers (GOC), among others[1]. Whereas the treatment for HER2-amplified breast cancer patients has been extremely successful, the treatment for GOC has been less so. In this review, we explore the mechanisms by which HER2 amplification contributes to cancer progression and prognosis, methods of targeting HER2 amplification, mechanisms of resistance to HER2 therapy, strategies to overcome resistance, biomarkers and future directions.

HER2 RECEPTOR AND ITS INTERACTIONS

HER2, encoded by the *ERBB2* oncogene on chromosome *17q21*[2], is a member of the epidermal growth factor receptor (EGFR) family associated with tumour cell proliferation, apoptosis, adhesion, migration and differentiation[3]. All studies investigating HER2 receptor interactions have been conducted in breast cancer cells, and a literature search did not reveal any studies of HER2 receptor interactions conducted specifically in GOC. Given the relatively disappointing results seen in GOC, we suggest it may be worthwhile exploring HER2 receptor interactions specifically in GOC, to investigate whether there are any mechanistic differences in HER2 binding and signalling between breast and GOC.

HER2 RECEPTOR OVEREXPRESSION AND ONCOGENIC MECHANISMS IN BREAST AND GOC

In both breast cancer and GOC, HER2 overexpression occurs in approximately 20%[4,5]. The Gastric Cancer Genome Atlas [part of The Cancer Genome Atlas (TCGA)] recently classified gastric cancer into four subtypes and found that HER2 overexpression occurs only in Epstein-Barr virus (EBV)-positive tumours, genomically-stable (GS) tumours and tumours with chromosomal instability (CIN) but not in microsatellite unstable (MSI-high) tumours[6]. Mechanisms by which HER2 overexpression can be oncogenic are complex, with activation of RAS-MAPK, c-jun and Akt-mTOR pathways[3] (Figure 1). HER2 overexpression may lead to formation of HER2 homodimers and ligand-independent downstream signalling[3]. The majority of studies investigating HER2 overexpression oncogenicity have been conducted in breast cancer, and mechanisms may differ in GOC.

INFLUENCE OF HER2 STATUS ON PROGNOSIS IN BREAST AND GASTRIC CANCER

In contrast to breast cancer, HER2 overexpression does not impact survival in GOC[2]. Large phase Ⅲ prospective randomised controlled trials such as ToGA[5], LOGiC[7] and TYTAN[8] demonstrate that patients with HER2 amplified GOC who receive the control arm (chemotherapy alone) have an overall survival (OS) similar to all-comers (Table 1)[5,7-9]. In the first-line ToGA and LOGiC trials, OS was 11.1 mo and 10.5 mo, respectively, in the control arms[5,7], compared to OS in all-comers of 9.9 mo in the Phase Ⅲ REAL2 trial[10]. In the 2nd-line TYTAN trial, OS was 8.9 mo in the control[8,9], which compared favourably to OS in all-comers treated with paclitaxel in the control arms of the RAINBOW (OS 7.4 mo)[11] and GOLD trials (OS 6.9 mo)[12]. This cross-trial comparison suggests that HER2 overexpression does not adversely affect GOC prognosis.

HER2 SCORING CRITERIA, DISCORDANCE AND HETEROGENEITY IN GOC AND BREAST CANCER

The HER2 scoring system in breast cancer was developed prior to the scoring system for GOC and was standardised in 2007 following an expert panel forum[13]. The ToGA trial used a new immunohistochemistry (IHC) scoring criteria developed by Hofmann[14] for gastric cancer due to inherent biological differences compared to breast cancer, such as tumour heterogeneity and baso(lateral) membrane staining[5,14]. Some criteria were

Table 1 Summary of selected randomized phase Ⅲ HER2 trials in HER2-amplified gastro-oesophageal cancer and breast cancer

Study title	Setting	*n*	Treatment arms	Primary endpoint	OS	PFS	HR and *P* value
Trastuzumab 1st line metastatic							
ToGA[5]	1st line metastatic GOC	594	Trastuzumab + chemotherapy *vs* chemotherapy alone	OS	Trastuzumab + chemotherapy: 13.8 mo (95%CI: 12-16) Chemotherapy alone: OS 11.1 mo (10-13)	Trastuzumab + chemotherapy: 6.7 mo (95%CI: 6-8) Chemotherapy alone: 5.5 mo (5-6)	HR = 0.74; 95%CI: 0.60-0.91; *P* = 0.0046)
Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2[19]	1st line metastatic breast cancer	469	Trastuzumab + chemotherapy *vs* chemotherapy alone	PFS	Trastuzumab + chemotherapy: 25.1 mo Chemotherapy alone: 20.3 mo	Trastuzumab + chemotherapy: 7.4 mo Chemotherapy alone: 4.6 mo	*P* = 0.046
Lapatinib 1st line metastatic							
LOGiC[7]	1st line metastatic GOC	545	Lapatinib + CAPOX *vs* Placebo + CAPOX	OS	Lapatinib + CAPOX: 12.2 mo (95%CI: 10.6-14.2) Placebo + CAPOX: 10.5 mo (9.0-11.3)	Lapatinib + CAPOX: 6 mo (95%CI: 5.6-7.0) Placebo + CAPOX: 5.4 mo (4.4-5.7)	HR = 0.91; 95%CI: 0.73-1.12 *P* value not significant (exact value not given)
Randomized trial of lapatinib *vs* placebo added to paclitaxel in the treatment of human epidermal growth factor receptor 2-overexpressing metastatic breast cancer[29]	1st line metastatic breast cancer	444	Lapatinib + paclitaxel *vs* Placebo + paclitaxel	OS	Lapatinib + paclitaxel: 27.8 mo (95%CI: 23.2-32.2 mo) Placebo + paclitaxel: 20.5 mo (17.9-24.3 mo)	Lapatinib + paclitaxel: 9.7 mo (95%CI: 9.2-11.1 mo) Placebo + paclitaxel: 6.5 mo (5.5-7.3 mo)	HR = 0.74; 95%CI: 0.58-0.94; *P* = 0.0124
Lapatinib 2nd line metastatic							
Tytan[8]	2nd line metastatic GOC	261	Lapatinib + Paclitaxel *vs* Paclitaxel alone	OS	Lapatinib + Paclitaxel: 11.0 mo Paclitaxel alone: 8.9 mo	Lapatinib + Paclitaxel: 5.5 mo Paclitaxel alone: 4.4 mo	HR = 0.84; 95%CI: 0.64-1.11 *P* = 0.1044
Lapatinib plus capecitabine for HER2-Positive advanced breast Cancer[30]	2nd line metastatic breast cancer	324 included in preliminary analysis	Lapatinib + capecitabine *vs* capecitabine alone	PFS	Not reported	Lapatinib + capecitabin: 8.4 mo Capecitabine alone: 4.4 mo	HR = 0.49; 95%CI: 0.34 to 0.71; *P* < 0.001
T-DM1 2nd line metastatic							
GATSBY[35]	2nd line metastatic GOC	345	T-DM1 *vs* taxane	OS	T-DM1: 7.9 mo Taxane: 8.6 mo	T-DM1: 2.7 mo Taxane: 2.9 mo	HR = 1.15, 95%CI: 0.87–1.51; *P* = 0 ·86
EMILIA[33]	2nd line metastatic breast cancer	991	T-DM1 *vs* lapatinib + capecitabine	PFS	T-DM1: 30.9 mo Lapatinib + capecitabine: 25.1 mo	T-DM1: 9.6 mo Lapatinib + capecitabine: 6.4 mo	HR = 0.65; 95%CI: 0.55 to 0.77; *P* < 0.001

HER2: Human epidermal growth factor receptor 2.

the same as breast cancer: HER2 positivity was defined as an IHC score of 3+ and/or *erbB*-2 amplification detected using fluorescent in-situ-hybridisation (FISH)[5,14]. Notably, GOC patients with highly amplified HER2 gene experience better response and survival than patients with lower HER2 gene amplification levels when treated with 1st-line trastuzumab plus chemotherapy for metastatic gastric cancer[15].

HER2 expression in primary and metastatic sites demonstrates heterogeneity more frequently in GOC than in breast cancer[16,17], and discordance between IHC and FISH results occur more frequently in GOC than in breast cancer[18]. This may explain the limited success of targeted anti-HER2 therapy in GOC. If only a small proportion of GOC cells shows HER2 overexpression and if our detection methods are unreliable, GOC cancer cells that do not overexpress HER2 will not be effectively targeted with anti-HER2 therapy, and we may be failing to treat adequately some patients with HER2 overexpression.

THERAPEUTIC AGENTS TARGETING THE HER2 SIGNALLING PATHWAY

Trastuzumab

The efficacy of trastuzumab (a monoclonal antibody against HER2) in breast cancer in combination with ch-

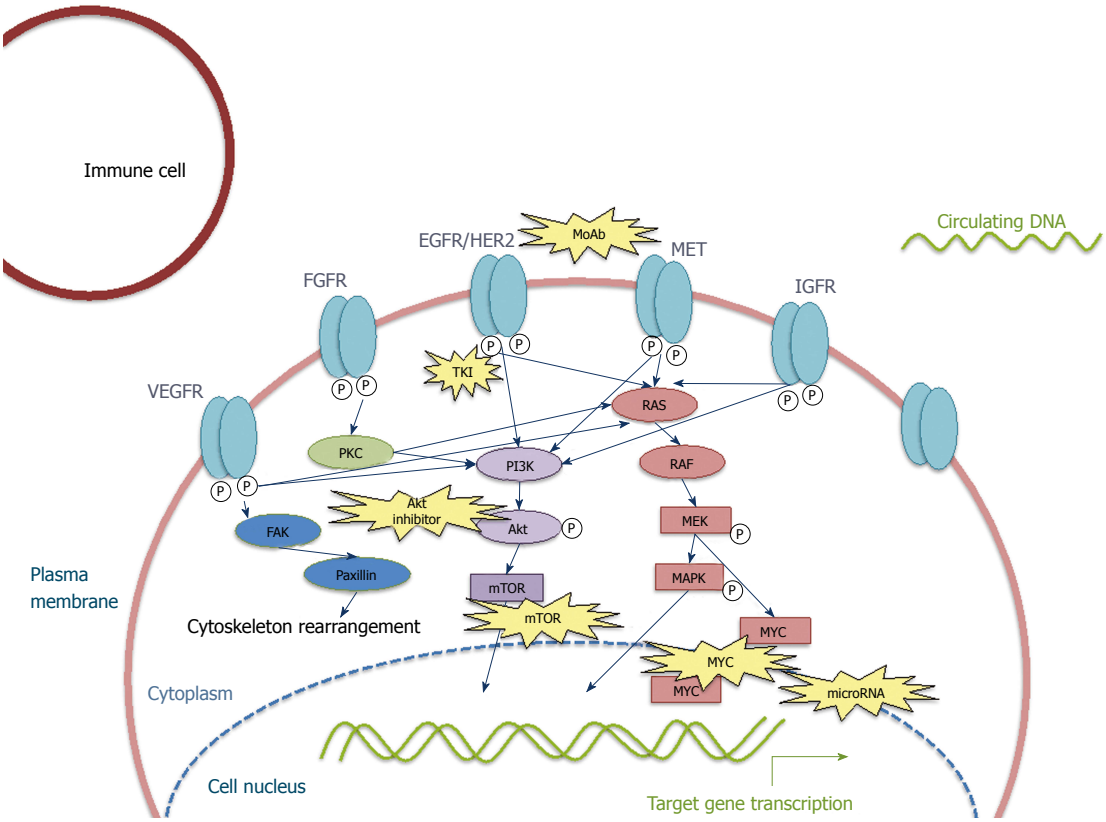

Figure 1 Simplified diagram showing signalling pathways related to human epidermal growth factor receptor 2 in gastric adenocarcinoma. VEGFR: Vascular endothelial growth factor receptor; FGFR: Fibroblast growth factor receptor; PKC: Protein kinase C; FAK: Focal adhesion kinase; HER2: Human epidermal growth factor receptor 2; MAPK: Mitogen-activated protein kinase; mTOR: Mammalian target of rapamycin.

emotherapy has been convincingly demonstrated in both metastatic (OS 25.1 mo in patients receiving trastuzumab + chemotherapy *vs* 20.3 mo in those receiving chemotherapy alone, Table 1)[19] and adjuvant settings[20]. Breast cancer OS is, however, influenced by the greater number of treatment options in the 2nd-line setting and beyond.

In GOC, trastuzumab is the only licensed anti-HER2 treatment, following positive results from the ToGA trial, an open-label, international, phase 3, randomised controlled trial evaluating trastuzumab plus platinum-fluoropyrimidine chemotherapy for 1st-line treatment of HER2 positive GOC (Table 1)[5]. Median OS was initially reported as 13.8 mo (95%CI: 12-16) in patients receiving trastuzumab plus chemotherapy *vs* 11.1 mo (10-13) in patients receiving chemotherapy alone (HR = 0.74; 95%CI: 0.60-0.91; P = 0.0046)[5]. This led to trastuzumab plus platinum-fluoropyrimidine chemotherapy followed by trastuzumab maintenance becoming the standard of care in 1st-line metastatic GOC patients[5]. Updated OS (after a further 1 year of follow-up) released by the United States Food and Drug Administration (FDA) in 2016 showed median OS of 13.1 mo (95%CI: 11.9-15.1) in the trastuzumab plus chemotherapy arm and 11.7 mo (95%CI: 10.3-13.0) in the control arm (HR = 0.8, 95%CI: 0.67-0.97)[21]. Subgroup analysis demonstrated that patients with IHC 3+ HER2 expression experienced the greatest benefit from trastuzumab (294 patients, HR = 0.66, 95%CI: 0.5-0.87). Patients with IHC 2+ HER2 expression gained less benefit from the addition of trastuzumab (160 patients, HR = 0.78, 95%CI: 0.55-1.10), and patients with IHC 1 or 1+ gained no benefit (133 patients, HR = 1.33, 95%CI: 0.92-1.92)[21]. Recent data on two different doses of trastuzumab in combination with chemotherapy in GOC found that a higher trastuzumab maintenance dose does not convey additional survival benefit (OS 12.5 mo in the 8 mg/kg + 6 mg/kg group *vs* 10.6 mo in the 8 mg/kg + 10 mg/kg group)[22].

It remains to be seen whether trastuzumab confers a survival benefit in the neo-adjuvant/perioperative/adjuvant setting in combination with chemotherapy + surgery ± radiotherapy, and several phase 2 trials are underway to address this question (UMIN 000016920, NCT01472029, NCT02250209, Table 2)[23,24]. Perioperative trastuzumab appears to be safe and well tolerated[25]. One Phase Ⅱ trial evaluating capecitabine + oxaliplatin with trastuzumab three cycles pre-operatively and post-operatively followed by 12 mo adjuvant trastuzumab reported an 18 mo DFS of 71% (95%CI: 53%-83%), a 24 mo DFS of 60% and a median follow-up of 24.1 mo (median DFS and OS not reached)[26]. Although a phase Ⅲ trial evaluating radiotherapy + chemotherapy ± trastuzumab is underway (NCT01196390, Table

Table 2 Selected perioperative (neoadjuvant + adjuvant) clinical trials currently underway targeting HER2 in HER2-amplified localised gastro-oesophageal cancer

Official study title	Stage and study number	Treatment arms	Estimated enrollment	Primary endpoint
Trastuzumab				
A randomized phase Ⅱ trial of systemic chemotherapy with and without trastuzumab followed by surgery in HER2-positive advanced gastric or esophagogastric junction adenocarcinoma with extensive lymph node metastasis: Japan Clinical Oncology Group study JCOG1301 (Trigger Study)[23]	Phase Ⅱ UMIN 000016920	Preoperative S-1 + cisplatin + trastuzumab *vs* S-1 + cisplatin Followed by adjuvant chemotherapy with S-1 for 1 yr	130	OS
Multicenter, explorative phase Ⅱ study of perioperative 5-FU, leucovorin, docetaxel, and oxaliplatin (FLOT) in combination with trastuzumab in patients with HER2-positive, locally advanced, resectable adenocarcinoma of the gastroesophageal junction or stomach (HerFLOT)	Phase Ⅱ NCT01472029	Pre-operative 5-FU + leucovorin + docetaxel + oxaliplatin (FLOT) + trastuzumab Post-operative trastuzumab monotherapy	53	pCR
Trastuzumab plus XELOX for HER2-positive stage Ⅲ gastric cancer after D2 gastrectomy: prospective observational Study[77]	Phase Ⅱ NCT02250209	Trastuzumab + capecitabine + oxaliplatin after D2 gastrectomy	40	3-yr DFS
A phase Ⅲ trial evaluating the addition of trastuzumab to trimodality treatment of HER2-overexpressing esophageal adenocarcinoma	Phase Ⅲ NCT01196390	Radiotherapy + paclitaxel + carboplatin + trastuzumab *vs* Radiotherapy + paclitaxel + carboplatin	591	DFS
Lapatinib				
A randomised phase Ⅱ/Ⅲ trial of peri-operative chemotherapy with or without bevacizumab in operable oesophagogastric adenocarcinoma and a feasibility study evaluating lapatinib in HER-2 positive oesophagogastric adenocarcinomas and (in selected centres) MRI and PET/CT sub-studies (STO3 trial)	Phase Ⅱ/Ⅲ NCT00450203	Epirubicin + cisplatin + capecitabine (ECX) + lapatinib *vs* ECX	40 (within lapatinib sub-study)	Safety
Pertuzumab + Trastuzumab				
INtegratioN of trastuzumab, with or without pertuzumab, into perioperatiVe chemotherApy of HER-2 positive stomach cancer: the INNOVATION-TRIAL[77]	Phase Ⅱ NCT02205047	Cisplatin/capecitabine or cisplatin/5-fluorouracil *vs* cisplatin/capecitabine + trastuzumab or cisplatin/5-fluorouracil + trastuzumab *vs* cisplatin/capecitabine + trastuzumab +pertuzumab or cisplatin/5-fluorouracil + trastuzumab + pertuzumab	220	Near complete pathological response rate
FLOT *vs* FLOT/Herceptin/Pertuzumab for perioperative therapy of adenocarcinoma of the stomach and gastroesophageal junction expressing HER-2 A phase Ⅱ/Ⅲ trial of the AIO. (PETRARCA study)	Phase Ⅱ/Ⅲ NCT02581462	5-FU + leucovorin + docetaxel + oxaliplatin (FLOT) *vs* FLOT + trastuzumab + Pertuzumab	404	pCR OS
Feasibility study of chemoradiation, TRAstuzumab and pertuzumab in resectable HER2+esophageal carcinoma: the TRAP study	Phase Ⅰ/Ⅱ NCT02120911	Pertuzumab + trastuzumab + standard chemoradiation with carboplatin and paclitaxel.	40	Safety

pCR: Pathological complete response; DFS: Disease-free survival.

2)[24], it is notable that trastuzumab is not being investigated in phase Ⅲ trials in the peri-operative GOC setting. This is likely due to the prohibitive number of patients (approximately 10000) that would require screening in order to recruit adequate numbers of patients for a sufficiently powered study, given that HER2 overexpression is around 20%[5], and a relatively small proportion of patients in Western countries are diagnosed with operable disease[27]. Only a proportion of these patients would have an adequate performance status to enter a clinical trial; therefore, trastuzumab will likely never be investigated in phase Ⅲ trials in the peri-operative setting.

In advanced GOC, trastuzumab is being investigated in combination with bevacizumab (NCT01359397, Table 3), afatinib (NCT01522768, Table 3) and *via* intr-

Table 3 Selected clinical trials currently underway targeting HER2 in advanced and metastatic HER2-amplified gastro-oesophageal cancer

Official study title	Stage and study number	Treatment arms	Estimated enrollment	Primary endpoint
Trastuzumab in combination with targeted therapies				
Phase Ⅱ study of docetaxel, oxaliplatin, capecitabine with bevacizumab and trastuzumab in case of HER2-positivity in patients with locally advanced or metastatic gastric cancer or adenocarcinoma of the gastro-oesophageal junction (B-DOCT study)	Phase Ⅱ NCT01359397	Docetaxel, oxaliplatin, capecitabine, bevacizumab *vs* Docetaxel, oxaliplatin, capecitabine, bevacizumab, trastuzumab	Information not available	PFS
A phase Ⅱ study of afatinib (BIBW 2992) and trastuzumab in patients with advanced HER2-positive trastuzumab-refractory advanced esophagogastric cancer	Phase Ⅱ NCT01522768	Afatinib (BIBW 2992) + trastuzumab	40	ORR
Intraperitoneal trastuzumab				
Phase Ⅰ trial of intraperitoneal ^{212}Pb-TCMC-trastuzumab for HER-2 expressing malignancy	Phase Ⅰ NCT01384253	^{212}Pb-TCMC-trastuzumab + trastuzumab	36	Safety
T-DM1				
A combination study of kadcyla (trastuzumab emtansine, T-DM1) and capecitabine in patients with HER2-positive metastatic breast cancer and patients with HER2-positive locally advanced/ metastatic gastric cancer (TRAX-HER2 study)[78]	Phase Ⅱ NCT01702558	Capecitabine + trastuzumab emtansine (T-DM1) *vs* T-DM1	235	Safety ORR
DS-8201				
Phase 1, two-part, multicenter, non-randomized, open-label, multiple dose first-in-human study of DS-8201A, in subjects with advanced solid malignant tumors[36]	Phase Ⅱ NCT02564900	Trastuzumab deruxtecan (DS-8201a)	198	Safety ORR
Lapatinib				
Safety and clinical activity of lapatinib in patients with HER2-positive refractory advanced cancer: a phase Ⅱ single arm prospective study	Phase Ⅱ NCT02342587	Lapatinib	25	ORR
New HER2 inhibitors				
A phase Ⅰ-Ⅱ study to assess the safety, efficacy and pharmacokinetic profile of HM781-36B combined with paclitaxel and trastuzumab in patients with HER-2 positive advanced gastric cancer	Phase Ⅰ/Ⅱ NCT01746771	HM781-36B(Poziotinib) (Other Names: NOV120101) + paclitaxel + trastuzumab	48	Safety DLT
A phase 1, dose escalation study of MGAH22 in patients with refractory HER2 positive breast cancer and patients with other HER2 positive carcinomas for whom no standard therapy is available	Phase Ⅰ NCT01148849	MGAH22 (margetuximab)	67	Safety
A phase Ⅰ multicenter, open-label, dose-escalation, and dose-expansion study to evaluate the safety, pharmacokinetics, immunogencity, and antitumor activity of MEDI4276 in subjects with select HER2-expressing advanced solid tumors	Phase Ⅰ NCT02576548	MEDI4276	120	Safety MTD
A phase Ⅰ study of pyrotinib in combination with docetaxel in patients with HER2 positive advanced gastric cancer	Phase Ⅰ NCT02378389	Pyrotinib + docetaxel	28	Safety
A two-part phase Ⅰ, open label, dose escalation study to evaluate the safety, tolerability and pharmacokinetics of pyrotinib in patients whose disease progressed on prior HER2 targeted therapy	Phase Ⅰ NCT02500199	Pyrotinib	70	Safety MTD
Neratinib				
An open-label, multicenter, multinational, phase 2 study exploring the efficacy and safety of neratinib therapy in patients with solid tumors with activating HER2, HER3 or EGFR mutations or with EGFR gene amplification	Phase Ⅱ NCT01953926	Neratinib	292	ORR
HER2-targeted immunotherapy				
A phase Ib/Ⅱ study of pembrolizumab and monoclonal antibody therapy in patients with advanced cancer (PembroMab)[77]	Phase Ⅰ/Ⅱ NCT02318901	Pembrolizumab + trastuzumab *vs* pembrolizumab + ado-trastuzumab emtansine (T-DM1) *vs* pembrolizumab + cetuximab	90	Safety and dose-finding
A phase Ⅰ study to evaluate the antitumor activity and safety of DUKE-002-VRP (HUHER2-ECD + TM), an alphaviral vector encoding the HER2 extracellular domain and transmembrane region, in patient with locally advanced or metastatic human epidermal growth factor receptor 2-positive (HER2+) cancers including breast cancer	Phase Ⅰ NCT01526473	AVX901	12	Safety

HER2-peptide vaccination of patients with solid tumors	Phase I NCT02276300	Cyclophosphamide sargramostim HER2-Peptid-Vakzine imiquimod	12	Safety

ORR: Overall response rate; HER2: Human epidermal growth factor receptor 2.

aperitoneal delivery (NCT01384253, Table 3)[24].

Lapatinib

Lapatinib is an oral tyrosine kinase inhibitor targeting EGFR and HER2[7,28]. In breast cancer, lapatinib demonstrated significant clinical benefit and is now a standard line of treatment[19,29,30]. In contrast, in GOC, although it showed promise in preclinical trials, lapatinib failed to translate into clinical benefit in both 1st-line (LOGiC)[7] and 2nd-line settings (TYTAN) (Table 1)[9]. The reasons for the disappointing results seen in GOC as compared to breast cancer may be related to lapatinib dosage, toxicities experienced, or different underlying HER2 signalling mechanisms in GOC and breast cancer. When lapatinib was combined with paclitaxel in a 1st-line breast cancer study and 2nd line GOC study (TYTAN), rates of AEs were broadly similar: 77% of patients in the lapatinib arm experienced diarrhoea in both the breast and TYTAN studies *vs* 29% of patients in the control arm in the breast study and 22% in the TYTAN study[8,29]. There was, however, a slightly higher rate of treatment discontinuation seen in GOC patients as compared to breast patients, with AEs resulting in treatment discontinuation in 16% in the lapatinib plus paclitaxel group *vs* 13% in the breast study)[8,29]. In the 1st-line GOC LOGiC trial (Table 1)[7], there were significantly higher toxicity rates in the lapatinib arm than the control arm, with 94% of patients experiencing adverse events (AEs) and 27% serious AEs (SAEs) in the lapatinib arm *vs* 88% AEs and 19% SAEs in the control arm. Diarrhoea occurred in 58% of patients receiving lapatinib *vs* 29% in the control arm, leading to lower relative drug exposure in the lapatinib arm[7]. Again lapatinib treatment resulted in higher rates of treatment discontinuation in GOC than breast cancer patients: 21% of patients in the lapatinib arm of LOGiC required treatment discontinuation *vs* 13% of breast cancer patients in the 2nd-line breast study receiving lapatinib plus capecitabine[30]. Overall, this suggests that the chemotherapy backbone with which to combine lapatinib is important, and chemotherapy drugs with overlapping toxicity may result in lower lapatinib dose-intensity and reduced efficacy in GOC. Additionally GOC patients frequently experience gastrointestinal side-effects prior to treatment and may be less able to tolerate lapatinib treatment. Another possible reason for the poor efficacy of lapatinib in GOC is that HER2 and EGFR signalling mechanisms may differ as compared to breast cancer.

Lapatinib is currently being investigated in Phase II/III trials in the peri-operative setting (STO3 trial, NCT00450203, Table 2)[24] and as monotherapy in the advanced setting (NCT02342587, Table 3)[24]. Safety data from the STO3 trial was presented at ESMO 2016 and suggested that administration of lapatinib at a dose of 1250 mg/d in combination with ECX chemotherapy (capecitabine 1000 mg/m^2) was feasible, although there was increased diarrhoea (21% in ECX + lapatinib group *vs* 0% in ECX group) and neutropenia (42% in ECX + lapatinib group *vs* 21% in ECX group), which did not appear to compromise operative management[31].

T-DM1

Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate that combines the HER2-targeted properties of trastuzumab with the cytotoxic activity of emtansine, enabling selective delivery of chemotherapy to HER2-overexpressing cells[32]. Although T-DM1 demonstrated significant clinical benefit in the EMILIA breast cancer trial in the 2nd-line setting (Table 1)[33], a similar study (GATSBY, Table 1) in GOC failed to meet its primary endpoint or any of its secondary endpoints[34,35]. It is worth noting that nearly half of the patients in the GATSBY trial were from the Asia-Pacific region. These patients are generally fit with a good performance status; and, therefore, it is likely that a significant proportion will have received post-study treatment[35]. T-DM1 monotherapy *vs* T-DM1 + capecitabine is being investigated in combination with capecitabine chemotherapy in GOC in pretreated patients (NCT01702558, Table 3) and recruitment has been completed[24].

Trastuzumab deruxtecan

Trastuzumab deruxtecan (DS-8201) is an antibody-drug conjugate comprising a humanised antibody against HER2 and a topoisomerase I inhibitor "payload" bound together by an enzyme-cleavable linker[36]. A phase I open label dose escalation study recently presented at ASCO[37] demonstrated an overall response rate (ORR) of 46.7% in HER2+ breast cancer patients pretreated with T-DM1 and pertuzumab and an ORR of 44.4% in gastric cancer patients pretreated with trastuzumab[37]. This high response rate demonstrates that the "payload" bound to the anti-HER2 antibody can make a significant difference to treatment success. For the first time, similar response rates were seen in both breast and gastric cancers pretreated with HER2 inhibitors, and responses were seen even in low HER2-expressing tumours[36]. Results of the currently planned phase 2 trials are eagerly awaited (NCT02564900, Table 3)[24], and whether these response rates can translate into improved overall survival remains to be seen.

Pertuzumab

Pertuzumab is a humanised monoclonal antibody targeting a different HER2 epitope to trastuzumab[38], preventing formation of HER2-HER3 heterodimers[39]. It can be administered concurrently with trastuzumab[40]. In the CLEOPATRA breast cancer study, pertuzumab demonstrated significant clinical benefit when added to trastuzumab plus taxane chemotherapy[40]. Disappointingly, in advanced GOC, the phase Ⅲ JACOB study of pertuzumab + trastuzumab failed to demonstrate a significant improvement in OS[41].

Pertuzumab is currently being explored in combination with trastuzumab and chemotherapy in the perioperative GOC setting in INNOVATION (NCT02205047) and PETRARCA trials (NCT02581462) and with the addition of radiotherapy in the TRAP trial (NCT02120911) (Table 2)[24].

Preclinical studies investigating pertuzumab in combination with T-DM1 in GOC cell lines and xenograft models found this combination caused growth inhibition but no tumour shrinkage[42]. A literature search did not reveal any clinical studies investigating this combination in GOC.

MM-111

MM-111 is a bispecific antibody fusion protein designed by Merrimack to inhibit HER3-ligand binding and signalling in HER2-amplified tumours by preventing formation of HER2-HER3 heterodimers[43,44]. Preclinical studies showed promise, leading to phase 1 and phase 2 studies in selected tumour types, including HER2-amplified breast and GOC[43,44]. However, the phase 2 study investigating MM-111 in HER2-amplified GOC patients was terminated early by the independent data monitoring committee when it was found that the addition of MM-111 to chemotherapy + trastuzumab resulted in a significantly poorer PFS and OS[43]. In light of the disappointing results seen in GOC[43], all further studies investigating MM-111 were withdrawn, and Merrimack announced that it does not plan to invest further in MM-111.

New HER2 inhibitors

Poziotinib is an oral pan-HER2 inhibitor whose role in combination with trastuzumab and paclitaxel is currently under investigation in advanced gastric cancer (NCT01746771, Table 3)[24]. Phase 1 studies in GOC are investigating MGAH22 (Margetuximab) (NCT01148849, Table 3)[24], a chimeric anti-HER2 monoclonal antibody similar to trastuzumab but engineered for increased binding[45]. Medimmune is investigating their HER2 inhibitor, MEDI4276, in a Phase 1 trial (NCT02576548, Table 3) in both breast and gastric cancers[24]. Pyrotinib is an oral tyrosine kinase inhibitor targeting both HER1 (EGFR) and HER2 and is currently being explored in phase 1 trials in GOC (NCT02378389, NCT02500199, Table 3)[24].

MECHANISMS THAT MAY AFFECT HER2 INHIBITION IN GOC

Resistance to HER2 therapy can be one of two types: primary (intrinsic) resistance occurs when there is no response to HER2 inhibitors and secondary (acquired) resistance occurs when there is an initial response followed by cessation of response[46]. Differentiating between these types of resistance is important, as it dictates the optimal timing of treatment strategies.

Alterations to the HER2 receptor

p95HER2: An aminotruncated form of HER2, known as p95HER2[46], lacks the region to which trastuzumab binds and is expressed in 20%-37% of breast cancer patients[47] and 60%-77% of GOC patients with HER2 amplified disease[48,49]. This may partly explain the poorer response to trastuzumab in GOC as compared to breast cancer.

HER2 mutation: Within the TCGA, 15 cases of *ERBB2* mutation in GOC were detected using RNA evidence out of 215 non-hypermutated tumours[6]. Evaluation of HER2 mutation across an array of tumour types revealed HER2 mutations in around 5% of gastric cancer patients[50]. Neratinib, a pan-HER tyrosine kinase inhibitor, is being explored in HER2-mutated cancer (NCT01953926, Table 3)[24].

Loss of HER2 expression

A recent study presented at ASCO found that 35% of GOC treated with trastuzumab lost HER2 positivity[51]. Similarly, in breast cancer, loss of HER2 positivity has been reported in patients treated with neoadjuvant trastuzumab + chemotherapy or chemotherapy alone, and loss of HER2 positivity was associated with an increased risk of disease relapse[52].

Signalling pathways

PIK3CA/PTEN/PI3K/AKT/mTOR pathway: The antitumour activity of HER2 inhibitors requires downstream inhibition of *PI3K/AKT*[46,53]. BOLERO-3 was a randomised, double-blind, placebo-controlled phase 3 trial in HER2 positive, trastuzumab-resistant, advanced previously-treated breast cancer patients that explored whether the mTOR inhibitor everolimus might restore sensitivity to trastuzumab[54]. It demonstrated significant improvement in PFS with the addition of everolimus [7 mo (95%CI: 6.74-8.18) in the everolimus group *vs* 5.78 mo (5.49-6.9) in the placebo group][54]. The randomised phase 3 BOLERO-1 trial compared everolimus plus trastuzumab plus paclitaxel to placebo plus trastuzumab plus paclitaxel in order to assess whether addition of everolimus at treatment outset might prevent intrinsic resistance: primary endpoint (PFS) was not met[55].

Phase 3 clinical trials have not been conducted specifically in HER2 positive GOC patients[49]. The phase 3 GRANITE trial randomised 656 patients with advanced pretreated gastric cancer to either everolimus or matching placebo[56]. HER2 status was not an inclusion or exclusion criteria, and we do not know the percentage of HER2 positive patients within this trial. The primary endpoint (OS) was not met, and everolimus was associated

with significant side-effects: 21.5% of patients receiving everolimus required drug discontinuation and 55.4% required dose adjustments/interruptions[56]. Such high rates of adverse events are concerning in the palliative setting, where quality of life is important.

IGF-1R expression

Insulin-like growth factor-1 receptor (IGF-1R) is involved in acquired resistance to HER2 blockade in breast cancer[46,57] and GOC[58] cells *in vitro* by forming heterodimers with HER2. Blockade of this heterodimer formation *in vitro* and *in vivo* restored sensitivity to HER2[57], and combination studies of HER2 blockade in combination with IGF-1R inhibitors were more effective than either agent alone[59]. Clinical studies exploring IGF-1R inhibitors in combination with HER2 inhibitors in breast cancer patients found no significant difference in PFS (NCT00684983)[49]; other studies evaluating this strategy were withdrawn, and there are no GOC studies[49].

MET overexpression

Clinical studies of MET inhibitors as monotherapy in HER2 negative breast cancer patients did not meet their primary endpoint[49,60]. In GOC, a randomized double-blind phase 3 clinical trial exploring MET inhibition in HER2 negative, MET positive GOC patients found no benefit from the addition of the MET inhibitor onartuzumab to chemotherapy[61]. Phase 2 results for an alternative MET inhibitor, tivatinib, similarly showed no survival advantage[62]. In light of these disappointing results, it is unlikely MET inhibition will be explored in the clinical setting in HER2-overexpressing breast or GOC patients.

HSP90

Combining HER2- and Heat shock protein (HSP90)-inhibition to overcome resistance to HER2 inhibitors showed promise preclinically in cell lines and mouse models in breast and GOC cell lines[63]. However, a phase 2 study in breast cancer has not yet released results[64], and a phase 2 study in gastric cancer was terminated (NCT01402401)[24].

MicroRNA

MicroRNAs (miRs) are small non-coding RNAs that control gene expression through messenger RNA degradation and post-transcriptional inhibition[65]. MiRs are tissue-specific, and different microRNA signatures may occur during resistance to HER2 inhibition in breast and GOC. In HER2 positive breast and gastric cancer cells, miR-21 overexpression leads to PTEN downregulation, suppression of trastuzumab-induced apoptosis and increased trastuzumab resistance[66,67]. MiRNA-542-3p downregulation promotes trastuzumab resistance in breast cancer *via* AKT activation[68]. MiR-7 functions as a suppressor of the oncogenic isoform of HER2, $HER2^{\Delta}16$, and reverses $HER2^{\Delta}16$-induced trastuzumab resistance in breast cancer[69]. The use of miRs not only as biomarkers but as targets for anticancer therapy may allow new therapeutic miR silencing in the future[70]. Inhibition of certain microRNAs may also enhance the effect of HER2 inhibition[71].

Immune response

Natural killer (NK) cells are required in order to exert trastuzumab's therapeutic effect[46]. Mice deficient in NK cells show trastuzumab resistance[72] and when numbers of innate and adaptive immune cells in the tumour microenvironment increase, there is increased tumour eradication[73]. Trials studying the immune environment in GOC are underway (NCT02318901, NCT01526473, NCT02276300, Table 3)[24].

Biomarkers

Specific-uptake positron emission tomography (PET) scans: Targeted PET scans using radiolabelled trastuzumab (89Zr-Trastuzumab) to demonstrate HER2 uptake can give real-time information on HER2 expression levels, visually displaying the development of resistance with the advantage of being relatively non-invasive and, therefore, preferable for patients[74].

Circulating DNA: Circulating DNA may represent a clinically useful biomarker that reduces the need for invasive biopsies. Plasma DNA digital PCR can detect HER2 status in metastatic breast cancer patients[75]. A systematic review and meta-analysis has suggested that serum HER2 is a potential surrogate for tissue HER2 status in gastric cancer[76].

CONCLUSION

Despite numerous HER2 inhibitors being investigated in a number of settings, trastuzumab in advanced disease is still the only HER2 inhibitor licensed for clinical use in the treatment of GOC. Even within this setting, the overall survival benefit is far less than that seen in breast cancer. Other HER2 inhibitors that have demonstrated success in breast cancer have failed to reach statistically significant endpoints in GOC clinical trials, and it remains to be seen whether clinical trials currently underway will show improved results. HER2 heterogeneity, amino-truncation loss of HER2 expression and differences in signalling pathways may contribute to the disappointing clinical trial outcomes seen in GOC. Different microRNA signatures and immune environments are also likely to play a role. Development of new HER2 inhibition strategies in conjunction with further research into how the role of HER2 differs in GOC as compared to breast cancer is required. Clinical trials utilizing biomarkers such as specific uptake PET scans and circulating DNA may provide early insight into whether patients are responding to HER2 inhibition. Only with improved understanding of HER2 inhibition in GOC can effective treatment be provided in order to improve clinical outcomes for patients.

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REVIEW

Advances in molecular, genetic and immune signatures of gastric cancer: Are we ready to apply them in our patients' decision making?

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Abstract

In the last few years we have witnessed a vast expansion of our knowledge regarding the molecular and genetic profile of gastric cancer. The molecular subtypes described have shed light on the pathogenesis of the disease, thus prompting the development of new therapeutic strategies and favoring a more individualized approach for treatment. Most of the clinical trials for so called targeted therapies could be considered, at best, partially successful. In addition, checkpoint inhibitors have recently been added to our armamentarium in later stages of the disease, and combinations with chemotherapy and targeted agents are currently under development. In view of the rapid advances of molecular oncology, a new challenge for the clinical oncologist arises: The appropriate patient selection for each new therapy, which can be made possible only through the implementation of predictive biomarkers in our therapy decision making.

Key words: Gastric cancer; Cancer Genome Atlas; Asian Cancer Research Group; Targeted therapy

Core tip: Despite recent advances in cancer therapeutics, the survival of gastric cancer patients with metastatic disease is dismal due to the complexity of the disease, the constant evolution of tumors and our still limited understanding of its biology. It is evident that a wide spectrum of prognostic and predictive biomarkers is needed in order to rationalize our decisions when managing patients with this specific tumor type and tailor our treatment to suit better the individual patient's unique needs.

Gkolfinopoulos S, Papamichael D, Papadimitriou K, Papanastasopoulos P, Vassiliou V, Kountourakis P. Advances in molecular, genetic and immune signatures of gastric cancer: Are we ready to apply them in our patients' decision making? *World J Gastrointest Oncol* 2018; 10(7): 172-183 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i7/172.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i7.172

INTRODUCTION

Gastric cancer (GC) is the fifth most common type of cancer and the third most common cause of cancer-related mortality worldwide[1]. Despite recent advances in cancer therapeutics, driven by the application of the findings of basic science in cancer genetics and host-tumor immune interactions, the prognosis of most patients with metastatic disease is dismal[2]. Indeed, in GC we seem to lack clear molecular targets based on key regulatory genes or the aberrant expression of growth factor receptors. Furthermore, the universal rise of immunotherapeutic approaches in various tumor types has only recently been incorporated in GC. It is evident that a wide spectrum of prognostic and predictive biomarkers is needed in order to rationalize our decisions when managing patients with this specific tumor type and tailor our treatment to suit better the individual patient's unique needs.

Genetic heterogeneity of GC

Our understanding in GC genetics was greatly expanded in 2014, when four main molecular subtypes of the disease were recognized in the context of the Cancer Genome Atlas (TCGA) project[3]. Further efforts were undertaken in order to relate molecular subtypes with the known histological subtypes that Lauren had proposed roughly half a century ago as well as with the location of the primary tumor and prognosis[4]. These efforts were met with moderate success, since it is now widely accepted that there is an important degree of overlap. Various basic studies and clinical trials followed, aiming to discover a clinically meaningful way of utilizing the findings of the TCGA project[5]. Unfortunately, thus far, the results have fallen short of the initial high expectations, although some success has been noted in subgroups of patients across trials that exhibited unique molecular characteristics. In 2015, another major molecular classification was proposed, this time from the Asian Cancer Research Group (ACRG), which shares similarities with TCGA yet has enough differences to be considered completely distinct (Table 1). The novelty with the ACRG was that the molecular subtypes discovered were associated with clinical outcomes[6]. A short review and comparison of both classification systems will be presented, followed by a brief and non-exhaustive analysis of the most important clinical trials employing target or immunotherapeutic strategies in this expanding area of oncology.

MOLECULAR SUBTYPES OF GC ACCORDING TO TCGA

The first and most comprehensive molecular characterization of gastric adenocarcinoma was reported by the TCGA Network. In this study, 295 (therapy naive) primary gastric adenocarcinoma samples were characterized using six different molecular platforms, including array-based somatic copy number analysis, whole-exome sequencing, array-based DNA methylation profiling, messenger RNA sequencing, microRNA sequencing, and reverse-phase protein array. No survival or racial differences were found among patients from each subgroup[3]. As mentioned before, there were four main subtypes discovered, which can roughly be categorized in the following groups.

Subtypes not inherently immunogenic

The following two subtypes are less likely to respond to immunotherapeutic strategies *per se*. Rather, combination approaches are probably required in order to attain a response using immunotherapy, such as adding chemotherapy to checkpoint inhibition or dual checkpoint inhibition. However, in cases with marked T-cells infiltration, we might expect that the checkpoints are probably up-regulated, and thus immunotherapy might still work. Apart from immunotherapy, targeted therapy with tyrosine kinase inhibitors (TKI) may prove to be another option in select subgroups of patients that carry specific driver mutations.

Chromosomal instability (50% of samples): The majority of the tumors analyzed in the project have fallen in this category. This subtype is found more frequently in the gastroesophageal junction (GEJ)/cardia (65%), is of intestinal histology, and affects mainly older (> 70 yo) individuals[7]. Genetically, it is characterized by marked aneuploidy and high frequency of *TP53* mutations (73%). Consequently, it features a high number of focal amplification of receptor tyrosine kinases, most importantly *VEGFA*, *EGFR* (10%), *ERBB2* (24%), *ERBB3* (8%), and *c-Met* (8%) as well as amplification of genes encoding cell cycle mediators, such as *CCNE1*, *CCND1*,

Table 1 Molecular subtypes of gastric cancer according to the Cancer Genome Atlas and Asian Cancer Research Group

Molecular subtypes of gastric cancer	
TCGA	ACRG
CIN (50%)	MSS/TP53− (35.7%)
MSI-H (21%)	MSS/TP53+ (26.3%)
GS (20%)	MSI-H (22.7%)
EBV + (9%)	MSS-EMT (15.3%)

TCGA: Cancer Genome Atlas; ACRG: Asian Cancer Research Group; CIN: Chrosomal instability; MSI-H: Microsatellite-high; GS: Genomically stable; EBV: Epstein-Barr virus; MSS: Microsatellite stable; TP53: Tumor protein p53; EMT: Epithelial-mesenchymal transition.

and *CDK6*[8]. These genetic aberrations contribute to making it the ideal candidate for application of targeted treatment, especially TKI inhibitors and monoclonal antibodies[9].

Genomically stable (20% of samples): The trademark characteristics of this subtype are diploidy and somatic mutations in *CDH1* (37%), which is also the gene that is mutated in hereditary diffuse GC syndrome[10]. Further common genetic aberrations are either *RHOA* mutations or *CLDN18-ARHGAP* rearrangements, both discovered in approximately 30% of tumors and usually mutually exclusive. All those mutations lead to disrupted intercellular cohesion and enhanced invasiveness, thus it is no surprise that most (73%) of these tumors belong to the diffuse histological variant. Most patients are of younger age (median 59 years), and there is no gender predominance[3]. The inherent relative lack of immunogenicity and targetable driver mutations may lead to increased difficulty in applying individualized treatment in this subtype. Perhaps this is the single molecular subtype in TCGA classification where classic cytotoxic chemotherapy will continue to retain the primary role in treatment.

Highly immunogenic subtypes

The other two subtypes are characterized by extensive infiltration of PD-L1(+) immune cells, which are dispersed throughout the tumor instead of being located in the invasive margin, as is common with other malignancies[11]. It is speculated that the patients who exhibit response to checkpoint inhibitors will belong to this particular subgroup, although this has not yet been proven[12].

Microsatellite-high (21% of samples): The second most common subtype in the TCGA classification is characterized by extensive DNA methylation and multiple somatic mutations. These types of tumors are diagnosed at an older age (median age 72 years), with a slightly higher preponderance in female patients (56%). The various and dispersed mutations across the genome are mostly a consequence of *MLH1* promoter hypermethylation. Other important genes, with potentially targetable products, which are found mutated, are *PIK3CA, EGFR, ERBB2*, and *ERBB3*[3].

The extensively mutated genetic material of these tumors creates an opportunity for immune system-oriented strategies. Indeed, the high amount of neoantigens, often presented in MSI-high tumors, elicit an immune response, manifested through extensive PD-L1 expression, which in this subtype reaches 33% and 45% on tumor and immune cells, respectively[13,14].

Epstein-Barr virus-positive (9% of samples): This subtype, whose main characteristic is the high Epstein-Barr virus (EBV) burden, was found to occur predominantly in the gastric fundus or body (62%), and is more common in men (81%). In TCGA, a recurrent amplification of 9p24.1 genetic locus is described, which is the site of genes *JAK2, CD274*, and *PDCD1LG2.* The first accounts for the aberrant activation of the JAK-STAT pathway, while the latter two encode PD-L1 and PD-L2, respectively. The 9p amplifications are found in at least 15% of EBV (+) tumors and lead to enhanced neoepitope presentation. It is also characterized by extreme DNA hypermethylation, most notably of the *CDKN2A* promoter, which leads to complete lack of p16 (p16INK4A) protein. It also features recurrent *PIK3CA* (80%), *ARID1A* (55%), and BCOR (23%) mutations[3]. These molecular alterations characterizing this particular subtype hint at the therapeutic potential of *JAK* inhibition, *PI3K/MTOR* inhibition and immunotherapeutic approaches.

MOLECULAR SUBTYPES OF GC ACCORDING TO ACRG

The ACRG analyzed 300 GC samples using gene expression, genome-wide copy number microarray and targeted sequencing. Partially overlapping with the TCGA classification and sharing some similarities but also exhibiting enough differences to be categorized as a completely distinct classification, four molecular subtypes are described. In this case, the foundations of this molecular classification are based on the basis of MSI status, *TP53* function, and epithelial-mesenchymal transition (EMT). In this classification the subtypes were associated with relevant clinical outcomes and revealed survival differences that were validated in independent cohorts[6].

The basis on which the first division took place was the loss of function of genes involved in the mismatch repair (MMR) system, thus distinguishing the MSI subtype. Then, the remaining tumors were divided depending on alterations in cell adhesion, angiogenesis, and motility, thus forming the MSS/EMT subtype. The rest were divided in two subtypes, depending on the loss of function of TP53, namely the microsatellite stable/TP53 intact (MSS/TP53+) and microsatellite stable/TP53 loss (MSS/TP53-) subtypes. Among these subtypes, the MSI showed the best overall prognosis, followed by

MSS/TP53+, MSS/TP53-, and MSS/EMT[6]. More extensively, the molecular subtypes and their main specific characteristics are:

Microsatellite stable/TP53 loss (35.7% of samples)

This subtype is characterized by the highest rate of *TP53* mutations (60%). Also, it features a greater aneuploidy and recurrent focal amplifications in *MDM2, ROBO2, GATA6, MYC. ERBB2, EGFR, CCNE1*, and *CCND1*[6].

Microsatellite stable/TP53 intact (26.3% of samples)

Compared to the rest, this subtype is characterized by a higher prevalence of EBV infection. In addition to exhibiting an active TP53 pathway, it is associated with APC, *ARID1A, KRAS, PI3KCA*, and *SMAD4* mutations[6].

Microsatellite-high (22.7% of samples)

This subtype occurred frequently in the antrum (75%), was mostly (> 60%) of intestinal-type histology, and was diagnosed more frequently at early stages (Ⅰ or Ⅱ), thus exhibiting the best overall survival. Genetically, it was associated with the presence of hypermutation, especially in genes encoding KRAS (23.3%), the PI3K-PTEN-mTOR pathway (42%), ARID1A (44.2%), ERBB2 (16.3%), ERBB3 (14%), and ALK (16.3%)[6].

Microsatellite stable/epithelial-mesenchymal transition (15.3% of samples)

This subtype was associated with diffuse type histology, as it was expected considering that it features aberrations in genes responsible for cell adhesion and motility. It presents at a significantly younger age with most of the patients diagnosed at advanced stages (Ⅲ/Ⅳ). Consequently, it carries the worst overall prognosis and a higher chance of recurrence. It is also characterized by higher rates of peritoneal spread, which can also be attributed to the above mentioned genetic changes[6,15].

Comparison between TCGA and ACRG classifications

It is evident that, when comparing the two classifications, certain similarities exist between the different subtypes. Apart from the obvious association between the MSI subtypes in both classifications, it can be argued that roughly the equivalent of the genomically stable (GS) subtype in the ACRG classification is the microsatellite stable/epithelial-mesenchymal transition (MSS/EMT) subtype, while analogies exist between the EBV and chromosomal instability (CIN) subtypes on one hand, and MSS/TP53+ and MSS/TP53- on the other, respectively[14]. However, as has been stated previously, there are certain major differences. For instance, while in the TCGA classification, EBV is a distinct subtype; ACRG EBV-infected tumors represent a part of the spectrum of the wider MSS/TP53+ subtype, which, moreover, is not characterized by hypermethylation or hypermutation. Another important difference is that in ACRG classification, *CDH1* and *RHOA* mutations did not occur as frequently in the MSS/EMT as in its approximately equivalent GS subtype[14]. It can be argued that these differences, among others, point also to the genetic heterogeneity of GC between different populations of different ethnic backgrounds, suggesting potentially different pathogenetic mechanisms for this disease in different parts of the globe.

CLINICAL TRIALS FOCUSING ON MOLECULAR AND IMMUNE BIOMARKERS

Targeting molecular pathways

HER2 inhibition: HER2 protein in GC is overexpressed mainly as a result of gene amplification. Its overexpression results in increased cell proliferation *via* its main target pathways, namely PI3K/Akt/mTOR and the RAS/MAPK[16]. Consequently, its blockade may potentially halt tumor progression, at least temporarily, until an alternative pathway is switched-on driving resistance.

HER2 amplification is mainly a characteristic of GEJ tumors (15%-32%) rather than distal ones (10%-15%)[14]. Also, the exact location of the protein in the cell differs, depending on the level of differentiation of the tumor. Well-differentiated tumors express the protein in the cell surface, whereas it is located mainly in the cytoplasm in poorly differentiated cancer cells[17]. HER2 targeting has been implemented in various lines of therapy, with both monoclonal antibodies and TKIs with variable success (Table 2).

Trastuzumab, a chimeric monoclonal antibody targeting the domain Ⅳ of HER2, has gained approval in first-line therapy when combined with fluopyrimidine/cisplatin chemotherapy doublet, after the positive results of the phase Ⅲ ToGA trial. A subset analysis of this trial has indicated that the provided survival benefit is narrowed only to the group of patients where HER2 is clearly overexpressed, as manifested by combined immunohistochemistry (IHC) (+2) and fluorescent *in situ* hybridization (FISH) positivity, or IHC (+3) positivity. As a result, Trastuzumab should be administered to a specific subset of patients fulfilling the criteria mentioned above[18].

In an attempt to replicate the positive results of CLEOPATRA, where another HER2-targeting monoclonal antibody Pertuzumab gained approval in the treatment of advanced breast cancer, the phase Ⅲ JACOB trial was initiated. In this trial, Pertuzumab was combined with chemotherapy doublet and Trastuzumab in stage Ⅳ treatment-naive GC patients. Although the mOS was numerically superior in the Pertuzumab arm by 3.3 mo, with a 16% reduction in the risk of death, the trial missed statistical significance only just barely (P = 0.0565). Furthermore, as opposed to the ToGA trial, the majority of subgroups were consistent with the overall analysis. The combination therapy also resulted in more

Table 2 Main targeted agents evaluated in metastatic gastric cancer

Biologic target	Targeted agent	Name/type of trial	Line of therapy	Study arms	Results	Ref.
c-MET	Rilutumumab	RILOMET-1 Phase Ⅲ	1st	ECX + Ril	Negative effect	[58]
		EXPAND Phase Ⅲ	1st	XP ± Cet	No benefit	[48]
	Cetuximab	AIO Phase Ⅱ	1st	FOLFOX + Cet	> 4 *EGFR* gene copies: Increased OS (log-rank P = 0.011; HR = 0.2, 95%CI: 0-0.8; P = 0.022)	[50]
EGFR	Panitumumab	REAL-3 Phase Ⅲ	1st	EOX ± Pani	No benefit	[49]
	Trastuzumab	ToGA Phase Ⅲ	1st	XP/FP ± H	OS: 13.8 *vs* 11.1, P = 0.0046 OS (IHC+3, IHC+2/FISH+): 16 mo *vs* 11.8 mo, P = 0.0036	[18]
	Pertuzumab	JACOB Phase Ⅲ	1st	FP + H ± Pert	No benefit	[19]
HER-2	Lapatinib	Tytan Phase Ⅲ	2nd	Pac w ± Lap	No benefit (unselected population) OS (IHC: 3+): 14 mo *vs* 7.6 mo, P = 0.0176	[21]
	Trastuzumab emtansine	GATSBY Phase Ⅱ-Ⅲ	2nd	TDM-1 *vs* taxane	No superiority	[22]
mTOR	Everolimus	GRANITE-1 Phase Ⅲ	2nd, 3rd	Everolimus *vs* placebo	No benefit	[55]
	Bevacizumab	AVAGAST Phase Ⅲ	1st	XP ± Bev	Primary endpoint (OS) was not met PFS: 6.7 mo *vs* 5.3 mo, P = 0.0037 ORR: 46% *vs* 37.4%, P = 0.0315	[25]
		REGARD Phase Ⅲ	2nd	Ram *vs* placebo	OS: 5.2 mo *vs* 3.8 mo, P = 0.047	[26]
VEGF, VEGFR	Ramucirumab	RAINBOW Phase Ⅲ	2nd	Pac w ± Ram	OS: 9.6 mo *vs* 7.4 mo, P = 0.017	[27]
		Phase Ⅱ	1st	FOLFOX ± Ram	No benefit	[28]
	Apatinib	Phase Ⅲ	beyond 2nd line	Apa *vs* placebo	OS: 6.5 mo *vs* 4.7 mo, P = 0.0149 PFS: 2.6 mo *vs* 1.8, mo, P < 0.001	[30]

ECX: Epirubicin-Cisplatin-Capecitabine; Ril: Rilutumumab; XP: Cisplatin-Capecitabine; Cet: Cetuximab; EOX: Epirubicin - Oxaliplatin - Capecitabine; Pani: Panitumumab; FP: Cisplatin - 5Fu; H: Herceptin; Pert: Pertuzumab; Pac w: Paclitaxel weekly; Lap: Lapatinib; TDM-1: Trastuzumab emtansine; Bev: Bevacizumab; Ram: Ramucirumab; Apa: Apatinib; OS: Overall survival; PFS: Progression free survival; ORR: Overall response rate.

incidents of diarrhea and hypokalemia[19].

Another attempt at HER2 inhibition in first line was the phase Ⅲ TRIO-013/LOGIC trial, where, in a selected population of HER2 positive patients, the addition of Lapatinib, a small intracellular TKI of ERBB1 and ERBB2, was evaluated on whether it would improve the survival benefit derived by Oxaliplatin/Capecitabine doublet chemotherapy. Unfortunately, the trial failed to demonstrate a statistically significant survival benefit. However, it did raise the question of the accuracy of the current method of appreciating HER2 positivity, since the observed clinical benefit closely correlated with the degree of gene amplification as well as with HER2 protein levels, implying that implementing a different scoring system where HER2 over-expressing tumors are defined by an IHC score of more than 3 (IHC) or 2 (FISH) values, may be more precise[20].

Lapatinib was also evaluated in the second line in the phase Ⅲ Asian TyTAN trial, where it was added to weekly Paclitaxel. It is interesting to note that the trial was performed in an unselected population, with 31% demonstrating weak (IHC: 1+) or none at all HER2 positivity. No survival benefit was noted in the study population, although in the subgroup with strong HER2 positivity (IHC: 3+), median survival improved to 14 mo *vs* 7.6 mo (P = 0.0176)[21].

Another negative phase Ⅲ trial compared a monoclonal antibody used in HER2(+) breast cancer, Trastuzumab Emtansine (TDM-1), and taxane monotherapy in HER2(+) patients (GATSBY trial). However, as in the TyTAN trial, HER2 expression was evaluated in archived samples, not taking into account the clonal heterogeneity and the possibility of tumoral evolution that may have occurred from the first to second line chemotherapy setting[22].

An attractive hypothesis regarding the etiology of the negative results of the above mentioned trials, apart from using archival samples, is the downregulation of HER2(+) tumors as a result of our targeting the HER2 protein in the first line setting. It is possible that HER2-directed therapies should be implemented preferably in the beginning of the treatment algorithm, with continuation or switch to another HER2 targeting agent, beyond progression, remaining an option for the select few who retain HER2 positivity. However, this is currently hypothesis-generating and should be confir-

med within a clinical trial.

Inhibition of angiogenesis: Neoangiogenesis has an established role in GC pathogenesis, mainly through vascular endothelial growth factor (VEGF)/VEGFR2 signaling, as there is evidence that VEGF serum levels correlate with increased stage and worse prognosis[23]. In animal models, VEGFR2 inhibition led to angiogenesis impairment and tumor regression[24].

Based on these data, targeting this pathway, either the receptor or the ligand, with monoclonal antibodies and TKIs has been studied in various clinical trials. In this case, targeting VEGFA with Bevacizumab in combination with traditional chemotherapy in first line has not provided a substantial survival benefit in a phase Ⅲ trial, although results showed a significant improvement in progression free survival (PFS) (6.7 mo *vs* 5.3 mo) and overall response rate (46% *vs* 37.4%)[25].

On the contrary, targeting the receptor has been more effective. In the phase Ⅲ REGARD trial, Ramucirumab, a monoclonal antibody blocking VEGFR2 demonstrated superior survival over placebo in second line[26]. Also, the same drug, when combined with a taxane in second line, also led to a statistically significant survival benefit of 2.2 mo[27]. The attempt to expand the use of Ramucirumab in first line in combination with FOLFOX in a phase Ⅱ trial did not produce the required results[28]. However, there is another ongoing phase Ⅲ trial of Ramucirumab combined with Cisplatin and a fluoropyrimidine in HER2 negative patients in first line (RAINFALL; NCT02314117) that may clarify its efficacy in this setting[29].

Inhibiting angiogenesis with TKIs also has a role in the management of advanced GC. Apatinib, a multikinase inhibitor mainly targeting VEGFR2, significantly improved OS over placebo in a phase Ⅲ trial in patients with heavily pretreated advanced GC, which led to its regulatory approval as monotherapy beyond second line[30]. Also, Regorafenib, another multikinase inhibitor targeting, among others, VEGFR2, is currently being tested in the same setting in a phase Ⅲ trial after successfully achieving its primary endpoint of superior PFS in a relevant phase Ⅱ trial[31,32]. Sorafenib resulted in disease stabilization and moderately good PFS in chemo-refractory patients in first- and second-line, but its addition to chemotherapy did not provide adequately encouraging results to justify a phase Ⅲ trial[33-36]. Therefore, it appears that inhibition of angiogenesis has a definite role in advanced GC. Still, there are only hints regarding the potential predictive biomarkers that would help in individualizing its use. For instance, the two less immunogenic subtypes in the TCGA classification, namely the CIN and GS, were associated with *VEGFA* gene amplification and elevated expression of angiogenesis-related pathways, respectively, providing some clues regarding the importance of angiogenic pathways as a driving force of progression in tumors with these molecular signatures[14]. It must also be noted that the positive results with angiogenesis inhibition have been produced in the later lines of treatment, which may imply that in the early stages of GC progression, angiogenesis has a less substantial role, while it is more predominant in later stages of the natural course of the disease. Lastly, it is important to note that targeting the receptor rather than the ligand seems to be the appropriate strategy, a phenomenon for which we have not yet reached a clear and robust explanation but may prove crucial for future anti-angiogenic strategies.

EGFR inhibition: Epidermal growth factor receptor (EGFR) or Erb-B1 is a transmembrane receptor found overexpressed in 30% of GC, while the *EGFR* gene is amplified in nearly 5%[37]. Increased EGFR signaling has been correlated with higher stage, poorly differentiated tumors, and increased invasiveness[38-40]. In preclinical models, Cetuximab, a chimeric anti-EGFR antibody, induces antibody-dependent cell-mediated cytotoxicity (ADCC)[41]. Phase Ⅱ trials with Cetuximab, Panitumumab, or Erlotinib combined with cytotoxics have yielded responses ranging between 41% and 65%, while second line Gefitinib or Erlotinib monotherapy has provided less impressive results, with responses between 9% and 11%, limited mostly to proximal GC[42-47].

These data have prompted testing of anti-EGFR targeting in phase Ⅲ trials. However, both EXPAND and REAL3 phase Ⅲ trials testing Cetuximab and Panitumumab in combination with Cisplatin-Capecitabine and EOX, respectively, did not show any PFS or OS benefit. Again, this may be attributed to poor patient selection, since the study population was not evaluated for EGFR expression or gene amplification[48,49]. The potential importance of this parameter has been made clear in at least two studies: in the phase Ⅱ study combining FOLFOX with Cetuximab, where the patients that exhibited greater than four *EGFR* gene copies demonstrated increased OS, and also in the TRANS-COG, where the subset of EGFR-amplified patients derived a statistically significant survival benefit with the addition of Gefitinib (HR = 0.19; $P = 0.007$)[50,51].

This appears to have been taken into account in a phase Ⅲ trial of second-line Nimotuzumab with Irinotecan (NCT01813253), which is currently recruiting patients that harbor EGFR-overexpressing (IHC: +2/3) tumors[52].

PI3K/Akt/mTOR inhibition: Resistance to targeted therapies often appears as a result of activation of downstream effectors by alternative molecular pathways. The PI3K/Akt/mTOR pathway in GC may become constitutively activated either through mutations in the *PI3K* gene, which occurs most often in EBV(+) and MSI tumors, or through inactivation of *PTEN* gene, the main negative regulator of the pathway, which is mostly found in the MSI subtype[3,53].

Targeting this pathway with an mTOR inhibitor, Everolimus, has produced encouraging results in a phase Ⅱ trial, producing a median PFS of 2.7 mo and OS of 10.1 mo[54]. However, the phase Ⅲ GRANITE-1 trial

that compared Everolimus to placebo in an unselected patient population, as second- or third-line therapy, failed to demonstrate any survival benefit. Once again, the study population was unselected for PI3K pathway activation[55]. Impairment of *Akt* function *via* allosteric inhibition in a phase Ⅱ study of the small molecule MK-2206, in unselected patients, did not produce any positive results either[56].

The above findings, rather than just annulling the findings of basic science, may be viewed as a further indication for the need of appropriate patient selection. PI3k/Akt/mTOR inhibition may still have a role where activation of this pathway is indeed the driver of cancer progression.

MET inhibition: The *MET* proto-oncogene encodes the c-MET receptor tyrosine kinase that has a crucial role in cell proliferation, angiogenesis, and migration. Its canonical activation pathway is *via* binding of its ligand, hepatocyte growth factor (HGF), but the activation can result independently of the binding through gene amplification or somatic mutation. The MET gene has been found amplified in 4%-10% of GC, while its protein product has been found overexpressed by IHC in up to 70%[57]. The implications of this deviation between gene amplification and protein overexpression have been made evident in the MET-targeted clinical trials.

All phase Ⅱ and Ⅲ trials that included patients based on MET overexpression *via* IHC provided negative results. A probable explanation is the vague definition of MET positivity by IHC. In the phase Ⅲ RILOMET study, the addition of Rilutumumab, an HGF-targeting monoclonal antibody, to triplet chemotherapy (ECX) proved detrimental. The study was terminated prematurely because of increased risk of death in the investigational arm[58]. The main targeted agents evaluated in various clinical settings in GC are presented in Table 2.

Targeting cancer stemness

A possible way in which tumors survive complete elimination from cytotoxic chemotherapy is the presence of cancer stem cells. Cancer "stemness" is frequently manifested through the activation of the STAT3 pathway, which induces the transcription of *Nanog* and *Myc* genes. The rationale for investigating this pathway in GC after failure of previous therapies in a large phase Ⅲ trial (BRIGHTER) was provided by encouraging response and disease control data from phase Ⅰ and Ⅱ trials, where the small molecule BBI608 (Napabucasin) was combined with Paclitaxel This trial is ongoing, however, interim analysis indicated diminished possibility of achieving the primary endpoint of OS[59,60].

Targeting DNA damage repair pathway

Poly (ADP-ribose) polymerase (PARP) is essential in correcting single-strand DNA breaks induced by cytotoxic agents. Inhibition of PARP has provided significant benefit in the subgroup of patients with breast and ovarian cancer that already exhibit a certain level of defect in the DNA repair mechanism, such as loss of function of *BRCA1/2* genes. Since *BRCA1/2* mutations in GC are rare, this strategy was implemented in tumors that are characterized by other defects in the repair pathway, like in the *ATM* gene, a quality termed "BRCAness"[61,62]. Preclinical and early clinical trials on tumors with *ATM* deficiency and *TP53* mutations were completed with significant success[63]. However, the phase Ⅲ GOLD trial failed to reveal a statistically significant, according to predetermined criteria, survival benefit in patients treated with Olaparib and Paclitaxel. This failure might once again be attributed to poor patient selection, since the study population was not selected based on *TP53* mutations, while furthermore only 18% of patients were *ATM* negative[64].

Targeting the tumor microenvironment

Andecaliximab, previously known as GS-5745, is a monoclonal antibody that targets matrix metalloproteinase (MMP) 9, an extracellular enzyme involved in matrix remodeling, angiogenesis, tumor growth, and metastasis. Encouraging results from the phase Ⅰ study, where it was combined with FOLFOX in patients both treatment naive and pretreated, have secured its evaluation in a phase Ⅲ trial (NCT02545504), where it is tested in first line in the same combination. The trial has completed accrual, and results are awaited. It is important to note that this strategy, if successful, has the potential to be implemented in a wide spectrum of patients with GC, without the need for a predictive biomarker. Also, since MMP inhibition affects the collagenous stroma of the tumor, not only will it clear the path for the chemotherapy drugs to reach cancer cells, but also it will enhance tumor immunogenicity, with obvious implications for a potential combination with immunotherapy[65].

Manipulating immune responses

Immunotherapy, mainly through the form of checkpoint inhibitors, has over the last few years been added to the armamentarium of various cancer therapeutic approaches, with serial approvals for the treatment of a wide spectrum of solid and hematologic malignancies. Unfortunately, the only single predictive biomarker we currently have at our disposal is PD-L1, which is far from being the most efficient in the field. Indeed, patients without PD-L1 expression can still respond, while others who express the biomarker do not derive benefit. In GC, contrary to melanoma or lung cancer, PD-L1 is expressed mostly in myeloid-derived immune cells and not in tumor cells[61]. The presence of MSI, as manifested through IHC or polymerase chain reaction (PCR), is considered predictive for response to immunotherapy, while other approaches, such as IFN-γ signature and immunoscore, have not yet been incorporated to clinical practice.

There is adequate evidence supporting the implementation of immunotherapy in GC management, both

preclinical and clinical. Firstly, there seems to be an association between PD-L1 and disease burden and, consequently, to limited survival[66]. In addition, according to the data from TCGA, as previously mentioned, elevated PD-L1 expression has been noted in the EBV(+) GC subtype, which correlates with the significant amount of the neoantigens produced as an effect of viral infection, as well as of amplification of 9p24[3]. Furthermore, it is well established that MSI-high tumors also mount a robust immune response, which predicts for clinical outcome and benefit of immune checkpoint blockade[67-69]. Clinical trials thus far have focused on checkpoint inhibitors, especially anti-PD-1/anti-PD-L1 and anti-CTLA4 antibodies, with the best results having been produced by the former.

The first trial to test an anti-PD1 inhibitor in advanced disease was the Keynote-12, where the safety and activity of Pembrolizumab in this setting was assessed. Only patients with PD-L1 positive tumors were enrolled. PD-L1 positivity was deemed as membrane staining in ⩾ 1% of cells, or alternatively as the presence of a distinctive PD-L1 positive pattern at the interface between neoplastic cells and their adjacent stroma. In this trial, no association between PD-L1 levels and response was observed. The results were similar to other trials of anti-PD-1 in various solid malignancies, with a response rate of 22% (95%CI: 10-39) and manageable toxicity profile, prompting the initiation of two large phase Ⅲ trials[70]. The Keynote-061 is evaluating Pembrolizumab *vs* Paclitaxel in the second line[71]. In the first-line setting, Keynote-062 has three arms comparing pembrolizumab as monotherapy and platinum/5-FU combination with or without pembrolizumab[72]. Finally, following the most recent trend of combining immunotherapy with targeted therapies or chemotherapy, two multicenter phase IB/Ⅱ studies are ongoing, determining activity and safety of Pembrolizumab in combination with anti-HER2 agents in patients with HER2 positive GC (NCT02901301 and NCT02689284)[73,74]. Their results are eagerly awaited.

Continuing with PD-1/PD-L1 inhibition, Nivolumab, another anti-PD-1 agent, was the first to gain approval in the third line setting, following the positive results of the pivotal phase Ⅲ trial ONO-4538/BMS-936558 (ATTRACTION 2). This trial, which employed an all-Asian study population, showed a statistically significant, albeit numerically small, survival benefit for Nivolumab over placebo in heavily pretreated patients with advanced/metastatic GC or GEJC. Median OS was 5.3 mo *vs* 4.1 mo (HR = 0.63, $P < 0.0001$,) and mPFS was 1.61 mo *vs* 1.45 mo (HR = 0.60, $P < 0.0001$) in the Nivolumab ($n = 330$) and placebo arms ($n = 163$), respectively[75]. This resulted in the Food and Drug Administration (FDA) approval of Nivolumab for GC or GEJC, in third line or beyond, irrespective of PD-L1 expression.

Finally, in the field of PD-1/PD-L1 axis inhibition, another promising agent is the anti-PD-L1 Avelumab, which has provided promising clinical activity in unselected patients, treated as first-line maintenance or second-line after progression, in the phase Ib trial JAVELIN. In this trial, patients were randomized after treatment with a first-line chemotherapy-based regimen by progression status: patients achieving disease control received Avelumab as switch maintenance, while those with progressive disease received the drug as second line. An acceptable safety profile, which was the primary endpoint of the trial, was demonstrated. Overall response rate was 9.0% and 9.7% in the two subgroups, respectively[76]. Following these positive results, two randomized phase Ⅲ trials were developed: JAVELIN Gastric 100, testing Avelumab as switch maintenance in the first line setting, and JAVELIN Gastric 300, in the third line[77,78]. Unfortunately, it was recently announced that JAVELIN Gastric 300, comparing single-agent Avelumab with physician's choice of chemotherapy, did not meet its primary endpoint of superior overall survival. The other phase Ⅲ trial is still ongoing.

Less encouraging has been the use of anti-CTLA4 inhibitors. Firstly, regarding Ipilimumab, the Phase Ⅱ trial (NCT01585987) that compared the drug to placebo in the second line was stopped prematurely when it became evident that the final analysis would procure no PFS benefit[79]. Also, no responses were reported with Tremelimumab, another anti-CTLA-4 inhibitor in the same setting[80]. It should also be noted that higher toxicity was observed in these trials, as compared to anti-PD-1/PD-L1 blockade. These differences might be attributed to the different targeting of these two classes of checkpoint inhibitors. While those targeting the PD-1 axis have an immediate effect in the tumor microenvironment, the anti-CTLA-4 modulates the immune response mainly in the lymph nodes.

In an attempt to enhance the activity of anti-CTLA-4 agents, combination treatment with anti-PD-1 was tested. The CheckMate-32 was a phase Ⅰ/Ⅱ trial with three arms: 160 pretreated patients were randomized to receive either Nivolumab monotherapy in the dose of 3 mg/kg, or Nivolumab plus Ipilimumab in the doses of 3-1 mg/kg in the second arm or 1-3 mg/kg in the third arm of the study. In all three arms, notable responses were observed, with an overall disease-control rate of 38%. The responses differed between PD-L1-positive (⩾ 1%) and PD-L1-negative (< 1%) tumors, reaching 27% and 12%, respectively. The highest overall response rate (26%) and overall survival (6.9 mo) were observed in arm 3 (Nivolumab 1 mg/kg and Ipilimumab 3 mg/kg), which prompted the launch of a phase Ⅲ trial[81]. The ongoing CheckMate-649 investigates Nivolumab plus Ipilimumab *vs* FOLFOX/XELOX in the first line, and a subgroup analysis regarding PD-L1 expression has already been planned[82].

Conclusively, immunotherapy could have a role in GC management, although, as in the management of other cancers, better predictive biomarkers are required. Moreover, it remains to be seen whether there is rationale for combining immunotherapy with targeted therapies and/or chemotherapy.

CONCLUSION

Even though most clinical trials investigating targeted agents have not produced the desired results so far, their failures might be attributed mostly to erroneous study planning and unscrupulous patient selection. The value of recognizing distinct molecular cancerous pathways goes far beyond mere classification purposes, and shall be better appreciated when these results could be applied in everyday practice with the purpose of providing clinically meaningful outcomes for our patients. Unfortunately, it is still unclear whether the clinical benefits of implementing next-generation sequencing and targeted therapies in the clinic will outweigh the economic burden of such a practice. Perhaps a way to tackle this issue is to create a panel of the main molecular and immune signatures of implemented pathways in order to categorize appropriately the patients in distinct prognostic and predictive subgroups. The results of the TCGA and ACRG classifications, among others, may provide the basis of such a molecular/immune signature panel that remains to be validated prospectively in large clinical trials providing the basis for rational stratification and design.

Health economics concerns aside, if our goal is to optimize outcomes for our GC patients, we probably need to implement these new molecular signatures in our daily practice. Due to the complexity of the disease, the constant evolution of tumors, and our still limited understanding of its biology, our mission to provide the best therapy to our patients is extremely difficult and challenging. However, through targeting tumorigenic drivers and awakening the immune system through immune-oriented strategies, it might be possible that we will at least be able to achieve the goal of life prolongation, while, at the same time, effectively alleviate cancer-related symptoms. A potential, hopefully not overly idealized, glimpse to the future of managing this disease, entails its multidisciplinary management by a variety of experts from diverse scientific backgrounds, towards an individualized approach for each unique patient.

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MINIREVIEWS

Prediction of malignancy and adverse outcome of solid pseudopapillary tumor of the pancreas

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Abstract

Since solid pseudopapillary tumor of the pancreas (SPTP) was officially classified by the World Health Organization in 1996, SPTP has recently received special attention in the literature. Studies have shown that SPTP is a heterogeneous tumor, with a small percentage of patients harboring aggressive behaviors. However, criteria for malignancy grade in SPTP have not been well established. The prognosis of SPTP is generally good, with cases having a chance for long-term survival even with recurrence and/or metastasis after surgical resection. The current American Joint Committee on Cancer/Union for International Cancer Control tumor, node, metastasis staging system is not specific to SPTP. The lack of a predictive staging classification that accurately describes the heterogeneity of this disease hinders meaningful research into optimal individualized therapy. Here we summarize and discuss the associated factors proposed for appraisal of the malignant potential and adverse outcome of SPTP.

Key words: Pancreas; Recurrence; Solid pseudopapillary tumor; Malignancy; Metastasis

Core tip: Solid pseudopapillary tumor of the pancreas (SPTP) is a heterogeneous tumor, with a small percentage of patients harboring aggressive behaviors. Its prognosis is generally good, with cases having a chance for long-term survival even with recurrence and/or metastasis after surgical resection. The lack of a predictive staging classification that accurately describes the heterogeneity of this disease hinders meaningful research into optimal individualized therapy. Here we summarize and discuss the associated factors proposed for appraisal of the malignant potential and adverse outcome of SPTP.

You L, Yang F, Fu DL. Prediction of malignancy and adverse outcome of solid pseudopapillary tumor of the pancreas. *World J Gastrointest Oncol* 2018; 10(7): 184-193 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i7/184.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i7.184

INTRODUCTION

Since solid pseudopapillary tumor of the pancreas (SPTP) was officially classified by the World Health Organization (WHO) in 1996, SPTP has been accepted worldwide. It had also been called Frantz tumor, papillary cystic tumor/neoplasm/carcinoma, solid and papillary neoplasm, solid and papillary epithelial neoplasm, solid and cystic tumor, and solid and cystic papillary epithelial neoplasm. Most of the tumors are found in young women in their second or third decade while it is rare in male patients, accounting for 12.05% of all cases. More than half of the patients are under the age of 25 years[1]. Occasionally, it occurs in children[2]. There was no significant difference in age between male and female patients. Approximately one-third of patients were asymptomatic, with the tumors incidentally discovered during physical examination or in work-up for unrelated diseases[1]. Although several genetic alterations such as somatic mutations in exon 3 of CTNNB1, and upregulated genes activated in Wnt/β-catenin, Hedgehog, and androgen receptor signaling pathways have been identified[3-5], the tumorigenesis of SPTP is still not clear. The incidence of SPTP seems to be increasing, and study of this rare tumor is thus of clinical significance.

Previously, SPTP was mostly considered as a benign tumor, but not until the 2010 version of the WHO classification was issued, all SPTPs are considered as low-grade malignant tumors. Studies have shown that SPTP is a heterogeneous tumor, with a small percentage of patients harboring aggressive behaviors[6-8]. Even if the tumor has no evidence of malignant potential, such as perineural invasion, vascular invasion, invasion of pancreatic parenchyma, and infiltration of peripancreatic tissue, it may metastasize to the liver or recur after surgery. Long-term survival can be achieved in SPTP patients with advanced or metastatic disease, which reveals that SPTP is a relatively indolent disease compared with other pancreatic carcinomas. It is difficult to elucidate the natural course of SPTP and to predict its malignancy and outcome after surgery due to limited follow-up studies. As such, SPTP remains a pancreatic surgical enigma and studies have failed to identify prognostic factors predicting its malignant behavior.

EPIDEMIOLOGIC TREND

The incidence of SPTP has increased markedly in recent years, possibly due to the ready use of modern imaging, diagnostic endoscopy, and physician awareness. Although epidemiologic trends have been documented for pancreatic cystic lesions[9-11], the true incidence and epidemiologic trend for SPTP are less clear. An understanding of its epidemiology has been hampered by the pervasive tendency to report the incidence along with other pancreatic tumors.

As the incidence of pancreatic tumors in China increases year by year[12], the number of patients with pancreatic diseases admitted to Huashan Hospital affiliated to Fudan University, Shanghai, China has continued to grow, so has the number of surgical procedures performed during the last decade. The number of patients with SPTP also increased during these years, with an average of more than six patients with this disease having been confirmed each year. Literature related to SPTP and the number of patients reported have rapidly grown since 1996 (Figure 1). A total of 390 cases were described in a previous systematic review of SPTP cases reported in China between 1996 and 2006[1]. Law *et al*[13] conducted a systematic review of English literature concerning SPTP published up to 2012, and identified 2744 cases of SPTP. A nationwide survey from South Korea showed that SPTP ranked as the third most common pancreatic cystic tumors (18.3%)[14]. These figures indicate that SPTP is not uncommon now worldwide. Given the population trend and the paucity of studies available to guide management of patients with SPTP, further research is imperative.

NATURE HISTORY AND TUMOR BIOLOGY

The origin, biological behavior and nature history of SPTP are not fully understood until now, leaving it as an enigmatic entity. SPTP was regarded as a borderline malignant tumor initially due to lack of evidence-based demonstration of true benign tumor. The WHO used the term "low-grade malignant" instead of "benign" in 2010. SPTP has a wide variability of tumor features from completely solid to almost completely cystic. Imaging studies have shown that smaller SPTPs usually appear as completely or mostly solid, while larger SPTPs typically appear as a large well-encapsulated heterogeneous mass with varying solid-cystic components due to necrosis, hemorrhage and degeneration[15]. A recent report revealed that evolution of liver metastasis from SPTP was relatively slow, with the metastatic lesions having a similar growth pattern of primary tumor characterized by a solid-cystic mass with pseudopapillary structures[16].

Parallel to the controversy regarding its histogenetic derivation, assessment of the malignant potential of SPTP remained a major controversial issue for decades. Although SPTP is considered as a tumor of low-grade malignancy, patients with this disease occasionally present with invasion into the portal/splenic vein (Figure 2) and/or adjacent organs or liver metastasis, mimicking pancreatic ductal adenecarcinoma. The prognosis of SPTP is generally good, with cases having a chance for long-term survival even with recurrence and/or meta-

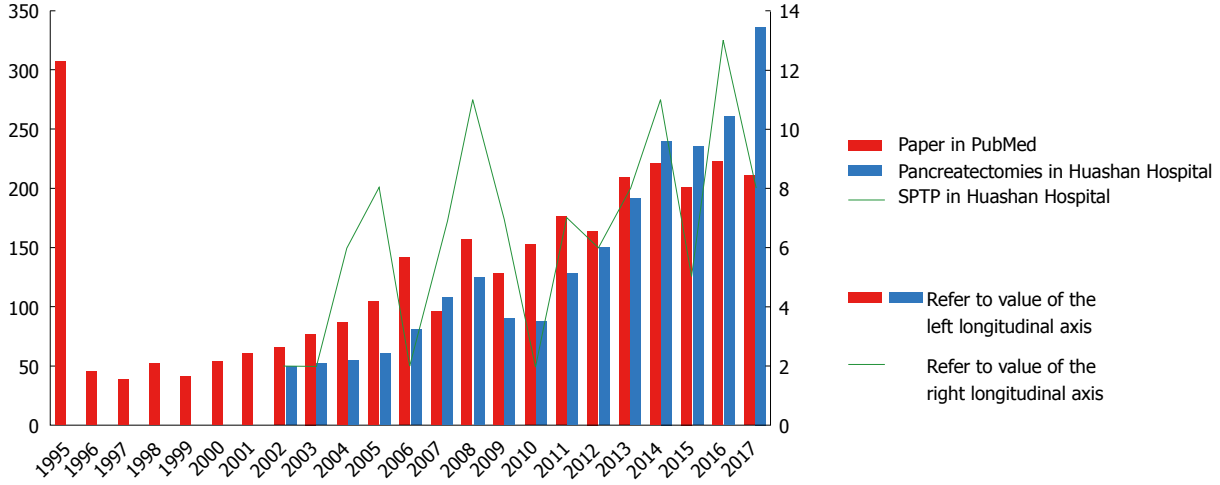

Figure 1 Publications concerning solid pseudopapillary tumor of the pancreas in PubMed, and number of pancreatectomies and patients undergoing surgery for solid pseudopapillary tumor of the pancreas in Huashan Hospital affiliated to Fudan University. Literature retrieved from PubMed (March 1, 2018) with the search terms "frantz tumor", "solid and cystic papillary epithelial neoplasm", "solid and cystic tumor", "papillary cystic tumor", "solid and papillary neoplasm", "papillary cystic carcinoma", "papillary and cystic tumor", "papillary and solid neoplasm", "solid and papillary epithelial neoplasm", "papillary cystic neoplasm", "solid pseudopapillary tumor", "pancreas", and "pancreatic" in "all fields". SPTP: Solid pseudopapillary tumor of the pancreas.

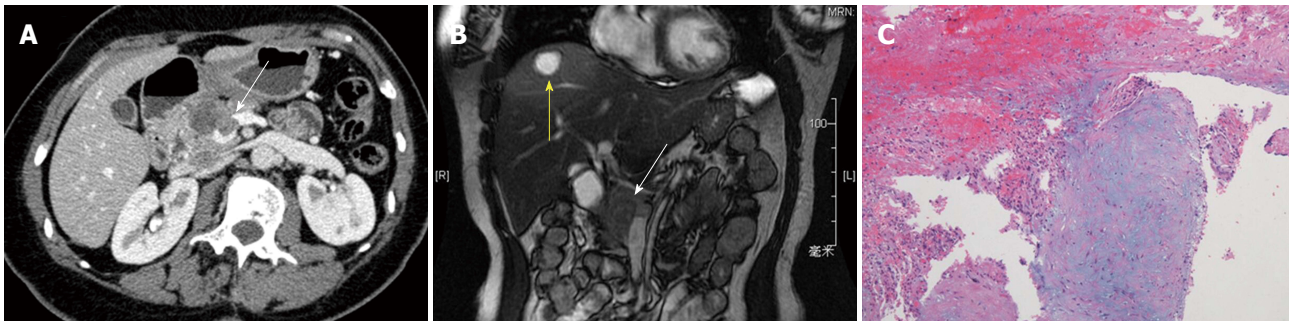

Figure 2 Solid pseudopapillary tumor of the pancreas presenting with invasion into the portal splenic confluence. A: Enhanced computed tomography scan revealed intraluminal filling defect in the portal splenic confluence (arrow); B: An abnormal signal of the pancreatic head (arrow) and high signal foci in the right anterior lobe of the liver (yellow arrow) can be readily delineated from the coronal magnetic resonance imaging (MRI) section; C: Splenic vein tumor thrombus was noted by hematoxylin-eosin staining (× 100).

stasis after surgical resection. Up to 10% of patients experienced a recurrence and/or metastasis of the disease after years of follow-up, and only a small subset of patients eventually died of this disease[6-8,17-37] (Table 1).

DIAGNOSIS OF MALIGNANT SPTP

Studies showed that tumor markers such as carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) were usually within normal ranges in patients with this disease. Thus, routine tumor markers are of no value to predict malignant SPTP[1]. Radiologically, SPTP typically appears as a well-capsulated heterogeneous mass with solid and cystic components, while small SPTP commonly represents a solid mass. Capsule and intratumoral hemorrhage are important clues to the diagnosis as they are rarely detected in other pancreatic neoplasms. In some cases, calcification may be present, whereas pancreatic duct dilatation is rarely found. Yang *et al*[16] reported that the liver metastatic lesions from SPTP increased in sizes gradually with cystic change. The relatively slow evolution of liver metastasis indicates its classic growth pattern. Although the proportion of solid component[38] and incomplete capsule[39,40] were shown to be associated with malignancy by a few reports, no consistent results were demonstrated. Rastogi *et al*[41] reported that tumors with greater enhancement assessed by contrast-enhanced computed tomography (CT) had aggressive characteristics. However, no correlations between malignancy and other radiological features including calcification were found. These findings indicate that diagnosis of malignant SPTP is difficult with imaging studies. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has been shown to be useful for preoperative definite diagnosis[42]. However, it may cause rupture of tumor and seeding of the needle tract by tumor cells during the procedure[43,44]. Although EUS-FNA has been used more frequently than ever in SPTP[13], its malignant nature is still difficult to confirm because of lack of specific markers.

Position emission tomography/computed tomogra-

Table 1 Reported series (> 20 cases) of solid pseudopapillary tumor of the pancreas in the English literature

Ref.	Country	Centers	F/M	Age (yr)	Size (cm)	Malignant, *n* (%)	Follow up (mo)	R/M (*n*)	Alive (*n*)
Peng *et al*[17], 2006	China	Single	25/0	33 (11-65)	9.3 (2.5-25)	3 (12)	3-111	0	25
Yu *et al*[18], 2007	China	Single	25/1	25.2 (13-57)	7.5 (3.8-15)	9 (34.6)	66 (10-237)	2	24
Machado *et al*[19], 2008	Brazil	Single	27/7	23 (10-72)	7 (1.5-15)	13 (38.2)	84 (3-170)	2	33
Lee *et al*[20], 2008	South Korea	Multi	57/5	30 (8-63)	6.5 (1.5-14)	9 (14.5)	47.5 (5.1-240.4)	2	62
Matos *et al*[21], 2009	United States/ Germany	Multi	20/1	33 (13-60)	5.5 (2.5-19.3)	3 (14.3)	55 (7-176)	0	21
Nguyen *et al*[22], 2011	Australia	Multi	30/4	33.3 (19.6-42.3)	6 (4.5-9)	9 (26.5)	70 (48-178)	2	32
Kim *et al*[23], 2011	South Korea	Single	98/16	36 (11-75)	4.2 (1.2-15)	26 (22.8)	57 (11-177)	4	114
Butte *et al*[24], 2011	United States	Single	38/7	38 (10-63)	4.9 (1.4-20)	9 (20)	44 (1-250)	5	38[1]
Cai *et al*[25], 2013	China	Single	30/3	29.2 (12-59)	4.9 (2-15)	17 (51.5)	45 (4-118)	1	32
El Nakeeb *et al*[26], 2013	Egypt	Single	22/2	24.83 (12-52)	9.2 (3-25)	6 (25)	71.6 (1-180)	2	22
Raman *et al*[27], 2013	United States	Single	43/8	29.3 (12.2-74.8)	5.3 (1.7-11.1)	11 (21.6)	37 (0-122)	1	50
Serrano *et al*[28], 2014	Canada	Single	26/6	36 (13-64)	4.7 (1.5-14)	15 (46.9)	43 (3-207)	3	31
Suzuki *et al*[29], 2014	Japan	Single	29/5	37.1 (15-68)	4.3 (1-11)	3 (8.8)	67 (3-326)	0	34
Kim *et al*[30], 2014	South Korea	Single	85/21	36 (10-65)	4.5 (1-15)	17 (16)	56.9 (37-93.4)	2	105
Kang *et al*[6], 2014	South Korea	Multi	317/34	36.8 ± 12.4	5.7 ± 3.3	98 (27.9)	> 6	9	316[2]
Estrella *et al*[7], 2014	United States	Single	54/10	33 (9-62)	5 (1.4-20)	49 (76.6)	76 (2-203)	10	53[3]
Yu *et al*[31], 2015	China	Multi	93/4	31.2 (16-57)	5.9 (1.5-14)	16 (16.5)	70.2 (3.5-221.5)	3	96
Zhang *et al*[32], 2015	China	Single	56/6	26 (8-66)	7.2 (3-15)	3 (4.8)	46 (2-135)	0	62
Yang *et al*[8], 2016	China	Single	58/13	31 (12-64)	5 (1-13)	13 (18.3)	45 (3-118)	3	70
Irtan *et al*[33], 2016	France	Multi	41/10	13.1 (8.7-17.9)	7 (2-12)	22 (43.1)	65 (0.3-221)	7	51
Marchegiani *et al*[34], 2016	Italy/United States	Multi	113/18	33 (7-68)	4 (0.7-20)	16 (12.2)	62 (12-304)	2	105[4]
Xu *et al*[35], 2017	China	Single	93/28	33.7 (11-68)	5 (1-13)	35 (28.9)	42.7 (6-97)	3	100[5]
Song *et al*[36], 2017	China	Single	46/7	35.4 (14-67)	6.4 (2-14)	10 (18.9)	48 (3-123)	2	45[6]
Lubezky *et al*[37], 2017	Israel	Single	29/3	28.4 ± 12.2	5.9 (0.9-14)	13 (40.6)	49.2 (1-228)	4	31

Note: We included data from the latest or most complete study in the case of duplicate reports on overlapping patients from the same institutions; [1]Three patients died of SPTP, and four patients died of other causes; [2]317 patients with more than 6 mo follow-up were reported for evaluation of oncologic outcome; [3]Follow-up information was available for 59 patients; [4]Follow-up information was available for 105 patients; [5]Follow-up information was available for 103 patients; [6]Follow-up information was available for 48 patients. F: Female; M: Male; R/M: Recurrence and/or metastasis.

phy (PET/CT) is a useful modality in the detection of malignant tumors and has been widely used in patients with pancreatic disease[45]. Limited data are available on PET/CT characteristics of SPTP, making the value of this modality controversial. It has been reported that SPTP has significantly higher tumor size-adjusted metabolic tumor volume and total lesion glycolysis compared with pancreatic ductal adenocarcinoma[46], which leads to a high rate of false positivity in F-18-fluorodeoxyglucose PET/CT when diagnosing this disease (Figure 3). However, this feature suggests that PET/CT may be helpful in detecting metastases of SPTP. Kang *et al*[47] categorized SPTP into five types according to the PET images and found no association between the fluorodeoxyglucose uptake and malignant potential. Until now, no definitive conclusions can be drawn about the clinical significance of PET/CT in SPTP due to limited cases reported. Thus, the clinical application value of PET scan in SPTP needs further investigation.

TREATMENT OF MALIGNANT SPTP

Surgical resection is curative in most of the patients with SPTP resulting in a five-year disease-specific survival rate of 98.5%[8]. Long-term survival can be achieved even in those with advanced or metastatic disease. It is interesting to note that patients who underwent limited resection with microscopically positive margins had similar outcomes as those who underwent extensive surgery with R0 resection[7]. The generally good prognosis of SPTP attributes to its relatively low malignant biological behavior. Therefore, aggressive surgical intervention is the optimal therapy for patients with advanced SPTP, even with metastasis. Wang *et al*[48] reported four patients with liver metastases undergoing aggressive surgery. All the patients received surgical resections for both the primary and metastatic lesions as completely as possible, and had good clinical outcomes during follow-up.

Adjuvant therapies such as chemotherapy (5-fluorouracil and gemcitabine as the main chemotherapeutic drugs) and radiotherapy have been reported in a few patients with a mean survival of 51.1 mo[13]. Sporadic reports found that neoadjvant chemotherapy or radiation therapy could benefit some patients with unresectable tumors[49-51]. Other therapeutic methods including radiofrequency ablation[52], transcatheter arterial chemoembolization[53], selective internal radiotherapy (SIRT)[54] and liver transplantation[55] have also been reported to achieve good results for patients with

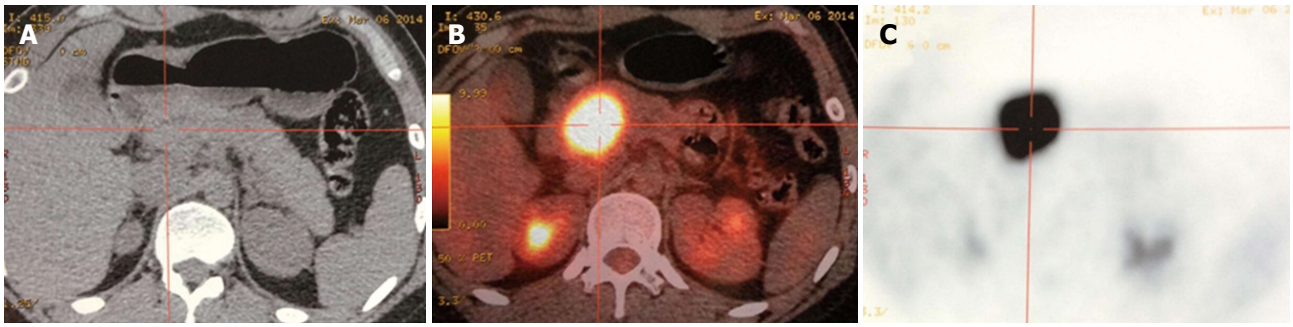

Figure 3 High uptake of F-18-fluorodeoxyglucose in a patient with solid pseudopapillary tumor of the pancreas. SPTP in a 25-year-old female patient with a T2 stage tumor. A: CT scan revealed a 5-cm isodense mass in the pancreatic head. B and C: Transaxial PET/CT (B) and PET (C) showed a hypermetabolic lesion with the maximum standardized uptake value of 33. She was disease free for 32 mo after surgical resection. SPTP: Solid pseudopapillary tumor of the pancreas; PET/CT: Position emission tomography/computed tomography.

liver metastasis from SPTP. However, despite a better understanding of this disease, individual treatment of unresectable or metastatic SPTP requires further study.

PREDICTORS OF MALIGNANCY

Malignant SPTP occurs in 18.3% of adult patients and in 43.1% of pediatric patients[8,33]. Preoperative differential diagnosis between benign and malignant SPTP is usually very difficult except in patients with tumor invasion to adjacent organs or with distant metastasis. There has been no consistency about the diagnostic criteria of malignant SPTP until today. Criteria for malignancy in SPTP have not been well established. Many researchers used the WHO-defined criteria for classification of solid pseudopapillary carcinoma, such as angioinvasion, perineural invasion, or deep infiltration into the surrounding tissue or metastasis to confirm the diagnosis of malignant SPTP[30]. Butte *et al*[24] defined malignant SPTP as locally unresectable tumor with macrovascular invasion, metastatic disease to regional or distant sites, or recurrence of disease after surgery. Ye *et al*[56] considered SPTP with incomplete capsules as malignant.

Due to the arbitrary criteria of malignancy used, and rarity of the disease with small proportion of malignancy, conflicting results have been reported about factors associated with malignant potential across institutions[8,20,23,24,26,27,30,31,35,36,38,39,56-58] (Figure 4). Butte *et al*[24] found that patients with malignant SPTP presented with larger tumor size ($P < 0.005$). Chung *et al*[39] explored differential imaging features between malignant and benign SPTP, and found that malignant SPTP more frequently had focal lobulated margins ($P = 0.027$) and focal discontinuity of capsule ($P = 0.005$). The study by Ye *et al*[56] revealed that SPTP with incomplete capsule had larger tumor size ($P=0.0015$) and mainly exophytic growth pattern ($P = 0.0194$). Yu *et al*[31] and Xu *et al*[35] showed that positive status for Ki-67 correlated with malignancy of SPTP, while Yang *et al*[8] did not demonstrate any association between the Ki-67 index and malignant SPTP. Most other studies[20,23,26,27,57,58] found no significant differences between benign and malignant SPTP, including age, sex, symptomatology, laboratory data, tumor marker, tumor size and location, tumor composition, growth pattern, and histopathology. Thus, malignancy cannot be easily predicted on the basis of preoperative findings and immunohistochemical patterns.

PREDICTORS OF ADVERSE OUTCOME

Most of the patients with SPTP have a good prognosis, while some have a less favorable prognosis because of recurrence and/or metastases. Studies on SPTP were characterized by case reports and small case series lacking of long-term follow-up. Kang *et al*[6] reported a low recurrence rate (2.8%) and excellent disease free survival and overall survival for SPTP after surgical resection in South Korea. The patients had a 5-year disease free survival of 95.4% and an overall survival of 98.8%. In a recent systematic review, the 5-year and 10-year recurrence free survival was 89.5% and 86.3%, respectively, with the 5-year and 10-year disease specific survival of 92.3% and 86.5%, respectively[8]. It is unclear whether factors associated with malignant potential are statistically significant predictors of adverse outcomes. Although a few recent studies have gathered significant series of SPTP, results are inconclusive with regard to predictors of prognosis[3,6-8,28,33-38,59,60] (Figure 5).

Estrella *et al*[7] showed that recurrent/metastatic SPTP was significantly associated with larger tumor size, invasion of muscular vessels, and the European Neuroendocrine Tumour Society (ENETS) tumor stage, but not with other clinicopathologic factors. In addition, muscular vessel invasion, ENETS T4 disease, and stage Ⅳ were important predictors of poor disease-specific survival after surgical resection. Kang *et al*[6] demonstrated that tumor size larger than 8 cm, microscopic malignant features, and stage Ⅳ were significant prognostic factors for tumor recurrence by multivariate analysis. Irtan *et al*[33] confirmed that the significant risk factors for recurrence in pediatric cases of SPTP were age < 13.5 years at diagnosis and positive surgical margins at initial tumor resection. It is interesting to note that many other studies[6-8,22,28,34,60] have shown that patients who

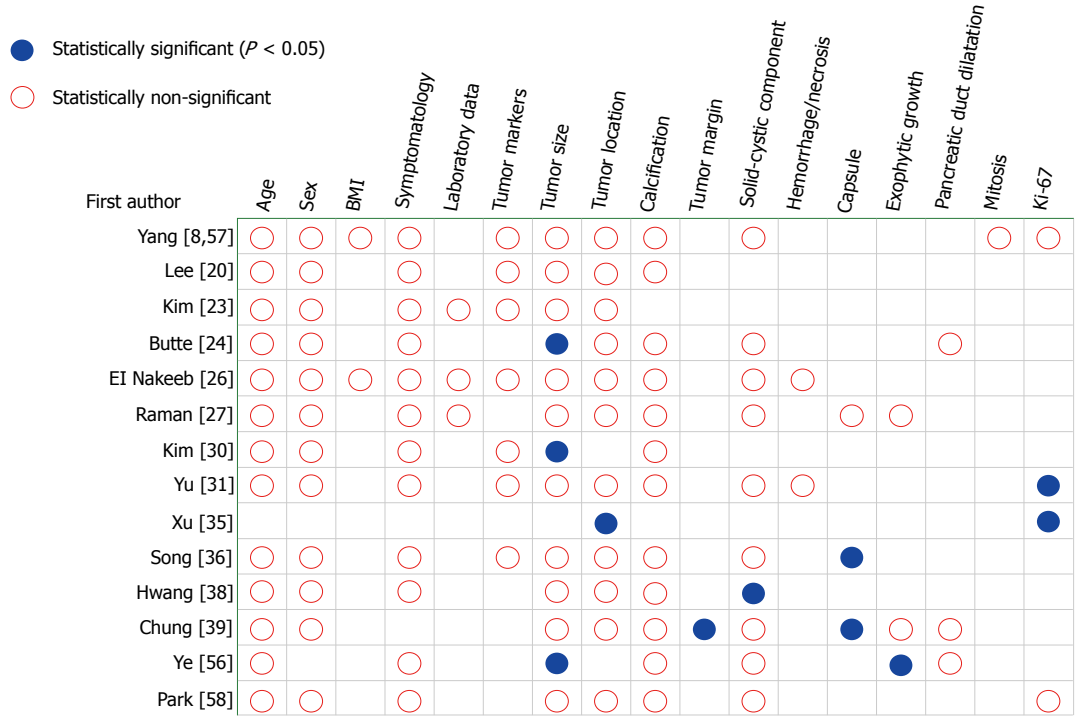

Figure 4 Factors associated with malignant solid pseudopapillary tumor of the pancreas by univariate analysis.

underwent limited resection and those with R1 resection had the same clinical outcomes as those who received more extensive resection with negative margin. Serrano *et al*[28] clarified that patients with stage Ⅳ or lymphovascular invasion more commonly developed recurrence. Both studies of Marchegiani *et al*[34] and Hwang *et al*[38] revealed that recurrence was more common in patients with malignant SPTP which fulfilled the WHO criteria. The study by Zhang *et al*[59] indicated that recurrence in malignant SPTP correlated with family malignant tumor history.

Several studies[3,57,61,62] have proposed Ki-67 as an additional support to histology for predicting tumor outcome, but conflicting results do exist. Yang *et al*[8] identified the most discriminating value of Ki-67 index using receiver operating characteristic curve analysis and demonstrated that the prognostic value of Ki-67 was maintained in both the Huashan cohort and the new historical cohort from literature. The result was consistent with a latest study by Kim *et al*[3]. However, similar to most studies, multivariate analysis could not be performed due to the small number of events. Nevertheless, a much larger number of patients is needed to validate the prognostic relevance of Ki-67.

CHALLENGES AND PERSPECTIVES

Recent studies have analyzed the biological behavior of SPTP, however reliable data on long-term follow-up are still needed. Case reports, small retrospective case series, and subjective views rather than facts dominate the available data. These studies have limitations including a small number of cases or events, no uniform parameters studied, lack of a gold standard for judging malignancy, and short length of follow-up. Although some studies adopted the WHO definition of malignancy, a considerable number of studies did not specify the proportion of malignant patients. The excellent prognosis makes overall survival difficult to be assessed, even if several studies have evaluated disease/recurrence free survival. In the light of these limitations, multicenter large-scale studies with long-term follow-up are needed to determine prognostic factors.

To date, no staging systems have been used to stratify patients in any international guidelines for management and follow-up of SPTP[63-65]. The American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) tumor, node, metastasis (TNM) staging system is a generally accepted standard for cancer staging with the principal aim of facilitating a uniform and standardized analysis of malignant tumors. While the current TNM staging system applies well to pancreatic carcinoma, it is not specific to SPTP. Tumors considered for the TNM system have potentials of local invasiveness (T-categorization) and spread *via* the lymphatic and blood vessels (N- and M-categories). In view of the rarity of lymphatic and hematogenous metastasis from SPTP, its usefulness in this condition was evidently limited.

The relative rarity of SPTP has delayed the development of evidence-based treatment guidelines. Patients with benign SPTP are still at risk of tumor recurrence or metastasis after surgical resection. Contemporary evidence supports surgery as the primary treatment for patients with operable metastatic SPTP[48,66]. One ob-

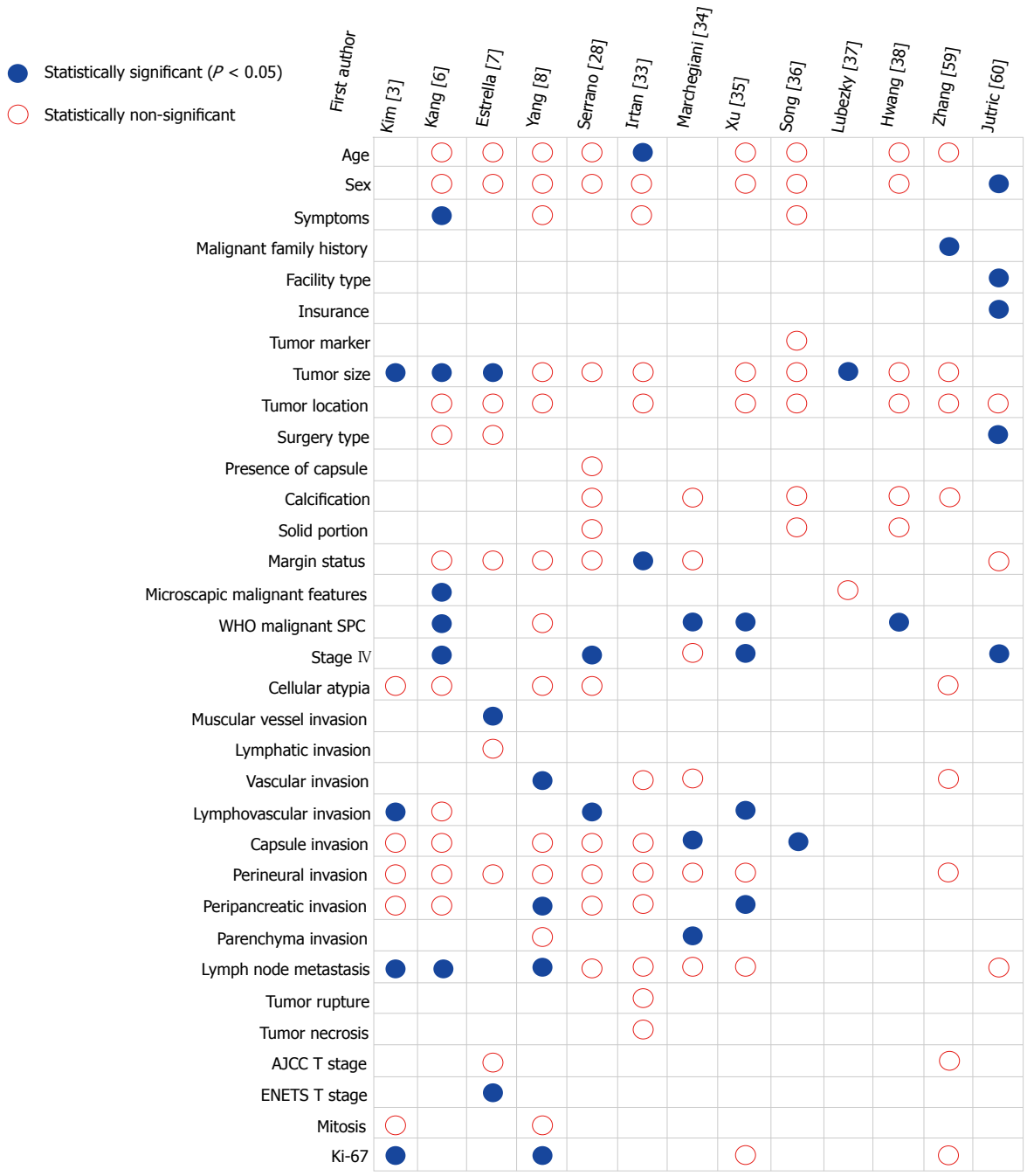

Figure 5 Predictors for adverse outcome of solid pseudopapillary tumor of the pancreas after surgical resection by univariate analysis. WHO: World Health Organization; SPC: Solid pseudopapillary carcinoma; AJCC: American Joint Committee on Cancer; ENETS: European Neuroendocrine Tumour Society.

stacle to better management of patients is the lack of a predictive classification that accurately describes the complexity and heterogeneity of this disease. In order to provide proper information to predict prognosis, a more specific and standardized histopathological evaluation of SPTP is needed. It is obvious that we urgently need an international consensus for collecting standardized data on SPTP. Better understanding of molecular mechanisms involved in SPTP tumorigenesis is important for improved management. It is probable that novel molecular prognostic variables for SPTP, which may be incorporated into classification systems, will emerge in future.

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ORIGINAL ARTICLE

Retrospective Study

Atypical anastomotic malignancies of small bowel after subtotal gastrectomy with Billorth II gastroenterostomy for peptic ulcer: Report of three cases and review of the literature

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Abstract

AIM

To present patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer, to review relevant literature, and to attempt to interpret the reasons those cancers developed to these postsurgical non-gastric sights.

METHODS
For the current retrospective study and review of literature, the surgical and histopathological records dated from January 1, 1993 to December 31, 2017 of our department were examined, searching for patients who have undergone surgical treatment of small-bowel malignancy to identify those who have undergone subtotal gastrectomy for benign peptic ulcer. A systematic literature search was also conducted using PubMed, EMBASE, and Cochrane Library to identify similar cases.

RESULTS
We identified three patients who had developed small-intestine malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy with Billroth Ⅱ gastroenterostomy for benign peptic ulcer-two patients with adenocarcinoma originated in the Braun anastomosis and one patient with lymphoma of the efferent loop. All three patients were submitted to surgical resection of the tumor with Roux-en-Y reconstruction of the digestive tract. In the literature review, we only found one case of primary small-intestinal cancer that originated in the efferent loop after Billroth Ⅱ gastrectomy because of duodenal ulcer but none reporting Braun anastomosis adenocarcinoma following partial gastrectomy for benign disease. We also did not find any case of efferent loop lymphoma following gastrectomy.

CONCLUSION
Anastomotic gastric cancer following distal gastrectomy for peptic ulcer is a well-established clinical entity. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon. The substantial diversion of the potent carcinogenic pancreaticobiliary secretions through the Braun anastomosis and the stomach hypochlorhydria, allowing the formation of carcinogenic factors from food, are the two most prominent pathogenetic mechanisms for those tumors.

Key words: Anastomotic cancer; Efferent loop; Braun anastomosis; Adenocarcinoma; Anaplastic large cell lymphoma

Core tip: Anastomotic gastric cancer following distal gastrectomy for peptic ulcer is a well-established clinical entity. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon. In this paper, three patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer are presented. The two most prominent pathogenetic mechanisms for those tumors are the stomach hypochlorhydria, allowing the formation of carcinogenic factors from food, and the substantial diversion of the potent carcinogenic pancreaticobiliary secretions through the Braun anastomosis.

Kotidis E, Ioannidis O, Pramateftakis MG, Christou K, Kanellos I, Tsalis K. Atypical anastomotic malignancies of small bowel after subtotal gastrectomy with Billorth II gastroenterostomy for peptic ulcer: Report of three cases and review of the literature. *World J Gastrointest Oncol* 2018; 10(7): 194-201 Available from: URL: http://www.wjgnet.com/1948-5204/full/v10/i7/194.htm DOI: http://dx.doi.org/10.4251/wjgo.v10.i7.194

INTRODUCTION

Small-bowel malignancies are among the rarest cancers, accounting for only 2% of all gastrointestinal cancers, even though the organ makes up more than 70% of the length and 90% of the surface area of the gastrointestinal tract[1]. Approximately 60% of small-bowel tumors are malignant, and among those, adenocarcinomas comprise 35% to 50% of all cases, carcinoid tumors 20% to 40%, sarcomas 15%, and lymphomas 10% to 15%[2-5]. Anastomotic gastric cancer following distal gastrectomy for peptic ulcer disease has long been recognized. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon.

In this paper, we present three patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer, and we attempt to interpret the reason that those cancers developed to these postsurgical non-gastric sights.

MATERIALS AND METHODS

For the current retrospective study and review of literature, the surgical and histopathological records dated from January 1, 1993 to December 31, 2017 of our department were examined, searching for patients who have undergone surgical treatment of small-bowel malignancy to identify those who have undergone subtotal gastrectomy for benign peptic ulcer. A systematic literature search was also conducted using PubMed, EMBASE, and Cochrane Library to identify similar cases.

RESULTS

Case 1

A 79-year-old white male presented at our hospital because of chronic anemia appearing as syncope episodes for the last 4-5 mo. He also developed early satiety during this period. In the past, the patient had undergone a subtotal gastrectomy followed by Billroth Ⅱ gastroenterostomy and Braun anastomosis for the treatment of peptic ulcer disease 22 years ago. His history also included hepatitis C, hypertension, type Ⅱ diabetes,

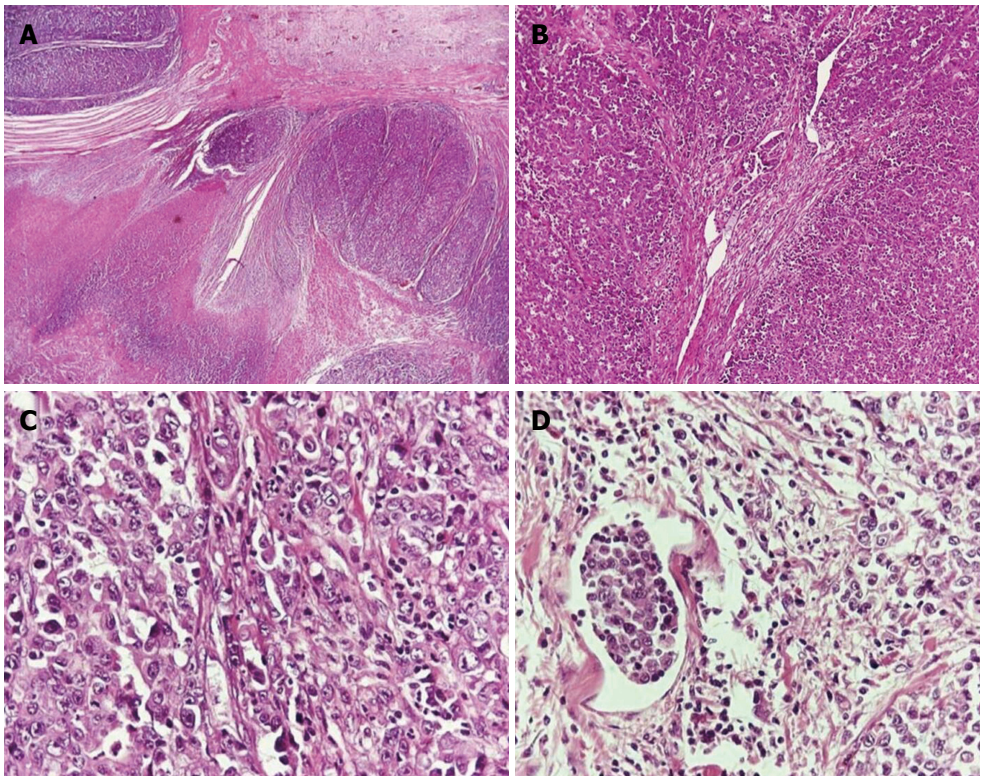

Figure 1 Adenocarcinoma of the small intestine. A: Low-power view shows the nodular formations of the carcinoma and foci of necrosis (H and E, × 25); B: High-power view shows the diffuse growth pattern and the presence of few tubular structures (H and E, × 100); C: High-power view shows the neoplastic cells with the hyperchromatic, irregular nuclei with prominent nucleoli; D: The presence of lymphatic tumor emboli (H and E, × 400).

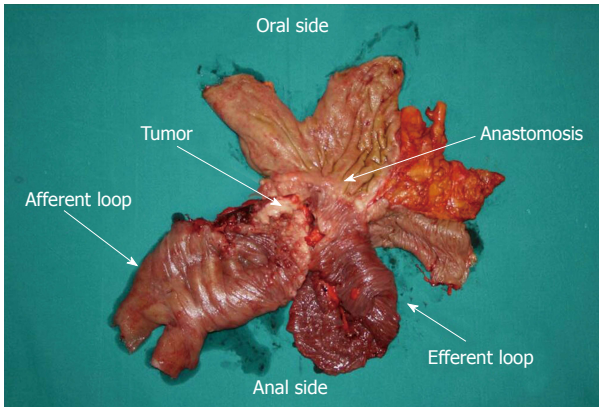

Figure 2 Surgical specimen after partial gastrectomy of the gastric pouch with resection of the Braun anastomosis.

and myocardial infarction. His physical examination revealed an enlarged liver, and his blood tests showed a hypochromic anemia and slightly deranged liver function. The upper gastrointestinal endoscopy showed a lesion with an uneven surface at the Braun anastomosis. The computed tomography (CT) scan demonstrated liver cirrhosis and a tumor at the level of Braun anastomosis.

Hematoxylin- and eosin-stained sections from the lesion revealed the presence of a high-grade adenocarcinoma infiltrating the entire bowel wall and extending into the surrounding mesenteric fat tissue. Fibrotic bands in the histological sample resulted in the formation of nodular configurations. Only focally few tubular structures were identified. Foci of necrosis and lymphatic tumor emboli were also present. The neoplastic cells had hyperchromatic, irregular nuclei with prominent nucleoli, and they arranged mainly in a diffuse growth pattern (Figure 1).

The tumor, about 4 cm in diameter, was resected en bloc with the previous gastrojejunal anastomosis, and the gastrointestinal continuity was restored with a Roux-en-Y gastrojejunal anastomosis. The patient was discharged and is free of disease until today, 9 mo after surgery.

Case 2

A 76-year-old man presented at our hospital with undefined abdominal discomfort and relapsing melenas for the last 2-3 mo. He also experienced a drop in hematocrit during this period. Prior surgical history included a subtotal gastrectomy because of a bleeding pyloric ulcer, followed by a Billroth Ⅱ Hofmeister–Finsterer anastomosis and a jejunojejunostomy (Braun), approximately 30 years ago. His physical examination and laboratory tests were unremarkable except for the presence of anemia. Endoscopy showed a tumor at the Braun anastomosis that ended up being an adenocarcinoma. The abdominal CT showed only the Braun anastomosis tumor. He underwent a partial gastrectomy of the gastric pouch with resection of the Braun anastomosis and tumor, measuring 5 cm × 2 cm, and a Roux-en-Y reconstruction (Figure 2). The patient had a smooth postoperative course and 2.5 years after treatment is free of relapse.

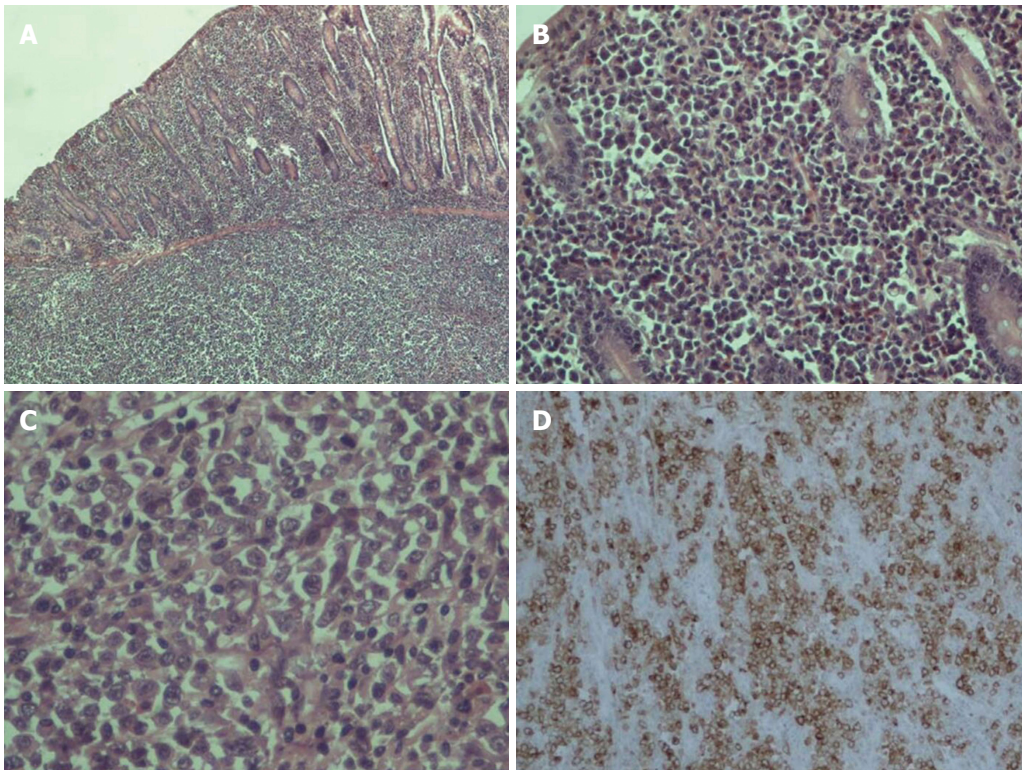

Figure 3 Anaplastic large cell lymphoma of the small bowel. A: H and E stain × 40; B: H and E stain × 200; C: H and E stain × 400; D: Ki-1 antigen (CD30) staining (+).

Case 3

A 78-year-old man was referred to our hospital for hematemesis and melena. The patient had undergone a partial gastrectomy with Billroth Ⅱ gastroenterostomy because of duodenal ulcer disease 30 years ago. His history, however, included splenectomy caused by trauma, cholecystectomy, and hypertension. His physical exam and blood tests were unremarkable except for the presence of a normocromic anemia. He underwent an upper-gastrointestinal endoscopy, which identified a sizable ulcer crater at the beginning of the efferent jejunal loop, about 4 cm from the anastomosis, with unsuccessful attempts of permanent hemostasis. A laparotomy was decided upon. A large tumor of the efferent jejunal loop was identified with multiple small infiltrations in the afferent loop of 15-20 cm. The rest of the small intestine was free. The *Helicobacter pylori* examination was positive. A segmental resection of the gastric pouch and the infiltrated jejunal loops was performed, followed by a Roux-en-Y reconstruction.

On histopathologic examination, the reported ulcer was part of a grayish intramural lesion that infiltrated the entire wall of the intestine. Microscopically, large undifferentiated neoplastic cells were widely disseminated. The cells contained a moderate amount of cytoplasm and sizable, oval, frequently irregular pleomorphic nuclei with multiple prominent nucleoli. Binucleate, abnormal multinucleate, and multilobed nuclei formats were observed (Figure 3). By immunohistochemistry, the large cells were strongly positive for CD30. They were also positive for vimentin, epithelial membrane antigen (EMA), CD7, CD43, and MUM1. Partial positivity was for the antigens CD138, p53, CD38, CD45RO (LCA), perforin, and AE1/AE3 (cytokeratin). The large cells were negative for the expression of CD2, CD3, CD5, CD4, CD8, ALK, CD56, CD20, CD79a, PAX5, CD45RA, TIA1, CD15, myeloperoxidase (MPO), lysozyme, and EBV-LMP1.

The findings are consistent with anaplastic large cell lymphoma (ALCL) ALK-negative (anaplastic lymphoma kinase), a rare type of non-Hodgkin lymphoma. The resection boundaries were free of neoplastic infiltration, and no lymph node involvement (17 in total) was found. The patient has been referred to the hematology department for further treatment and follow-up.

DISCUSSION

Small-bowel adenocarcinomas are relatively uncommon and have a slight male preponderance (3:2), and their peak incidence is the seventh decade of life[1]. They are believed to arise from premalignant adenomas[6]. They also have a predilection for the duodenum, with a marked decrease in frequency moving axially along the small bowel[1]. Knowing from physiology that this is exactly the effect of the distribution of ingested chemicals and the effect of gastric and pancreaticobiliary secretion on intestinal mucosa may indicate that these substances may have carcinogenic properties[4,5]. Furthermore, small-bowel adenocarcinoma is associated with Crohn's disease (up to 100-fold risk), celiac disease,

and familial polyposis syndrome, none of which were included in our patients' history. There is not a specific complex of symptoms diagnostic for small-bowel cancer, but the most common are abdominal pain, nausea, obstruction symptoms, and weakness. Bleeding, either occult as melena like our first and second case or acute in the form of hematemesis like our 3rd case, is more uncommon[2,6].

Following distal gastrectomy for peptic ulcer disease, gastric cancer can develop, usually after years, in the gastric remnant[7]. A gastric remnant carcinoma is defined as a primary carcinoma arising in the stomach, remnant at least 5 years after previous partial gastrectomy for benign disease, most frequently peptic ulcer disease. The 5-year interval is necessary to avoid confusion with cancer recurrence after initial misdiagnosis. Several large prospective studies with long-term follow-up indicate that the relative risk for this gastric neoplasm development is not increased for up to 15 years after gastric resection[8,9], likely because of surgical removal of mucosa at risk for gastric cancer development, followed by modest increases in cancer risk (three times the control value) observed only after 20 years[10-12]. Recently, conservative medical therapy has displaced partial gastrectomy for the treatment of ulcer. Nevertheless, since surgical therapy was still used frequently for the treatment of gastroduodenal ulcer disease until the late 1970s and early 1980s and gastric remnant carcinoma develops with a time interval of 20–40 years, the surgeon will be confronted with this disease regularly until at least 2020[13]. Stage for stage, the prognosis for gastric stump cancer is similar to proximal gastric cancer[14].

Additionally, Ravi Thiruvengadam *et al*[15] had attempted to estimate the risk of cancer at gastrointestinal spots other than the stomach, such as the small and large intestines, the esophagus, and the gallbladder, after gastric surgery for benign disease. There was no strong evidence for an increased risk of any gastrointestinal cancer following gastric surgery. However, after 10 years, Staël von Holstein *et al*[16] showed that there is an increased risk for nongastric gastrointestinal cancer, but similar to gastric remnant carcinoma, that risk emerges only 20 years postoperatively. The abovementioned studies concluded that all patients should be screened after an interval of 15-20 years after the distal gastrectomy.

With regard to the pathogenesis of gastric remnant cancer, the predominant factors presumed to be responsible for it are duodenogastric reflux and hypochlorhydria. Chronic duodenogastric reflux causes various histological alterations at the gastric stump, such as intestinal metaplasia, dysplasia, and adenoma. The gastrojejunal anastomosis is considered the most common site of gastric remnant carcinoma because the quantity and concentration of gastroduodenal reflux are highest here. Both bile acids and pancreatic juice seem to be carcinogenic factors, even though we do not know exactly which components are responsible. Bile acids, such as deoxycholic bile acid and nitrated derivatives of glycocholic and taurocholic bile acids, seem to have a carcinogenic influence at the gastric stump mucosa[5,17-19]. Braun, in 1893, introduced the jejunojejunal anastomosis between the afferent and efferent small intestine loops immediately distal to a gastrointestinal anastomosis. Using radionuclide biliary scanning, Vogel *et al*[20] found that Braun enteroenterostomy adequately diverts a substantial amount of bile from the stomach in patients undergoing gastroenterostomy or Billroth Ⅱ resection. Hence, because of the skipping of the ascending (or afferent) and descending (or efferent) jejunal loop (approximately 50 cm), the pancreaticobiliary fluids come less in contact with the gastric stump and more with the Braun anastomosis and the efferent limb distal to it. Therefore, the increased exposure of the latter surfaces to carcinogenic bile acids, not only for the gastric stump mucosa but also for the small intestine[1,4], is most likely the underlying pathophysiologic mechanism that enables the Braun anastomosis mucosa to become dysplastic and neoplastic before the gastric stump mucosa does.

Because of the resection of the gastrin-producing cells after a distal gastrectomy, the gastric stump mucosa usually becomes atrophic. This atrophy causes hypochlorhydria, and thus, the pH value rises, resulting in bacterial population growth. Some of these bacteria reduce dietary nitrates to nitrites, which, in the presence of substrates, such as food proteins, can lead to the formation of potent carcinogens[13,21,22]. If those carcinogens are absorbed systemically, then that supports the observation of Staël von Holstein *et al*[16] that after a gastrectomy for ulcer, there is an increased risk of developing a carcinoma in a location other than the gastric stump, just like in our patients.

We reviewed the literature and found some similar cases of gastrointestinal cancer near but not on the anastomosis after partial gastrectomy for benign disease. Takebayashi *et al*[23] presented a case of primary small-intestinal cancer that originated in the efferent loop after the Billroth Ⅱ gastrectomy that occurred 32 years earlier because of duodenal ulcer. Rose *et al*[24] reported a case of gastric adenocarcinoma arising at the duodenal stump 40 years after a Billroth Ⅱ partial gastrectomy for benign condition. Table 1 summarizes the reported cases in the literature and our cases with atypical anastomotic malignancies of small bowel after subtotal gastrectomy with Billorth Ⅱ gastroenterostomy for peptic ulcer. To our knowledge, our patients are the first reported cases of Braun anastomosis adenocarcinoma following partial gastrectomy for benign disease.

Lymphomas affect the small bowel as a manifestation of systemic disseminated disease, or they may be primarily present[3]. Non-Hodgkin lymphomas (NHLs) of the gastrointestinal tract represent 4% to 20% of all NHLs[25]. Of all gastrointestinal NHLs, 25% to 35% of cases occur within the small bowel, in which lymphomas parallel the distribution of lymphoid follicles, resulting in

Table 1 Synopsis of reported cases with atypical anastomotic malignancies of small bowel after subtotal gastrectomy with Billorth II gastroenterostomy for peptic ulcer

Case	Sex	Age	Tumor	Origin	Clinical data	Laboratory data	Treatment	Outcome	Ref.
1	M	79	Small intestine adenocarcinoma	Braun anastomosis after 22 yr from gastrectomy	Syncope episodes, early satiety	Hypochromic anemia	En block resection and Royx-en-Y gastrojejunal anastomosis	Disease free 9 mo	Kotidis, 2018
2	M	76	Small intestine adenocarcinoma	Braun anastomosis after 30 yr from gastrectomy	Abdominal Discomfort, Melenas	Anemia	En block resection and Royx-en-Y gastrojejunal anastomosis	Disease free 2.5 yr	Kotidis, 2018
3	M	78	Anaplastic large cell lymphoma	Efferent loop after 30 yr from gastrectomy	Hematemesis, melena	Normochromic anemia	En block resection and Royx-en-Y gastrojejunal anastomosis	Referred to hematology department	Kotidis, 2018
4	M	79	Small intestine adenocarcinoma	Efferent loop after 32 yr from gastrectomy	Asymptomatic	Anemia	Jejunectomy	Disease free at 17 mo	[23]
5	F	79	Duodenal adenocarcinoma	Duodenal stamp 40 yr after gastrectomy	Fatigue and weakness for 3 mo	Anemia	Resection of afferent limp	Disease free at 12 mo	[24]

the ileum being the most common site of involvement[1]. Anaplastic large cell lymphoma (ALCL) is a distinctive subtype of NHL. It accounts for approximately 2% of all cases of NHL. It belongs to the NHL subcategory of peripheral T-cell lymphomas (PTCL). It is made up of either malignant T-cells or "null-lymphocytes" (lack both B- and T-cell markers). The presence of the protein CD30 antigen on the surface of lymphoma cells is the hallmark of the disease[26]. Usually, ALCL is negative for cytokeratin. The positive cytokeratin AE1/AE3 cells in our case were considered remnant epithelial cells. The ALK-negative subtype of ALCL appears more commonly in the elderly, is more aggressive, and belongs to the systemic form of ALCL[27], which typically presents with painless enlarged lymph nodes and extranodal site involvement, most commonly including the skin, bones, soft tissues, and lungs. The gastrointestinal tract being involved in our case is rare[27], and even though, in the literature, rare cases of gastrointestinal ALCL at various spots, including the small intestine, have been documented[28-30], as far as we know, this is the first case reported at the efferent loop of a Billroth Ⅱ gastroenterostomy decades after operation.

In conclusion, the "by-pass" path of the bile made by a Braun anastomosis added to a Billroth Ⅱ gastrectomy is the most prevalent hypothesis for the development of the two adenocarcinomas at the specific spot. Much more needs to be discovered about the ALCL ALK-negative type of lymphoma to make assumptions since it has not been studied for more than 20 years. However, we cannot be certain that the appearance of those small-intestinal tumors at those spots were directly related to the operations that occurred decades ago.

ARTICLE HIGHLIGHTS

Research background

Despite the fact that the small intestine makes up more than 90% of the surface area and 70% of the length of the gastrointestinal tract, small bowel malignancies are among the rarest cancers. Anastomotic gastric cancer following distal gastrectomy for peptic ulcer is a well-established clinical entity. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon.

Research motivation

To present patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer.

Research objectives

In this paper, we present three patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer, to review relevant literature, and to interpret the reason that those cancers developed to these postsurgical nongastric sights.

Research methods

For the current retrospective study and review of literature, the surgical and histopathological records of our department were examined, searching for patients who have undergone surgical treatment of small-bowel malignancy to identify those who have undergone subtotal gastrectomy for benign peptic ulcer. A systematic literature search was also conducted using PubMed, EMBASE, and Cochrane Library to identify similar cases.

Research results

We identified three patients who had developed small-intestine malignancy at the level of the gastrointestinal anastomosis decades after a subtotal

gastrectomy with Billroth Ⅱ gastroenterostomy for benign peptic ulcer-two patients with adenocarcinoma originated in the Braun anastomosis and one patient with lymphoma of the efferent loop. All three patients were submitted to surgical resection of the tumor with Roux-en-Y reconstruction of the digestive tract. In the literature review, we only found one case of primary small-intestinal cancer that originated in the efferent loop after Billroth Ⅱ gastrectomy because of duodenal ulcer but none reporting Braun anastomosis adenocarcinoma following partial gastrectomy for benign disease. We also did not find any case of efferent loop lymphoma following gastrectomy.

Research conclusions

Anastomotic gastric cancer following distal gastrectomy for peptic ulcer is a well-established clinical entity. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon. The substantial diversion of the potent carcinogenic pancreaticobiliary secretions through the Braun anastomosis and the stomach hypochlorhydria, allowing the formation of carcinogenic factors from food, are the two most prominent pathogenetic mechanisms for those tumors.

Research perspectives

The "by-pass" path of the bile made by a Braun anastomosis added to a Billroth Ⅱ gastrectomy is the most prevalent hypothesis for the development of the two adenocarcinomas at the specific spot. Much more needs to be discovered about the ALCL ALK-negative type of lymphoma to make assumptions since it has not been studied for more than 20 years. However, we cannot be certain that the appearance of those small-intestinal tumors at those spots had a direct relation to the operations that occurred decades ago.

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