

Review

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# Predictive biomarkers for immunotherapy in gastric cancer

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## Abstract

Gastric cancer remains a significant global health burden, and while immunotherapy offers promising therapeutic avenues, its efficacy varies greatly among patients. The key challenge is accurately identifying treatment responders, while alternative strategies are necessary for non-responders. Biomarkers such as PD-L1 expression, tumor mutational burden, mismatch repair status, and Epstein-Barr virus infection have shown predictive potential, yet the quest for more reliable markers continues to be challenging. Emerging technologies, including liquid biopsy, single-cell sequencing, and artificial intelligence, present novel approaches to enhancing individualized research and improving predictive capabilities. This review provides a comprehensive analysis of current biomarkers and introduces emerging candidates from recent studies, thereby contributing to the ongoing efforts to refine patient stratification and treatment strategies.

**Keywords:** Gastric Cancer, immunotherapy, immune checkpoint inhibitor, predictive biomarker



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## INTRODUCTION

Gastric cancer (GC) ranks as the fifth most prevalent malignancy and the fourth leading cause of cancer-related mortality worldwide, posing a significant global health burden<sup>[1]</sup>. Historically, treatment options for GC were limited, with chemotherapy serving as the mainstay despite offering only modest survival benefits<sup>[2]</sup>. However, the advent of immunotherapy has revolutionized the therapeutic landscape for advanced GC, demonstrating remarkable antitumor efficacy and offering new hope for patients. Current immunotherapeutic strategies for advanced GC include immune checkpoint inhibitors (ICIs), adoptive cell transfer, cancer vaccines, and chimeric antigen receptor (CAR) T-cell therapy<sup>[3]</sup>.

Comprehensive genomic analyses have revealed that GC is a highly heterogeneous disease, comprising distinct subtypes, each characterized by unique molecular profiles. While these molecular subtypes have offered some insights into treatment strategies and prognostication for GC, there remains a critical need for more robust predictive biomarkers, particularly in identifying populations that are more likely to benefit from immunotherapy. Currently, traditional biomarkers such as programmed death ligand-1 (PD-L1) expression, tumor mutational burden (TMB), Epstein-Barr virus (EBV) infection status, and mismatch repair (MMR) deficiency are widely used to predict immunotherapy response. However, their predictive power is often limited. Emerging technologies such as liquid biopsy, single-cell sequencing, deep neural networks, and machine learning present novel tools for advancing individualized research.

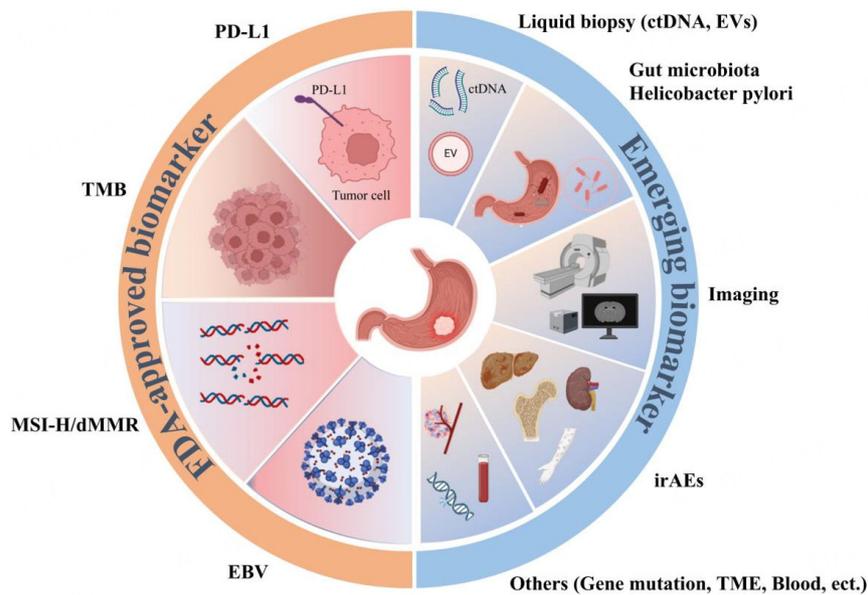
In this review, we provide a comprehensive overview of current and emerging predictive biomarkers for immunotherapy in GC [Figure 1]. We also discuss their clinical implications, limitations, and future directions, with the aim of guiding patient stratification and advancing the field of precision medicine in GC treatment.

## FDA-APPROVED BIOMARKERS

### PD-L1

Programmed death-1 (PD-1) is a negative co-stimulatory receptor predominantly expressed on activated T cells, functioning to attenuate excessive immune responses through its interaction with ligands, PD-L1, and programmed death ligand-2 (PD-L2). Upregulation of PD-L1 has been observed in approximately 40% of GC, primarily localized to myeloid cells at the invasive margin<sup>[4]</sup>. Immunohistochemistry (IHC) remained the standard method for assessing PD-L1 protein expression, with the combined positive score (CPS) and tumor proportion score (TPS) being widely utilized metrics for quantifying PD-L1 expression.

Numerous clinical studies have assessed the value of PD-L1 expression in relation to immunotherapy efficacy. In the third-line treatment, the KEYNOTE-059 trial demonstrated a higher objective response rate (ORR) in GC patients with CPS  $\geq 1$  compared with PD-L1-negative (CPS  $< 1$ ) (15.5% vs. 6.4%)<sup>[5]</sup>. The CheckMate-032 trial confirmed the clinically significant antitumor activity of nivolumab and the combination of nivolumab plus ipilimumab in metastatic GC following failure of second-line chemotherapy. Post hoc exploratory analyses from the trial indicated a trend toward improved efficacy when PD-L1 expression was assessed using CPS rather than TPS, particularly at higher cutoffs of  $\geq 5$  and  $\geq 10$ , in the pooled analysis of all treatment regimens<sup>[6]</sup>. In first-line treatment, the ORIENT-16 trial, which investigated sintilimab combined with chemotherapy for advanced GC in China, reported statistically significant overall survival (OS) benefits with sintilimab plus chemotherapy in participants with CPS  $\geq 5$ , as well as in the overall randomized population<sup>[7]</sup>. Similarly, the CheckMate-649 trial demonstrated that the combination of nivolumab and chemotherapy significantly improved both OS and progression-free survival (PFS) in patients with CPS  $\geq 5$  compared to chemotherapy alone, and improved OS in patients with CPS  $\geq 1$ <sup>[8]</sup>. However, in the KEYNOTE-062 study, the combination of pembrolizumab with 5-FU and



**Figure 1.** Predictive biomarkers of gastric cancer immunotherapy. PD-L1: Programmed cell death-ligand 1; TMB: tumor mutation burden; MSI-H: microsatellite instability-high; dMMR: mismatch repair deficiency; EBV: Epstein-Barr virus; ctDNA: circulating tumor DNA; EVs: extracellular vesicles; irAEs: immune-related adverse events; TME: tumor microenvironment.

cisplatin or capecitabine did not show statistically significant benefits for patients with CPS  $\geq 1$ <sup>[9]</sup>. Relevant studies on the role of PD-L1 as a biomarker in gastric cancer immunotherapy are summarized in [Table 1](#).

From third-line therapy to neoadjuvant therapy, PD-L1 expression has demonstrated predictive value for immunotherapy in numerous studies, as a critical biomarker and a key reference point for clinical decision making in GC. Overall, PD-L1 remains a principal predictor of immunotherapy efficacy, with CPS  $\geq 5$ , and particularly CPS  $\geq 10$ , serving as a more definitive indicator of therapeutic benefit. Beyond GC, antibodies targeting PD-1 or PD-L1 have revolutionized the treatment landscape for other advanced-stage cancers. In non-small cell lung cancer (NSCLC), higher PD-L1 TPS ( $\geq 50\%$ ) correlates with improved outcomes for anti-PD-1 monotherapy, as seen in the KEYNOTE-024 trial<sup>[24]</sup>. In urothelial carcinoma, PD-L1 expression guides second-line ICI use, though its predictive reliability remains debated<sup>[25]</sup>. Similarly, in head and neck squamous cell carcinoma (HNSCC), PD-L1 CPS  $\geq 20$  identifies patients most likely to benefit from pembrolizumab monotherapy<sup>[16]</sup>. In urothelial carcinoma, the IMvigor210 trial linked PD-L1 positivity (IC2/3) to improved response rates with atezolizumab<sup>[26]</sup>, though the IMvigor211 trial failed to confirm OS benefits in PD-L1-high patients, raising questions about its reliability as a standalone biomarker<sup>[27]</sup>. These findings highlight the context-dependent utility of PD-L1 while emphasizing the need for complementary biomarkers to refine predictive accuracy. Therefore, advancing and standardizing diagnostic approaches to identify key immune suppressive mechanisms in individual tumors may pave the way for more effective, patient-tailored therapies.

## TMB

Tumor mutations can produce neoantigens that enable the immune system to recognize and attack tumors. TMB is a biomarker that quantifies the number of mutations in cancer and has been shown to correlate with the efficacy of immunotherapy in GC. In June 2020, the FDA approved pembrolizumab for patients with metastatic TMB-high (TMB-H) solid tumors, including GC, based on results from the KEYNOTE-158 trial<sup>[28]</sup>. An exploratory analysis of the KEYNOTE-061 trial demonstrated a positive association between TMB and clinical outcomes in GC patients treated with pembrolizumab, with those having TMB  $\geq 10$

**Table 1. Clinical trials of the treatment of ICIs in GC**

Line	ICI	Study or clinical trial	Phase	Cancer type	Study design	Number	Outcome	Biomarker	Significant association
Third line	Pembrolizumab	KEYNOTE-012 <sup>[10]</sup>	Ib	Advanced GC	Pembrolizumab alone	39	ORR 22%	TPS; MIDS	-
	Pembrolizumab	KEYNOTE-059 <sup>[5]</sup>	II	Advanced GC/GEJC	Pembrolizumab alone	259	ORR: 11.6% DOR: 8.4 m OS: 5.6 m PFS: 2.0 m	CPS; MSI status	Higher ORR in CPS $\geq$ 1 patients; higher ORR in MSI-H/dMMR patients
	Nivolumab $\pm$ ipilimumab	CheckMate-032 <sup>[11]</sup>	II	Metastatic GC/EC/GEJC	Group 1: Nivolumab alone Group 2: Nivolumab 1 mg/kg + ipilimumab 3 mg/kg Group 3: Nivolumab 3 mg/kg + ipilimumab 1 mg/kg	160	ORR: 12% vs. 24% vs. 8% 12-month OS rate: 39% vs. 35% vs. 24%	CPS	Longer OS in CPS $\geq$ 5 patients
	Nivolumab	ATTRACTION-02 <sup>[12]</sup>	III	Metastatic GC/GEJC	Nivolumab vs. placebo	493	OS: 5.26 vs. 4.14 m year OS rate: 26.2% vs. 10.9% year OS rate: 10.6% vs. 3.2%	TPS	-
	Avelumab	JAVELIN Gastric 300 <sup>[13]</sup>	III	Advanced GC/GEJC	Avelumab vs. chemotherapy	371	Negative results	TPS	-
Second line	Pembrolizumab	KEYNOTE-061 <sup>[14]</sup>	III	Advanced GC/GEJC	Pembrolizumab vs. paclitaxel	592	Negative results	CPS; TMB	Higher 24-month OS rate in patients with CPS $\geq$ 5 and $\geq$ 10; longer OS in patients with TMB $\geq$ 10
First line	Sintilimab	ORIENT 16 <sup>[15]</sup>	III	Advanced GC/GEJC	Sintilimab + XELOX vs. placebo + XELOX	650	OS: 15.2 vs. 12.3 m	CPS	Longer OS in all patients and in patients with CPS $\geq$ 5
	Nivolumab $\pm$ ipilimumab	CheckMate-649 <sup>[11]</sup>	III	Non-HER2-positive metastatic GC/GEJC	Nivolumab + ipilimumab vs. nivolumab + chemotherapy vs. chemotherapy	1,581	OS: 11.2 vs. 14.1 vs. 11.1 m	CPS	OS interaction analysis by PD-L1 CPS cutoffs: significant at CPS $\geq$ 5, not at CPS $\geq$ 1
	Pembrolizumab	KEYNOTE-062 <sup>[16]</sup>	III	Metastatic GC/GEJC	Pembrolizumab vs. pembrolizumab + chemotherapy vs. chemotherapy + placebo	763	No statistically significant benefit in OS and PFS	CPS; MSI status	Pembrolizumab monotherapy is effective in gastric cancer with PD-L1 CPS $\geq$ 10 and MSI-high
	Nivolumab	ATTRACTION-4 <sup>[17]</sup>	II	HER2-negative metastatic GC/GEJC	Nivolumab + SOX vs. nivolumab + CapeOX	724	ORR: 57.1% vs. 76.5% PFS: 9.7 vs. 10.6 m	TPS	-
	Pembrolizumab	KEYNOTE-659 <sup>[18]</sup>	IIb	CPS $\geq$ 1 and HER2-negative advanced GC/GEJC	Cohort 1: pembrolizumab + SOX cohort 2: pembrolizumab + SP	100	Cohort 1 and cohort 2: ORR: 72.2% and 80.4% DOR: 10.6 and 9.5 m DCR: 96.3% and 97.8%	CPS	-

	Pembrolizumab	KEYNOTE-859 <sup>[19]</sup>	III	Locally advanced or metastatic HER2-negative GC/GEJC	Pembrolizumab + chemotherapy vs. placebo + chemotherapy	1,579	PFS: 9.4 and 8.3 m OS: 16.9 and 17.1 m OS: 12.9 vs. 11.5 m	CPS	Longer OS in CPS ≥ 1 and CPS ≥ 10 patients (13.0 and 15.7 m); minimal OS benefit and no PFS benefit in CPS < 1 patients
	Pembrolizumab	KEYNOTE-811 <sup>[20]</sup>	III	HER2-positive advanced GC	Pembrolizumab + trastuzumab + chemotherapy vs. placebo + trastuzumab + chemotherapy	698	PFS: 20.0 vs. 16.8 m	CPS	Minimal benefit in CPS < 1 patients
Neoadjuvant therapy	Camrelizumab	NCT03878472 <sup>[21]</sup>	II	cT4a/bN+ GC	Camrelizumab + apatinib + chemotherapy (S-1 ± oxaliplatin)	25	PCR: 15.8% MPR: 26.3%	MSI status; CPS; TMB	Pathological responses correlate significantly with MSI status, PD-L1 expression, and TMB
	Camrelizumab	Neo-PLANET <sup>[22]</sup>	II	Locally advanced GC/GEJC	Camrelizumab + chemoradiotherapy	36	PCR: 33.3% MPR: 44.4% RO resection rate: 91.7%	CPS	-
	Nivolumab ± relatlimab	NCT03044613 <sup>[23]</sup>	Ib	Resectable EC/GEJC	Arm A: nivolumab arm B: nivolumab + relatlimab	32	Arm A and arm B: PCR: 40.0% and 21.4% MPR: 53.5% and 57.1%	CPS	Not linked to pCR/MPR; trend for pCR in CPS ≥ 5; CPS ≥ 5 associated with longer RFS

CPS: Combined positive score; DOR: duration of response; EC: esophageal cancer; GC: gastric cancer; GEJC: gastroesophageal junction cancer; MIDS: mononuclear inflammatory density score; MPR: major pathological response; MSI: microsatellite instability; ORR: overall response rate; OS: overall survival; PCR: pathological complete response; PD-L1: programmed death ligand-1; PFS: progression-free survival; RFS: recurrence-free survival; TPS: tumor proportion score; TMB: tumor mutation burden.

mutations per megabase (mut/m) showing better OS compared to paclitaxel<sup>[29]</sup>. A clinical trial assessing the safety and efficacy of toripalimab in advanced GC (NCT02915432) showed significant improvement in OS for the TMB-H group compared to the TMB-low group (14.6 vs. 4.0 months, HR = 0.48, 96%CI: 0.24-0.96,  $P = 0.038$ )<sup>[29]</sup>. In the PLANET phase II trial, whole exome sequencing (WES) of treatment samples identified a higher pCR rate in patients with a pretreatment TMB above the median level (4.04 mut/m) compared to those with TMB below the median<sup>[28]</sup>. In clinical practice, TMB has been approved by the FDA as a tumor-agnostic biomarker for pembrolizumab in patients with metastatic TMB-high (TMB-H, ≥ 10 mutations per megabase) solid tumors.

However, the correlation between TMB and ICI responsiveness is not consistently evident. Although TMB has proven to be a valuable predictor of response to immunotherapy, the optimal TMB threshold for GC remains unclear, with considerable variability in the gene panels employed across different studies. The measurement of TMB demands comprehensive genomic profiling, which is costly and technically challenging, thereby restricting its accessibility in settings with limited resources. Two non-genome sequencing assays, MSK-IMPACT<sup>®</sup> and FoundationOne CDx, have been approved by the FDA<sup>[30]</sup>. To improve the predictive utility of TMB in immunotherapy for GC, researchers are now exploring combinations of TMB with other molecular markers, as well as refining the contextual interpretation of TMB<sup>[31]</sup>.

### EBV status

Epstein-Barr virus-associated gastric cancer (EBVaGC) has been identified as a distinct molecular subtype, accounting for approximately 9% of GC. Previous studies have reported a response rate of around 25% to ICIs in EBVaGC patients<sup>[32,33]</sup>. EBV infection induces an immune-active tumor microenvironment. In addition to cellular neoantigens produced from tumor-specific DNA alterations, EBVaGC expresses foreign viral antigens, which serve as prime targets for T cell responses<sup>[34]</sup>. The presence of these non-self viral antigens is likely a crucial factor influencing the heightened antitumor immune response and altered tumor microenvironment<sup>[35]</sup>. However, the low prevalence of EBVaGC limits its applicability as a broad biomarker for GC immunotherapy. The relationship between EBV positivity and response to immunotherapy remains a subject of ongoing debate. In a prospective phase 2 clinical trial, advanced GC patients treated with pembrolizumab as salvage therapy demonstrated a remarkable ORR of 100% in EBVaGC<sup>[36]</sup>. However, in another study, Sun *et al.* evaluated camrelizumab as salvage therapy in EBVaGC (NCT03755440), and none of the six patients achieved an objective response, leading to the discontinuation of the trial<sup>[37]</sup>. The significant variability in treatment efficacy may stem from several factors, including patient heterogeneity, differences in trial design, tumor microenvironment (TME) diversity, and host immune characteristics. To address these challenges, future studies should focus on optimizing patient selection, trial design, and combination therapy strategies.

Integrating multi-omics analyses to evaluate the tumor microenvironment and genetic profiles of patients could facilitate the development of more precise immunotherapy approaches. Qiu *et al.*, through dynamic single-cell transcriptome sequencing and paired immune repertoire analysis (scTCR/BCR-seq), provided a detailed characterization of the tumor immune microenvironment before and after immunotherapy in EBV-positive and EBV-negative GC. They identified a key ISG-15+CD8+ T-cell subset associated with immunotherapy response, offering a novel therapeutic direction for EBVaGC<sup>[38]</sup>. The mechanisms linking EBV to improved immunotherapy outcomes in GC require further exploration. Future research should focus on understanding the characteristics of the immune microenvironment in EBVaGC and how to leverage insights to enhance immunotherapy efficacy.

### Microsatellite instability status and dMMR

The MMR system is a highly conserved DNA repair mechanism that preserves genomic integrity during replication. Deficient MMR (dMMR) leads to the accumulation of genetic errors in microsatellite sequences, resulting in a microsatellite instability-high (MSI-H) phenotype, which is characterized by genomic instability, elevated somatic mutation rates, enhanced immunogenicity, and distinct responses to treatment and prognosis. MSI-H/dMMR status serves as a strong predictive biomarker for ICI treatment, driven by a high neoantigen load, abundant tumor-infiltrating lymphocytes, and elevated PD-L1 expression. The FDA granted the first tumor-agnostic approval for pembrolizumab in May 2017 for the treatment of MSI-H/dMMR tumors. In the phase III KEYNOTE-062 and KEYNOTE-061 trials, ICI monotherapy consistently demonstrated superior OS compared to chemotherapy from the onset of treatment, with higher ORR in patients with MSI-H/dMMR GC<sup>[9,14]</sup>. The efficacy of ICIs in MSI-H/dMMR GC has also been corroborated by multiple meta-analyses<sup>[39,40]</sup>. MSI-H/dMMR status is emerging as a key molecular hallmark, indicating significant sensitivity to ICI treatment, with ORR ranging from 29% to 60% and disease control rates (DCR) between 48% and 89%<sup>[41]</sup>. Recent studies have reported high responsiveness of dMMR/MSI-H locally advanced GC to immunotherapy, significantly improving the pathological response rate<sup>[42,43]</sup>. However, approximately 20%-50% of MSI-H/dMMR GC patients do not benefit from immunotherapy, highlighting the need for further research into resistance mechanisms. Despite the favorable response of dMMR/MSI-H GC patients to immunotherapy, further research is required to determine the optimal treatment strategy, whether through dual immunotherapy or a combination of chemotherapy and immunotherapy, as no definitive answer currently exists. In summary, MSI-H/dMMR status is widely

recognized as a strong predictor of response to ICIs in GC.

## EMERGING BIOMARKERS

### Liquid biopsy-derived predictive biomarkers

Liquid biopsy is a technique involving the collection and analysis of non-solid biological tissues. The fundamental principle relies on the release of tumor-related substances into the blood or other bodily fluids, allowing for the assessment of these components to detect tumor activity and provide a quantitative analysis of tumor burden<sup>[44,45]</sup>. It is safe, convenient, repeatable, and enables real-time monitoring of treatment response, demonstrating significant potential in personalized cancer therapy and efficacy prediction. Currently, the primary biomarkers used in liquid biopsy studies to predict the efficacy of immunotherapy in GC include circulating tumor DNA (ctDNA) and extracellular vesicles.

Multiple studies have highlighted the dual role of ctDNA in predicting the response to immunotherapy in GC and monitoring treatment efficacy. ctDNA analysis has demonstrated significant potential in stratifying patients who are likely to benefit from immunotherapy. Maron *et al.* conducted ctDNA and tissue molecular profiling on 61 GC patients treated with pembrolizumab, and found that a decrease in ctDNA concentration by the 6th week of continuous monitoring can predict the benefit of immunotherapy<sup>[46]</sup>. Additionally, mutations in genes such as CEBPA, FGFR4, MET, and KMT2B correlated with a higher incidence of immune-related adverse events (irAEs)<sup>[47]</sup>. Furthermore, regulatory factors related to ctDNA methylation were shown to be linked to improved immunotherapy efficacy, highlighting the importance of epigenetic modifications in predicting response to immune checkpoint inhibitors (ICIs)<sup>[48]</sup>. Several studies have highlighted the utility of circulating tumor DNA (ctDNA) in real-time monitoring of treatment response during immunotherapy. A study involving 200 patients with advanced gastric adenocarcinoma utilized next-generation sequencing (NGS) to analyze genomic alterations in ctDNA from blood samples and revealed that dynamic changes in ctDNA levels could serve as a potential biomarker for monitoring treatment efficacy in advanced GC<sup>[49]</sup>. Similarly, another clinical study involving 46 GC patients treated with ICIs showed that a reduction in the maximum variant allele frequency in ctDNA by more than 25% was associated with longer median PFS and higher ORR<sup>[47]</sup>. These findings suggest that ctDNA dynamics during treatment could serve as an early indicator of therapeutic efficacy, potentially guiding timely adjustments to treatment strategies. Several ongoing clinical trials, including NCT05594381, NCT04817826, NCT04484636, and NCT03409848, are investigating the predictive role of ctDNA in the immunotherapy of GC. Future studies should aim to validate these findings in larger, multicenter trials and explore the integration of ctDNA with other biomarkers for comprehensive patient stratification.

Extracellular vesicles (EVs), including exosomes, microvesicles, and apoptotic bodies, are membrane-bound vesicles released by cells that carry proteins, lipids, and nucleic acids, which could be classified as tumor-derived or non-tumor-derived and serve as biomarkers for predicting responses to immunotherapy<sup>[50]</sup>. Exosomes carrying PD-L1 can enhance the immune evasion capabilities of tumor cells, and eliminating circulating exosomes containing PD-L1 may potentially improve the efficacy of ICIs<sup>[51]</sup>. Li *et al.* found that exosomes from M1 macrophages contain miRNA-16-5p, which can downregulate PD-L1 expression in GC cells, thereby enhancing T cell-dependent immune responses<sup>[52]</sup>. The interplay of nicotinamide metabolism between macrophages and fibroblasts modulates the GC microenvironment. By using extracellular vesicles to regulate nicotinamide metabolism, the cytotoxicity of CD8+ T cells and the response to ICIs in GC can be restored<sup>[53]</sup>. Additionally, engineered EVs offer novel strategies for GC treatment by enhancing tumor targeting, improving tumor-killing capabilities, and activating antitumor immune responses. For instance, engineered EVs loaded with siRNA or shRNA have been shown to significantly inhibit cancer growth and improve survival rates in mouse models, leading to the initiation of a phase I clinical trial

(NCT03608631)<sup>[54]</sup>. EVs can also indirectly kill tumors by activating antitumor immune responses. Tumor-derived EVs exhibit tumor antigens on their surface, and they can be engineered to incorporate adjuvants, effectively creating vaccines that initiate antitumor immunity<sup>[55]</sup>. In summary, EVs possess significant potential and promise, presenting more effective and precise therapeutic strategies through mechanisms such as targeted delivery, gene regulation, and immune system activation.

As a biomarker for gauging the efficacy of immunotherapy in GC, liquid biopsy encounters several limitations and challenges. For instance, the short half-life, low abundance, and uneven distribution of ctDNA in peripheral circulation can significantly hinder the reproducibility of liquid biopsy, leading to sensitivity limitations<sup>[45]</sup>. Variability in detection outcomes poses a significant obstacle to its advancement, especially considering the pronounced heterogeneity of GC. Additionally, the lack of standardization in molecular sample collection, preservation, processing, and detection and characterization techniques impacts the specificity of liquid biopsy<sup>[56]</sup>. The current evidence supporting the use of liquid biopsy in GC is of limited quality, primarily due to the small sample sizes in most studies, which are frequently conducted at single centers. For broader clinical applications, liquid biopsy urgently needs validation through extensive, multicenter, and long-term clinical trials. Future research directions in liquid biopsy should focus on developing more sensitive and specific detection methods and establishing standardized protocols for sample collection and analysis.

### **Gut microbiota and helicobacter pylori infection**

The gut microbiome is intricately connected to immune function, with a complex interplay between its various microorganisms and the tumor microenvironment, which is crucial for maintaining immune homeostasis. In recent years, there has been a growing focus on understanding the influence of the microbiome and its metabolites on the cancer immune system and their implications for response to ICIs<sup>[57]</sup>. For instance, research on patients with advanced gastrointestinal cancers undergoing PD-1/PD-L1 therapy revealed that those who responded to immunotherapy exhibited a higher relative abundance of *Prevotella* and *Bacteroides*<sup>[58]</sup>. A higher abundance of *Lactobacillus* may enhance the efficacy of immunotherapy in GC indirectly by fostering a more diverse gut microbiota<sup>[59]</sup>. The secretion of SagA by *Enterococcus faecium* can bind to NOD2, thereby enhancing host immune responses through various pathways and augmenting the antitumor effects of anti-PD-L1 therapy<sup>[60]</sup>. *Bifidobacterium* exerts antitumor effects by inducing dendritic cell maturation, activating IFN- $\alpha$  and IFN- $\beta$  signaling pathways, and stimulating cytotoxic CD8+ T cells<sup>[61]</sup>. Lee *et al.* found that bacterially derived butyrate may reduce PD-L1 expression by modulating signaling pathways such as NF- $\kappa$ B and STAT3, thereby inhibiting tumor immune evasion<sup>[62]</sup>. Gut bacteria-derived short-chain fatty acids (SCFAs) can potentially diminish the anticancer activity of CTLA-4 by inhibiting the accumulation of relevant T cells and reducing IL-2 infiltration<sup>[63]</sup>. This highlights the potential of gut microbiota-derived metabolites as key modulators of immune checkpoint pathways, offering new avenues for therapeutic intervention.

GC is characterized by substantial heterogeneity, with tumors varying in location and molecular subtypes having distinct gut microbiomes. Yang *et al.* have reported distinct microbiome and metabolite profiles in proximal and distal GC<sup>[64]</sup>. In distal GC, the level of *Methylobacterium-methylorubrum* was found to be significantly higher, correlating positively with pro-carcinogenic metabolites and negatively with anti-carcinogenic ones. In contrast, *Rikenellaceae\_Rc\_gut\_group* showed a significant increase in proximal GC, with a positive correlation to pro-carcinogenic metabolites<sup>[64]</sup>. The microbial composition and metabolic profiles of MSI-H gastrointestinal tumors differ markedly between immunotherapy-resistant and non-resistant patients. Four microbial biomarkers - *Bacteroides caccae*, *Veillonella parvula*, *Veillonella atypica*, and *Clostridiales* bacteria - have been identified as predictors of immunotherapy response<sup>[65]</sup>. Future research should investigate the interplay between microbial metabolites and immune modulation in distinct

GC subtypes to identify novel therapeutic targets.

A common infectious microorganism in GC patients is *Helicobacter pylori*, which could alter systemic antitumor immune responses by reducing pro-inflammatory cytokines and enhancing the secretion of anti-inflammatory cytokines<sup>[66]</sup>. Recent retrospective analyses have established a link between *H. pylori* infection and poorer survival outcomes in GC patients treated with ICIs<sup>[58,67]</sup>. However, prospective studies on the prognostic impact of *H. pylori* in immunotherapy of GC are lacking. The specific mechanisms through which *H. pylori* infection affects tumor immunotherapy are not fully understood. *H. pylori*-positive GC exhibits a “hot” tumor microenvironment characterized by a higher density of PD-L1+ cells and non-exhausted CD8+ T cells. Transcriptomic studies indicate that *H. pylori*-positive GC shares molecular characteristics with immunotherapy-responsive GC<sup>[68]</sup>. *H. pylori* and its associated factors are capable of inducing the upregulation of PD-L1 in gastric epithelial cells, thereby disrupting immune homeostasis<sup>[69]</sup>. Additionally, *H. pylori* infection has been implicated in altering the composition of the gastrointestinal microbiota<sup>[70]</sup>. Beyond its impact on immune cells, *H. pylori* may also influence the efficacy of tumor immunotherapy by modulating the gastrointestinal microbiome.

With the growing understanding of the gut microbiome in recent years, several potential microbial interventions for cancer treatment have been proposed, including fecal microbiota transplantation, biotherapy, nanotechnology-based approaches, probiotic or antibiotic treatments, and dietary interventions<sup>[71]</sup>. However, most of these techniques have been applied primarily to solid tumors other than GC. Future research should focus on validating these strategies in GC-specific settings and integrating microbiome modulation with other immunotherapeutic approaches to improve patient outcomes.

### Imaging biomarkers

Imaging biomarkers, by analyzing medical imaging data to extract tumor characteristics such as morphology, texture, and signal intensity, provide new insights into the assessment of immunotherapy efficacy in GC. CT radiomic features have been validated as predictors of immunotherapy response in GC<sup>[72]</sup>. Nuclear medicine molecular imaging techniques, such as <sup>89</sup>Zr-labeled anti-PD-L1 antibodies and anti-CD8 single-domain antibodies, have shown potential in visualizing immune-related markers *in vivo*, offering new methods for monitoring tumor immune responses<sup>[73]</sup>. <sup>68</sup>Ga-FAPI-04 PET/CT imaging enables non-invasive, *in vivo* depiction of cancer-associated fibroblasts within the immunosuppressive tumor microenvironment, potentially serving as a prognostic indicator for survival and antitumor immune responses in patients receiving ICIs<sup>[74]</sup>. Our research team has contributed to this domain, establishing an association between radiomic imaging biomarkers and both prognosis and immunotherapy response in GC<sup>[72]</sup>. Furthermore, by converging radiology with deep learning analysis, we have devised a non-invasive predictive methodology for tumor microenvironment status from radiographic images, capable of anticipating the efficacy of immunotherapy and elucidating the biological basis for these predictions<sup>[75]</sup>.

While radiomics has exhibited considerable promise in forecasting the efficacy of immunotherapy for GC, the absence of standardized protocols and robust validation is a significant hurdle. The “black box” nature of prediction biomarkers and models established through deep learning and machine learning poses challenges in interpretability, making them difficult to accept for clinical decision making<sup>[76]</sup>. Further studies are warranted to integrate imaging histological features with clinical data, thereby validating the clinical utility of imaging biomarkers for clinical practice adoption.

### Predictive biomarkers of immunotherapy-related adverse events

Immune-related adverse events (irAEs) represent a distinctive side effect of ICIs, akin to autoimmune reactions. These irAEs can affect nearly every organ system, with the skin, gastrointestinal tract, lungs,

endocrine, musculoskeletal, and other systems being the most frequently involved<sup>[77]</sup>. While ICIs induce a sustained antitumor response by immune cells, they can also disrupt immune system balance, leading to irAEs that differ from the toxicities typically associated with conventional chemotherapy<sup>[78]</sup>. Theoretically, the occurrence of irAEs may suggest a more favorable response to ICIs. However, whether irAEs can predict the response to ICIs in GC remains controversial. Several clinical studies have confirmed an association between the occurrence of irAEs and the survival outcomes of patients receiving ICI therapy. Typically, patients who experience irAEs exhibit a more favorable OS<sup>[79,80]</sup>. Conversely, a large meta-analysis suggests a weak correlation between the efficacy of ICIs and the occurrence of specific irAEs with OS across multiple solid tumors, including GC. Mild irAEs, rather than severe ones, have been associated with better efficacy<sup>[81]</sup>. Ongoing debate persists regarding the specific conclusions of these studies, likely attributable to heterogeneity in cancer types within study cohorts and variability in treatment regimens. Larger clinical cohorts are necessary for further validation to establish reliable predictive biomarkers for ICI efficacy.

### Other biomarkers

Mutations, deletions, and other alterations in certain oncogenes and tumor suppressor genes are closely associated with the efficacy of immunotherapy. ARID1A mutations<sup>[82]</sup>, NF- $\kappa$ B-related metabolic genes<sup>[83]</sup>, interferon (IFN)- $\gamma$  signaling pathways and T-cell activation-related genes<sup>[84]</sup> have been identified as potential biomarkers for predicting the efficacy of immunotherapy in GC. Research on gene mutations in the context of immunotherapy for GC remains relatively underexplored, underscoring the need for comprehensive clinical trials and translational studies to fully elucidate their clinical potential.

The TME plays a crucial role in regulating tumor progression and the response to treatment. Scoring the phenotypes of immune cells within the immune microenvironment can effectively predict the efficacy of immunotherapy in GC<sup>[35,85]</sup>. Tumor-infiltrating lymphocytes (TILs)<sup>[86]</sup> and tertiary lymphoid structures (TLS)<sup>[87]</sup> have been associated with the efficacy and prognosis of immunotherapy in GC. Recently, Chen *et al.* reported a correlation between the spatial distribution of tumor-infiltrating immune cells and the response to immunotherapy, offering new insights into predicting responses to ICIs<sup>[88]</sup>. The application of multi-omics technologies, including genomics, transcriptomics, and metabolomics, has identified various TME-related biomarkers that can serve as effective predictors of the response to immunotherapy in GC<sup>[89,90]</sup>. However, the limited adoption of these technologies and reliance on small cohorts or database-derived data have constrained their reliability, preventing widespread clinical implementation.

Additionally, a range of routinely evaluated clinical markers, such as gender<sup>[52]</sup>, body mass index<sup>[91]</sup>, and peripheral blood biomarkers<sup>[92-94]</sup>, are also explored to predict the efficacy of immunotherapy in GC.

### CONCLUSION

Identifying GC patients who are likely to benefit from immunotherapy is of paramount importance. We provide a comprehensive review of the various biomarkers currently reported in the literature for assessing the efficacy of immunotherapy, including PD-L1 expression, TMB, EBV infection, and MMR status, which are widely applied clinical biomarkers for evaluating the therapeutic efficacy of immunotherapy. Further large-scale clinical studies are required to validate these biomarkers. Liquid biopsies, gut microbiota, and adverse event profiling are emerging as potential predictive biomarkers for the efficacy of immunotherapy. Given the variability in standard treatment regimens, tumor heterogeneity, and differences in detection methodologies, studies have yielded inconsistent and even contradictory results. In this context, the field of immunotherapy research in GC is in urgent need of further exploration to develop more reliable and effective biomarkers.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Li S, Liang H

Performed data acquisition, as well as providing administrative, technical, and material support: Li G

### Availability of data and materials

Not applicable.

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### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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