Review Article

Clinical and molecular prognostic markers of survival after surgery for gastric cancer: tumor-node-metastasis staging system and beyond

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Abstract: For accurately predicting prognosis and for effectively describing cancer states at a certain point during treatment to other care providers and patients, various staging systems have been utilized in gastric cancer. Among these, the UICC/AJCC tumor-node-metastasis (TNM) staging system is most widely used. However, even within the same substage, gastric cancers can vary substantially in regards to prognosis after treatment. For more accurate and individualized prognostication, staging systems have been found to benefit from including molecular markers and genomic subtypes, in addition to clinicopathological parameters, such as age, sex, tumor size, tumor location, Lauren classification, number of lymph nodes resected, extent of surgical resection, lymphovascular invasion, and adjuvant chemotherapy. In this review article, we review and summarize relevant biomarkers for gastric cancer that can be incorporated into the current anatomy-based TNM staging system, as well as results from validation studies thereof.

Keywords: Biomarker; gastric cancer; TNM staging system; prognosis

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Introduction

While the incidence and death rates of gastric cancer have declined globally since 1975, gastric cancer remains the fifth most frequently diagnosed cancer and the third leading cause of cancer-related death worldwide (1). Classically, the prediction of oncologic outcomes and treatment recommendations for gastric cancer have primarily been guided by the tumor-node-metastasis (TNM) staging system (*Table 1*) (2). However, given that clinical outcomes have been found to vary substantially even within the same stage group, more detailed and individualized prediction models have been constructed. By incorporating prognostic clinicopathological parameters, such as age, sex, tumor size, tumor location, Lauren histotype, number of lymph nodes resected, extent of surgical resection, lymphovascular invasion, and adjuvant chemotherapy, these models have been shown to provide more individualized information on survival prediction adjunct to TNM staging (3-7).

Meanwhile, recent molecular and genomic analyses of cancer cells have identified biomolecular markers and genomic subtypes that can be utilized to predict cancer prognosis and adjuvant treatment responses, complementing the traditional anatomy-based TNM system. For breast cancer, the eighth edition of the American Joint Committee on Cancer (AJCC) incorporated a pathological prognostic

Table 1 Clinical stage (cTNM) and 1, 3, 5-year and median survivals in patients with gastric cancer, based on NCDB/Shizuoka Cancer Center data and stratified by clinical stage grouping (cTNM)

Clinical stage group	Patients, n 7,306/4,091	1-year survival, %	3-year survival, %	5-year survival, %	Median survival, mon
I (T1-2, N0)	1,418/2,318	80.6/98.9	64.9/95.0	56.7/90.2	84.93/–
IIa (T1-2, N+)	296/161	74.2/96.8	53.7/83.6	47.3/75.2	46.06/–
IIb (T3-4a, N0)	783/566	68.9/87.8	41.4/67.7	33.1/59.3	23.82/98.73
III (T3-4a, N0)	1,427/758	66.4/82.9	33.1/55.1	25.9/43.4	19.12/45.07
IV (T4b & M+)	3,382/288	28.3/51.7	7.8/22.1	5.0/14.1	6.24/13.3

NCDB, National Cancer Database (USA).

stage system into the classical TNM staging system (8). The newly incorporated prognostic and predictive biomarkers include estrogen receptor, progesterone receptor, human epidermal growth factor receptor-2 (HER2), histologic grade, and multigene expression assay (e.g., Oncotype Dx®). Several validation studies have shown improved stage stratification upon combination of this prognostic staging system, compared to the anatomic stage alone (9-11). Likewise, extensive molecular and genomic studies on gastric cancer have brought new understanding of its molecular heterogeneity and the use of markers thereof in developing prognostic and predictive biomarkers. Based on gene expression profiles, The Cancer Genome Atlas (TCGA) research network, the Asian Cancer Research Group (ACRG), and other study groups have subclassified gastric cancer into several molecular subtypes of more biologically homogeneous signatures (12-15). Indeed, patient stratification based on molecular information has been found in several cohort studies to have clinical implication in the prediction of both survival outcomes (12,16-19) and responsiveness to adjuvant therapies for gastric cancer (17-19). Also, biomarkers predictive of responses to immunotherapy have been widely searched and investigated for their use in guiding the selection of appropriate immune checkpoint therapy (20-22).

Herein, we review and summarize relevant biomarkers for gastric cancer, including immune-related markers for immunotherapy, as well as results from validation studies performed over the last decade. Overall, this review suggests that, for better patient stratification and more targeted selection of treatment, incorporating molecular aspects into anatomy-based TNM classification may improve the prognostic value of the current staging system for gastric cancer.

HER2

HER2 (encoded by proto-oncogene ERBB2 on chromosome 17q21) is a cell membrane receptor tyrosine kinase belonging to the epidermal growth factor receptor (EGFR) family (EGFR or HER1, HER2, HER3, and HER4). Not having a ligand binding site, HER2 serves as a preferred heterodimerization partner for other members of the HER family. HER-2-containing heterodimers transduce signals that are remarkably stronger than signals from other HER combinations, and overexpression of HER2 driven by ERBB amplification has been shown to play roles in cell proliferation, apoptosis, migration, and differentiation (23,24). Interestingly, ERBB amplification or HER2 overexpression has been reported in 7–22% of gastric cancer patients, predominantly in intestinal-type and in gastroesophageal junction tumors (24-27).

Concerning the prognostic value of HER2, results are conflicting. Some studies have suggested that overexpression of HER2 is associated with worse prognosis (28-32), while others found no prognostic significance for HER2 positivity (26,33,34). The contrasting results may stem from methodological differences in measuring HER2 expression and intra-tumoral heterogeneity (35).

Despite the controversy surrounding HER2 as a prognostic marker, its predictive value was delineated in the landmark ToGA (The Trastuzumab for Gastric cancer) study, which investigated the efficacy of trastuzumab, a HER2-targeting monoclonal antibody, in patients with HER2-positive advanced gastric or gastroesophageal junction cancer (25). In this open-label, international, phase III, randomized controlled trial, 524 patients with unresectable, metastatic gastric cancer or gastroesophageal junction cancer showing HER2 overexpression or gene

amplification were randomly assigned to treatment with trastuzumab plus chemotherapy (cisplatin plus fluorouracil or capecitabine) or chemotherapy alone. Therein, median overall survival (OS) was longer in patients treated with trastuzumab plus chemotherapy in comparison with chemotherapy-alone group (13.8 versus 11.1 months, HR 0.74; 95% CI, 0.60–0.91; P=0.0046). In addition, post-hoc analysis of subgroups stratified according to levels of HER2 expression revealed that patients with high expression of HER2 protein [immunohistochemistry (IHC) scoring 3+ or 2+ and fluorescence in-situ hybridization positive] had a longer OS (median OS 16.0 months, HR 0.65; 95% CI, 0.51–0.83). With the results from the ToGA trial, trastuzumab in combination with cisplatin and a fluoropyrimidine was approved by the Food and Drug Administration (FDA, USA) as a first-line treatment for patients with metastatic gastric cancer and HER2 overexpression. Meanwhile, the multicenter, randomized phase III, Lapatinib Optimization Study in HER2-positive Gastric Cancer (LOGiC) trial assessed the predictive value of HER2 by adding lapatinib, a tyrosine kinase inhibitor of both EGFR and HER2, to capecitabine and oxaliplatin as a first-line treatment in advanced gastroesophageal adenocarcinoma (36). Despite failing to show a statistically significant OS benefit, subgroup analysis revealed an improvement in OS in Asian patients (HR 0.68; 95% CI, 0.48–0.96) and patients younger than 60 years old (HR 0.69; 95% CI, 0.51–0.94). Another clinical trial investigated the efficacy of lapatinib plus paclitaxel in comparison to paclitaxel alone in a second-line setting for Asian patients with HER2-positive advanced gastric cancer (37). In this trial, median OS was better in patients treated with lapatinib plus paclitaxel (11.0 versus 8.9 months), although the difference did not reach statistical significance (P=0.1044). Interestingly, however, a clinically meaningful prolongation of OS was observed in lapatinib plus paclitaxel group versus paclitaxel alone in IHC3+ patients (HR 0.59; 95% CI, 0.37–0.93; P=0.0176), not in IHC0/1+ or IHC2+ patients. Other anti-HER2 agents, such as pertuzumab, a monoclonal antibody blocking heterodimerization of HER2/HER3 receptors, and trastuzumab emtansine (T-DM1), an antibody-drug conjugate, have also been assessed in clinical trials in patients with HER2 overexpression (38,39), finding no differences in survival outcomes.

As anti-HER2 therapeutic strategies other than trastuzumab have shown limited survival benefits, current interests in HER2-positive gastric cancer lie in analyzing inter-patient and intratumoral heterogeneity, as well as concomitant alterations in genes related to downstream signaling pathways, which have been proposed as underlying mechanisms of primary or acquired resistance to anti-HER2 therapy (40-45). Further stratifying HER2-positive patients according to molecular heterogeneity and integrating genomic profiles of relevant oncogenic mediators may refine the therapeutic outline and enhance the predictive value of HER2.

Microsatellite instability (MSI)

MSI is a tumor phenotype of hypermutability resulting from impaired DNA mismatch repair. In sporadic gastric cancer, MSI is associated with CpG island methylation phenotype and is primarily caused by hypermethylation of mutL homolog 1 (MLH1) promoter and subsequent MLH1 silencing in the early stages of tumorigenesis (46). MSI tumor accounts for 5.6% to 33.3% of all gastric cancers and has been shown to be associated with female sex, relatively old age at diagnosis, intestinal subtype, location at the mid or distal stomach, and early diagnostic stage (TNM stage I/II) (12,13,47). MSI is now recognized as a robust marker of a subgroup of gastric cancer in both of the molecular classifications proposed by the TCGA project and the ACRG group.

In two recent meta analyses, gastric cancer with MSI at high frequency (MSI-H) have been found to have better prognosis than MSI-low and/or microsatellite stable (MSI-L/MSS) tumors (47,48). Concerning chemotherapy responses, however, among resected gastric cancers, MSI-H groups have not been found to show survival benefits, compared to MSI-L/MSS groups, with some studies suggesting even worse outcomes, implying possible negative effects for cytotoxic drugs to MSI-H tumors (49-52). One retrospective study of the long-term outcomes of patients with stage II/III gastric cancer showed that patients with MSI-H did not show a statistically significant benefit from adjuvant chemotherapy and that, instead, chemotherapy elicited detrimental effects in patients with stage III disease (49). Similarly, in post hoc analysis of the United Kingdom Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial, deficient mismatch repair (dMMR) and MSI-H were associated with negative prognostic effects in patients who received perioperative chemotherapy (50). These results are similar to those for colon cancer, wherein MSI-H is accepted as a predictive marker for non-responsiveness to fluorouracil-based adjuvant treatment, the assessment of which for

stage II colon cancer is recommended in the National Comprehensive Cancer Network and European Society for Medical Oncology guidelines (53,54).

In an immunologic context, however, recent studies have shown that patients with MSI-H-positive solid tumors could derive benefits from immunotherapy due to a highly immunogenic microenvironment that frequently expresses immune-regulatory molecules, namely programmed cell death ligand 1 (PD-L1) (55-57). As a result, the FDA has approved pembrolizumab, an anti-PD-1 monoclonal antibody that blocks PD-1/PD-L1 interactions, for the treatment of patients with unresectable or metastatic solid tumors with MSI or dMMR. For gastric cancer, pembrolizumab has been further approved for the treatment of refractory advanced gastric cancer expressing PD-L1 [combined positive score (CPS) ≥1%] based on the phase II KEYNOTE-059 trial (58).

A recent post hoc analysis of the Capecitabine and Oxaliplatin Adjuvant Study in Stomach Cancer (CLASSIC) trial reported on the clinical implications of MSI-H status and PD-L1 expression in adjuvant settings for stage II/III gastric cancer (52). In this study, the prognosis of patients treated with chemotherapy was significantly better only in MSS individuals, among whom only those with PD-L1 negativity, not PD-L1 positivity, showed significant survival benefits therefrom, compared to a surgery only group.

These findings suggest that MSI could serve as a prognostic marker and, together with PD-L1, a predictive marker for identifying patients who might benefit most from PD-1 inhibitors and for stratifying patients with stage II/III gastric cancer in relation to adjuvant chemotherapy responses.

Epstein-Barr virus (EBV)

EBV positivity is found in about 10% of gastric cancer patients worldwide and is characterized by a higher prevalence in men, predominance in the proximal stomach (fundus and body), lower lymph node metastasis, better prognosis (59-64), and massive infiltration by proliferating CD8+ T-lymphocytes (65,66). The good prognosis of EBV-associated gastric cancer (EBVaGC) is thought to be explained by an increased local host immune response that inhibits the outgrowth of metastasis in the lymph nodes, as reflected by increased CD8+ T-lymphocytes and mature dendritic cells (67). At the same time, however, more dominant CD4+CD25+FoxP3+ regulatory T cells and upregulated expression of key immune suppressive cytokines, such as interleukin (IL)-1b and IL-10 in EBVaGC enables tumor cells to evade the immune response (60).

Constituting a separate subtype in the molecular classification system by TCGA, EBVaGC exhibits extreme DNA promoter hypermethylation, PIK3CA mutation, and PD-L1/L2 overexpression (13). Studies have suggested that, along with a massive T-cell inflamed microenvironment, PD-L1/L2 overexpression possibly renders EBVaGC a high sensitivity to PD-1 checkpoint blockage-based immunotherapy (21,68). In a recent phase II clinical trial that performed molecular profiling of 61 patients with metastatic gastric cancer treated with pembrolizumab monotherapy as a salvage treatment, all of six patients with EBVaGC achieved a partial response, with a median response duration of 8.5 months (21). Of the 55 patients with PD-L1 CPS positivity, overall response rates (ORR) were significantly higher in PD-L1-positive tumors than PD-L1-negative tumors (50.0% versus 0.0%, P<0.001). Meanwhile, a more dramatic response was also observed in patients with MSI-H tumors (ORR of 85.7%, 6 of 7 MSI-H patients). Reporting the case of patient with EBVaGC, but no high mutation burden or mismatch repair defect who derived a meaningful clinical benefit from treatment with avelumab, an anti-PD-L1 antibody, Panda *et al.* analyzed TCGA data from an immunological point of view (68). The study reported that EBVaGC had low mutation burden and stronger evidence of an immune infiltrating profile, compared with MSI tumors, as well as higher expression of checkpoint pathway genes, such as PD-1, PD-L1/L2, CTLA-4, CD80, and CD86, compared with MSS tumors. They suggested that EBV-positive gastric cancers with low mutational burden ought to comprise a subset of MSS gastric cancers that may respond to immune checkpoint therapy.

Meanwhile, in a recent retrospective post hoc analysis of the CLASSIC trial that evaluated EBV as a marker to predict prognosis and responsiveness of adjuvant chemotherapy for stage II/III gastric cancer, EBV-negative individuals showed statistically significant survival benefits from adjuvant chemotherapy (P=0.001), whereas EBV-positive individuals did not (P=0.687) (51).

Overall, these data suggest that though EBVaGC and MSI-H tumors are mutually exclusive entities, both subtypes exhibit immunogenic states and low or no sensitivity to adjuvant chemotherapy. Further prospective studies are warranted to validate EBVaGC as a predictor of responsiveness to immune checkpoint therapeutics and adjuvant chemotherapy.

PD-L1 and immune-related markers

Immune checkpoint inhibition has been the most studied immunotherapy for treating solid tumors, including gastric cancer. By blocking negative feedback signaling from cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or PD1 on T lymphocytes, treatment with antibody-mediated inhibitors, such as ipilimumab (anti-CTLA-4 antibody) or pembrolizumab and nivolumab (anti-PD1 antibody), has elicited clinical responses in a subset of patients with advanced gastric cancer (58,69-71).

With FDA approval of the tumor-agnostic pembrolizumab for unresectable or metastatic solid tumors with MSI or dMMR, it has since garnered approval for recurrent or metastatic gastric or gastroesophageal junction adenocarcinoma expressing PD-L1. Evidence for its use was derived from documentation of durable ORRs in a multicenter, open-label, multicohort trial (KEYNOTE-059/Cohort1). The trial reported an ORR of 13.3% (95% CI, 8.2–20.0), with 1.4% of patients achieving a complete response among the 55% (n=143) of enrolled patients with tumors expressing PD-L1 and either MSS or undetermined MSI or mismatch repair status (58). In a multicenter, open-label, phase Ib KENOTE-012 trial that assessed efficacy of pembrolizumab in 39 patients with PD-L1-positive recurrent or metastatic adenocarcinoma of the stomach or gastroesophageal junction, the ORR for evaluable patients was 22% (95% CI, 10–39) at central review (71).

Although the value of PD-L1 as a predictive marker for responsiveness to immune checkpoint inhibitor in advanced gastric cancer was shown in the clinical trials mentioned above, the observed modest response rate for pembrolizumab aroused studies in search of immune-relevant biomarkers beyond PD-L1 and MSI-H. The immune-related markers that have been suggested are associated with either high tumor neo-antigenicity or tumor mutational burden (TMB), such as MSI-H, or related to activated T-cell infiltrates in the tumor microenvironment (TME), such as PD-L1 expression on tumor and immune cells. By evaluating more than 300 patient samples across 22 tumor types from four KEYNOTE clinical trials, Cristescu *et al.* reported TMB and a T cell-inflamed gene expression profile jointly predicted responders and nonresponders to pembrolizumab, regardless of tumor type (20). In a phase Ib/II clinical trial conducted by Wang *et al.*, high TMB was associated with survival benefits in chemorefractory gastric cancer treated with toripalimab, a humanized PD-1 antibody. In the study, patients with high TMB (n=12, TMB-H) responded significantly better than patients with low TMB (n=42, TMB-L) (ORR 33.3% versus 7.1%, P=0.017), and the TMB-H group showed meaningful survival benefits in regards to OS, compared to the TMB-L group (14.6 versus 4.0 months, HR 0.48; 95% CI, 0.24–0.96, P=0.038) (72). These studies suggest that TMB status could be used in addition to PD-L1 to predict responsiveness to anti-PD-1 therapy.

As infiltration of immune cells into the TME is a prerequisite for the action of PD-L1 blockade, cancer classification based on T-cell infiltration along with PD-L1 has been suggested. Although the prognostic value of PD-L1 alone in predicting survival benefit remains controversial, in a study that divided TMEs for gastric cancer into four immune microenvironment subtypes according to PD-L1 and tumor infiltrating lymphocytes (TIL) measured by intratumoral CD8 density, the prognosis of TME type 1, which expressed PD-L1+/TIL+, showed the best survival in regards to both disease-free survival (HR 2.044) and OS (HR 1.993), while the PD-L1-/TIL- subtype showed the worst survival (73). Similarly, Koh *et al.* assessed PD-L1 expression and CD8+ TILs in stage II/III gastric cancer, with the PD-L1-/CD8-low type showing the worst OS (P<0.001) (74).

In summary, these studies emphasized the significance of immune infiltration status in TME, represented by PD-L1 and immune-relevant markers, such as TMB, CD8+ TILs, MSI-H, and EBV positivity, in predicting responses to immune therapy and survival benefits.

Circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA)

CTCs and ctDNA have been recognized as promising biomarkers via liquid biopsies for prognostic evaluation of various cancer types, including gastric cancer. Compared to tissue-based biomarkers, liquid biopsy enables longitudinal assessment of therapeutic efficacy and monitoring of evolving tumor genomes as a repeatable and minimally invasive method.

CTC counts are found to be larger in metastatic gastric cancer than in nonmetastatic gastric cancer (75) and CTC positivity correlated with worse progression-free survival (PFS) and OS (76,77). Moreover, tracing of CTC levels during chemotherapy revealed that CTC counts measured at 4 weeks after chemotherapy were shown to be predictive of disease control rate (78), median PFS and OS (79).

CtDNA is fragmented DNA in bloodstream, a type of

cell-free DNA but originated from tumor cells or CTCs. CtDNA analysis, recently available with the advent of modern genomic techniques is becoming a promising tool in monitoring tumor progression, residual disease, and drug responses. Several studies have revealed that ctDNA levels measured before surgery or over the treatment course have significant prognostic value comparable to known tumor marker CEA or CA 19-9 (80,81) and are indicative of disease recurrence or regression (21,80-82). Hamakawa *et al.* longitudinally quantified ctDNA levels targeting TP53 mutation in 3 patients who underwent gastrectomy for advanced gastric cancer and reported that TP53-ctDNA levels were correlated with disease status, decreasing with surgical resection and increasing after recurrence (80). Like CTC counts, ctDNA levels were reported to be higher in patients with stage IV gastric cancer, and patients with high ctDNA levels showed worse 5-year OS than those with low ctDNA levels (P=0.039). Among 244 patients who received curative surgery, patients with high preoperative ctDNA levels were more likely to experience peritoneal recurrence than those with low ctDNA levels (P=0.044) (81). Another study group assessed the dynamics of cell-free EBV DNA in 153 patients who underwent surgery for gastric cancer including 14 patients with EBVaGC. Plasma EBV DNA levels were positively-associated with the size of EBVaGC tumors, and preoperative detection of plasma EBV DNA disappeared after surgery in all nine cases, suggesting that plasma EBV DNA levels may reflect tumor burden (83). In the aforementioned study by Kim *et al.*, longitudinal genomic profiling of ctDNA mutational load and ctDNA levels during treatment with pembrolizumab was correlated with clinical responses, and patients stratified to the upper tertile of ctDNA mutational load experienced an improved ORR compared to the lower two tertiles (83% versus 7.7%, P=0.0014) (21).

Another promising application of ctDNA analysis is real-time monitoring of targeted therapy resistance and identification of acquired mutations in the progress of gastric cancer progression, where a relatively high discordance of gene aberrations between primary and metastatic tumors is often detected (45,84,85). Pectasides *et al.* reported significant discrepancies in multicopy amplifications of clinically relevant genes between primary tissue and metastases in 10 of 28 patients with advanced gastric cancer (36%). Notably, in the discordant primary and metastatic lesions, targetable alterations in ctDNA and metastatic tissue were concordant in seven of eight cases (87%), suggesting that ctDNA profile has better representation of the property of the metastatic portion of the disease over the primary lesion in gastroesophageal cancer (84). In a prospective phase II trial of capecitabine/oxaliplatin plus lapatinib as a first-line therapy for 32 patients with metastatic and/or recurrent gastric cancer with HER2-overexpression, serial ctDNA next-generation sequencing testing revealed that ERBB2 copy number alterations in ctDNA were predictive of treatment responses and that plasma-detected genomic aberrations, such as MYC, EGFR, FGFR2, and MET amplifications, were associated with lapatinib sensitivity and/or resistance (45).

These studies have demonstrated that monitoring of CTCs/ctDNA levels perioperatively and during the treatment course could provide real-time prognostic information in gastric cancer and that genomic profiling of ctDNA could be of use for early detection of recurrence and identification of therapeutic resistance. This will allow for more precise description of disease status and proper adjustment of therapy for patients on an individual and timely basis.

Conclusions

While the current TNM system for gastric cancer provides important prognostic information reflective of the anatomic extent of the disease, survival prediction and treatment decisions relying solely on TNM staging have been confronted with clinical heterogeneity detected across the same stage. Accordingly, in an effort to improve the accuracy of prognostication, the 8th edition of the TNM gastric cancer staging system includes modifications intent on refining the homogeneity of patients belonging to the same stage groups. In this review article, we presented modern data on several candidate markers that could be incorporated in future TNM systems for gastric cancer (*Table 2*). Providing adjunctive prognostic information, some of these markers are already being routinely described in pathology reports in some countries, and some are under validation studies or are being recruited for clinical translation in prospective trials. Nevertheless, in order for these markers to be incorporated into daily clinical practice as additive factors of TNM in the treatment landscape of gastric cancer, many remaining issues need to be resolved.

First, researchers should take into consideration that many recent studies on biomarkers and gene expression classifiers have been based on retrospective analyses of clinical cohorts and tumor specimens and have rarely included prospective cohorts of patients. Moreover,

Table 2 Prognostic and predictive features of molecular markers in gastric cancer

Biomarkers	Clinical feature/use	Prognostic value	Predictive value
HER2	• Targeted therapy • Co-targeting related genes	Not consistent	Therapeutic response to anti-HER2 therapy (e.g., trastuzumab and lapatinib)
MSI	• Immunogenicity • PD-L1 expression	Better prognosis than MSI-L/MSS	• Response to immune checkpoint therapy (pembrolizumab) • Possible negative effect for adjuvant chemotherapy
EBVaGC	• Immunogenicity • PD-L1/2 overexpression	Better prognosis than EBV negative	• Response to immune checkpoint therapy (pembrolizumab) • Possible negative effect for adjuvant chemotherapy
Immune-related markers (e.g., PD-L1)	• Targetable grouping • Response to immune checkpoint inhibition	Not consistent	Therapeutic response to immune checkpoint inhibitors (e.g., pembrolizumab, nivolumab, ipilimumab)
CTCs/ctDNA analysis	• Reflection of tumor burden • Early detection of recurrent disease • Identification of resistant mutations during targeted therapy	Poor prognosis with CTCs/ctDNA-high status	• High post-therapy CTC/CtDNA status associate with a poor therapeutic response • Response to target therapy based on ctDNA gene profiling

HER2, human epidermal growth factor receptor 2; MSI, microsatellite instability; EBVaGC, Epstein-Barr virus associated gastric cancer; PD-L1, programmed death-ligand 1; CTCs, circulating tumors cells; ctDNA, circulating tumor DNA.

many validation cohorts have not included individuals of different races or regions. Hence, accumulation of data from prospectively designed studies, as well as retrospective studies performed in larger cohorts involving different populations, surgical technique, and adjuvant chemotherapies, is needed for verification of biomarkers and subsequent robust classification of patients.

Second, more studies need to be conducted in adjuvant settings beyond a metastatic frame considering that TNM stage provides less prognostic information for stage II/III cancers than for stage I and IV gastric cancers. While cytotoxic chemotherapy constitutes the backbone of contemporary adjuvant therapy in stage II/III resected gastric cancer, studies have shown that further stratification of patients is important in selecting the most beneficial adjuvant therapy among chemotherapy, targeted therapy, immunotherapy, or a combination thereof. However, most data on biomarkers have been obtained in a metastatic setting, and all targeted therapies approved this far [e.g., trastuzumab, ramucirumab (a VEGFR 2 antibody), and pembrolizumab] have been recommended for refractory or metastatic gastric cancer. Therefore, biomarker-driven clinical trials designed for stratifying patients after surgery are needed to enable more accurate prognostication and, thereby, hopefully, lead to survival benefits.

Third, comprehensive understanding of the similarities and differences of existing or upcoming gene-based classifiers is needed for optimization of patient stratification and prediction of therapeutic responses. Most gene profiles and risk score systems have been developed based on somatic mutations or unique expression of several key genes, DNA hypermethylation (e.g., MSI), transcriptional phenotype (e.g., mesenchymal or epithelial), and immunologic characteristics of the TME. Introducing added complexity, a combination of gene-based subtypes with target molecules could optimize selection of systemic therapy. More studies will be needed to guide clinicians for proper translation of molecular subtypes into the clinic.

Lastly, achieving standardization of molecular techniques for determining marker expression status and reaching a consensus on prognostic threshold values are required to ensure reproducibility. Just as there are standard guidelines

for detecting and defining the expression status of approved biomarkers, [i.e., HER2 (86), MSI-H (87) and PD-L1 (58)], emerging biomarkers should be described under unified methodology with proper cutoff values for prognostication, thereby facilitating universal applicability, as well as providing clear-cut criteria for clinical trials.

While establishing biology-based staging is complex and many issues remain, accumulating evidence supports further development of the TNM system for gastric cancer, the process of which will also broaden understanding of oncogenic mechanisms and lead to the development of new therapeutic strategies.

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Footnote

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