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REVIEW

Advances and challenges in gastric cancer testing: the role of biomarkers

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ABSTRACT Advances in the identification of molecular biomarkers and the development of targeted therapies have enhanced the prognosis of patients with advanced gastric cancer. Several established biomarkers have been widely integrated into routine clinical diagnostics of gastric cancer to guide personalized treatment. Human epidermal growth factor receptor 2 (HER2) was the first molecular biomarker to be used in gastric cancer with trastuzumab being the first approved targeted therapy for HER2-positive gastric cancer. Programmed death-ligand 1 positivity and microsatellite instability can guide the use of immunotherapies, such as pembrolizumab and nivolumab. More recently, zolbetuximab has been approved for patients with claudin 18.2-positive diseases in some countries. More targeted therapies, including savolitinib for MET-positive patients, are currently under clinical investigation. However, the clinical application of these diagnostic approaches could be hampered by many existing challenges, including invasive and costly sampling methods, variability in immunohistochemistry interpretation, high costs and long turnaround times for next-generation sequencing, the absence of standardized and clinically validated diagnostic cut-off values for some biomarkers, and tumor heterogeneity. Novel testing and analysis techniques, such as artificial intelligence-assisted image analysis and multiplex immunohistochemistry, and emerging therapeutic strategies, including combination therapies that integrate immune checkpoint inhibitors with targeted therapies, offer potential solutions to some of these challenges. This article reviews recent progress in gastric cancer testing, outlines current challenges, and explores future directions for biomarker testing and targeted therapy for gastric cancer.

KEYWORDS Gastric cancer; testing; diagnosis; biomarkers; precision therapy

Introduction

Gastric cancer is one of the most common cancers and a leading cause of cancer-related mortality[1]. According to the 2022 GLOBOCAN estimates, gastric cancer ranked fifth for both incidence and mortality globally among all cancers[2]. The incidence and mortality rates of gastric cancer are expected to show a small but persistent decrease through 2040 worldwide. However, this decrease will be offset by the growing and aged population, resulting in a net increase of new cases and deaths globally in the upcoming years[3]. A population-based study predicted that by 2040, the annual burden of gastric cancer will increase to approximately 1.8 million new cases and 1.3 million deaths if the current incidence and mortality rates remain unchanged. In fact, if the incidence and mortality rates decrease by 2% each year, there will still be an annual burden of approximately 1.18 million new cases and 0.85 million deaths by 2040[4].

Because early-stage gastric cancer is typically asymptomatic, patients with gastric cancer are frequently diagnosed in advanced stages, making curative resection unlikely[5]. Locally advanced and metastatic gastric cancers generally have a poor prognosis despite chemotherapy and personalized treatment, such as targeted therapy or immunotherapy, is needed to improve patient outcomes[6]. Gastric cancer testing and diagnosis have traditionally relied on the histopathologic classifications and conventional tumor markers (**Figure 1**). Widely used histopathologic classifications include

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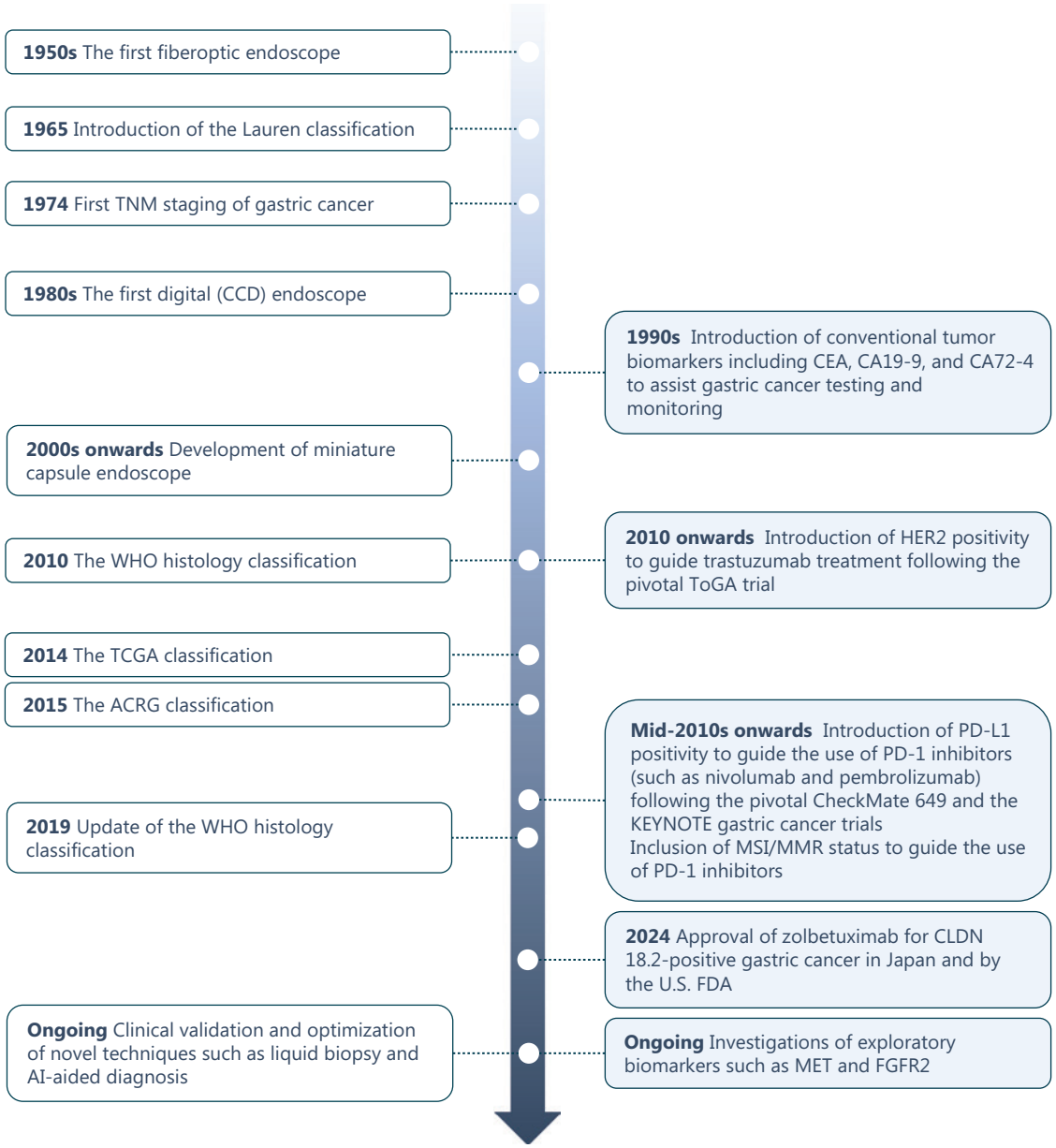

Figure 1 Timeline of selected milestones in gastric cancer testing[7-12]. Gastric cancer testing and diagnosis have traditionally relied on histopathologic classifications, such as the Lauren classification and conventional tumor markers (e.g., CEA and CA19-9). Since the 2010s, advancements in molecular biomarkers and targeted therapies have greatly transformed gastric cancer testing. HER2, PD-L1, and MSI/MMR are now integral to routine clinical diagnostics for gastric cancer, allowing personalized treatment strategies. Anti-CLDN 18.2 therapy has been approved in Japan. More investigations of novel biomarkers, such as MET, and diagnostic techniques are currently underway. AI, artificial intelligence; ACRG, Asian Cancer Research Group; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4; CCD, charge coupled device; CEA, carcinoembryonic antigen; CLDN18.2, claudin 18.2; FDA, Food and Drug Administration; FGFR2, fibroblast growth factor receptor 2; HER2, human epidermal growth factor receptor 2; MSI/MMR, microsatellite instability/mismatch repair; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; TCGA, The Cancer Genome Atlas; TNM, tumor-node-metastasis; WHO, World Health Organization.

the Lauren classification (introduced in 1965)[13] and the more recent World Health Organization classification (updated in 2019)[14]. Both histopathologic classification schemes provide important guidance for surgery and chemotherapy selection. Conventional tumor markers, such as carcinoembryonic antigen, carbohydrate antigen (CA) 19-9, and CA 72-4, are also

commonly used in gastric cancer diagnosis, staging, and monitoring[7,15]. However, these conventional histopathologic classifications and biomarkers render insufficient information to guide targeted therapy and immunotherapy.

As such, molecular biomarkers are becoming increasingly important in the diagnosis and treatment decision of gastric cancer (**Figure 1**). For example, human epidermal growth factor receptor 2 (HER2) is the first molecular biomarker to be used in gastric cancer, and trastuzumab, an anti-HER2 antibody, is the first approved targeted therapy for gastric cancer[8]. Programmed death-ligand 1 (PD-L1) is another established biomarker for gastric cancer, and pembrolizumab, an anti-PD-L1 antibody, has been recommended for patients with PD-L1-expressing tumors[16]. More recently, zolbetuximab has been approved for patients with claudin 18.2 (CLDN18.2)-positive, unresectable advanced or recurrent gastric cancer in Japan (March 2024) and the US (October 2024)[17,18]. Of note, ramucirumab, a vascular endothelial growth factor receptor 2 (VEGFR2) antibody, is also approved for advanced gastric cancer[16]. However, the use of ramucirumab does not require detection of specific biomarkers. Therefore, VEGFR2 is not discussed in this review. In addition to the established biomarkers, exploratory biomarkers, such as MET and fibroblast growth factor receptor 2 (FGFR2), and the corresponding targeted therapies are currently being studied with several biomarkers showing promise in predicting treatment responsiveness to specific targeted therapies[12].

However, despite these advances, significant challenges remain in biomarker testing. For example, the commonly used sampling method of tissue biopsy is invasive and costly[19,20]. Essential testing methods also have limitations, such as inter-observer variability in immunohistochemistry (IHC) and the high cost and lengthy turnaround for next-generation sequencing (NGS)[21]. The absence of standardized, clinically validated diagnostic cut-off values for exploratory biomarkers may hinder clinical application. Finally, tumor heterogeneity, both spatial and temporal, can impact biomarker testing results and compromise the efficacy of biomarker-guided therapies. Addressing these challenges is crucial for the broader application of biomarker-based approaches in the management of gastric cancer.

The current progress in gastric cancer testing based on established and exploratory molecular biomarkers is reviewed herein, and the challenges and prospects of biomarker testing and biomarker-guided therapy in gastric cancer are discussed.

Established biomarkers in gastric cancer

HER2

HER2 is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (RTKs) and is encoded by *ERBB2* on chromosome 17[12,22]. HER2 activates downstream pathways through heterodimerization and tyrosine phosphorylation-mediated signal transduction[23]. Major signaling pathways activated by HER2 include the rat sarcoma/rapidly accelerated fibrosarcoma/mitogen-activated protein kinase kinase/extracellular signaling related kinase (RAS/RAF/MEK/ERK) and phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathways, which regulate cell proliferation, differentiation, and survival and participate in the tumorigenesis of many types of cancer, including breast, gastric, and colorectal cancers (**Figure 2**)[23]. HER2 is overexpressed and/or amplified in approximately 20% of patients with gastric cancer[22] and HER2-positive tumors are typically more aggressive and more likely to recur[8]. The prognostic significance of HER2 expression in gastric cancer, unlike breast cancer, has not been established[16]. Some studies have suggested that HER2-positivity is associated with a poorer prognosis in gastric cancer patients[24]. As early as 2000, Allgayer et al.[25] reported that increasing HER2 expression is associated with shorter disease-free ($P = 0.023$) and overall survival (OS) ($P = 0.0160$) in a consecutive prospective series of 203 gastric cancer patients. Allgayer et al.[25] also showed that HER2 is an independent prognostic factor for OS among patients who received curative resection [risk ratio (RR) = 1.54, 95% confidence interval (CI): 1.08–1.67, $P = 0.049$] and among all patients (RR = 1.33, 95% CI: 1.28–1.38, $P = 0.028$). However, there are also studies that have shown HER2 is not an independent prognostic factor of patient outcome, except in a very small subset of patients with intestinal histology[26-28].

IHC and *in situ* hybridisation (ISH) are currently recommended to detect HER2 overexpression/amplification[6,12]. The US National Comprehensive Cancer Network (NCCN) guidelines for gastric cancer recommend HER2 testing for all gastric cancer patients at the time of diagnosis if metastatic disease is documented or suggested[16]. An IHC score of 3+ (strong complete, basolateral, or lateral membranous reactivity in ≥ 10% of cancer cells) is positive for HER2 overexpression[16]. For patients with an IHC score of 2+ (considered equivocal), ISH

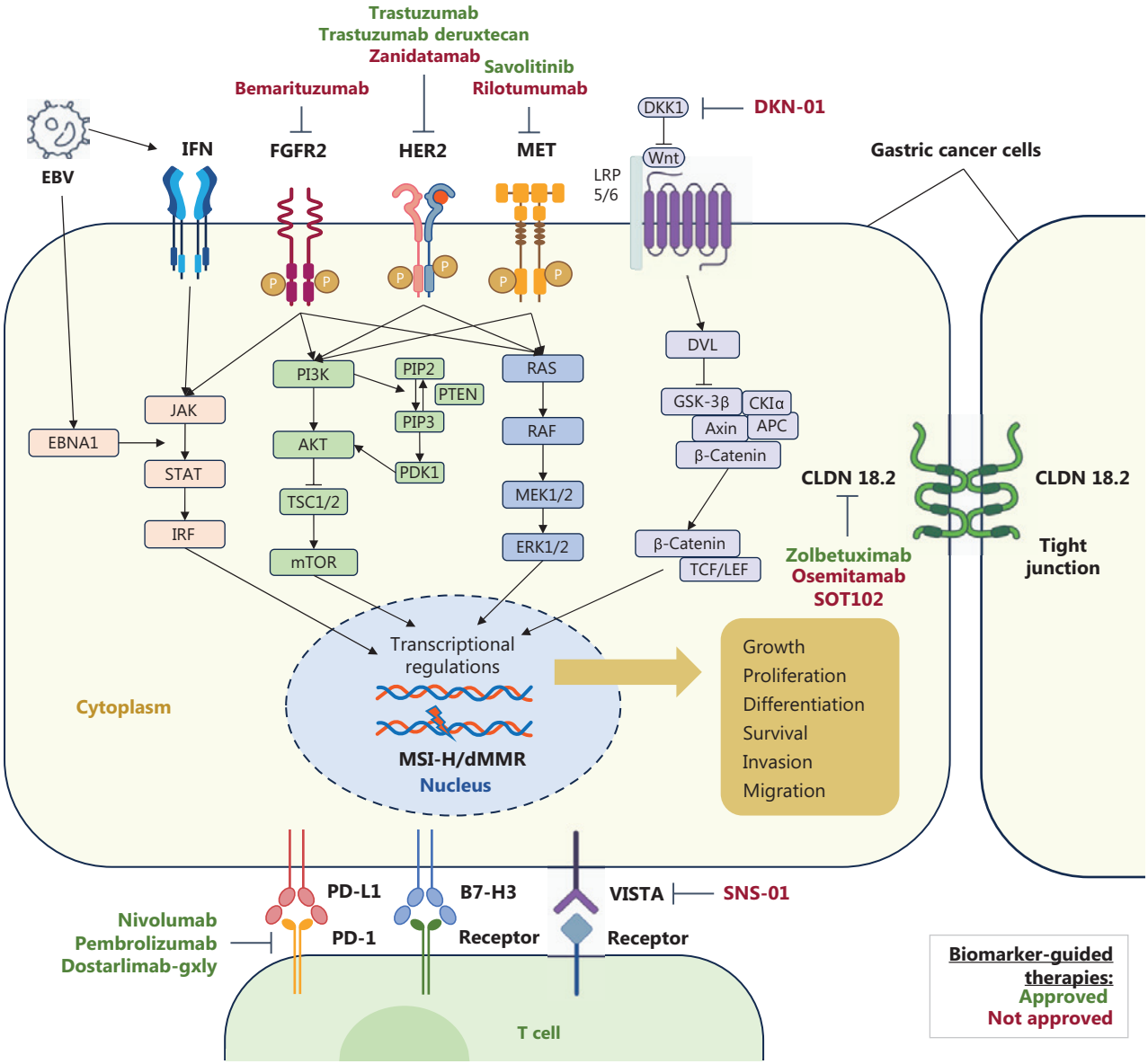

Figure 2 Key molecular alterations and signaling pathways involved in the pathogenesis and progression of gastric cancer, along with selected targeted therapies (adapted from Lei et al.[8]). Many biomarkers have been associated with gastric cancer, such as molecules in growth factors pathways and immune checkpoint control modulators. Growth factor receptors (e.g., HER2, MET, and FGFR2) typically activate essential downstream pathways through dimerization and tyrosine kinase signaling. The downstream pathways, including PI3K/AKT/mTOR, RAS/RAF/MEK/ERK, and JAK/STAT/IRF, mediate essential cellular processes, including growth, proliferation, differentiation and survival, and participate in the tumorigenesis and progression of many cancer types. DKK1 regulates the Wnt/β-catenin signaling pathway, which is also an essential pathway involved in cell proliferation, migration, and death. PD-L1 binds to PD-1 and suppresses T-cell receptor signaling, and this mechanism is commonly hijacked by cancer cells to escape immune recognition. On a related note, dysregulated expression of the MMR genes can impair cellular repair function during DNA replication, resulting in the MSI-H/dMMR phenotype. This phenotype contributes to gastric cancer through different mechanisms, including an upregulated PD-L1 expression. Likewise, EBV is associated with different oncogenic effects and it is known to increase PD-L1 expression through the JAK/STAT/IRF pathway. B7-H3 and VISTA are also immune checkpoint proteins and have been associated with immune invasion in gastric cancer. Another notable biomarker is CLDN18.2, a tight junction protein commonly expressed in differentiated gastric mucosa cells. CLDN18.2 may become more exposed when tight junctions are disrupted upon malignant transformation of gastric epithelial cells. In terms of biomarker-guided treatments for gastric cancer, trastuzumab and trastuzumab deruxtecan are recommended for patients with HER2-positivity. PD-L1-positivity and MSI-H/dMMR can guide the use of immunotherapies, such as pembrolizumab. Zolbetuximab has been approved for patients with CLDN18.2-positive disease in some countries. More targeted

therapies, including savolitinib for MET-positive patients, are currently under clinical investigation. AKT, protein kinase B; APC, adenomatous polyposis coli; CLDN18.2, claudin 18.2; CKIα, casein kinase Iα; DKN-01, Dikkopf-1 monoclonal antibody 1; DKK1, Dikkopf-1; DVL, disheveled; EBV, Epstein-Bar virus; EBNA, Epstein-Bar nuclear antigen; ERK1/2, extracellular signal-regulated kinase 1/2; FGFR2, fibroblast growth factor receptor 2; HER2, human epidermal growth factor receptor 2; GSK-3β, glycogen synthase kinase 3β; IFH, interferon; IRF, interferon regulatory factor; JAK, Janus kinase; LRP5/6, low-density lipoprotein receptor-related protein 5/6; MEK1/2, mitogen-activated protein kinase 1/2; MSI-H/dMMR, microsatellite instability high/defective mismatch repair; mTOR, mammalian target of rapamycin; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PDK1, phosphoinositide-dependent protein kinase 1; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol diphosphate; PIP3, phosphatidylinositol 3-phosphate; PTEN, phosphatase and tensin homolog; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; STAT, signaling transducer and activator of transcription; TCF/LEF T-cell factor/lymphoid enhancer factor; TSC1/2, tuberous sclerosis complex 1/2; VISTA, V-domain immunoglobulin-containing suppressor of T-cell activation.

should be performed and patients with an *HER2*/centromere enumerator probe 17 (*CEP17*) ≥ 2 or an average *HER2* copy number ≥ 6.0 signals/cell are considered positive[16]. These definitions are widely adopted in real world clinical practice[29,30].

As early as 2010 the randomized phase III ToGA trial demonstrated the efficacy of trastuzumab in HER2-postive advanced gastric/gastric-oesophageal junction (GEJ) cancer patients, with the trastuzumab plus chemotherapy arm showing a longer median OS than the chemotherapy only arm [13.8 months *vs.* 11.1 months, hazard ratio (HR) = 0.74, 95% CI: 0.60–0.91, $P = 0.0046$][31]. **Table 1** is a summary of important clinical trials for key molecular biomarkers with published results. This combination treatment is now the standard first-line therapy for patients with HER2-positive gastric/GEJ cancer[16,39]. The phase II DESTINY-Gastric 01 trial explored the use of trastuzumab deruxtecan, an antibody drug conjugate, in HER2-positive gastric patients in whom disease progressed after two lines of previous therapy (including trastuzumab) and demonstrated significant improvement in tumor response and OS compared to the physician's choice of chemotherapy[29]. DESTINY-Gastric 02, a single-arm phase II study, further confirmed the efficacy and safety of trastuzumab deruxtecan in patients with disease progression on or after first-line therapy with a trastuzumab-containing regimen[32]. The use of other anti-HER2 therapies, such as pertuzumab and margetuximab, have also been studied[23,40,41]. Zanidatamab, a novel bispecific anti-HER antibody, is currently being investigated in an open-label, active-comparator, phase III study (HERIZON-GEA-01)[42].

PD-L1

PD-L1 is the second predictive biomarker to be used for gastric cancer[43]. PD-L1 is encoded by *CD274* on chromosome 9 and is the ligand of programmed death-1 (PD-1), an inhibitor checkpoint receptor expressed on cytotoxic T-cells and other immune cells[12,44,45]. Binding of PD-L1 to PD-1 suppresses T-cell receptor signaling. Importantly, this signaling pathway is commonly hijacked by cancer cells to escape immune surveillance (**Figure 2**)[12,46,47]. Elevated expression of PD-L1 has been reported in up to 65% of gastric/GEJ cancers and is associated with subtypes of tumors with high mutational burden[48]. A meta-analysis of 10 studies involving a total of 1,901 patients showed that PD-L1 expression is significantly associated with larger tumor sizes [odds ratio (OR) = 1.87, 95% CI 1.25–2.78, $P = 0.002$], a higher likelihood of lymph node metastasis (OR = 2.17, 95% CI 1.04–4.52, $P = 0.04$), and shorter OS (HR = 1.64, 95% CI 1.11–2.43, $P = 0.01$)[49].

PD-L1 is expressed more often on immune cells in the tumor microenvironment than the tumor cells[12,50]. As such, PD-L1 expression in gastric cancer is assessed using IHC and indicated using the combined positive score (CPS), which is calculated as the number of PD-L1-staining cells (i.e., tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells, then multiplied by 100[16,51]. A specimen is considered PD-L1-positive if the specimen has a CPS ≥ 1[16].

Currently, nivolumab and pembrolizumab (PD-1 inhibitors) have been widely recommended for advanced gastric cancer patients who are PD-L1-positive[16,52,53]. Other PD-L1-guided immune checkpoint inhibitors (ICIs) have also been approved for gastric cancer in some regions of the world, such as sintilimab and tislelizumab in China[53]. The pivotal phase III CheckMate 649 trial demonstrated that nivolumab plus chemotherapy significantly improved the OS (HR = 0.71, 95% CI 0.59–0.86, $P < 0.0001$) and progression-free survival (PFS) (HR = 0.68, 95% CI 0.56–0.81, $P < 0.0001$) compared to chemotherapy alone in previously untreated, unresectable, non-HER2-positive gastric/GEJ/oesophageal adenocarcinoma patients with a PD-L1 CPS ≥ 5[33]. The use of pembrolizumab in PD-L1-positive gastric cancer patients has been studied in several important clinical trials,

Table 1 Important clinical trials for key molecular biomarkers

Biomarker	Trial	*n*	Patients	Testing method	Biomarker cut-off value	Treatment	Efficacy outcomes		
							Median OS	Median PFS	ORR
Established biomarkers									
HER2	ToGA[31] Phase III	594	Gastric or GEJ cancer	IHC & FISH	IHC3+ or *HER2:CEP17* ratio ≥ 2	Trastuzumab + chemo *vs.* PL + chemo	13.8 m *vs.* 11.1 m (HR = 0.74, 95% CI: 0.60–0.91, *P* = 0.0046)	6.7 m *vs.* 5.5 m (HR = 0.71, 95% CI: 0.59–0.85, *P* = 0.0002)	47% *vs.* 35% (*P* = 0.0017)
	DESTINY-Gastric 01[29] Phase II	187	Gastric or GEJ cancer	IHC & ISH	IHC3+ or IHC2+/ISH+	Trastuzumab deruxtecan *vs.* chemo	12.5 m *vs.* 8.4 m (HR = 0.59, 95% CI: 0.39–0.88, *P* = 0.01)	5.6 m *vs.* 3.5 m (HR = 0.47, 95% CI: 0.31–0.71, *P*: NR)	51% *vs.* 14% (*P* < 0.001)
	DESTINY-Gastric 02[32] Phase II	79	Gastric or GEJ cancer	IHC & ISH	IHC3+ or IHC2+/ISH+	Trastuzumab deruxtecan	12.1 m (95% CI: 9.4 m–15.4 m)	5.6 m (95% CI: 4.2 m–8.3 m)	42%
PD-L1	CheckMate 649[33] Phase III	1,581	Gastric or GEJ or oesophageal cancer	IHC	CPS ≥ 5	Nivolumab + chemo *vs.* chemo	14.4 m *vs.* 11.1 m (HR = 0.71, 98.4% CI: 0.59–0.86, *P* < 0.0001)	7.7 m *vs.* 6.05 m (HR = 0.68, 98% CI: 0.56–0.81, *P* < 0.0001)	60% *vs.* 45% (*P*: NR)
	KEYNOTE-859[34] Phase III	1,579	Gastric or GEJ cancer	IHC	CPS ≥ 1 and CPS ≥ 10	Pembrolizumab + chemo *vs.* PL + chemo	CPS ≥ 1: 13.0 m *vs.* 11.4 m (HR = 0.74, 95% CI: 0.65–0.84, *P* < 0.0001) CPS ≥ 10: 15.7 m *vs.* 11.8 m (HR = 0.65, 95% CI: 0.53–0.79, *P* < 0.0001)	CPS ≥ 1: 6.9 m *vs.* 5.6 m (HR = 0.72, 95% CI: 0.63–0.82, *P* < 0.0001) CPS ≥ 10: 8.1 m *vs.* 5.6 m (HR = 0.62, 95% CI: 0.51–0.76, *P* < 0.0001)	CPS ≥ 1: 52% *vs.* 43% (*P* = 0.0004) CPS ≥ 10: 61% *vs.* 43% (*P* < 0.0001)
CLDN18.2	SPOTLIGHT[35] Phase III	565	Gastric of GEJ cancer	IHC	≥ 75% of cells with moderate-to-strong membrane staining	Zolbetuximab + mFOLFOX6 *vs.* PL + mFOLFOX6	18.23 m *vs.* 15.54 m (HR = 0.75 m, 95% CI: 0.60–0.94, *P* = 0.0135)	10.61 m *vs.* 8.67 m (HR = 0.75, 95% CI: 0.60–0.94, *P* = 0.0066)	48% *vs.* 48% (*P*: NR)
	GLOW[36] Phase III	507	Gastric or GEJ cancer	IHC	Same as SPOTLIGHT	Zolbetuximab + CAPOX *vs.* PL + CAPOX	14.39 m *vs.* 12.16 m (HR = 0.771, 95% CI: 0.615–0.965, *P* = 0.0118)	8.21 m *vs.* 6.80 m (HR = 0.687, 95% CI: 0.544–0.866, *P* = 0.0007)	42.5% *vs.* 40.3% (*P*: NR)
Exploratory biomarkers									
MET	VIKTORY[37] Phase II	20[†]	Gastric cancer	NGS or FISH	*MET* amplification	Savolitinib	NR	NR	50%
FGFR2	FIGHT[38] Phase II	155	Gastric or GEJ cancer	IHC or NGS	IHC2+/3+ or amplification by ctDNA NGS	Bemarituzumab + mFOLFOX6 *vs.* PL + mFOLFOX6	Not reached *vs.* 12.9 m (HR = 0.58, 95% CI: 0.35–0.95, *P* = 0.027)	9.5 m *vs.* 7.4 m (HR = 0.68, 95% CI: 0.44–1.04, *P* = 0.073)	47% *vs.* 33% (*P* = 0.11)

[†]The VIKTORY umbrella trial assigned a total of 105 patients to different biomarker-specific trials. We only described the 20 *MET*-amplified patients assigned to savolitinib treatment. CAPOX, capecitabine and oxaliplatin; chemo, chemotherapy; CI, confidence interval; CLDN18.2, claudin 18.2; CPS, combined positive score; FGFR2, fibroblast growth factor receptor 2; FISH, fluorescence *in situ* hybridisation; GEJ, gastro-oesophageal junction; HR, hazard ratio; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, *in situ* hybridization; m, month; mFOLFOX6, modified 5-fluorouracil, leucovorin and oxaliplatin; NGS, next-generation sequencing; NR, not reported; ORR, objective response rate; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PL, placebo.

such as KEYNOTE-059 (phase II, later-line), KEYNOTE-061 (phase III, later-line), and KEYNOTE-062 (phase III, first-line), which yielded mixed results[54-56]. A subsequent integrated analysis of these three studies demonstrated consistent improvements toward more favourable clinical outcomes with pembrolizumab across lines of therapy in patients with a PD-L1 CPS ≥ 10[57]. The phase III KEYNOTE-811 trial investigated the addition of pembrolizumab to first-line trastuzumab plus chemotherapy for patients with HER2-positive, locally advanced or recurrent gastric cancer. The addition of pembrolizumab significantly improved the median PFS in patients with a PD-L1 CPS ≥ 1 compared to placebo (10.8 months *vs.* 7.2 months, HR = 0.70, 95% CI: 0.58–0.85) but not for patients with a PD-L1 CPS < 1 (9.5 months *vs.* 9.6 months, HR = 1.17, 95% CI: 0.73–1.89)[58]. More recently, the double-blind, placebo-controlled, randomized phase III KEYNOTE-859 trial showed that the median OS was longer in the pembrolizumab group than the placebo group among patients with a PD-L1 CPS ≥ 1 (13.0 months *vs.* 11.4 months, $P < 0.0001$) or a PD-L1 CPS ≥ 10 (15.7 months *vs.* 11.8 months, $P < 0.0001$)[34].

Microsatellite instability (MSI)/mismatch repair (MMR)

Mounting evidence suggests that in addition to PD-L1 expression, response to ICIs may also be predicted by other biomarkers, including MSI-high (MSI-H) or defective MMR (dMMR)[12]. In approximately 10% of gastric and GEJ cancer patients, dysregulated expression of MMR genes disrupts cellular repair function during DNA replication, leading to the MSI-H/dMMR phenotype (**Figure 2**)[47]. Testing for MSI-H/dMMR can be based on polymerase chain reaction (PCR) or NGS for MSI, or IHC for MMR[16]. According to the 2024 NCCN guidelines for gastric cancer, patients in whom ≥ 30% of the MSI markers exhibit instability or ≥ 2 of the 5 National Cancer Institute (NCI) or mononucleotide markers exhibiting instability are considered to be MSI-H[16]. Patients with loss of nuclear expression of ≥ 1 MMR proteins are considered to be dMMR[16].

Several studies have demonstrated that MSI-H/dMMR status can be used as a marker to guide the use of ICIs for advanced gastric cancer patients. For example, a *post hoc* analysis of data from KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 demonstrated that pembrolizumab or pembrolizumab plus chemotherapy conferred durable antitumor activity compared to chemotherapy alone in patients with MSI-H tumors[59]. A meta-analysis that included 2,545 advanced gastric cancer patients from KEYNOTE-061, KETNOTE062, CheckMate-649, and JAVELIN Gastric 100 (avelumab *vs.* chemotherapy as maintenance treatment) showed that with reference to chemotherapy, the HR for OS benefit with anti-PD-1 treatment was 0.34 (95% CI: 0.21–0.54) for patients with MSI-H tumors and 0.85 (95% CI: 0.71–1.00) for patients with microsatellite stable tumors[60]. Importantly, based on data from 149 patients with MSI-H tumors across several clinical trials, the U.S. Food & Drug Administration (FDA) granted an accelerated approval for pembrolizumab in 2017 to treat patients with advanced MSI-H tumors, regardless of tumor site or histologic features[61]. This finding marked the first approval of a tissue/site-agnostic, biomarker-guided treatment[61]. Currently, pembrolizumab, nivolumab plus ipilimumab, and dostarlimab-gxly have been recommended by the NCCN guidelines for unresectable locally advanced, recurrent, or metastatic gastric cancer patients with MSI-H/dMMR tumors, independent of PD-L1 status[16].

CLDN18.2

The claudin family of proteins are important components of tight junctions[62]. CLDN18.2 is encoded by the *CLDN18* gene located at chromosome 3q22[62]. Under normal physiologic conditions, CLDN18.2 is almost exclusively expressed in differentiated gastric mucosal membrane epithelial cells, although CLDN18.2 may also be expressed in gastric, lung, oesophageal, and pancreatic tumor cells[62,63]. In normal tissues, CLDN18.2 regulates permeability to the Na^+ and H^+ in gastric acid by maintaining the barrier function of the gastric mucosa (**Figure 2**)[62,63]. Importantly, CLDN18.2 is retained and becomes more exposed when tight junctions are disrupted upon malignant transformation of gastric epithelial tissue, such CLDN18.2 can serve as a potential target for antibodies and other targeted therapies[12,63]. The role of CLDN18.2 in gastric cancer development and progression remains elusive, with both downregulation and overexpression being reported in varying proportions of patients across studies[64]. For example, Sanada et al.[65] reported that downregulation of CLDN18.2 was observed in 57.5% of gastric cancer and correlated with poorer survival in advanced gastric cancer. Other studies have reported overexpression of CLDN18.2 in gastric cancer, ranging from 29.4%–87% of patients[63]. CLDN18.2 expression may be associated with multiple factors, including cancer stage and subtype[63,64].

The only approved compendium diagnostic assay for CLDN18.2 IHC is the VENTANA CLDN18 (43-14A; Ventana

Medical Systems, Inc./Roche Diagnostics, Oro Valley, Arizona, USA), although other IHC methods have also been used in previous studies[63]. Previous studies have used different scoring methods, such as the immunoreactivity and H-scores, and a wide range of cut-off values[63]. Two recent pivotal phase III trials (SPOTLIGHT and GLOW) defined IHC-positivity as moderate-to-strong CLDN18.2 membrane staining in at least 75% of tumor cells[35,36]. A more detailed discussion of detection methods and cut-off values for CLDN18.2-positivity is provided by Mathias-Machado et al.[63].

Zolbetuximab is a monoclonal antibody targeting CLDN18.2[35]. The global, randomized, double-blind, placebo-controlled phase III SPOTLIGHT trial determined the efficacy and safety of zolbetuximab plus the modified folinic acid (or levofolinate), fluorouracil, and oxaliplatin regimen (mFOLFOX6) *vs.* placebo plus mFOLFOX6 in CLDN18.2-positive, HER2-negative, advanced gastric or GEJ adenocarcinoma in the first-line setting[35]. Zolbetuximab treatment achieved a significant reduction in the risk of disease progression or death compared to placebo (HR = 0.75, 95% CI: 0.60–0.94, $P = 0.0066$)[35]. Another global, randomized, double-blind phase III trial (GLOW) compared zolbetuximab plus capecitabine and oxaliplatin (CAPOX) *vs.* placebo plus CAPOX in a similar patient population as SPOTLIGHT and concluded that zolbetuximab significantly improved the PFS (median: 8.21 months *vs.* 6.8 months; HR = 0.687, 95% CI: 0.544–0.866, $P = 0.0007$) and OS (median: 14.39 months *vs.* 12.16 months; HR = 0.771, 95% CI: 0.615–0.965, $P = 0.0118$) compared to placebo[36]. The Japan Pharmaceuticals and Medical Devices Agency approved zolbetuximab for treating CLDN18.2-positive, unresectable advanced or recurrent gastric cancer in March 2024[17]. The FDA approved zolbetuximab with chemotherapy for treating gastric or GEJ adenocarcinoma in October 2024, together with approval for the companion diagnostic test using the VENTANA CLDN18 (43-14A) RxDx assay[18]. Other anti-CLDN18.2 agents, such as osemitamab and SOT102, are also under development[63]. **Figure 2** summarizes all biomarkers reviewed herein.

Exploratory biomarkers in gastric cancer

MET

MET is a receptor tyrosine kinase with hepatocyte growth factor (HGF) as the ligand. HGF/MET signaling activates downstream PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathways and contributes to important processes, including embryogenesis, tissue regeneration, survival, and migration (**Figure 2**)[8,66,67]. However, mounting evidence suggests that MET also participates in tumor proliferation, invasion, and metastasis in multiple cancer types[66,68]. The most common types of MET alterations in gastric cancer are protein overexpression (39%–60%) and gene amplification (4%–7%)[69-72]. These alterations are adverse prognostic factors for gastric cancer. A meta-analysis that included 2,258 gastric cancer patients from 14 studies showed that compared to patients without MET overexpression or *MET* amplification, the HR for mortality was 2.42 (95% CI: 1.66–3.54) for patients with MET overexpression and 2.82 (95% CI: 1.86–4.27) for patients with *MET* amplification[73].

MET overexpression is determined using IHC, while *MET* amplification can be assessed using either fluorescence ISH (FISH) or NGS. However, despite numerous past and ongoing studies on anti-MET therapies in gastric cancer, the lack of established thresholds for predicting responsiveness to targeted therapies remains a problem for MET overexpression and *MET* amplification[67]. Neither the definitions of MET overexpression IHC scores nor the cut-off values have been standardized, as highlighted by Peng et al.[67]. The same is true for *MET* amplification, with varying criteria, such as MET/centromere 7 ≥ 2.0 or ≥ 2.2, being used in different studies[72].

Savolitinib, an anti-MET tyrosine kinase inhibitor (TKI), has demonstrated favourable efficacy and safety in advanced gastric cancer patients with *MET* amplification in several phase II trials[37,74]. For example, savolitinib monotherapy achieved a 50% objective response rate (ORR) among 20 *MET*-amplified patients in the VIKTORY umbrella trial, which was the highest across the different biomarker-specific treatment arms in the umbrella trial. For patients with a *MET* gene copy number > 10, an even higher ORR of 70% was achieved[37]. The Chinese National Medical Products Administration granted a breakthrough therapy designation in August 2023 for savolitinib in patients with *MET*-amplified locally advanced or metastatic gastric or GEJ cancer who failed at least two lines of standard therapies[75]. In the ongoing phase II NCT04923932 trial of savolitinib in *MET*-amplified locally advanced/metastatic gastric or GEJ cancer, pre-specified interim analyses demonstrated a confirmed ORR by independent review committee of 45%, which reached 50% in the 16 patients with high *MET* gene copy number[74]. In addition to savolitinib, other anti-MET

therapies, such as rilotumumab, are also being studied in advanced gastric cancer[72].

FGFR2

FGFR2 is a transmembrane tyrosine kinase receptor encoded by the *FGFR2* gene located on chromosome 10q26[76]. The FGFR family of proteins drive downstream pathways, including the Janus kinase/signal transducer and activator of transcription/interferon regulatory factor (JAK/STAT/IRF), PI3K/mTOR/AKT, and RAS/RAF/MEK/ERK pathways. These pathways regulate important processes, such as cell survival, differentiation, and proliferation; dysregulation is associated with tumorigenesis and cancer progression[6,12,77] (**Figure 2**). As such, the FGFR pathway has emerged as a potential treatment target in several cancers[77]. Of note, *FGFR2* amplification is the most common type of FGFR gene aberration and has been associated with gastric cancer, especially the diffuse subtype[77]. Depending on the testing methods used and the study population, the proportion of gastric cancer patients with *FGFR2* amplification ranges from 2%–9%[77]. Meta-analyses have shown that patients with *FGFR2* amplification or FGFR2 overexpression have significantly worse survival than patients without *FGFR2* amplification or FGFR2 overexpression (amplification: HR = 2.09, 95% CI: 1.68–2.59, $P < 0.00001$; overexpression: HR = 1.4, 95% CI: 1.25–1.58, $P < 0.00001$)[76].

ISH and NGS can be used to detect *FGFR2* amplification and IHC can be used to detect FGFR2 overexpression[12]. Due to the low frequency of *FGFR2* amplification and the time and expense involved for genetic testing, it may be more efficient in clinical practice to stratify gastric cancer patients who may benefit from anti-FGFR2 therapies based on IHC[12]. *FGFR2* amplification and FGFR2 overexpression have been used as inclusion criteria in clinical trials of anti-FGFR2 therapies, with varying definitions and cut-off values[77]. For example, the FIGHT trial for bemarituzumab, a monoclonal antibody against FGFR2b, enrolled patients with FGFR2b overexpression (defined as IHC2+/3+) or *FGFR2* amplification [by circulating tumor DNA (ctDNA)][38], while the earlier SHINE trial for AZD4547, an FGFR1/2/3 TKI, included gastric cancer patients displaying *FGFR2* polysomy or amplification (defined as an *FGFR2*/centromere 10 ratio ≥ 2 or *FGFR2* gene clusters in ≥ 10% of tumor cells)[78].

Bemarituzumab is a first-in-class monoclonal antibody against FGFR2b[38]. In the exploratory phase II FIGHT trial enrolling FGFR2b-selected gastric or GEJ adenocarcinoma patients who had not been treated with FGF-FGFR pathway inhibitors, the 77 patients treated with bemarituzumab had a numerically longer median PFS compared to the 78 patients treated with matched placebo plus mFOLFOX6 (9.5 months *vs.* 7.4 months, HR = 0.68, 95% CI: 0.44–1.04, $P = 0.073$)[38]. Although this result was not statistically significant, a confirmatory phase III trial (FORTITUDE-101, NCT05052801) with sufficient statistical power is currently underway[79].

HER2-low

While past studies mainly focused on HER2-positive (IHC3+ or IHC2+/ISH+, also known as HER2-high) patients, a significant proportion of gastric cancer patients are HER2-low (IHC1+ or IHC2+/ISH-) and exhibit distinct clinicopathologic features compared to HER2-negative and -positive patients[80,81]. A retrospective analysis of the DESTINY-Gastric01 trial reported an estimated HER2-low prevalence of 28.3%, ranging from 19.1%–40.6% across different study centres[82]. Higher proportions (40%–60%) of HER2-low expression have also been reported in other studies[80]. Importantly, among the 45 patients with HER2-low (cohort 1: IHC2+/ISH-, $n = 21$; cohort 2: IHC1+, $n = 24$), locally advanced or metastatic gastric or GEJ adenocarcinoma enrolled in DESTINY-Gastric01, trastuzumab deruxtecan treatment achieved 26.3% and 9.5% confirmed ORRs in cohorts 1 and 2, respectively; 68.4% and 60.0% of patients in cohorts 1 and 2 experienced tumor size reductions, respectively[83]. These results have provided preliminary evidence for the clinical activity of anti-HER2 therapy, even in HER2-low gastric cancer patients, warranting additional randomized controlled trials in larger cohorts to explore the potential benefit of anti-HER2 therapy in this group of patients. On a related note, a recent development in breast cancer has been the recognition by the American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) guidelines of the clinical benefit of trastuzumab deruxtecan as demonstrated in HER2-low metastatic breast cancer patients in DESTINY-Breast04[84]. The 2023 update of the guidelines acknowledges a new indication for trastuzumab in breast cancer patients with HER2 IHC1+ or IHC2+/ISH- and highlight the relevance of distinguishing between HER2 IHC1+ and IHC0[85].

Epstein-Barr virus (EBV)

EBV-positive gastric cancer is a distinct gastric cancer subtype and accounts for approximately 10% of gastric cancer

cases[16,86]. EBV may contribute to gastric cancer through different oncogenic effects. Of particular importance, EBV-positive gastric cancer typically shows increased expression of PD-L1, which is mediated through the JAK/STAT/IRF pathway (**Figure 2**)[8]. The standard detection method for EBV is EBV-encoded RNA (EBER) ISH[86], while other methods, such as NGS and quantitative PCR, are also being explored[87,88]. Patients with EBV-associated gastric cancer tend to display distinct immune characteristics, including elevated PD-L1 expression and heightened expression of immune checkpoint markers, such that patients with EBV-associated gastric cancer are likely to benefit from treatment with ICI[6,47,86]. Importantly, recent data suggest that EBV status may be predictive of treatment responses to immunotherapy. For example, in a prospective phase II clinical trial involving 61 patients with metastatic gastric cancer, pembrolizumab as salvage therapy achieved a 100% ORR among the six patients with EBV-positive tumors, with a median response duration of 8.5 months[89]. A prospective observational study conducted in China enrolled nine patients with EBV-positive gastric carcinoma who were treated with immunotherapy[90]. Three of these patients achieved a partial response, five had stable disease, while the remaining patient with no measurable lesions had decreases in ascites and tumor marker levels[90]. The longest response duration was 18 months at the time of the last follow-up evaluation[90]. In a more recent study, Bai et al.[87] reported that EBV was as effective as dMMR in predicting responses to ICIs. An ORR of 54.5% was achieved among EBV-positive/MRR proficient gastric cancer patients on immunotherapy[87]. While testing for EBV status is not currently recommended for routine clinical care[16], these results have demonstrated the potential of EBV status in the clinical value of immunotherapy.

Tumor-infiltrating lymphocytes (TILs)

TIL is an emerging biomarker that can be assessed in conjunction with PD-L1[6,12]. Increased TIL expression is often identified in EBV-positive and MSI-high gastric cancer patients[6,12]. It has been shown that the TIL profile may help predict treatment responses to immunotherapy. For example, Chen et al.[91] established a multi-dimensional TIL signature based on the presence of $CD4^+FoxP3^-PD\text{-}L1^+$, $CD8^+PD\text{-}1^-LAG3^-$, and $CD68^+STING^+$ cells and the spatial organisation of $CD8^+PD\text{-}1^+LAG3^-$ T cells, and reported that the TIL signature was associated with treatment responses to anti-PD-1/PD-L1 treatment and patient survival. A recent *post-hoc* analysis of the CLASSIC trial showed that TIL status also predicted the benefit of adjuvant chemotherapy[92]. Stage II-III gastric cancer patients with low TIL density achieved longer disease-free survival if treated with adjuvant chemotherapy in addition to surgery compared to surgery alone[92]. Therefore, TIL may be a valuable biomarker for future research. TIL therapy, an adoptive therapy based on TIL isolated from resected tumor specimens, is currently being explored[6].

Other novel biomarkers

Apart from the abovementioned biomarkers, several other novel biomarkers have emerged as potential targets for the development of new targeted therapies for gastric cancer, including dickkopf-1 (DKK1), V-domain immunoglobulin-containing suppressor of T-cell activation (VISTA), B7-H3, and aquaporin-5 (**Table 2**)[94,97,98,103]. DKK1, a secretory protein first identified as a head inducer during embryogenesis[105], can bind to low-density lipoprotein receptor-related protein 5/6 and Frizzled to inhibit Wnt/β-catenin signaling, which is an essential pathway involved in cell proliferation, migration, and death[8]. DKK1 has also been found to promote epithelial-to-mesenchymal transition and cisplatin resistance in gastric cancer by activating the phosohatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway[93]. Additionally, DKK1 promotes tumor immune invasion and hinders anti-PD-1 therapy by inducing immunosuppressive macrophages in gastric cancer[94]. DKN-01 is a DKK-1 neutralizing antibody. The combination of DKN-01 with pemprolizumab was reported to be well-tolerated in a previous phase Ib study[95]. In the recent phase IIa open-label trial, DKN-01 in combination with chemotherapy and tislelizumab was also well-tolerated in patients with advanced gastroesophageal adenocarcinomas[96]. A randomized phase II trial of DKN-01 is currently ongoing[96]. VISTA is expressed on several types of immune cells, such as macrophages and dendritic cells, and VISTA-induced T-cell activation is non-redundant from the PD-1/PD-L1 pathway, making VISTA a promising target for immunotherapy[98]. A phase I/II trial of a novel anti-VISTA monoclonal antibody is currently ongoing[99]. The discovery of novel biomarkers will reveal more opportunities to advance care for gastric cancer patients.

Table 2 Summary of selected novel biomarkers for gastric cancer

Biomarkers	Key preclinical evidence	Targeted therapy in clinical trials
DKK1	– Promotes epithelial-to-mesenchymal transition and contributes to cisplatin resistance[93] – Promotes tumor immune invasion and impede anti-PD-1 treatment[94] – DKK1 blockade reduced the growth of human gastric cancer tumors with high DKK1 expression in a xenograft model[94]	DKN-01 – Well-tolerated in phase Ib and IIa trials[95,96] – Phase II trial ongoing (NCT04363801)[96]
VISTA	– Expression associated with PD-L1 expression[97] – Predominantly expressed on tumor-associated macrophages in gastric cancer[98] – VISTA blockade promoted T cell-medicated antitumor immunity and enhanced the efficacy of PD-1 inhibitor in *ex vivo* tumor inhibition assay[98]	SNS-101 – Phase I/II study ongoing (NCT05864144)[99]
B7-H3	– High level associated with low intra-tumoral $CD8^+$ T cell density[100] – Promotes stemness characteristics to gastric cancer cells by promoting glutathione metabolism[101] – B7-H3-directed CAR-T cells showed anti-tumor effect in xenograft model[102]	None identified
Aquaporin-5	– Specifically highly expressed by gastric cancer stem cells[103] – Coordinates with LGR5 to determine the fates of gastric cancer stem cells[103] – Overexpression associated with lymph node metastasis[104]	None identified

CAR-T cells, chimeric antigen receptor T cells; DKK1, dickkopf-1; LGR5, leucine-rich repeat-containing G protein-coupled receptor 5; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; VISTA, V-domain immunoglobulin-containing suppressor of T-cell activation.

Existing challenges and future directions of biomarker testing and biomarker-guided treatment

Sampling method: tissue *vs.* liquid biopsy

Despite being the gold standard for gastric cancer testing and diagnosis, tissue biopsy has several notable drawbacks, including invasiveness, pain, and cost[19,20]. Additionally, a single tissue biopsy is typically insufficient to capture tumor heterogeneity, thus limiting the information tissue biopsy can provide (**Figure 3**)[19,20].

Liquid biopsy as a non-invasive and inexpensive alternative

Liquid biopsies have emerged as a novel, non-invasive sampling method in recent years[106]. Originally, liquid biopsy referred to the investigation of circulating tumor cells (CTCs) in the blood[106], but liquid biopsy has since expanded to include analysis of ctDNA, circulating free DNA (cfDNA), non-coding RNAs, exosomes, and microRNAs[19,20,106]. In addition to being non-invasive and inexpensive, liquid biopsies can reveal spatial and temporal tumor heterogeneity and can be performed repeatedly to monitor treatment response and disease recurrence over time[107,108]. Although not routinely used in gastric cancer testing and monitoring at this time[12], liquid biopsy is increasingly utilised in patients with advanced diseases, especially those who are unfit for conventional tissue biopsy[16].

Several studies have underscored the potential of liquid biopsy in detecting molecular markers and enhancing testing efficiency. For example, Willis et al.[109] reported that cfDNA-based MSI testing had an overall accuracy of 98.4% with a positive predictive value of 95%. Nakamura et al.[110] compared trial enrollment in the SCRUM-Japan GOZILA study utilizing ctDNA sequencing *vs.* the GI-SCREEN study utilizing tissue sequencing. Nakamura et al.[110] found that in patients with gastrointestinal cancer ctDNA genotyping significantly reduced screening duration (11 d *vs.* 33 d, $P < 0.0001$) and improved trial enrollment rate (9.5% *vs.* 4.1%, $P < 0.0001$) without compromising treatment efficacy compared to tissue genotyping[110]. Nevertheless, diagnostic testing based on liquid biopsy is far from optimized and challenges, such as low sensitivity, lack of standardized operational procedures, and limited clinical validations need to be addressed in future studies[20]. In addition to liquid biopsy, non-invasive techniques utilizing novel urinary and fecal metabolic biomarkers for gastric cancer are also under exploration[5]. These sampling methods can procure large quantities of samples and do not require specialized personnel[5], making the sampling methods potentially valuable for mass population screening.

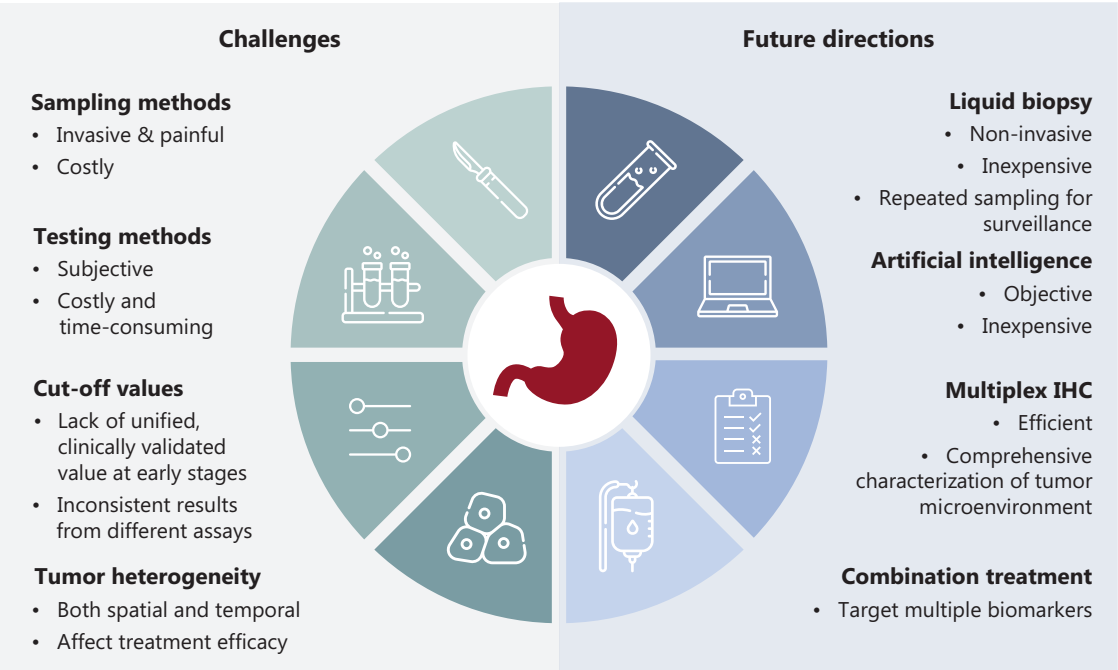

Figure 3 Challenges and future directions of biomarker testing and biomarker-guided treatment. Molecular testing and targeted therapies for gastric cancer face several important challenges: 1) Traditional tissue biopsy is invasive and costly. 2) Essential testing methods may be limited by inter-observer variability (such as for IHC) or high cost and long turnaround time (such as for NGS). 3) Lack of unified, clinically validated cut-off values may hinder the effective application of some biomarkers. 4) Tumor heterogeneity, both spatial and temporal, may affect the efficacy of biomarker-guided treatment. Novel testing techniques and treatment strategies may help overcome some of these challenges: 1) Lipid biopsy offers a non-invasive alternative to tissue biopsy. 2) AI may be employed to improve image analysis and minimize subjectivity. 3) Multiplex IHC allows the simultaneous detection of multiple biomarkers, providing a more comprehensive tumor profile. 4) Ongoing clinical trials are also exploring combination treatment to address heterogeneity and treatment resistance. AI, artificial intelligence; IHC, immunohistochemistry; NGS, next-generation sequencing.

Testing methods and diagnostic cut-off values

The success of biomarker-guided treatment hinges heavily on accurately identifying patients with specific biomarker expression[111]. Currently recommended testing methods include IHC, ISH, PCR, and NGS[16]. According to the NCCN guidelines, IHC, ISH, and targeted PCR should be considered first for identification of biomarkers before NGS, while comprehensive genomic profiling *via* a validated NGS assay should be considered if limited tissue is available or if the patient is unable to undergo a traditional biopsy, such that sequential testing of single biomarkers and limited diagnostic panels will exhaust the sample[16]. However, significant challenges persist in both the testing methods and determination of cut-off values for biomarker expression. For example, IHC is commonly used to assess protein overexpression based on a semiquantitative scoring system (0, 1+, 2+, and 3+)[21], but the definitions of values in this four-point scale may vary across studies and need to be further specified[67]. Even when scores are well-defined, the semiquantitative method implicates subjective judgment, which can lead to substantial inter- and intra-observer variability and often requires well-trained pathologists with years of experience[21]. Apart from the inherent accuracy and reliability of testing methods, the accessibility and timeliness of such molecular testing techniques may also limit utility.

Moreover, the cut-off values for biomarker positivity by IHC and/or genetic testing, such as FISH, NGS, and PCR, need to be clinically validated and often take time to evolve. Using the established biomarker, HER2, as an example, earlier studies, such as the ToGA trial (published in 2010) defined HER2-positivity as IHC3+ or an *HER2*:*CEP17* ratio of ≥ 2 by FISH[31], whereas more recent studies, such as DESTINY-Gastric01 and DESTINY-Gastric02, and current guidelines have defined HER2-positivity as IHC3+ or IHC2+/ISH+[16,29]. Similarly, for PD-L1, although

the NCCN guidelines for gastric cancer recommend the use of CPS, the tumor proportion score (TPS) is also commonly used in research and/or clinical practice, especially in non-small cell lung cancer[112,113]. Additionally, the use of different assays for the same biomarker construct may yield disparate results[113]. Not surprisingly, this lack of unified, clinically validated cut-off values is even more pronounced for emerging biomarkers. Peng et al.[67] provided an extensive discussion of the varying definitions of MET overexpression by IHC used in clinical studies over the years, highlighting the ongoing research for an optimal, unified threshold. This lack of unified cut-off values may lead to inconsistent results across different studies and render the evidence generated ineffective for informing biomarker-guided treatment decisions. Furthermore, given the high molecular heterogeneity and complex immunologic profiles of gastric cancer[39], the expression of a single biomarker may be inadequate to guide treatment decisions.

Artificial intelligence (AI) to assist image analysis

In response to the challenges in testing methods and diagnostic cut-off values, researchers are actively seeking solutions. Indeed, investigations into the potential applications of the fast-developing AI technologies have gained momentum. For example, Bencze et al.[21] conducted a study comparing traditional semiquantitative scoring *vs.* AI-aided image analysis of IHC. The findings showed that AI-aided software, following appropriate training, can accurately identify cells of interest, distinguish among organelles, and recognize protein-specific chromogenic labelling and nuclear counterstaining, which potentially provides a more accurate alternative to semi-quantitative scoring[21]. Kapil et al.[114] also described an image analysis-based method for quantitative continuous scoring (QCS) of digital whole-slide images acquired from baseline HER2 IHC-stained breast cancer tissue. QCS-based patient stratification predicted patient responses to trastuzumab deruxtecan better than manual scoring[114]. Several groups of researchers have used machine learning techniques to assess MSI and/or EBV status, which yielded promising results[115-120]. For example, Su et al.[115] utilized a deep learning system to recognise MSI status based on hematoxylin-eosin staining whole-slide images, achieving patient-level accuracy rates of 86.36% in annotated slides and 83.87% in slides with no tumor contour annotation. Jiang et al.[116] developed a deep-learning radiomics model based on preoperative abdominal dynamic contrast-enhanced computer tomography to non-invasively evaluate MSI status. Notably, both examples are inexpensive and do not require additional wet lab tissue testing. While further clinical validations are essential to ascertain the independent diagnostic efficacy of these novel techniques, the novel techniques are undoubtedly valuable as confirmation tests or as preliminary screening tools before a confirmatory molecular test.

Multiplex IHC (MIHC) to capture immunologic profiles

Another noteworthy advance in gastric cancer testing and diagnosis is the utilization of MIHC to simultaneously detect multiple antigens[121-123]. This approach enables a more comprehensive characterization of tumor features, including the immunologic profiles of the tumor immune microenvironment. For example, Jia et al.[121] used MIHC on CD4/CD8/CD20/CD66b/CD68/CD163/PD-1/PD-L1/TIM-3/LAG-3/FoxP3/CTLA-4/HLA-DR/STING and CLDN18.2 to decipher the spatial distribution of immune cells in CLDN18.2-positive gastric cancer, and found that the proportions of $CD8^+PD\text{-}L1^-$, $CD8^+LAG3^-$, and $CD8^+TIM\text{-}3^-$ T cells were significantly elevated in CLDN18.2-positive tumors compared to CLDN18.2-negative tumors. Similarly, the abovementioned study on multidimensional TIL signatures conducted by Chen et al.[91] also employed MIHC. Such insights into the tumor immune microenvironment may offer valuable information to tailor specific treatments for gastric cancer patients in the future.

Tumor heterogeneity and treatment resistance

Tumor heterogeneity is one of the most fundamental features of malignancies[124]. Past studies have underscored the significant spatial and temporal heterogeneity observed in gastric cancer[39,124,125]. For example, in the phase II expansion-platform trial PANGEA, 49% of patients experienced a shift to a different biomarker group from baseline upon progression after first-line treatment, with an additional 48% undergoing a change in the assigned treatment group after second-line treatment[126]. This extensive tumor heterogeneity may have contributed to treatment resistance and failures in biomarker-guided clinical trials[39,125], and needs to be addressed to improve the prognosis of patients with gastric cancer.

Combination treatment to improve treatment efficacy

In recent years combination therapies targeting multiple biomarkers have been increasingly explored[30,41,58,127]. As

mentioned above, the randomized, double-blind, phase III trial KEYNOTE-811 explored adding pembrolizumab to first-line trastuzumab plus chemotherapy in patients with HER2-postive, advanced gastric or GEJ[30,58]. To date, protocol-specified interim analyses of KEYNOTE-811 have shown that compared with placebo, pembrolizumab significantly improved PFS when combined with first-line trastuzumab and chemotherapy for metastatic HER2-positive gastric or GEJ patients, specifically in patients with a PD-L1 CPS score ≥ 1[30,58]. This combination has already been recommended in the NCCN guidelines[16].

Investigations of other combination therapies are currently underway. For example, the ongoing phase III HERIZON-GEA-1 trial is investigating zanidatamab plus chemotherapy with or without tislelizumab in first-line HER2+ advanced/metastatic gastroesophageal adenocarcinoma[42]. A phase II study investigating the combination of ceralasertib (a Rad3-related protein kinase inhibitor) and durvalumab (an anti-PD-L1 antibody) in previously treated advanced gastric cancer patients showed promising antitumor activity, warranting further confirmation in biomarker-driven trials[127]. A phase II trial assessing savolitinib in combination with durvalumab in *MET*-amplified advanced gastric cancer patients who failed primary chemotherapy is currently ongoing[128].

Conclusions

Advances in molecular biomarkers and molecular-guided therapies have transformed the treatment landscape of cancers in the past decades. Gastric cancer biomarkers, such as HER2, PD-L1, and MSI have become integral to guiding targeted therapies and CLDN18.2-guided treatment has been approved in some regions. Explorative markers, such as MET and FGFR2, hold great promise for future clinical applications with several targeted therapies under development. These biomarkers and targeted therapies are invaluable additions to our armamentarium against gastric cancer and significantly expand treatment options. However, challenges remain in the accurate detection and consistent application of these biomarkers across diverse patient populations and clinical settings. For example, PD-L1 testing can yield variable results depending on the testing platform and tumor context, and MSI testing, though helpful, is not universally applicable. Other factors that could influence the application of biomarker testing also include sample quality and availability, test accessibility and cost, pathologist technical expertise, as well as regulatory and guideline variability. All of these factors must be addressed to enhance the clinical application of molecular testing.

As mentioned in this review, researchers are exploring techniques, such as liquid biopsy, AI-aided image analysis, and MIHC to better identify patients with specific biomarker profiles. These efforts may help to mitigate some of the existing challenges, such as the need for non-invasive sampling and standardized image reading, and facilitate greater use of precision medicine with targeted therapies. Additionally, as targeted therapies evolve, the combination of targeted therapies with immunotherapy and chemotherapy may offer a more comprehensive treatment approach for patients with gastric cancer. Personalized combinations of these therapies could enhance treatment efficacy, minimize resistance, and ultimately improve patient survival. However, much work remains to be done in refining these strategies and addressing their potential side effects and toxicities. On a related note, given the high prevalence and late diagnosis of gastric cancer worldwide, it is also important to improve the diagnosis of gastric cancer through early screening.

In conclusion, while substantial progress has been made in the molecular testing and treatment of gastric cancer, ongoing research is essential to fully realize the potential of these biomarkers and therapies. By continuing to improve diagnostic precision and therapeutic options, the future of gastric cancer treatment will likely be more individualized and effective, driving the field toward precision medicine.

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