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REVIEW

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Advancements and challenges in gastric cancer: epidemiology, biomarkers, and therapeutic strategies

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Abstract

Gastric cancer is the fifth most common cause of cancer-related deaths globally, with a decreasing but still high number of cases. Although there have been improvements in treatment choices, the expected survival rates have not yet been achieved. In addition to the challenges associated with developing effective therapies, there is an urgent need to establish diagnostic and predictive biomarkers to guide treatment selection. Therefore, this review summarizes key aspects of gastric cancer, including its epidemiology, associated risk factors, and underlying pathogenesis. It also discusses the main biomarkers involved in this disease, such as PD-L1, HER − 2, Epstein-Barr virus (EBV), Claudin 18.2, FGFR2, and the current standard and targeted therapies. Molecular testing for these changes is gaining significance in the context of gastric cancer. By incorporating detailed biomarker analysis into clinical practice, we can provide more effective and personalized treatment options, ultimately improving clinical management and enhancing survival rates for gastric cancer patients.

Keywords Gastric cancer, Biomarkers, MSI, HER2, EBV, PD-L1, Claudin, FGFR, Immunohistochemistry, Target therapy

Epidemiology, risk factors, and pathogenesis

Gastric cancer, including gastroesophageal junction cancer, is the fifth most common malignancy and remains the fifth leading cause of cancer-related deaths worldwide. Incidence rates have been declining, but remain high, particularly in Eastern Asia, Central America, and Latin America. Conversely, some geographic regions, such as North America, Northern Europe, and Africa, exhibit lower incidence rates (< 15 per 100,000 population) (Globocan 2022).

The most common risk factors associated with GC include infections (*Helicobacter pylori* and *Epstein-Barr virus*) tobacco use, dietary factors (consumption of salty food, nitrosamines, red meat), high alcohol intake, elevated body mass index, and genetic polymorphism. The progressive decline in incidence rates may be attributed to improved hygiene standards, better food preservation, increased intake of fresh fruits and vegetables, and *H. pylori* eradication (Thrift et al. 2023).

While most cases of gastric cancers are sporadic, approximately 10% exhibit familial clustering. Familial gastric cancer comprises at least three major syndromes: hereditary diffuse gastric cancer, gastric adenocarcinoma and proximal polyposis of the stomach, and familial intestinal gastric cancer. Germline mutations in CDH1 and CTNNA1 are implicated in the familial form of diffuse gastric cancer (Marwitz et al. 2020).

Other syndromes are also involved, such as Lynch, Li-Fraumeni, Peutz-Jeghers, hereditary breast-ovarian

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cancer syndromes, familial adenomatous polyposis, and juvenile polyposis.

Adenocarcinoma is the predominant histological type of gastric and gastroesophageal junction cancer, accounting for more than 90% of cases. The World Health Organization (WHO) system classifies gastric adenocarcinoma into four subtypes: papillary, tubular, mucinous, and poorly cohesive (Nagtegaal et al. 2019). Alternatively, the Lauren classification distinguishes gastric cancer into two major subtypes - diffuse and intestinal - each characterized by distinct epidemiological, morphological, and molecular features (Oliveira et al. 2015).

In the Cancer Genome Atlas (TCGA) analysis, the genome and proteome of gastric cancer have been extensively characterized to uncover molecular subtypes and identify dysregulated pathways. Investigators proposed four molecular subtypes: Epstein–Barr virus (EBV) positive, microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN) tumors (Sohn et al. 2017). Each molecular subtype is enriched with distinct genomic features, potentially allowing for personalized treatment strategies.

In summary, approximately two-thirds of patients are diagnosed at an advanced stage of the disease. Despite advancements in treatment options, the desired survival rates remain unreached. In addition to the ongoing challenges in developing effective therapies, there is an urgent need to establish diagnostic and predictive biomarkers to guide treatment selection (Sohn et al. 2017; Liu and Meltzer 2017).

Molecular biomarkers

Defective DNA mismatch repair (dMMR)

Defective DNA mismatch repair (MMR) leads to alterations in the length of repetitive sequences, a molecular phenomenon known as microsatellite instability (MSI). The primary cause of DNA MMR defects is the inactivation of MMR genes, often due to hypermethylation and epigenetic silencing of MLH1 in most sporadic tumors. MSI has been identified as a distinct molecular subgroup in gastric cancer (GC) and is mostly caused by hypermethylation of the MLH1 promoter (Talari et al. 2023; Cancer Genome Atlas Research Network 2014).

Testing for MMR deficiency is typically performed using immunohistochemistry, while MSI testing is conducted via PCR or next-generation sequencing (NGS). Immunohistochemistry serves as an excellent first-line testing method, analyzing the nuclear expression of four repair proteins (MLH1, PMS2, MSH2, and MSH6) and demonstrating a high agreement rate (> 90%), similar to MSI detection using PCR (Shia et al. 2004).

In test interpretation, the presence of these proteins, indicated by the retention of nuclear expression of MMR proteins, suggests that the MMR system is proficient (pMMR). Conversely, the loss of expression (negative nuclear expression) of MMR proteins indicates a deficient MMR system (dMMR). Finally, as previously mentioned, for MSI assessment, the PCR test compares the allelic position of the microsatellite loci in tumors (with or without normal tissue), while NGS allows the efficient identification of many loci to establish MSI status. The exact number of loci analyzed depends on the specific pipeline used (Puliga et al. 2021) (Figs. 1).

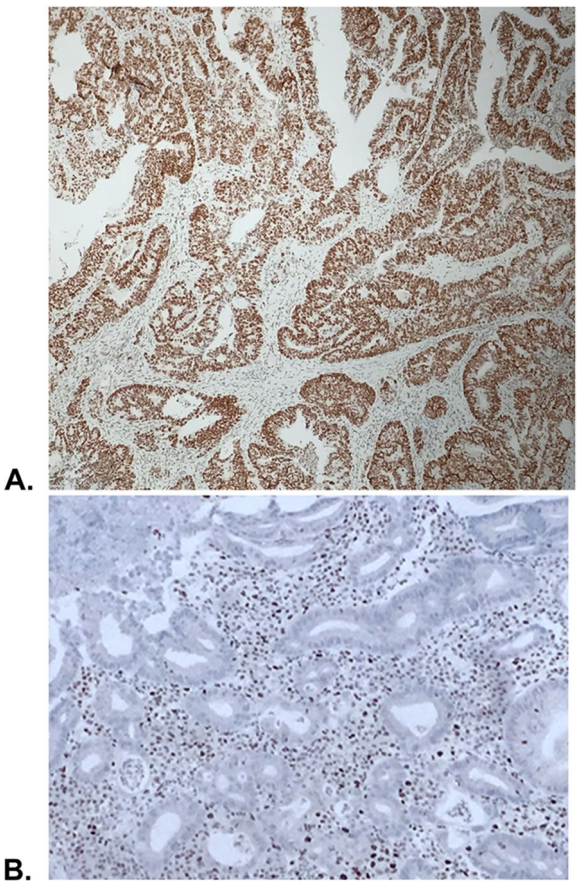

Fig. 1 **A** Different immunohistochemistry patterns of Mismatch repair protein. IHC for MSH2 shows a strong immune reaction with intact nuclear staining in neoplastic cells indicating a proficient system (pMMR); **B** IHC for MLH1 reveals a loss of tumor cell nuclear staining with intact positive internal control, in a deficient system (dMMR)

MSI-H-related gastric tumors were associated with the female sex, older ages, distal stomach location, and intestinal subtype of the Lauren classification and a favorable prognosis in patients with Stage II and III gastric cancer treated only with surgery (Park et al. 2023; Nakashima et al.1995).

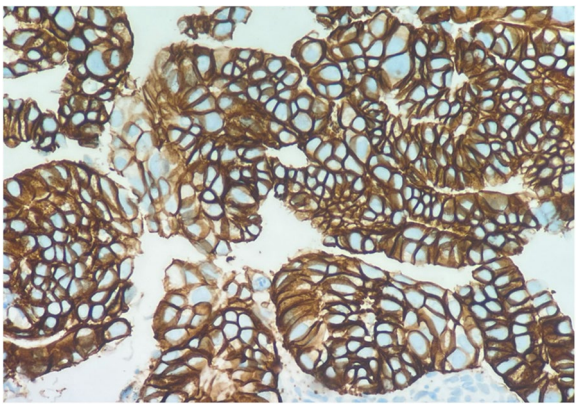

Fig. 2 Gastric cancer - HER2 immunohistochemical staining with strong complete basolateral membranous reactivity, score 3+ (positive). 40x magnification

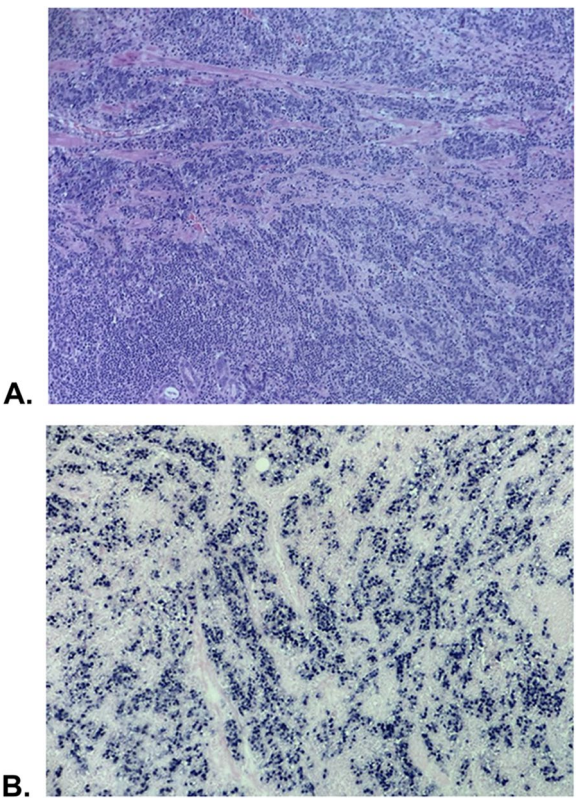

Fig. 3 **A** Poorly differentiated gastric carcinoma with an exuberant lymphoid stroma (H&E,10x); **B** EBV-encoded small RNA ISH. Epstein-Barr virus (EBV) Infected carcinoma cells exhibited nuclear dark blue staining (EBVaGC) (H&E, 10x)

MSI-H patients with resectable GC had a favorable survival compared with those with MSI-low/microsatellite stable (MSS) disease (5-year OS, 77.5% vs. 59.3%). Conversely, an individual patient data (IPD) meta-analysis, which combined four randomized trials (MAGIC, CLASSIC, ARTIST, and ITACA-S) involving gastric cancer patients with a deficient mismatch repair (dMMR), showed that they did not benefit from chemotherapy in addition to surgery compared to surgery alone (Kim et al. 2015). On the other hand, another meta-analysis that evaluated patients with resected gastric cancer found opposite results, suggesting that adjuvant chemotherapy extended the survival of dMMR/MSI-H patients compared to surgery alone (Pietrantonio et al. 2019). Therefore, new studies are emerging to investigate the conflicting evidence regarding the efficacy of perioperative chemotherapy in GC patients with dMMR/MSI- H.

In addition, MSI-H or MMR-deficient (dMMR) protein expression has been proven to be a biomarker for response to immune checkpoint inhibitors in advanced solid cancers (Kim et al. 2020). Previous studies described that mismatch-repair deficient cancers are related to numerous mutation-associated neoantigens that the immune system might recognize, once these tumors all harbor hundreds to thousands of somatic mutations, regardless of their cell of origin (Le et al. 2017).

The U.S. Food and Drug Administration has approved the anti-PD-1 antibody pembrolizumab as a cancer type- or site-agnostic treatment for patients with advanced MSI-H or dMMR solid tumors (Dolcetti et al. 1999).

Currently, by NCCN guidelines: universal testing for microsatellite instability (MSI) by PCR/next-generation sequencing (NGS) or MMR by IHC is recommended in all newly diagnosed patients (NCCN, 2024).

PD-L1 CPS

PD-L1 expression has been investigated as a predictive biomarker of response to immunotherapy in several tumors and is reported to be positive in up to 40–65% of gastric cancer (Bang et al. 2018).

Combined positive score (CPS) and tumor proportion score (TPS) are proposed scoring PD-L1 immunostaining; however, CPS has been used as a stratification factor in clinical trials. The expression is based on immunohistochemistry staining of formalin-fixed tumor samples. Only histology specimens (biopsy and resection) are considered suitable. Cytology samples should not be considered to be tested (AgilentDako). Antibodies 22C3 and 28 – 8 are incorporated to detect PD-L1 expression in gastric cancer.

Standardized IHC PD-L1 antibody assays (e.g., Dako 22C3 and Dako 28 – 8) have been used as crucial biomarkers in patient selection to predict treatment response to immune checkpoint inhibitor therapies (such as pembrolizumab and nivolumab) in gastric cancer. Although both share the same essential function of identifying PD-L1 expression, they present some important

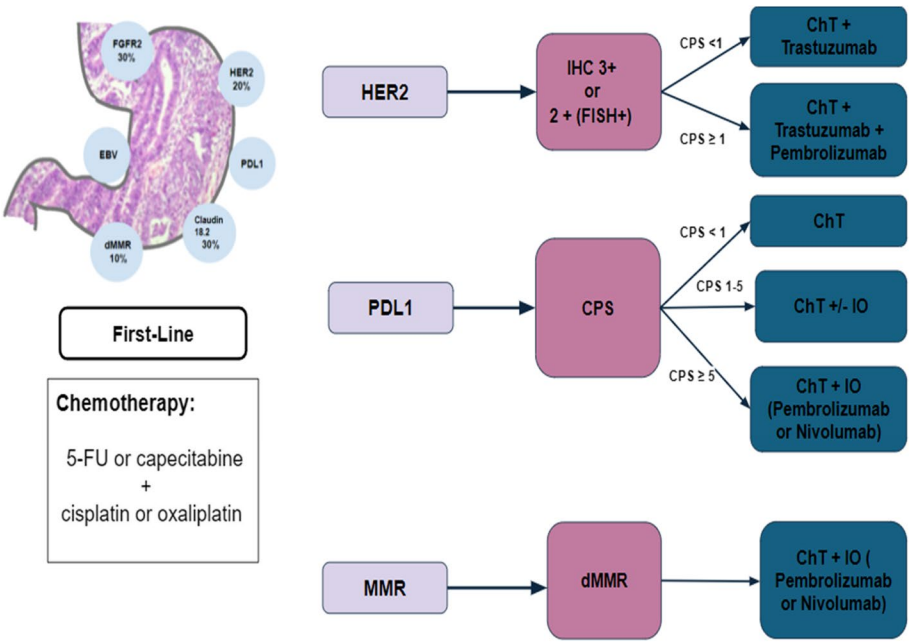

Fig. 4 The choice of first-line treatment according to biomarkers. Adapted from Diretriz Estômago: Doença avançada. Brazilian Society of Clinical Oncology (Diretriz SBOC. 2024)

differences in their clinical application. Both have their specificities and are used in different testing platforms. Antibody 22C3 is often associated with pembrolizumab, while 28 – 8 is related to nivolumab (Soomin et al. 2021).

The choice between using the 22C3 or 28 – 8 antibodies may depend on several factors, including the planned treatment. Pembrolizumab generally uses 22C3, while nivolumab can use 28 – 8. Another important factor is test availability. The presence of specific antibodies and testing platforms in the laboratory can influence the choice.

The combined positive score is calculated as the number of PD-L1–staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100; a minimum of 100 viable tumor cells must be present for a sample to be considered evaluable (AgilentDako; Kulangara et al. 2019).

Tumor cells may exhibit convincing partial or complete membrane staining of varying intensities, as long as lymphocytes and macrophages within the tumor nests or adjacent supporting stroma may also display convincing membrane and cytoplasmic staining of any intensity. The formula for calculating staining positivity should exclude non-staining tumor cells, tumor cells with only cytoplasmic staining, in situ carcinoma, and any inflammatory cells that are not located within the tumor nests or adjacent to the supporting stroma (AgilentDako).

Four pivotal phase III trials (KEYNOTE-062, CheckMate 649, ATTRACTION-4, ORIENT-16) have been published evaluating the efficacy of immune checkpoint inhibitors combined with first-line chemotherapy for unresectable advanced gastric cancer. These trials differed in population, backbone chemotherapy, trial design, and findings (Narita and Muro 2023).

The proportion of CPS ≥ 5 in general gastric cancer patients in clinical trials was reported to be approximately 30–40%(Narita et al. 2021; Ahn and Kim 2021). According to the data from the KEYNOTE-062 trial, patients with a CPS ≥ 10 appeared to benefit from pembrolizumab alone. This trial evaluated pembrolizumab, chemotherapy, or pembrolizumab plus chemotherapy in patients with advanced unresectable or metastatic gastric. Pembrolizumab monotherapy was not superior to chemotherapy in patients with CPS of 1 or greater. Pembrolizumab prolonged OS vs. chemotherapy in patients with CPS of 10 or greater (median, 17.4 vs. 10.8 months; HR, 0.69; 95% CI, 0.49–0.97), but this difference was not statistically tested. For patients with CPS ≥ 10, the 24-month OS rate was greater in the pembrolizumab arm (28.3%) versus the chemotherapy arm (21.2%) (Shitara et al. 2020b).

On the other hand, the phase 3 Checkmate 649 global trial of nivolumab in combination with chemotherapy versus chemotherapy alone in patients with untreated, advanced unresectable or metastatic gastric cancer improved overall survival (OS) and progression-free survival (PFS) with the combination treatment in patients with CPS ≥ 5 (Janjigian et al. 2021). Therefore, a cutoff

value of CPS 5 was approved in Europe to select those who benefit from adding nivolumab to chemotherapy as a first-line treatment. In other locations, including Brazil, the approval of the combination is regardless of the PD-L1 CPS. However, the level of PD-L1 expression should be used in the decision to combine nivolumab with the chemotherapy regimen in the first line (Diretriz SBOC. 2024).

In the refractory setting, The FDA granted pembrolizumab accelerated approval for patients with recurrent, locally advanced or metastatic gastric cancer who experienced disease progression to 2 or more lines of therapy and had PD-L1 expression (CPS ≥ 1). Regarding the interchangeability between PD-L1 assays by 28 − 8 and 22C3 Yeong et al. suggested in 2022 that 28–8 assay may result in higher PD-L1 scores and a higher proportion of PD-L1 positivity compared to 22C3, with only moderate concordance between the 22C3 and 28–8 and recommended being caution in treating the assays as equivalent (Yeong et al. 2022). Recent publications suggested that this discordance may be associated with unfavorable efficacy outcomes in patients treated with nivolumab plus chemotherapy (Kim et al. 2024).

ERBB2 (HER2)

Human epidermal growth factor receptor 2 (HER2) is a member of the ErbB family, which comprises a group of four transmembrane glycoproteins with tyrosine kinase activity: epidermal growth factor 1 (EGFR or ErbB1), ErbB2 (human EGF receptor 2 [HER2]), ErbB3, and ErbB4. These tyrosine kinases are crucial for transmitting cellular signals that regulate normal cell growth and differentiation. The HER2 receptor is encoded by the ErbB2 gene, a proto-oncogene located on chromosome 17q21, whose amplification is associated with a variety of tumors, including gastroesophageal cancer (Rubin et al. 2024).

In gastric cancer, HER2 (ERBB2/HER2) amplification or overexpression occurs in approximately 20% of advanced gastric or gastroesophageal junction adenocarcinomas, 30% in the intestinal subtype, and 5% in the diffuse subtype. The prognostic significance of HER-2 in gastric cancer remains contentious due to inconsistent results across studies (Park et al. 2006; Zhou et al. 2012).

Several drugs are approved for the treatment of patients with HER2-positive gastric cancer. Consequently, it is recommended that this biomarker be evaluated in all patients with metastatic disease who are being considered for systemic treatment. Her-2 is generally assessed by immunohistochemistry (IHC).

Testing can be performed on archived paraffin-embedded tissue from endoscopic biopsies, resection specimens (e.g., ESD or gastrectomy), biopsies of metastatic sites, and cytology samples with cell block preparations. The success of a high-quality test depends on several factors: tissue fixation (10% neutral-buffered formalin), cold ischemia time (less than 1 h), and adequate fixation time. Additionally, the pathologist's experience is crucial. The Her-2 IHC evaluation may require expertise to determine the appropriate area, as non-neoplastic cells (normally with membrane positivity) and dysplastic epithelium should not be considered overexpressing (Kumarasinghe et al. 2023; Yamashita-Kashima et al. 2014; Hofmann et al. 2008). HER2 positivity is defined as IHC 3 + or IHC 2 + with positive in situ hybridization (ISH) or fluorescence in situ hybridization (FISH). ISH and FISH positivity is defined as a ratio of ≥ 2.0 for the number of HER2 copies to the number of signals for CEP17; a ratio of < 2.0 is considered positive if the HER2 copy number was > 6 Fig. 2.

In 2010, the phase III ToGA (Trastuzumab for Gastric Cancer) trial was published, marking the first randomized trial to assess the clinical efficacy and safety of trastuzumab added to chemotherapy for first-line treatment of advanced gastric or gastroesophageal junction cancers with overexpression of HER2. Eligible patients received a chemotherapy regimen consisting of capecitabine plus cisplatin or fluorouracil plus cisplatin in combination with trastuzumab. The combination of trastuzumab and chemotherapy improved the median overall survival to 13.8 months compared to 11.1 months with chemotherapy alone (hazard ratio [HR] 0,74; IC 95%: 0,60–0,91; $p = 0{,}0046$) establishing it as the standard of care first-line treatment for these patients (Bang et al. 2010).

Until recently, no HER2-targeted agents beyond trastuzumab had demonstrated a significant benefit in patients with HER2-positive gastric cancer. Pertuzumab, combined with trastuzumab and chemotherapy, in the context of first-line therapy, did not further prolong overall or progression-free survival (Tabernero et al. 2018), nor did lapatinib combined with chemotherapy in a cohort predominantly consisting of patients who had not previously received trastuzumab in first-line (Hecht et al. 2016) or second-line treatment, as compared with placebo or chemotherapy alone, respectively (Satoh et al. 2014). The heterogeneity of HER2 expression may be a critical factor limiting the efficacy of HER2-targeted treatments in gastric cancer (Janjigian et al. 2018). Preclinical studies have shown that combining tumor-targeting antibodies with PD-1 inhibitors can improve immune infiltration and T-cell responses, potentially overcoming tolerogenic dendritic cells. This has translated into meaningful responses with pembrolizumab, trastuzumab, and chemotherapy in patients with HER2-positive gastroesophageal cancer (Janjigian et al. 2020; Rha et al. 2020).

The KEYNOTE-811 evaluated the efficacy and safety of adding pembrolizumab or placebo to trastuzumab plus fluoropyrimidine and platinum-based chemotherapy (fluorouracil plus cisplatin or capecitabine plus oxaliplatin) in patients with untreated HER2-positive metastatic gastric or gastroesophageal junction cancer. The first interim analysis showed that the combination of pembrolizumab plus trastuzumab and chemotherapy significantly improved the objective response rate compared with trastuzumab and chemotherapy (74.4% vs. 51.9%) leading to the US Food and Drug Administration granting accelerated approval for this combination (Janjigian et al. 2021).

The addition of pembrolizumab significantly improved PFS compared to placebo plus standard of care (SOC) in all patients (median PFS: 10.0 months vs. 8.1 months; HR 0.72; 95% CI 0.60–0.87; $p=0.0002$) and in those with a PD-L1 combined positive score ≥ 1 (10.8 months vs. 7.2 months; HR 0.70; 95% CI 0.58–0.85) at a median follow-up of 28.4 months). In the third interim analysis after 606 PFS events and at a median follow-up of 38.5 months, 24-month PFS rates were 24% and 15%, respectively. The final overall survival analysis is awaited to confirm the long-term therapeutic effect of this regimen (Janjigian et al. 2021).

The antibody-drug conjugate trastuzumab deruxtecan (T-DXd) has emerged as the second anti-HER2 agent to demonstrate benefit in HER2-positive gastric cancer. The rational strategy for ADCs is to improve efficacy and reduce systemic adverse events by using antibodies selectively to deliver a potent cytotoxic agent directly to tumor cells, thereby enhancing the therapeutic index of chemotherapeutic agents. The released cytotoxic payload could exert a bystander effect, which has great potential against HER2-expressing cancers.

In the phase 2 DESTINY-Gastric01 study, trastuzumab deruxtecan demonstrated improved response rates and overall survival compared to chemotherapy in Asian patients with locally advanced or metastatic HER2-positive gastric or gastroesophageal junction cancer who had progressed after two lines of previous treatment including trastuzumab. An objective response was reported in 51% of the patients in the trastuzumab deruxtecan group, compared with 14% of those in the physician's choice group ($P<0.001$). Overall survival was longer with trastuzumab deruxtecan than with chemotherapy (median, 12.5 vs. 8.4 months; hazard ratio for death, 0.59; 95% confidence interval, 0.39 to 0.88; $P=0.01$, which crossed the prespecified O'Brien-Fleming boundary [0.0202 based on number of deaths]). Myelosuppression and interstitial lung disease were the notable toxic effects (Shitara et al. 2020b).

The phase 2 DESTINY- Gastric02 trial aimed to assess trastuzumab deruxtecan in patients from the USA and Europe with unresectable or metastatic gastric or gastroesophageal junction cancer, progressive disease on or after first-line therapy with a trastuzumab-containing regimen. At the data cutoff for the updated analysis (median follow-up 10·2 months [IQR 5·6–12·9]), a confirmed objective response was reported in 33 (42% [95% CI 30·8–53·4]) of the 79 patients (Van Cutsem et al. 2023).

These clinically meaningful results support the use of trastuzumab deruxtecan as second-line therapy in patients with HER2-positive advanced gastric or gastroesophageal junction cancer. The ongoing phase 1b/2 DESTINY-Gastric03 trial is evaluating the safety and efficacy of T-DXd in combination with chemotherapy and immunotherapy in HER2-expressing gastric cancer (Ph1b/2 Study of the Safety and Efficacy of T-DXd Combinations in Advanced HER2-expressing Gastric Cancer (DESTINY-Gastric03).

In summary, the importance of this biomarker is undeniable. Her-2 evaluation, conjointly with PD-L1 expression and DNA mismatch repair assessment, is crucial for optimizing treatment planning for patients with gastric cancer.

EBV

Epstein-Barr virus (EBV)-associated gastric cancer (EBVaGC) accounts for approximately 10% of all gastric cancers worldwide. The presence of EBV has been recognized as a potential biomarker in gastric cancers.

EBV-associated gastric cancers have distinct clinicopathologic characteristics, including male predominance, preferential location in the gastric cardia or postsurgical gastric stump, lymphocytic infiltration, a lower frequency of lymph node metastasis, more favorable prognosis, and a diffuse type of histology in most series (Iizasa et al. 2022).

The cohort study data from TCGA also reported that EBVaGC has the best recurrence-free period and overall survival compared to MSI, GS, and CIN subtypes (Sohn et al. 2017). In addition, in part due to the overexpression/amplification of programmed cell death ligand1 (PD-L1) and PD-L2 in EBVaGCs, these tumors have high immunogenicity and are good candidates for therapy with immune checkpoint inhibitors. Deregulated immune response genes in this tumor's subtype affect the tumor immune microenvironment (Kim et al. 2015), leading to a unique spatial arrangement of tumor cells within exuberant lymphoid stroma: so-called "lymphoepithelioma-like carcinoma". Prominent tumor-infiltrating lymphocytes, the characteristic morphologic feature in

EBV-positive gastric cancer, can be a surrogate indicator of tumor behavior and prognosis (Jeong et al. 2022).

EBV detection can be performed using at least five methodologies such as Southern blotting, immunohistochemistry, western blotting, PCR, and EBER ISH assay, being the last one widely used and considered the gold standard for detecting and localizing latent EBV in tissue samples. This technique can be performed on tissues fixed in formalin and embedded in paraffin (Kim M and Seo AN, 2022).

The detection of Epstein–Barr virus (EBV) in gastric cancer patients is crucial for clinical decision-making, as it is related to specific treatment responses and prognosis Fig. 3.

FGFR2

The FGF (fibroblast growth factor) signaling pathway regulates a variety of cellular functions including cell proliferation, migration, and differentiation. Dysregulation of Aberrant fibroblast growth factor receptor (FGFR) signaling can culminate in tumorigenesis and cancer progression (Eswarakumar et al. 2005). FGFR2 overexpression occurs in approximately 30% of gastric cancer, specifically the diffuse subtype. It correlates with aggressive features including higher grade T stage, more frequent lymph node dissemination, and inferior progressive free survival (PFS) and overall survival (OS) in patients receiving platinum and fluoropyrimidine chemotherapy. The role of FGFR signaling, specifically FGFR2, is less established in esophageal squamous cell cancer (ESCC) with a paucity of evidence for clinical benefit in these patients (Gordon et al. 2022).

FGFR2 and HER2 amplifications are generally mutually exclusive (Klempner et al. 2019) Nevertheless, approximately 40% of FGFR2-altered gastroesophageal cancers harbor other mutations that may render them resistant to FGFR pathway targeted therapy. FGFR2 amplification occurs in microsatellite stable (MSS) tumors and is not enriched for PD-L1 expression. Currently, there is no approved FGFR inhibitor for FGFR2-positive gastroesophageal cancer (Su et al. 2014).

The protein can be detected in the cytoplasm and at the cell membrane, in both intestinal and diffuse type GC. There are two major FGFR2 isoforms. In GC, FGFR2-IIIb is the predominantly overexpressed isoform (Ooki and Yamaguchi 2021). FGFR2 protein expression in GC was most commonly studied in Asian populations and data based on white patients are scarce. Schrumpf et al. focused on validating the expression and putative tumor biological significance of FGFR2 in a large non-Asian GC cohort. They used intensity IHC to categorize the positivity of the reaction, anyway, FGFR2 is currently being explored for the treatment of GC; however, no standardized test algorithm has been developed yet. FGFR2 can also be tested by amplification using chromogenic in situ hybridization (CISH) (Röcken 2023).

The randomized phase 2 FIGHT trial assessed the effectiveness and safety of the humanized monoclonal antibody IgG1 FGFR2b bemarituzumab in combination with chemotherapy (mFOLFOX6) in patients with FGFR2b-selected gastric or gastroesophageal junction adenocarcinoma. Patients underwent prescreening via tumor biopsy for immunohistochemistry analysis of FGFR2b overexpression and plasma next-generation sequencing of cell-free circulating tumor DNA (ctDNA) to detect FGFR2 amplification. Positive FGFR2b overexpression status was determined by moderate (2+) to strong (3+) membranous staining in more than 0% of tumor cells. The study found a 4% prevalence of FGFR2 amplification, which is consistent with that reported in previous studies (2.5–7.4%) and a 29% prevalence of FGFR2b overexpression at the upper end of that reported in previous studies (2.7–31.1%).

After 24 months, bemarituzumab plus chemotherapy showed slightly longer median PFS and OS without statistical significance. Median PFS was 9.5 months with bemarituzumab versus 7.4 months with placebo (HR, 0.72 [95% CI, 0.49–1.08), and median OS was 19.2 months versus 13.5 months (HR, 0.77 [95% CI, 0.52–1.14]), respectively. Adverse events such as stomatitis and corneal issues were noted with bemarituzumab, which are class effects related to inhibition of the FGF–FGFR pathway. Patients with ≥10% of tumor cells showing 2+/3 + FGFR2b IHC staining intensity exhibited better efficacy. Randomized phase 3 trials focusing on these patients are underway to confirm bemarituzumab's clinical benefit. (Wainberg et al. 2022)

The confirmatory FORTITUDE-101 study is evaluating bemarituzumab plus mFOLFOX6 in patients with untreated, advanced or metastatic gastric or GEJ adenocarcinoma (Smyth et al.2022) and the concurrent phase 1b/3 FORTITUDE-102 study will assess the efficacy and safety of bemarituzumab + mFOLFOX6 + nivolumab versus placebo + mFOLFOX6 (Wainberg et al. 2022).

Claudin 18.2

Claudin 18 isoform 2 (Claudin 18.2) is a tight-junction molecule member of the claudin family, which plays pivotal roles in regulating tissue permeability, paracellular transport, and signal transduction. The protein is detectable in both the cytoplasm and at the cell membrane in intestinal and diffuse types of gastric cancer. This protein is predominantly localized in the non-malignant gastric epithelium but becomes accessible on the tumor cell surface during malignant transformation, making CLDN18.2

a compelling target for therapeutic intervention in gastric cancer. (Nakayama et al. 2024).

Moreover, Claudin 18.2 is known to be overexpressed in various other cancer types, including oesophageal, pancreatic, colorectal cancer, non-small-cell lung cancer (NSCLC), and ovarian cancer (Nakayama et al. 2024).

In gastric cancer, a higher prevalence of claudin 18.2 positivity has been observed in tumors with diffuse-type histology (48.3%) compared to those of intestinal-type histology (38.8%) (Shitara et al. 2023). Data from a previous study reported lower rates of claudin 18.2 positivity among Asian patients (24%) in comparison to those in Europe (32%) or North America (34%) (Moran et al. 2018).

Bioinformatic analysis of several publicly available datasets indicates higher levels of *CLDN18* in EBV-positive and MSS, *TP53*-positive or *TP53*-negative gastric cancer subtypes (Li et al. 2020). Despite these associations, no distinct molecular profile for claudin 18.2-overexpressing gastric cancer has emerged from these analyses. Since it lacks a distinctive molecular profile, if a claudin 18.2 targeted agent is approved, broad screening for claudin 18.2 expression across all gastric cancer subtypes would be recommended. This observation may suggest that the overexpression of claudin18.2 results from hypomethylation of promoter CpG islands (Sahin et al. 2008).

Testing for Claudin 18.2 can be conducted through immunohistochemical (IHC) analysis of formalin-fixed paraffin-embedded tissue specimens, where positivity is defined as ≥75% of tumor cells exhibiting moderate-to-strong membranous staining for CLDN18 (Kubota et al. 2023). Additionally, CLDN18-ARHGAP gene fusion has been identified in GC, particularly within genomically stable and diffuse carcinoma subtypes. This fusion can be assessed by RNA sequencing and quantitative PCR in patients with adequate archival tissue samples. However, the relationship between the fusion gene and the expression of CLDN18.2 detected in immunohistochemistry requires further investigation (Pellino et al. 2021).

Zolbetuximab is a first-in-class immunoglobulin G1 monoclonal antibody that specifically targets CLDN18.2 and mediates antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity in CLDN18.2-positive gastric and gastroesophageal junction adenocarcinoma. Data from two phase III trials investigating zolbetuximab in combination with chemotherapy have demonstrated significant benefits for this combination in patients with advanced-stage, CLDN18.2-positive gastric cancers.

The Phase 3 SPOTLIGHT trial evaluated the efficacy and safety of first-line zolbetuximab in combination with mFOLFOX6 versus placebo combined with mFOLFOX6 in patients with claudin 18.2 positive, HER2-negative, locally advanced unresectable or metastatic gastric or gastroesophageal junction.

Zolbetuximab plus mFOLFOX6 significantly prolonged progression-free survival and overall survival compared to the placebo group. Specifically, the median PFS was 10.61 months in the zolbetuximab group versus 8.67 months in the placebo cohort (HR 0.751; $P=0.0066$). The median OS was 18.23 months in the zolbetuximab group vs. 15.54 months in the placebo group (HR 0.75, 95% CI 0.60–0.94; $p=0.0053$). The most common all-grade adverse events with zolbetuximab plus chemotherapy included nausea, vomiting, and decreased appetite.

The GLOW study was conducted simultaneously with SPOTLIGHT to further confirm the efficacy of incorporating zolbetuximab into chemotherapy in the first-line treatment setting. Patients with claudin 18.2 positive, HER2-negative, locally advanced unresectable or metastatic gastric or gastroesophageal junction cancers were randomized to receive zolbetuximab plus CAPOX versus placebo + CAPOX. PFS was statistically significantly prolonged in the zolbetuximab group versus placebo (median, 8.21 months versus 6.80 months, respectively; HR = 0.687; 95% CI, 0.544–0.866; $P=0.0007$). The median OS was 14.39 months in the zolbetuzimab group compared to 12.16 months in the placebo cohort (HR = 0.771; 95% CI, 0.615–0.965; $P=0.0118$).

The consistent survival benefits observed in both trials validate CLDN18.2 as a new target and demonstrate that zolbetuximab enhances PFS and OS when combined with chemotherapy in affected patients. Despite the disparities in the representation of countries within these studies, both GLOW and SPOTLIGHT reported the same prevalence rate of screened patients with tumors assessable for CLDN18.2 expression, at 38,4%, demonstrating that CLDN18.2 is a prevalent biomarker in HER2-negative, locally advanced unresectable or mG/GEJ adenocarcinoma.

Previous retrospective studies have suggested no significant correlation between CLDN18.2 positivity and the expression of biomarkers such as HER2 and PD-L1 (Pellino et al. 2021); In GLOW, 21.9% of assessed patients had tumors with a PD-L1 CPS ≥ 5, whereas in SPOTLIGHT, 13.2% of assessed patients exhibited PD-L1 CPS ≥ 5. Collectively, these studies establish CLDN18.2 as a prevalent and unique biomarker delineating a population of patients with CLDN18.2-positive tumors who appear to derive benefit from targeted therapy in combination with chemotherapy, although it should be noted that zolbetuximab has not yet been approved for commercial use. Furthermore, other agents targeting claudin 18.2 are currently being tested in early-phase clinical trials (Pellino et al. 2021).

Agnostic approved biomarkers: NTRK, BRAF and RET

NTRK

There are few studies on the prevalence and detection methods of fusion of neurotrophic tyrosine receptor kinase (*NTRK)* fusions in GC.

NTRK gene fusion is a molecular event driving tumorigenesis, where the chimeric oncoprotein containing the TRK tyrosine kinase domain is constitutively activated, activating downstream pro-oncogenic pathways. Fusions were found in about 0.3% of solid tumors, with varying frequencies across different types of cancer (Drilon et al. 2018). Clinicopathological characteristics of gastric cancer (GC) with oncogenic NTRK alterations are not well known. According to Pu et al.'s study in 2023, the hepatoid or enteroblastic differentiation type of GC demonstrated enrichment in NTRK gene alterations, unlike dMMR-type GC (Pu et al. 2023).

Detection methods for NTRK gene fusion include immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), reverse transcription polymerase chain reaction (RT-PCR), and next-generation sequencing (NGS), but their performance in gastric cancer remains understudied due to the low frequency in this type of cancer. (Kun et al. 2024). In November 2018 and August 2019 respectively, Larotinib and entrectinib were approved for a " tissue agnostic" indication by the Food and Drug Administration (FDA) as Trk inhibitors for metastatic or locally advanced solid tumors with NTRK gene fusion.

Larotrectinib's approval was based on results from three multi-center clinical studies (a phase 1 trial (*NCT02122913*), SCOUT, and NAVIGATE (Drilon et al. 2018; Hong et al. 2019). In these studies, larotrectinib exhibited a response rate of 75% (according to an independent review) in patients with TRK fusion-positive tumors across 17 cancer types.

Entrectinib's approval was based on results from the following multi-center, single-arm trials: ALKA-372-001 (EudraCT 2012-000148-88), STARTRK-1, and STARTRK-2 (Rolfo et al. 2020; Doebele et al. 2020; Drilon et al. 2017). Results from these trials revealed a response rate of 57% in patients with TRK fusion-positive solid tumors across 10 different tumor types. Both inhibitors have shown efficacy and tolerability in clinical trials (Garcia-Foncillas et al. 2022), thus, entrectinib and larotrectinib are recommended as second-line or subsequent therapies for individuals with NTRK gene fusion-positive GC.

BRAF

The RAS/RAF/MEK/ERK pathway plays a crucial role in various cellular functions including proliferation, migration, survival, angiogenesis, and cell cycle regulation and alteration in the B-Raf proto-oncogene (BRAF) can lead to the activation of this pathway. BRAF mutations are found in approximately 7–15% of all cancers, with the most common mutation occurring at position V600 (Subbiah et al. 2023).

However, in gastric cancer (GC), BRAF mutations are infrequently observed, with only 2.2% (7/319) of GC patients exhibiting such mutations, primarily of the BRAF V599M type. This raises the question if BRAF mutations play a significant driving role in the pathogenesis of GC (Choi et al. 2016).

Numerous DNA-based molecular assays are employed to detect BRAF mutations. The FDA has approved several diagnostic methods, including Sanger sequencing, pyrosequencing, mutation-specific Polymerase Chain Reaction (PCR), real-time PCR, digital PCR (dPCR), High-Resolution Melting curve analysis (HRM), Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry (MALDI-TOF MS; Sequenom), and Next-Generation Sequencing (NGS) based assays. These essays have high sensitivity and specificity (~ 85–100%) in detecting genomic alterations, including *BRAF* gene mutations (Vranics et al. 2022).

A promising treatment for patients with BRAF mutations involves the combination of the BRAF Trk inhibitor dabrafenib and the MEK inhibitor trametinib. This dual therapy effectively blocks oncogenic MAPK pathway signaling, suppresses the growth and survival of BRAFV600-mutant cells, and enhances anti-tumor activity. In June 2022, the FDA granted Accelerated Approval to this combination for adult and pediatric patients with unresectable or metastatic BRAFV600E-mutant solid tumors that have progressed post-treatment and lack alternative treatment options. This approval was based on significant efficacy and safety outcomes, as demonstrated in the Rare Oncology Agnostic Research (ROAR) (Subbiah et al. 2023).

RET

Activating receptor-tyrosine kinase rearranged during transfection (*RET*) mutations and fusions have been recognized as potent drivers of oncogenesis. These alterations are most commonly found in papillary thyroid cancer (10–20%) and non-small cell lung cancer (2%), while in other solid tumors such as GC, the prevalence is 3.3% (Andreev-Drakhlin et al. 2019).

Several studies have evaluated the effectiveness of RET immunohistochemistry (IHC) as a surrogate marker for RET activation. However, the absence of specific RET antibodies increases the risk of false-positive results, making IHC currently unsuitable for detecting RET mutations. Although fluorescence in situ hybridization (FISH) can identify RET rearrangements, its clinical

utility is limited due to a high rate of false positives and poor specificity. While quantitative PCR (qPCR) techniques offer rapid and cost-effective solutions, they are limited in detecting novel fusion variants, restricting their use to the most common aberrations. The most effective way to identify RET alterations is through comprehensive next-generation sequencing, ideally incorporating DNA and RNA analysis for fusions as well. (Belli et al. 2020; Skórzewska et al. 2023).

Targeted therapies for RET-dependent cancers have progressed from older multikinase inhibitors to selective RET inhibitors such as selpercatinib and pralsetinib. The international phase I/II ARROW trial evaluated pralsetinib efficacy and safety in twenty-nine patients with 12 different *RET* fusion-positive solid tumor types, excluding non-small-cell lung cancer and thyroid cancer, who had previously received or were not candidates for standard therapies. The highly selective RET inhibitor was well tolerated, demonstrating an overall response rate of 57% and a disease control rate of 83% in 23 patients across cancer types. Consequently, in 2022, the US FDA approved the first tumor-agnostic selective RET inhibitor (Subbiah et al. 2022). Furthermore, it is essential to consider molecular testing for this marker in GC Fig. 4.

Conclusion

Gastric cancer is a heterogeneous disease characterized by variations in histology, tumor location, and molecular markers, all of which represent distinct prognostic and predictive factors. The thorough evaluation of biomarkers is crucial for achieving successful therapy outcomes, and identifying novel biomarkers and understanding the mechanisms of already known markers is a promising area that can improve patient outcomes through personalized medicine.

We review that clinical trials with therapies anti-HER2 and anti-PD-L1 have shown a benefit in overall survival and are approved for clinical management of these tumors. While studies into targeted therapies for FGFR2 and Claudin 18.2 remain ongoing, the preliminary results are promising. Particularly for BRAF, NTRK, and RET, although rare in GC, the biomarker evaluation can guide personalized treatment strategies and influence prognosis, once they are approved agnostic markers. Molecular testing for these alterations is increasingly pivotal in the management of gastric cancer. By integrating comprehensive biomarker analysis into clinical practice, we can achieve more effective and tailored therapeutic interventions, ultimately improving survival rates and quality of life for gastric cancer patients.

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References

AgilentDako, The Dako PD-L1 IHC 22C3 pharmDx Interpretation Training Program. Disponível em: https://pdl122c3-learning.dako.com. [(Accessed on march 2024)].

Ahn S, Kim K-M. PD-L1 expression in gastric cancer: interchangeability of 22C3 and 28–8 pharmDx assays for responses to immunotherapy. Mod Pathol. 2021;34:1719–27.

Andreev-Drakhlin, Alexander Y, Roszik J, and Vivek Subbiah. The landscape of RET alterations from 56,970 adult patients with cancer: Clinical implications. Journal of Clinical Oncology (2019): n. pag.

Bang YJ, Ruiz EY, Van Cutsem E et al. Phase III, randomized trial of avelumab versus physician's choice of chemotherapy as third-line treatment of patients with advanced gastric or gastro-oesophageal junction cancer: primary analysis of JAVELIN Gastric 300. Ann Oncol. 2018.

Belli C, Anand S, Gainor JF, Penault-Llorca F, Subbiah V, Drilon A, Andrè F, Curigliano G. Progresses toward Precision Medicine in RET-altered solid tumors. Clin Cancer Res. 2020;26:6102–11.

Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014;513:202–9.

Choi YY, Noh SH, Cheong JH. Molecular dimensions of gastric Cancer: translational and clinical perspectives. J Pathol Transl Med. 2016;50(1):1–9. https://doi.org/10.4132/jptm.2015.09.10. Epub 2015 Oct 26.

Desilets A, Repetto M, Yang SR, Sherman EJ, Drilon A. *RET*-Altered Canctumor agnosticnreviewReview of Biodiagnosisgnositargetedrtherapyhactivityt-ivity. Cancers (Basel). 2023;15(16):4146.

Doebele RC, Drilon A, Paz-Ares L, Siena S, Shaw AT, Farago AF, Blakely CM, Seto T, Cho BC, Tosi D, et al. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: Integrated analysis of three phase 1–2 trials. Lancet Oncol. 2020;21:271–82. https://doi.org/10.1016/S1470-2045(19)30691-6.

Dolcetti R, Viel A, Doglioni C, Russo A, Guidoboni M, Capozzi E, Vecchiato N, Macrì E, Fornasarig M, Boiocchi M. High prevalence of activated intraepithelial cytotoxic T lymphocytes and increased neoplastic cell apoptosis in colorectal carcinomas with microsatellite instability. Am J Pathol. 1999;154:1805–13.

Drilon A, Siena S, Ou SI, Patel M, Ahn MJ, Lee J, Bauer TM, Farago AF, Wheler JJ, Liu SV, et al. Safety and Antitumor Activity of the Multitargeted Pan-TRK, ROS1, and ALK inhibitor entrectinib: combined results from two phase I trials (ALKA-372-001 and STARTRK-1. Cancer Discov. 2017;7:400–9.

Drilon A, Laetsch TW, Kummar S, DuBois, et al. Efficacy of Larotrectinib in TRK Fusion-positive cancers in adults and children. N Engl J Med. 2018;378(8):731–9.

Eswarakumar VP, Lax I, Schlessinger J. Cellular signaling by fibroblast growth factor receptors. Cytokine Growth Factor Rev. 2005;16:139–49.
Garcia-Foncillas J, Bokemeyer C, Italiano A, Keating K, Paracha N, Fellous M, Marian M, Fillbrunn M, Gao W, Ayyagari R, Lassen U. Indirect treatment comparison of Larotrectinib versus Entrectinib in treating patients with TRK Gene Fusion cancers. Cancers (Basel). 2022;14(7):1793.
Globocan. 2022 [(accessed on march 2024)]. Disponível online: https://gco.iarc.who.int/media/globocan/factsheets/populations/900-world-fact-sheet.pdf
Gordon A, Johnston E, Lau DK, Starling N. Targeting FGFR2 positive gastroesophageal Cancer: current and clinical developments. Onco Targets Ther. 2022;15:1183–96.
Hanrahan AJ, Chen Z, Rosen N, et al. BRAF — a tumour-agnostic drug target with lineage-specific dependencies. Nat Rev Clin Oncol. 2024;21:224–47.
Hecht JR, Bang Y-J, Qin SK, et al. Lapatinib in combination with capecitabine plus oxaliplatin in human epidermal growth factor receptor 2–positive advanced or metastatic gastric, esophageal, or gastroesophageal adenocarcinoma: TRIO-013/LOGiC — a randomized phase III trial. J Clin Oncol. 2016;34:443–51.
Hofmann M, Stoss O, Shi D, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology. 2008;52(7):797–805.
Hong DS, Bauer TM, Lee JJ, Dowlati A, Brose MS, Farago AF, Taylor M, Shaw AT, Montez S, Meric-Bernstam F, et al. Larotrectinib in adult patients with solid tumours: a multi-centre, open-label, phase I dose-escalation study. Ann Oncol. 2019;30:325–31.
Iizasa H, Kartika AV, Fekadu S, Okada S, Onomura D, Wadi AFAA, Khatun MM, Moe TM, Nishikawa J, Yoshiyama H. Development of epstein-barr virus-associated gastric cancer: Infection, inflammation, and oncogenesis. World J Gastroenterol. 2022;28(44):6249–57. https://doi.org/10.3748/wjg.v28.i44.6249.
Janjigian YY, Sanchez-Vega F, Jonsson P, et al. Genetic predictors of response to systemic therapy in Esophagogastric Cancer. Cancer Discov. 2018;8(1):49–58. https://doi.org/10.1158/2159-8290.CD-17-0787. Epub 2017 Nov 9. PMID: 29122777; PMCID: PMC5813492.
Janjigian YY, Maron SB, Chatila WK, et al. First-line pembrolizumab and trastuzumab in HER2-positive oesophageal, gastric, or gastro-oesophageal junction cancer: an open-label, single-arm, phase 2 trial. Lancet Oncol. 2020;21:821–31.
Janjigian YY, Shitara K, Moehler M, et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): a randomised, open-label, phase 3 trial. Lancet. 2021;398(10294):27–40.
Jeong Y, Cho CE, Kim JE, et al. Deep learning model to predict Epstein–Barr virus associated gastric cancer in histology. Sci Rep. 2022;12:18466.
Kim M, Seo AN. Molecular Pathology of Gastric Cancer. J Gastric Cancer. 2022;22(4):273–305. https://doi.org/10.5230/jgc.2022.22.e35. PMID: 36316106; PMCID: PMC9633931.
Kim SY, Choi YY, An JY, Shin HB, Jo A, Choi H, et al. The benefit of microsatellite instability is attenuated by chemotherapy in stage II and stage III gastric cancer: results from a large cohort with subgroup analyses. Int J Cancer. 2015;137:819–25.
Kim JW, Cho S-Y, Chae J, et al. Adjuvant chemotherapy in microsatellite instability–high gastric cancer. Cancer Res Treat. 2020;52:1178–87.
Kim HD, Shin J, Song IH, Hyung J, Lee H, Ryu MH, Park YS. Discordant PD-L1 results between 28 – 8 and 22C3 assays are associated with outcomes of gastric cancer patients treated with nivolumab plus chemotherapy. Gastric Cancer. 2024 Apr 22.
Klempner SJ, Madison R, Pujara V, et al. FGFR2-altered gastroesophageal adenocarcinomas are an uncommon clinicopathologic entity with a distinct genomic landscape. Oncologist. 2019;24(11):1462–8.
Kubota Y, Kawazoe A, Mishima S, et al. Comprehensive clinical and molecular characterization of claudin 18.2 expression in advanced gastric or gastroesophageal junction cancer. ESMO Open. 2023;8(1):100762.
Kulangara K, et al. Clinical utility of the combined positive score for programmed death ligand-1 expression and the approval of pembrolizumab for treatment of gastric cancer. Arch Pathol Lab Med. 2019;143:330–7.
Kumarasinghe MP, Houghton D, Allanson BM, Price TJ. What therapeutic biomarkers in gastro-esophageal junction and gastric cancer should a pathologist know about? Surg Pathol Clin. 2023;16(4):659–72. https://doi.org/10.1016/j.path.2023.05.004.
Kun Dong L, Yin Y, Wang L, Jia X, Diao X, Huang L, Zhou D, Lin Y, Sun. Prevalence and detection methodology for preliminary exploration of NTRK fusion in gastric cancer from a single-center retrospective cohort, Human Pathology, 148, 2024, Pages 87–92, ISSN 0046-8177, https://doi.org/10.1016/j.humpath.2024.04.011
Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357:409–13.
Li J, Zhang Y, Hu D, Gong T, Xu R, Gao J. Analysis of the expression and genetic alteration of CLDN18 in gastric cancer. Aging. 2020;12(14):14271–84. https://doi.org/10.18632/aging.103457. Epub 2020 Jul 15.
Liu X, Meltzer SJ. Gastric cancer in the era of precision medicine. Cell Mol Gastroenterol Hepatol. 2017;3:348–58.
Marwitz T, Hüneburg R, Spier I, Lau JF, Kristiansen G, Lingohr P, Kalff JC, Aretz S, Nattermann J, Strassburg CP. Hereditary diffuse gastric Cancer: a comparative cohort study according to pathogenic variant status. Cancers (Basel). 2020;12(12):3726. https://doi.org/10.3390/cancers12123726. PMID: 33322525; PMCID: PMC7763201.
Massarrat S, Stolte M. Development of gastric cancer and its prevention. Arch Iran Med. 2014;17(7):514–52.
Moran D, Maurus D, Rohde C, Arozullah. A. 103P. Prevalence of CLDN18.2, HER2 and PD-L1 in gastric cancer samples. Ann Oncol. 2018;29:viii32.
Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA, WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020;76(2):182–8. https://doi.org/10.1111/his.13975.
Nakashima H, et al. Microsatellite instability in multiple gastric cancers. Int J Cancer. 1995;64:239–42.
Narita Y, Muro K. Updated immunotherapy for gastric Cancer. J Clin Med. 2023;12(7):2636.
Narita Y, Sasaki E, Masuishi T, Taniguchi H, Kadowaki S, Ito S, Yatabe Y, Muro K. PD-L1 immunohistochemistry comparison of 22C3 and 28 – 8 assays for gastric cancer. J Gastrointest Oncol. 2021;12:2696–705.
National Comprehensive Cancer Network. Gastric Cancer. (Guidelines Version 2.2024). https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf. Accessed April 10, 2024.
Nakayama I, Qi C, Chen Y, Nakamura Y, Shen L, Shitara K. Claudin 18.2 as a novel therapeutic target. Nat Rev Clin Oncol. 2024;21(5):354–69. https://doi.org/10.1038/s41571-024-00874-2.
Oliveira C, Pinheiro H, Figueiredo J, Seruca R, Carneiro F. Familial gastric cancer: genetic susceptibility, pathology, and implications for management. Lancet Oncol. 2015;16(2):e60–70. https://doi.org/10.1016/S1470-2045(14)71016-2.
Ooki A, Yamaguchi K. The beginning of the era of precision medicine for gastric cancer with fibroblast growth factor receptor 2 aberration. Gastric Cancer. 2021;24(6):1169–83.
Park DI, et al. HER-2/neu amplification is an independent prognostic factor in gastric cancer. Dig Dis Sci. 2006;51(8):1371–9.
Park Y, Nam SK, Seo SH, Park KU, Oh HJ, Park YS, Suh YS, Ahn SH, Park DJ, Kim HH, Lee HS. Comprehensive study of microsatellite instability testing and its comparison with immunohistochemistry in gastric cancers. J Gastric Cancer. 2023;23(2):264–74.
Pellino A, Brignola S, Riello E, Niero M, et al. Association of CLDN18.2 protein expression with clinicopathological features and prognosis in advanced gastric and gastroesophageal junction adenocarcinomas. J Pers Med. 2021;11:1095.
Ph1b/2. Study of the Safety and Efficacy of T-DXd combinations in Advanced HER2-expressing gastric Cancer (DESTINY-Gastric03.
Pietrantonio F, Miceli R, Raimondi A, et al. Individual patient data meta-analysis of the value of microsatellite instability as a biomarker in gastric cancer. J Clin Oncol. 2019;37(35):3392–3400. https://doi.org/10.1200/jco.19.01124.
Pu X, Fu Y, Sun Q, Li L, Kwasi A, Ma Z, Fan X, Sun B. NTRK gene alterations were enriched in hepatoid or enteroblastic differentiation type of gastric cancer. J Clin Pathol. 2024;77(9):608–613. https://doi.org/10.1136/jcp-2023-208865. PMID: 37451841.
Puliga E, Corso S, Pietrantonio F, Giordano S. Microsatellite instability in gastric Cancer: between lights and shadows. Cancer Treat Rev. 2021;95:102175.
Rha SY, Lee C-K, Kim HS, et al. Targeting HER2 in combination with anti-PD-1 and chemotherapy confers a significant tumor shrinkage of gastric cancer: a multi-institutional phase Ib/II trial of first-line triplet regimen (pembrolizumab, trastuzumab, chemotherapy) for HER2-positive advanced

gastric cancer (AGC). Proc Am Soc Clin Oncol. 2020;38(suppl):3081. (abstr).
Röcken C. Predictive biomarkers in gastric cancer. J Cancer Res Clin Oncol. 2023;149(1):467–81. https://doi.org/10.1007/s00432-022-04408-0. Epub 2022 Oct 19.
Rolfo C, De Braud F, Doebele R, Drilon A, Siena S, Patel M, Cho B, Liu S, Ahn M, Chiu C, et al. Efficacy and safety of entrectinib in patients (pts) with NTRK-fusion positive (NTRK-fp) solid tumors: an updated integrated analysis. J Clin Oncol. 2020;38:3605.
Rubin E, Shan KS, Dalal S, Vu DUD, Milillo-Naraine AM, Guaqueta D, Ergle A. Molecular Targeting of the human epidermal growth factor Receptor-2 (HER2) genes across various cancers. Int J Mol Sci. 2024;25:1064. https://doi.org/10.3390/ijms25021064.
Sahin U, Koslowski M, Dhaene K, Usener D, Brandenburg G, Seitz G, Huber C, Türeci O. Claudin-18 splice variant 2 is a pan-cancer target suitable for therapeutic antibody development. Clin Cancer Res. 2008;14(23):7624–34.
Satoh T, Xu R-H, Chung HC, et al. Lapatinib plus Paclitaxel versus paclitaxel alone in the second-line treatment of HER2-amplified advanced gastric cancer in Asian populations: TyTAN — a randomized, phase III study. J Clin Oncol. 2014;32:2039–49.
Shia J, Ellis NA, Klimstra DS. The utility of immunohistochemical detection of DNA mismatch repair gene proteins. Virchows Arch. 2004;445:431–41.
Shitara K, Van Cutsem E, Bang Y-J, Fuchs C, Wyrwicz L, Lee K-W, Kudaba I, Garrido M, Chung HC, Lee J, et al. Efficacy and safety of Pembrolizumab or Pembrolizumab Plus Chemotherapy vs Chemotherapy alone for patients with First-line, Advanced Gastric Cancer: the KEYNOTE-062 phase 3 Randomized Clinical Trial. JAMA Oncol. 2020;6:1571–80.
Shitara K, Bang YJ, Iwasa S, Sugimoto N, Ryu MH, Sakai D, Chung HC, Kawakami H, Yabusaki H, Lee J, Saito K, Kawaguchi Y, Kamio T, Kojima A, Sugihara M. Yamaguchi. Trastuzumab Deruxtecan in previously treated HER2-Positive gastric Cancer. N Engl J Med. 2020b;382(25):2419–30.
Shitara K, Xu RH, Ajani JA, et al. Global prevalence of claudin 18 isoform 2 in tumors of patients with locally advanced unresectable or metastatic gastric or gastroesophageal junction adenocarcinoma. Gastric Cancer. 2024;27:1058–68. https://doi.org/10.1007/s10120-024-01518-1.
Skórzewska M, Gęca K, Polkowski WP. A clinical viewpoint on the use of targeted therapy in Advanced Gastric Cancer. Cancers. 2023;15:5490.
Smyth EC, Chao J, Muro K, Yen P, Yanes RE, Zahlten-Kumeli A, Rha SY. Trial in Progress: Phase 3 Study of Bemarituzumab + MFOLFOX6 versus Placebo + MFOLFOX6 in Previously Untreated Advanced Gastric or Gastroesophageal Junction (GEJ) Cancer with FGFR2b Overexpression (FORTITUDE-101) J. Clin. Orthod. 2022.
Sociedade Brasileira de Oncologia Clínica (SBOC). Diretrizes de tratamentos oncológicos recomendados pela Sociedade Brasileira de Oncologia Clínica: Estômago: Doença avançada. 2024.
Sohn BH, Hwang JE, Jang HJ, Lee HS, Oh SC, Shim JJ, Lee KW, Kim EH, Yim SY, Lee SH, Cheong JH, Jeong W, Cho JY, Kim J, Chae J, Lee J, Kang WK, Kim S, Noh SH, Ajani JA, Lee JS. Clinical significance of four molecular subtypes of gastric cancer identified by the Cancer Genome Atlas project. Clin Cancer Res. 2017;23:4441–9.
Soomin Ahn, Kyoung-Mee Kim, PD-L1 expression in gastric cancer: interchangeability of 22C3 and 28-8 pharmDx assays for responses to immunotherapy. Mod Pathol. 2021;34(9):1719–27. https://doi.org/10.1038/s41379-021-00823-9.
Su X, Zhan P, Gavine PR, et al. FGFR2 amplification has prognostic significance in gastric cancer: results from a large international multicentre study. Br J Cancer. 2014;110(4):967–75.
Subbiah V, Cassier PA, Siena S, et al. Pan-cancer efficacy of pralsetinib in patients with *RET* fusion–positive solid tumors from the phase 1/2 ARROW trial. Nat Med. 2022;28:1640–5.
Subbiah V, Kreitman RJ, Wainberg ZA, et al. Dabrafenib plus Trametinib in *BRAFV600E*-mutated rare cancers: the phase 2 ROAR trial. Nat Med. 2023;29:1103–12.
Tabernero J, Hoff PM, Shen L, et al. Pertuzumab plus Trastuzumab and chemotherapy for HER2-positive metastatic gastric or gastro-oesophageal junction cancer (JACOB): final analysis of a double-blind, randomized, placebo-controlled phase 3 study. Lancet Oncol. 2018;19:1372–84.
Talari FF, Bozorg A, Zeinali S, et al. Low incidence of microsatellite instability in gastric cancers and its association with the clinicopathological characteristics: a comparative study. Sci Rep. 2023;13:21743.
Thrift AP, Wenker TN, El-Serag HB. Global burden of gastric cancer: epidemiological trends, risk factors, screening and prevention. Nat Rev Clin Oncol. 2023;20:338–49. https://doi.org/10.1038/s41571-023-00747-0.
Van Cutsem E, di Bartolomeo M, Smyth E, Chau I, Park H, Siena S, Lonardi S, Wainberg ZA, Ajani J, Chao J, Janjigian Y, Qin A, Singh J, Barlaskar F, Kawaguchi Y, Ku G. Trastuzumab Deruxtecan in patients in the USA and Europe with HER2-positive advanced gastric or gastroesophageal junction cancer with disease progression on or after a trastuzumab-containing regimen (DESTINY-Gastric02): primary and updated analyses from a single-arm, phase 2 study. Lancet Oncol. 2023;24(7):744–56.
Vanni I, Tanda ET, Spagnolo F, Andreotti V, Bruno W, Ghiorzo P. The current state of Molecular Testing in the BRAF-Mutated Melanoma Landscape. Front Mol Biosci. 2020;7:113.
Vranic S, Basu GD, Hall DW, Gatalica Z, Tumor-Type, Agnostic. Targeted therapies: BRAF inhibitors join the Group. Acta Med Acad. 2022;51(3):217–31. https://doi.org/10.5644/ama2006-124.392. Epub 2022 Dec 30.
Wainberg ZA, Enzinger PC, Kang YK, et al. Bemarituzumab in patients with FGFR2b-selected gastric or gastro-oesophageal junction adenocarcinoma (FIGHT): a randomised, double-blind, placebo-controlled, phase 2 study. Lancet Oncol. 2022;23(11):1430–40.
Yamashita-Kashima Y, Shu S, Yorozu K, Hashizume K, Moriya Y, Fujimoto-Ouchi K, Harada N. Importance of formalin fixing conditions for HER2 testing in gastric cancer: immunohistochemical staining and fluorescence in situ hybridization. Gastric Cancer. 2014;17(4):638–47.
Yeong J, Lum HYJ, Teo CB, Tan BKJ, Chan YH, Tay RYK, Choo JR, Jeyasekharan AD, Miow QH, Loo LH, Yong WP, Sundar R. Choice of PD-L1 immunohistochemistry assay influences clinical eligibility for gastric cancer immunotherapy. Gastric Cancer. 2022;25(4):741–50.
Zhou F, et al. Prognosis significance of HER-2/neu overexpression/amplification in Chinese patients with curatively resected gastric cancer after the ToGA clinical trial. World J Surg Oncol. 2012;10:274.

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