ABSTRACT

Gastric cancer (GC) is one of the most common lethal malignant neoplasms worldwide, with limited treatment options for both locally advanced and/or metastatic conditions, resulting in a dismal prognosis. Although the widely used morphological classifications may be helpful for endoscopic or surgical treatment choices, they are still insufficient to guide precise and/or personalized therapy for individual patients. Recent advances in genomic technology and high-throughput analysis may improve the understanding of molecular pathways associated with GC pathogenesis and aid in the classification of GC at the molecular level. Advances in next-generation sequencing have enabled the identification of several genetic alterations through single experiments. Thus, understanding the driver alterations involved in gastric carcinogenesis has become increasingly important because it can aid in the discovery of potential biomarkers and therapeutic targets. In this article, we review the molecular classifications of GC, focusing on The Cancer Genome Atlas (TCGA) classification. We further describe the currently available biomarker-targeted therapies and potential biomarker-guided therapies. This review will help clinicians by providing an inclusive understanding of the molecular pathology of GC and may assist in selecting the best treatment approaches for patients with GC.

Keywords: Gastric cancer; Human genome project; Molecular diagnostic testing; Diagnostic molecular pathology; Biomarker; Molecular targeted therapy

INTRODUCTION

Gastric cancer (GC) is one of the most common cancers and the third leading cause of cancer-related deaths worldwide, with more than 768,793 estimated deaths in 2020 [1], despite the ongoing decline in incidence and mortality in recent decades. Unfortunately, there is a significant difference in both the incidence and mortality of GC patients between East Asian and Western countries owing to the lack of validated screening programs for GC and the quality of global therapeutic approaches [2,3]. Most patients in Western countries are diagnosed late with advanced GC disease stages, and these patients show poor prognosis with a high mortality rate. Although targeted drugs in combination with conventional chemotherapeutic agents have improved the overall survival (OS) of patients with GC, the long-term survival of patients with advanced and/or metastatic disease remains poor [4-6].
Currently, therapeutic regimens follow a “one-size-fits-all” approach and do not consider the highly heterogeneous nature of GC. From morphological and molecular viewpoints, GC is a heterogeneous disease [7]. Moreover, GC is a multifactorial disease influenced by both genetic and environmental risk factors [8]. For these reasons, it may be difficult to target the entire tumor despite better control over known risk factors and improvements in new therapeutic strategies, including new chemotherapeutic and targeted agents such as trastuzumab and ramucirumab.

With respect to patient stratification, although numerous morphologic classifications for GC have been proposed, the Lauren, Japanese Gastric Cancer Association (JGCA), and World Health Organization (WHO) classification systems are the most popular [9]. Nevertheless, their clinical utility in guiding precise treatment for individual patients is doubtful [10,11]. Therefore, new alternative schemes for patient stratification are required. Recently, remarkable advances in genomic technology and high-throughput analysis have made it possible to study GC at a molecular level. Integrative analyses of large-scale genomic and proteomic profiling data have facilitated the identification of candidate driver alterations in GC pathogenesis [11]. Understanding these candidate driver alterations may lead to the discovery of potential biomarkers and therapeutic targets [11-14]. Taken together, the molecular genetic classification of GC may help define future personalized therapy plans by providing opportunities for patient stratification and the development of new biomarkers for clinical trials. For example, recent molecular data offer a rationale for examining the importance of Epstein-Barr virus (EBV) and mismatch repair (MMR) systems in predicting immunotherapy efficacy in GC [15].

The aim of this review is to help clinicians understand the molecular classification of GCs, particularly focusing on The Cancer Genome Atlas (TCGA) classification [16], which has paved the way for targeted therapies in GC. We discuss established biomarker-guided therapies for GC, including human epidermal growth factor receptor 2 (HER2)-targeted, immune checkpoint inhibitor (ICI), and tropomyosin receptor kinase (TRK) inhibitor therapies. We also discuss the potential biomarkers of targetable alterations. Owing to the increasing demand for the revision of the ‘Standardized Pathology Report for Gastric Cancer,’ the Committee of the Gastrointestinal Pathology Study Group of the Korean Society of Pathologists was organized in 2022, and after several discussions and consensus meetings, the Standardized Pathology Report for Gastric Cancer, 2nd edition, will be released. Thus, detailed principles and guidelines for the interpretation of biomarker testing in routine pathology laboratories will be described in the Standardized Pathology Report for Gastric Cancer, 2nd edition.

This comprehensive review will assist medical trainees and clinicians dedicated to GC treatment in selecting the best precision management approaches for patients with GC by improving the understanding of the molecular pathology of GC.

MOLECULAR CLASSIFICATION OF GC

TCGA molecular classification of GC
The advent of molecular techniques has accelerated the molecular classification of various human cancers [12,17]. TCGA groups have categorized GC into four subtypes using large-scale genome sequencing analysis: EBV-positivity, microsatellite instability
EBV-positive GC (8.8% of GC cases in the TCGA cohort) is more prevalent in men than in women and commonly occurs in the gastric fundus and body (in the upper and middle parts of the stomach) [16,18-20]. EBV-positive GC displays extreme CpG island methylator phenotypes (CIMP), which are distinct from those of MSI GC [16,21]. These are characterized by cyclin-dependent kinase inhibitor 2A (CDKN2A) promoter hypermethylation and MLH1 promoter hypermethylation deficiency [16,22]. Genes that are frequently mutated in EBV-associated gastric carcinomas (EBVaGCs) include phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), AT-rich interactive domain-containing protein 1A (ARID1A), and BCL6 corepressor (BCOR) [16,23]. Interestingly, PIK3CA mutations were frequently found in 80% of EBV-positive GC cases and 42% of MSI GC cases in the TCGA cohort. However, PIK3CA mutations in EBV-positive GC displayed a more dispersed pattern than those localized in the kinase domain (exon 20) in EBV-negative GC [16]. Although not as high as 80% as observed in other studies, a significant association was observed between EBV-positive status and PIK3CA mutations [24,25]. PIK3CA regulates the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway, and its mutations have been found in various cancers [24,26]. Clinical trials evaluating the efficacy of PI3K/AKT/mammalian target of rapamycin (mTOR) axis inhibitors in PIK3CA-mutated patients with advanced cancers are currently ongoing [27]. Recently, the Food and Drug Administration (FDA) has approved the use of alpelisib plus fulvestrant for patients with hormone receptor-positive, HER2-negative, PIK3CA-mutated, advanced or metastatic breast cancer [28-30]. However, in a recent study on EBV-positive GC, intratumoral and intertumoral heterogeneity of PIK3CA mutations was identified using hot spot pyrosequencing and next-generation sequencing (NGS) [24]. Furthermore, research showed that intratumoral heterogeneity of EBV-encoded small RNA (EBER)-positivity with juxtaposition of EBER-negative and EBER-positive tumor areas was assessed using the EBER in situ hybridization (ISH) assay [24]. Although its utility as a
potential biomarker is being investigated, these findings may attenuate the effects of targeted therapies for \textit{PIK3CA} mutations. Tumor protein p53 (\textit{TP53}) mutations are known to rarely occur in EBV-positive GC owing to EBV-induced p53 stabilization [16,31]; however, based on our experience, \textit{TP53} mutations are not as infrequent as previously reported, particularly in EBV-positive GC with intestinal-type histology (unpublished data). EBV-positive GC exhibits a high frequency of amplifications for programmed death ligands 1 and 2 (PD-L1 and PD-L2), Janus kinase 2 (\textit{JAK2}), and erb-b2 receptor tyrosine kinase 2 (\textit{ERBB2}; \textit{HER2}) [16]. The intensity of IL-12-mediated signals in EBV-positive GC suggests the presence of strong immune cells [16,32]. Combined with an evidence of PD-L1/2 overexpression, these findings provide a rationale for testing immune checkpoint inhibitors as therapeutic agents for EBV-positive GC [16,33,34]. As such, EBV-positive GC has distinct genetic and epigenetic molecular characteristics as well as distinct histologic features, which will be discussed later.

MSI in GCs (21.7% of GC cases in the TCGA cohort) was more prevalent in females and older patients and was mainly localized in the gastric antrum (in the distal part of the stomach) [16]. MSI GC shows a very high number of mutations (hypermutation) and MSI-associated gastric-CIMP methylation with epigenetic silencing of mutl. homolog 1 (\textit{MLH1}) [16]. \textit{MLH1} promoter methylation is the most frequent mechanism leading to MSI-H in GC [35,36], although MSI-H phenotypes occur via various mechanisms [37]. MSI GC is noted to be associated with mutations of \textit{PIK3CA}, erb-b2 receptor tyrosine kinase 3 (\textit{ERBB3}; \textit{HER3}), \textit{ERBB2}, and \textit{EGFR}, along with major histocompatibility complex I; moreover, MSI GC is generally rare, with targetable gene amplifications [16]. In contrast, B-Raf proto-oncogene and serine/threonine kinase (\textit{BRAF}) V600E mutations were not found in colorectal cancer with high MSI (MSI-H) [16,38]. MSI-H is an FDA-approved biomarker used to predict responses to pembrolizumab, an anti-PD1 antibody [39]. Neoantigens derived from hypermutated genes in MSI-H tumors elicit enhanced antitumor responses to ICIs [40]. Therefore, the detection of MSI-H GCs is needed to assess their eligibility for immunotherapy. Histological data of MSI-H GCs revealed diverse patterns, usually accompanied by dense lymphocytic infiltration, similar to EBV GC [37,41].

Genomically stable (GS) GC (19.7% of GC cases in the TCGA cohort) are associated with a diffuse type of Lauren histology and are diagnosed at a relatively younger age [16,42,43]. The somatic mutational burden of GS GC is the lowest among the four subtypes; accordingly, fewer targetable genetic alterations were observed in GS GC than in other subtypes [16]. Among them, mutations in cadherin 1 (\textit{CDH1}), ras homolog family member A (\textit{RHOA}), and \textit{ARID1A} were commonly observed [16,44,45]. In addition, 15% of GS GC cases harbored a \textit{CLDN18--ARHGAP26} translocation, which was mutually exclusive with somatic \textit{RHOA} alterations, contributing to dysregulated Rho signaling and enhanced cell motility. In line with this, GS GC demonstrated elevated syndecan-1 mediated signaling and angiogenesis-related pathways, in addition to enhanced expression of B1/B3 integrins, which may serve as potential therapeutic targets [16,46,47].

CIN in GC (49.8% of GC cases in the TCGA cohort) represents the largest group and is characterized by marked aneuploidy, either broadly or focally [13,16]. This subtype preferentially occurs in GEJ and cardia, and is associated with the intestinal type of Lauren histology [16,32]. \textit{TP53} is most frequently altered in this subtype (71% of CIN GC cases) [48]. Additionally, genomic amplification of receptor tyrosine kinase (RTKs), including \textit{ERBB2} (\textit{HER2}), \textit{ERBB3} (\textit{HER3}), \textit{EGFR}, \textit{FGFR2}, \textit{MET}, \textit{JAK2}, \textit{KRAS} proto-oncogene, GTPase (\textit{KRAS}) or \textit{NRAS} proto-oncogene, GTPase (\textit{NRAS}), and vascular endothelial growth factor A (\textit{VEGFA}),
as well as cell cycle mediators (cyclin E1 [CCNE1], cyclin D1 [CCND1], and cyclin-dependent kinase 6 [CDK6]), have been observed, some of which are therapeutic targets [16, 49-53]. During reverse-phase protein array analysis, phosphorylation of EGFR and expression of p53 appeared to be significantly increased, consistent with EGFR amplification and frequent TP53 mutations in this subtype [16]. In a subsequent study, it was reported that GC patients with CIN experienced the greatest benefit from adjuvant chemotherapy (CTx), whereas those with GS experienced the least benefit from adjuvant CTx [54]. As CIN occurs in nearly 50% of GC patients and is associated with frequent targetable genetic alterations, further studies on the respective targeted drugs are urgently needed.

Although the TCGA group did not investigate the association between molecular subtypes and prognosis, subsequent studies demonstrated that the prognosis of EBV-positive GC and MSI-H GC was better than that of the other subtypes, whereas the prognosis of GS GC was the worst [54-56].

OTHER MOLECULAR CLASSIFICATIONS

In addition to the TCGA molecular classification of GC, which is the most frequently used, other molecular classifications of GC have been proposed using different molecular methods and analysis tools. Of these, we briefly review the molecular classification suggested by the Asian Cancer Research Group (ACRG) and the Singapore groups. Furthermore, we summarize a simplified algorithm that can reproduce the recently presented molecular subgroups of GC using immunohistochemical (IHC) and ISH assays.

ACRG classification

The ACRG group employed array-based gene expression profiling data and applied principal component analysis to the expression profiling dataset [57]. They then classified GC into 4 subtypes associated with distinct patterns of molecular alterations, disease progression, clinical outcomes, MSI, microsatellite stable (MSS)/epithelial-mesenchymal transition (EMT), MSS/TP53+, and MSS/TP53− [57]. The ACRG group first molecularly separated GC into two large categories, MSI and MSS. MSS tumors were stratified based on the presence or absence of an epithelial-mesenchymal gene signature (MSS/EMT). The remaining tumors were divided into final categories, MSS/TP53+ and MSS/TP53−, according to the presence or absence of TP53 activity [57]. The MSI subtype is consistent with that in the TCGA classification, but there are distinct differences in terms of molecular mechanisms and driver genes [57]. Although the MSS/EMT, MSS/TP53+, and MSS/TP53− subtypes are enriched with GS, EBV, and CIN subtypes of TCGA classification, respectively, they do not overlap perfectly [32]. The CIN and GS subtypes are present in all ACRG subtypes [32,57]. Furthermore, CDH1 and RHOA mutations, which appeared with high frequency in GS subtypes in TCGA classification, were infrequently observed in the MSS/EMT subtype [57]. These differences may be due to variations in ethnicity, the larger inclusion rate for diffuse Lauren histology types (ACRG, 45% vs. TCGA, 24%), and a lower proportion of GEJ cancers and proximally located GC in the ACRG cohort [32,57].

The MSI subtype (22.7% of GC cases in the ACRG cohort) shared similar molecular and clinical features with MSI-H GC in the TCGA cohort. This subtype was significantly associated with the presence of hypermutations in ALK (16.3%), KRAS (23.3), and ARID1A (44.2%), and was involved in the PI3K/phosphatase and tensin homolog (PTEN)/mTOR
pathway (42%) with loss of MLH1 expression [32,57]. In the MSI subtype, PIK3CA H1047R mutations are frequently observed [57]. The MSI subtype showed the best prognosis and lowest frequency of recurrence (mostly liver metastasis) among the four subtypes [57]. This subtype was also associated with an early stage (I/II) at the time of diagnosis (>50%), intestinal-type Lauren histology (>60%), and GC located in the antrum (75%) [32,57].

The MSS/EMT subtype (15.3% of GC cases in the ACRG cohort) occurs at a significantly younger age, with the majority (>80%) showing a diffuse subtype of Lauren histology at advanced stages (III/IV) [57]. Notably, this subtype showed the worst prognosis and highest recurrence frequency (mostly peritoneal seeding) among the four subtypes [57]. The MSS/EMT subtype harbored fewer somatic mutations and copy number alterations than the other subtypes [57].

The MSS/TP53− subtype (35.7% of GC cases in the ACRG cohort), as its name implies, displayed a functional loss of TP53 and somatic TP53 mutations. Copy number analysis revealed that the MSS/TP53− subtype showed recurrent amplification of ERBB2, EGFR, MYC, cyclin E1 (CCNE1), MDM2 proto-oncogene (MDM2), cyclin D1 (CCND1), roundabout guidance receptor 2 (ROBO2), and GATA binding protein 6 (GATA6). However, the MSS/TP53+ subtype (26.3% of GC cases in the ACRG cohort) displayed the second-best prognosis among the 4 subtypes and a relatively higher frequency of mutations in KRAS, PIK3CA, APC regulator of the WNT signaling pathway (APC), ARID1A, and SMAD4 compared to the MSS/TP53− subtype.

'Singapore-Duke' classification
The Singapore group (also called the ‘Singapore-Duke’ classification) used microarray-based gene expression profiling to classify subtypes of GC with biological properties and sensitivity for chemotherapy and targeted agents via unsupervised hierarchical clustering [58]. They categorized GC into three subtypes: mesenchymal, proliferative, and metabolic [58].

The mesenchymal subtype showed high expression of cadherin 2 (CDH2) mRNA and low expression of CDH1 mRNA, consistent with the highly upregulated EMT [58]. Cancer stem cell-related pathways are also activated in this subtype, with increased CD44 (CD44) and decreased CD24 (CD24) expression [58]. This subtype is associated with transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), nuclear factor κ-light-chain enhancer of activated B cells (NFκB), mTOR, and sonic hedgehog (SHH) pathways [32,58]. In addition, the mesenchymal subtype is enriched in tumors with low CNAs and strongly correlated with a diffuse type of Lauren histology and poorly differentiated GC [32,58]. In an in vitro study, GC cell lines of the mesenchymal subtype showed significant sensitivity to PI3K/ AKT/mTOR inhibitors [32,58].

The proliferative subtype is enriched in gene sets associated with the gene cycle [58]. This subtype frequently harbors TP53 mutations and higher levels of CNAs than other subtypes, which are primarily caused by copy number gains. This subtype was associated with increased oncogenic pathway activity, including E2F, MYC, and RAS pathways [32,58]. It showed enrichment for the amplification of several oncogenes such as CCNE1, MYC, ERBB2, and KRAS [58]. Hypomethylated sites in the proliferative subtype displayed a much higher proportion of aberrantly methylated CpG islands than those in the other 2 subtypes [58]. Based on the hypothesis that DNA hypomethylation may play a role in promoting chromosomal instability, this may be related to high levels of CNAs [32,58-60]. This subtype was strongly correlated with the intestinal type of Lauren histology and low tumor grade [32,58].
The metabolic subtype showed increased activity in pathways associated with spasomolytic-polypeptide-expressing metaplasia, which has been suggested to be an intermediate step in GC tumorigenesis [58]. GC cell lines of the metabolic subtype are more sensitive to 5-fluorouracil (5-FU) than GC cell lines of other subtypes [58]. In the Singaporean and Australian cohorts, patients with the metabolic subtype treated with 5-FU displayed improved survival compared to patients who received surgery alone [58]. This sensitivity may be related to the lower levels of thymidylate synthase and dihydropyrimidine dehydrogenase in the metabolic subtype compared to that in other subtypes [32].

However, no significant differences in cancer-specific survival or disease-free survival, tumor, node, metastasis (TNM) stage, or tumor size were observed among the three subtypes [58]. Interestingly, Singaporean patients with the proliferative subtype displayed worse disease-free survival in multivariate analysis, whereas Australian patients did not [58].

**Simplified algorithm using IHC and ISH assays**

Advanced methods and high-throughput technology are required to classify the molecular subtypes of GC; however, these methods are not effective or cost-effective in daily routine diagnostic practice [32]. In routine pathological examination, IHC staining of MMR proteins, p53, and E-cadherin combined with EBER ISH may be helpful in categorizing molecular subtypes of GC [61-63]. This algorithm divides GC into 5 subgroups according to EBV status, deficient mismatch repair (dMMR), and aberrant E-cadherin and p53 expression [61,63]. Aberrant E-cadherin and p53 expression is observed when there is a complete loss of cytoplasmic/granular staining and complete loss of diffuse and strong staining, respectively [61].

A previous study analyzed 146 GC cases from a Western cohort and reported that the proportions of GC cases with EBER positivity, dMMR, aberrant expression of E-cadherin, and aberrant expression of p53 were 5%, 16%, 21%, and 51%, respectively [61]. In another study using the same algorithm, 349 GC cases were analyzed from an Asian cohort, and the proportions of GC cases with EBER positivity, dMMR, aberrant expression of E-cadherin, and aberrant expression of p53 were 7.4%, 6.9%, 15.2%, and 50%, respectively [63]. Interestingly, the proportion of GC cases with dMMR (Western, 16% vs. Asian, 6.9%) and normal p53 expression (Western, 7% vs. Asian, 21.4%) differed significantly between cohorts, which may be due to geographical and ethnic differences [61,63]. Unlike the study conducted in the Western cohort using 4 MMR proteins (MLH1, PMS1 homolog 2, mismatch repair system component [PMS2], mutS homolog 2 [MSH2], and mutS homolog 6 [MSH6]), the study conducted in an Asian cohort only confirmed MLH1 protein expression [61,63]. However, both studies used tissue microarrays, which consider the intra-patient heterogeneity of biomarker expression in GC [63].

The clinical and molecular characteristics of each subtype are summarized in Table 2. GC with aberrant p53 expression was associated with the CIN subtype of TCGA, MSS/TP53+ subtype of ACRG, and proliferative subtype of Singapore-Duke classification [61,63]. GC with normal p53 expression was correlated with the MSS/EMT subtype of ACRG and metabolic subtype of Singapore-Duke classification [61,63]. As expected, GC with aberrant E-cadherin expression was linked to the GS subtype of TCGA, MSS/EMT subtype of ACRG, and mesenchymal subtype of Singapore-Duke classification [61,63]. However, these subtypes are not perfectly matched; therefore, a suitable reference is recommended.
Table 2. Clinical and molecular characteristics of a protein and mRNA expression-based classification [61,63]

| Associated histology | EBV tumors | MSI tumors | EMT tumors | Aberrant p53 expression tumors | Normal p53 expression tumors |
|----------------------|------------|------------|------------|-------------------------------|-----------------------------|
| Prognosis            | Best prognosis | Better prognosis | Worst prognosis | Intermediate prognosis | Intermediate prognosis |
| Molecular features   |  • PIK3CA, ARID1A, and BCOR mutations |  • MLH1 promoter hypermutation |  • CDH1 and RH0A mutations |  • High TP53 mutation |  • Intermediate level of mutations (APC, KRAS, ARID1, PI3K, and SAMD4) |
|                      |  • CDKN2A (p16) promoter hypermethylation |  • Hyper-mutation (occasional mutations in PIK3CA, ERBB2, ERBB3, and EGFR) |  • Low number of mutations |  |  |
| Characteristic findings |  • Male predominance |  • Old age |  • Young age |  • Higher N category |  • MUC6 over-expression |
|                      |  • Body location |  • Distal location |  • Higher T and N category |  |  |

Comparison with TCGA classification

| EBV | MSI | GS | CIN | None |

Comparison with ACRG classification

| None | MSI | MSS/EMT | MSS/TP53 | MSS/TP53 |

EBV = Epstein-Barr virus; MSI = microsatellite instability; EMT = epithelial-mesenchymal transition; T = tumor; N = node; TCGA = The Cancer Genome Atlas; GS = genomically stable; CIN = chromosomal instability; ACRG = Asian Cancer Research Group; MSS = microsatellite stable; TP53 = tumor protein p53.

Molecular Targeted Therapies for GC

The NCCN Guidelines for Gastric Cancer Version 2.2022 state that IHC and/or molecular testing for HER2/ERBB2 status, MSI or MMR status, PD-L1 expression, tumor mutation burden-high (TMB-H) status, and neurotrophic tropomyosin-related kinase (NTRK) gene fusions are involved in the clinical management of advanced GC [64]. The recent development and introduction of ICIs to cancer patients has greatly influenced the therapeutic landscape of malignant tumors [65-68]. Programmed death-1 receptor (PD-1) inhibitors (such as pembrolizumab and nivolumab), PD-L1 inhibitors (including durvalumab), and CTLA-4 inhibitors (including ipilimumab) have been approved for certain cancers [67,69]. Key factors including MSI, PD-L1 expression, and tumor mutational burden affect the treatment response to ICIs [33,70,71]. However, the relationship between these biomarkers is complex, and it remains unclear whether using a combination of biomarkers is better than relying on a single marker [72-75]. In a recent clinical trial (NCT02589496), patients with EBV-positive metastatic GC showed dramatic response rates to pembrolizumab, providing a clinical evidence of potential sensitivity to ICIs [76].

HER2-targeted therapy

HER2 is a member of the EGFR family of tyrosine kinases, which are involved in cell proliferation, differentiation, apoptosis, adhesion, and migration [77-80]. HER2 overexpression/HER2 amplification is found in a subset of cancers, including breast cancer [81], GC [82], and colorectal cancer [83]. Although HER2 overexpression/HER2 amplification are associated with aggressive disease and poor prognosis in breast cancer [84,85], their prognostic value in GC is controversial [79,80,86-95]. The overall frequency of HER2 overexpression/HER2 amplification ranged from 6% to 35% in GC [79,80,90,96,97], with a relatively higher frequency in GEJ cancer than in GC, in Lauren intestinal-type than in diffuse or mixed type, and in tumors with moderate differentiation than in tumors with poor differentiation [64,79,80,90,98,99]. Trastuzumab plus chemotherapy is the first-line therapy for HER2-amplified GC [4]. However, GC did not respond as well to HER2-targeted therapies as did HER2-amplified breast cancer [100-103]. Heterologous HER2 expression as
well as high levels of intra- and intertumoral heterogeneity in GC can contribute to intrinsic and acquired resistance to HER2-targeted agents [96,104,105]. Unfortunately, intratumor heterogeneity of HER2 expression is estimated to be present in up to 30% of HER2-positive GC cases [99,106-108]. To overcome this resistance, combinations of anti-HER2 antibodies with other drugs with various mechanisms of action have been developed [109-111]. Recently, pembrolizumab plus trastuzumab and chemotherapy have been added as first-line therapies for HER2-amplified GC, given the synergistic antitumor effect of ICIs and HER2-targeted therapy [112]. Trastuzumab deruxtecan, an antibody-drug conjugate composed of anti-HER2 antibody linked to a topoisomerase I inhibitor, can be used as third-line therapy for patients who previously received HER2-targeted therapy [113]. Deruxtecan, a cytotoxic topoisomerase I inhibitor, acts by killing the surrounding tumor cells (bystander antitumor effect) and may be effective for GC patients with heterogeneous or low levels of HER2 expression [114].

HER2 positivity was defined as IHC 3+ or IHC 2+ and ISH positivity (Tables 3 and 4, Fig. 1) [115,116]. HER2 testing is recommended for all patients with advanced, recurrent, or metastatic GC or GEJ cancer (G/GEJ cancer) [64,108,115]. HER2 IHC is recommended as the initial test [64,108,115]. HER2 IHC was scored using a 4-tiered system based on an evaluation area cutoff of ≥10% stained tumor cells for resection specimens and a small single cluster consisting of ≥5 tumor cells, irrespective of the percentage of tumor cells stained for biopsy specimens, and interpreted as follows [96]: negative (0 or 1+), equivocal (2+), or positive (3+). Only membranous staining and not nuclear or cytoplasmic staining was counted for scoring. In breast cancer, only a complete membranous staining pattern indicates positivity [117], but in GC, incomplete, basolateral, or lateral membranous staining patterns are often observed and indicative of positivity, as well as complete membranous staining [96,108,115]. As such, there are inherent differences in HER2 expression between breast cancer and G/GEJ cancer; therefore, gastroenterologists and gastrointestinal pathologists should always be aware of this and should not apply the scoring criteria for breast cancer [79]. Additionally, it is important to consider the difference in scoring criteria according to the GC specimen type (surgically resected versus biopsy specimen) [79,96,115]. Although this 4-tiered scoring system was observed to be reproducible among different pathologists in a validation study [118], occasionally, the assessment of staining intensity may lead to inter- and intra-observer variability [119,120].

**Table 3.** HER2 scoring criteria for gastric cancer using IHC assays [96,115]

| IHC score | Staining pattern | Interpretation |
|-----------|------------------|---------------|
| 0         | Membranous staining in ≤10% of tumor cells (surgical specimen) or no membranous staining in any of tumor cells (biopsy specimen) | Negative |
| 1+        | Faint/barely perceptible membranous staining (reactive only in part of membrane) in ≥10% of tumor cells (surgical specimen) or tumor cell cluster irrespective of the percentage of tumor cells stained (biopsy specimen) | Negative |
| 2+        | Weak to moderate complete, basolateral, or lateral membranous staining in ≥10% of tumor cells (surgical specimen) or tumor cell cluster irrespective of the percentage of tumor cells stained (biopsy specimen) | Equivocal; proceed to ISH |
| 3+        | Strong complete, basolateral, or lateral membranous staining in ≥10% of tumor cells (surgical specimen) or tumor cell cluster irrespective of the percentage of tumor cells stained (biopsy specimen) | Positive |

**Table 4.** HER2 scoring criteria for gastric cancer using ISH assays [96,115]

| HER2 ISH result | ISH scoring criteria (at least 20 tumor cell nuclei should be counted) |
|-----------------|---------------------------------------------------------------------|
| Negative        | HER2/CEP17 <2.0 or <4 HER2 signals when ≥3 CEP17 signals and HER2/CEP17 <2.0 |
| Equivocal       | 4 to 6 HER2 signals with ≥3 CEP17 signals and HER2/CEP17 <2.0. Another 20 cancer cells should be counted. |
| Positive        | HER2/CEP17 ≥2.0 or ≥6 HER2 signals when ≥3 CEP17 signals and HER2/CEP17 ≥2.0 |

HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemical; ISH = in situ hybridization.

*Tumor clusters were defined as ≥5 cancer cells.

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Cases with IHC 2+ or indeterminate results of any cause should be confirmed using ISH (fluorescence ISH [FISH] or silver ISH [SISH]) to determine the final HER2 status [64,96,115]. In clinical practice, SISH is preferred over FISH for assessing HER2 IHC 2+ samples, as it allows the histologic evaluation of tumors, easily identifies HER2-positive tumors using light microscopy while considering marked intratumoral heterogeneity, leaves permanent signals for storage, and can be completely automated [108,116,117].

FISH/SISH results were assessed using the ratio of the number of HER2 signals to that of the centromere of chromosome 17 signals (CEP17) within the nucleus counted in at least 20 non-overlapping cancer cells (HER2:CEP17). Sometimes, cases with three or more signals of CEP17 in tumor cells on average are observed, which are usually due to the segmental duplication of CEP17 (or referred to as “polysomy”) [115]. Alternatively, FISH/ISH results can be presented as the average ERBB2 copy number per cell [64]. Therefore, a HER2:CEP17 ratio of ≥2.0, or average HER2 copy number ≥6 signals/cell was considered positive for an ISH test (HER2 amplification), despite a HER2:CEP17 ratio <2, whereas HER2:CEP17 ratio <2.0, or average HER2 copy number <4 signals/cell was considered negative [64,115]. When cases are observed with 4–6 HER2 signals on average, another 20 tumor cells should be counted in a different target area [115]. Notably, the discordant HER2 status between primary lesions and synchronous or metachronous locoregional/distant metastatic lesions is also observed owing to intertumoral heterogeneity in GC, which ranges from 2% to 14% [121-126]. Repeat HER2 testing may be considered at the initial diagnosis and in the diagnosis of recurring or metastatic GC [64,108].

Recently, NGS has been considered instead of sequential testing for a single biomarker if limited diagnostic tissue is available, or if the patient is unable to undergo a traditional biopsy [64]. The use of IHC/ISH should be considered first, and NGS should be performed where appropriate [64].
**MSI or MMR**

MSI is a hypermutable phenotype characterized by the accumulation of mutations in monomorphic microsatellites (short tandem repeats) caused by the loss of DNA MMR activity [32,72]. DNA MMR is a highly conserved mechanism designed to recognize and replace/repair mismatched nucleotides during DNA replication [72]. Repetitive DNA sequences are prone to spontaneous DNA insertion and deletion mutations [70]. Impairments in MMR proteins frequently introduce frameshift-inactivating mutations [127]. Although MSI is the hallmark of Lynch syndrome and constitutional MMR deficiency syndrome, it is found in various sporadic cancers [70,72,128]. Approximately 6.9%–22.7% of sporadic GC cases display MSI-H depending on the detection method and ethnicity [16,57,61,63]. Of note, MSI-H GC with Lynch syndrome constitutes 6%–13% of GC cases [129,130], thereby warranting familial surveillance. As mentioned earlier, GC with MSI-H is a distinct molecular classified subtype and has specific clinical characteristics, such as higher morbidity in females, occurrence at a relatively older age, located in the antrum (distal) of the stomach, significant association with the intestinal type of Lauren histology, early disease stage, and favorable prognosis [16,61,63,131,132]. MSI-H is associated with poor therapeutic response to 5-FU-based cytotoxic chemotherapy, whereas MSI-H is considered a predictive biomarker of benefit from immunotherapy [39,76,133-139]. In particular, post hoc analysis of the phase 2 KEYNOTE-059 and phase 3 KEYNOTE-061 and KEYNOTE-062 randomized trials suggested that MSI-H may be a biomarker for pembrolizumab therapy in patients with advanced G/GEJ cancer, regardless of the line and therapy received [140].

The NCCN Guidelines for Gastric Cancer Version 2.2022 state that universal testing for MSI using polymerase chain reaction (PCR)/NGS or MMR by IHC analysis should be performed for all patients with newly diagnosed GC [64]. PCR, IHC, and NGS analyses have been widely used [70,72,108,141,142]. A recently developed peptide nucleic acid probe-mediated real-time PCR-based method using 5 quasi-monomorphic mononucleotide repeat markers was used [143]. Surprisingly, artificial intelligence has been employed in combination with whole-slide imaging techniques, allowing the prediction of MSI status by deep machine learning based on hematoxylin and eosin-stained slides [144-146]. However, artificial intelligence technology is expensive, requires specialized equipment, and has a low sensitivity.

IHC analysis directly evaluates the nuclear expression of 4 MMR proteins (MLH1, PMS2, MSH2, and MSH6) involved in MMR. (Fig. 2) [64,147]. This method showed a high concordance rate (>90%), similar to MSI detection using PCR [148,149]. If all 4 of these proteins are expressed, it means that the MMR process is intact (proficient MMR [pMMR]) [108,133]. However, the loss of expression of any of these four proteins indicates dMMR [108,133]. Generally, dMMR is considered equivalent to MSI-H. Mutations in MLH1 and MSH2 result in the subsequent proteolytic degradation of the mutated protein and their respective dimeric partners, PMS2 and MSH6, respectively [72]. Conversely, mutations in PMS2 and MSH6 may not result in proteolysis of their primary partners [72]. For this reason, loss of MLH1 expression is accompanied by loss of PMS2 expression, whereas loss of MSH2 expression accompanies loss of MSH6 expression. However, the reverse is not true [127,133]. In more than 90% of MSI-GC cases, loss of MLH1 and/or PMS2 by hypermethylation of the MLH1 gene has been observed [61,63]. Notably, in IHC testing, IHC staining results may be misinterpreted owing to technical or biological reasons and may present false information that does not reflect the true MMR state [72]. In the past, when interpreting MMR IHC test results, loss of expression was regarded as a complete absence (or less than 1%) of tumor cells [150]. However, occasionally, heterogeneous IHC staining results showing mixtures of
weakly stained/non-stained and strongly stained areas within the same tumor can be found [151-153]. Furthermore, aberrant cytoplasmic or membranous staining has been observed [148,154,155]. Therefore, interpretation of IHC staining results is difficult.

PCR compares the allelic position of the microsatellite locus in tumors with that in normal tissue [70,108,133,156]. Fluorescence multiplex PCR and capillary electrophoresis were used to evaluate MSI status by measuring the gene expression levels of microsatellite markers after fluorescence-labeled PCR amplification [64,70]. The National Cancer Institute panel consists of two mononucleotide repeat loci (BAT-25 and BAT-26) and three dinucleotide repeat loci (D2S123, D5S346, and D17S250) [157,158]. As a newly developed alternative panel, the Pentaplex panel (Promega, Madison, WI, USA), which includes quasi-monomonucleotide markers (BAT-25, BAT-26, NR-21, NR-27 [or Mono-27], and NR-24), can detect MSI-H without control DNA samples [159]. If the tumor showed instability at two or more of these markers compared to the paired normal sample, it was termed MSI-H (Fig. 3). In current clinical practice, MSS and MSI-low tend to be regarded as one subtype, based on several clinical studies [160,161].

Finally, NGS interrogates nearly 100 microsatellite loci to determine MSI status [162]. Compared to PCR-based tests, NGS methods do not require corresponding normal controls and can also be used to determine tumor mutation burden (TMB; also referred to as tumor mutational load) and identify other potentially targetable alterations suitable for personal and precision therapy [72]. Although the sensitivity and specificity of NGS methods are reportedly very high (>95%) [163-165], they require stringent DNA quality and lack standard and validated parameters for detecting dMMR/MSI in tumors.
Each detection method has its own advantages and disadvantages. Furthermore, PCR/NGS analysis of MSI and IHC analysis of MMR proteins measure different biological effects related to dMMR function [64]. For example, IHC test can yield false-negative results if missense mutations in the MMR gene cause dMMR [166]. Moreover, MSI-H tumors caused by a defective $MSH6$ gene can be interpreted as MSS in PCR analysis [167,168]. Furthermore, PCR analysis may be compromised by sample confusion [158]. Taken together, co-testing using different MSI detection methods increases sensitivity and improves patient response to biomarker-guided therapy [169,170].

**PD-L1**

Tumor cells can evade immune surveillance via various mechanisms [171-173]. PD-L1, a transmembrane protein encoded by $CD274$, is upregulated in tumor cells and binds to PD-1, a co-inhibitory receptor expressed on the cytotoxic cluster of differentiation 8 (CD8)-positive T cells [174]. The PD-1/PD-L1 interaction is one of the major mechanisms of immune modulation, which inhibits T-cell function by inducing T-cell exhaustion to promote immune evasion [33]. Cancer cells use this brake system to avoid apoptotic effects induced by T cells [175]. PD-L1 overexpression induces an immunosuppressive tumor microenvironment in several cancers [175]. PD-1/PD-L1 blocking agents successfully restore the immune function of T cells [176-178]. The NCCN Guidelines for Gastric Cancer Version 2.2022 suggest that PD-L1 testing may be considered for locally advanced, recurrent, or metastatic GC in patients receiving PD-1 inhibitor treatment [64]. PD-L1 expression in tumor cells and surrounding immune cells can be quantified and used to predict the response of cancer patients to ICIs. Four FDA-registered PD-L1 antibodies (PD-L1 IHC 22C3 pharmDx, PD-L1 IHC 28-8 pharmDx,
VENTANA PD-L1 [SP263], and VENTANA PD-L1 [SP142]) were used in IHC analyses of various solid tumors. These antibodies are available as pre-packaged kits for use on approved platforms [179]. The NCCN Guidelines for Gastric Cancer Version 2.2022 mentioned that a US FDA-approved companion diagnostic test, which provides the required and essential information necessary for safe and effective use of the corresponding drug, should be used to identify patients for treatment with PD-1 inhibitors [64]. This companion diagnostic test is a qualitative IHC assay using an anti-PD-L1 antibody to detect PD-L1 protein levels in formalin-fixed, paraffin-embedded tumor tissues [64]. FDA-approved pembrolizumab, a third-line therapy in patients with G/GEJ cancer, displayed a combined proportion score (CPS) of ≥1 using the PD-L1 IHC 22C3 pharmDx assay on the Autostainer Link 48 platform based on the findings of the phase 2 KEYNOTE-059 trial [180]. Recently, nivolumab plus chemotherapy was also approved as a first-line therapy for G/GEJ cancers with CPS ≥5 using the PD-L1 IHC 28-8 pharmDx assay on the Autostainer Link 48 platform based on the findings of the phase 3 CheckMate-649 [181]. CPS, one of the two scores to identify PD-L1 expression, is measured as the number of PD-L1 stained cells (including tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells and then multiplied by 100 (Fig. 4) [182,183]. Samples containing at least 100 viable tumor cells were required for accurate evaluation, and if the calculation result exceeded 100, the maximum score was considered CPS 100. In some trials on other solid tumors, such as metastatic non-small cell lung cancers and melanomas, a tumor proportion score (TPS) has been reported and considered [64,65,184]. The TPS method counts the percentage of PD-L1 expressing tumor cells among total tumor cells [185,186]. However, as TPS does not include tumor-infiltrating immune cells when calculating the score, it may not be efficient in identifying ICI responders [179]. Pre-analytical issues such as tissue fixation and processing significantly affect the outcome of IHC reactions and may lead to erroneous PD-L1 IHC test results [179,187,188]. In addition, manual scoring of PD-L1 immunostained slides by a pathologist may introduce potential errors in reproducibility [179]. Several recent studies have demonstrated that automated digital image analysis provides accuracy and consistency comparable to manual scoring of PD-L1 expression [179,189-191] in various solid tumors.

Fig. 4. PD-L1 immunohistochemical staining using 22C3 anti-PD-L1 antibodies. Almost all tumor cells demonstrated membranous or granular membranous staining, and the recruited immune cells (lymphocytes and macrophages) displayed both cytoplasmic and membranous staining. A combined proportion score of 70 was assigned to this field. Original magnification: ×200.

PD-L1 = programmed death ligand 1.
**TMB**

TMB, usually measured by the total number of somatic coding mutations per megabase (Mb) of genome sequenced [192], has been actively investigated to predict ICI responses [193]. Whereas whole exome sequencing is considered optimal for estimating TMB, several commercially available targeted NGS platforms also provide TMB values, showing a good correlation with TMB values from whole exome sequencing [71]. Targeted gene panels larger than up to 1 Mb of the coding genome are generally recommended for the enumeration [71,193]. However, the adoption of TMB as a biomarker in current clinical practice are faced with major limitations, namely the lack of harmonization in panel-based TMB quantification, adequate methods to transform TMB estimates across different panels, and robust predictive cutoffs [193]. The FDA approved pembrolizumab for patients with metastatic solid tumors with TMB-H (≥10 mutations/Mb) who had progressed after previous treatment and had no satisfactory alternative treatment options based on the KEYNOTE-158 trial [64,194]. In the exploratory analysis of the KEYNOTE-062 trial, the association between TMB and clinical efficacy of first-line pembrolizumab-based therapy was demonstrated in patients with advanced G/GEJ cancer, but the clinical usefulness of TMB weakened after the exclusion of MSI-H [195].

**NTRK gene fusion**

Fusions of NTRK genes (NTRK1, NTRK2, and NTRK3) induce the expression of constitutively active chimeric TRK proteins (TRKA, TRKB, and TRKC), which serve as potential oncogenic drivers across a range of tumor types [196-198]. Fusion of NTRK to an unrelated gene results in the overexpression and permanent activation of the TRK fusion protein [199,200]. As transmembrane proteins, these kinases have a ligand-dependent function by transducing extracellular signals to the nucleus to activate cell growth, proliferation, and survival pathways, such as the mitogen-activated protein kinase/extracellular signal-regulated kinase and PI3K/AKT pathways [199-201]. Although NTRK fusions are frequently reported in secretory breast carcinoma, mammary analog secretory carcinoma, and congenital mesoblastic nephroma, they occur at a very low frequency in common solid cancers [196,202-204]. In GC, NTRK fusions are extremely rare, but may be associated with an aggressive phenotype [205-207]. Larotrectinib and entrectinib—TRK inhibitors—demonstrated excellent responses to NTRK-fusion tumors regardless of the tumor type (“tumor-agnostic”) [202,203,208,209]. Based on this, the FDA recently approved select TRK inhibitors, larotrectinib and entrectinib, for the treatment of NTRK fusion-positive solid tumors [64,210]. The NCCN Guidelines for Gastric Cancer V.2.2022 recommend entrectinib and larotrectinib as second-line or subsequent therapies for NTRK fusion-positive tumors [64]. Therefore, NTRK fusions must be identified during clinical practice of advanced GC. As a screening tool, pan-TRK IHC can be used first, and targeted reverse transcription PCR (RT-PCR) and/or RNA-based NGS are considered diagnostic methods for detecting NTRK fusions [208,211]. Although pan-TRK IHC can detect TRK proteins A, B, and C, this assay was not optimized to differentiate between wild-type TRK and chimeric fusion proteins. This is because protein expression may not result from gene fusion events [212]. FISH may have limited utility in elucidating NTRK fusions because it is not designed for multiplexing. To comprehensively evaluate the 3 NTRK fusions, three separate FISH tests are required unless multicolor approaches are developed [213]. RT-PCR is designed to detect only known translocation partners and breakpoints, and not new breakpoints or fusion partners [213]. Taken together, NGS may be the most promising tool for investigating NTRK fusions, along with other possibly targetable alterations, using minimal samples.
EBV
EBV, a herpesvirus formally called human gammaherpesvirus 4, is a double-stranded DNA virus that infects B lymphocytes [214]. It is a ubiquitous virus that infects more than 90% of the adults worldwide in their life-time [214,215]. EBV leads to infectious mononucleosis in adolescents and young adults [215] and causes neoplasms of various cell origins, such as B-cell, NK/T cells, epithelial cells, and mesenchymal cells [216,217]. EBVaGCs account for approximately 5%-40% of GC cases worldwide, with variable frequencies between geographic regions [16,20,61,63,216,218]. EBVaGCs have distinct clinicopathological features and molecular characteristics and are one of the TCGA subtypes. The clinical characteristics of EBVaGCs are as follows: proximal locations and remnant stomach, early disease stage, and favorable prognosis [20,54,132,219]. Recent retrospective studies have revealed that the frequency of lymph node metastasis is very low in submucosal invasive EBVaGCs (T1b category of TNM stage) [220,221]. Prominent infiltration of lymphocytes is considered a typical histologic feature of EBVaGCs (Fig. 5A). GC with similar histologic patterns is ‘gastric carcinoma with lymphoid stroma (GCLS)’ of the WHO classification, which has been referred to as lymphoepithelioma-like carcinoma or medullary carcinoma [216,222]. GCLS is significantly associated with EBV infection, which is identified in 20%-90% of GCLS cases [216,222-225]. However, GCLS is also observed in MSI GC, and EBV and MSI are mutually exclusive in GC owing to mutually exclusive methylation in the MLH1 promoter [222,226]. Interestingly, other histologic patterns in EBVaGCs have been reported, including conventional intestinal-type adenocarcinoma-like [227] and Crohn's disease-like [227], or rarely, chronic granulomatous inflammation-like [228] and signet ring-cell carcinoma-like patterns [229,230]. EBV can be detected using several methods, such as Southern blotting, IHC staining, western blotting, PCR, and EBER ISH assay [108,231-233]. In GC, the EBER ISH assay is the most widely used method and is regarded as the gold standard for detecting and localizing latent EBV in tissue samples (Fig. 5B) [108,132]. EBER1 and 2 are produced in large amounts in the nucleus of each EBV-infected cell, that is, 106-107 copies [216]. The EBER ISH assay is a highly sensitive detection method and provides relatively stable results even in formalin-fixed paraffin-embedded tissue blocks; however, quantitative analysis of viral particles cannot be performed. Recently, droplet digital PCR has been used to quantitatively detect EBV with high sensitivity [234].

Upcoming biomarker-targeted therapies
There are several ongoing clinical trials on GC harboring potential targetable alterations. As mentioned earlier, oncogenic PIK3CA mutations in breast cancer are an indication for alpelisib plus fulvestrant treatment in advanced or metastatic breast cancer [29]. PIK3CA

Fig. 5. Histologic appearance of EBV-associated gastric cancer (A) and EBV-encoded small RNA ISH (B). EBV-infected carcinoma cells exhibited nuclear dark blue staining. Original magnification: ×40. EBV = Epstein-Barr virus; ISH = in situ hybridization.
mutations, which are found in GC, with higher frequencies in EBV-positive and MSI-H GC of TCGA subtypes, may serve as potential therapeutic targets [16]. Interestingly, PIK3CA mutations are mostly hot-spot mutations located in exon 9 (E542K and E545K) and exon 20 (H1047R) in various solid tumors, but PIK3CA mutations are more dispersed in EBV-positive GC [16,25]. Although no relationship between PIK3CA mutations and patient outcomes has been found in GC [235-237], it has been reported that activation of the PI3K/AKT/mTOR signaling pathway may have a discriminatory negative effect on OS and progression-free survival in advanced GC treated with trastuzumab-based chemotherapy [238]. Other studies have suggested that PIK3CA mutations in exon 9 may be associated with worse prognosis than PIK3CA mutations in exon 20 in MSI-H GC and EBV-positive GC [25,239]. Unfortunately, in randomized phase III trials (RADPAC trial; NCT01248403), everolimus in combination with paclitaxel did not lead to improved outcomes in pretreated metastatic G/GEJ cancer [240]. Additional studies on this biomarker are currently underway.

Because they showed disappointing results for outcomes in several clinical trials [32,241,242], protein overexpression and/or amplification of RTKs, such as FGFR2, EGFR, and MET, have not received much interest recently, but may still be investigated as candidates for biomarker-guided therapy [242-244]. In particular, monotherapy using bemarituzumab, which is a fucosylated monoclonal antibody against the FGFR2b receptor, has demonstrated clinical activity in patients with late-line G/GEJ cancer with FGFR2b overexpression and/or FGFR2 amplification [245,246]. FGFR2 amplification occurs in approximately 4%–10% of GC cases with both GS and CIN of TCGA subtypes [16,245,247-250]. FGFR2 amplification tends to be related to advanced stage, lymphatic invasion, and worse prognosis [247,250], suggesting that FGFR2 inhibition may be an important therapeutic strategy [245]. In tumors with FGFR2 amplification, the FGFR2B splice variant is almost always expressed on the cell surface [245].

Because some GCs secrete angiogenic factors and display increased angiogenesis, inhibiting angiogenic growth factors may provide an avenue for future therapeutic targets [251,252]. Treatments targeting using claudin 18.2 (CLDN18.2), which is almost always expressed in GS GC harboring CLDN18 the ARHGAP26/6 fusion [253], are being investigated [254].

Poly (ADP-ribose) polymerase (PARP) inhibitors are emerging as key therapeutic options for certain cancers with BRCA1/2 mutations, homologous recombination-related genes, or homologous recombination deficiency (HRD) [255]. To date, the clinical relevance of HRD in GC remains unclear. HRD-related GC is found in approximately 10% of GC cases. In a randomized phase II clinical trial (NCT01063517), olaparib, an oral PARP inhibitor, in combination with paclitaxel, showed activity in the treatment of metastatic GC, resulting in greater OS in patients with GC lacking ATM mutations [256]. However, in a randomized phase III clinical trial (NCT01924533), olaparib/paclitaxel treatment did not result in significant improvements in OS in an entire ATM-negative cohort of Asian patients with advanced GC [257].

**CONCLUSION**

Here, we review the molecular classification and biomarker-targeted therapies for GC. Currently used molecular classifications have some limitations associated with molecular techniques, ethnicity, and tumor heterogeneity. Moreover, there are limited options of molecular-targeted therapies for GC, including Herceptin and trastuzumab deruxtecan (for
HER2-positive GC) and ICIs (for MSI-H, PD-L1 high-expression, and TMB-high GC along with EBVaGC). In addition, the application of new techniques, such as circulating tumor DNA sequencing [258] and single-cell RNA sequencing [259], in combination with deep learning approaches [260], will expand our knowledge regarding the molecular heterogeneity, resistance mechanisms, and complex tumor microenvironment of GC. These applications will lead to a better molecular classification of GC as well as targeted therapies in the era of precision medicine.

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