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REVIEW

Recent Advances in Systemic Treatments for HER-2 Positive Advanced Gastric Cancer

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Abstract: Gastric cancer (GC) is the fifth most common cancer worldwide. Despite recent improvements in treatment quality and options, advanced gastric cancer remains one of the hardest to cure cancers, with a median overall survival (OS) of 10–12 months and a 5-year OS of approximately 5–20%. There is an unmet need for further efforts to palliate disease-related symptoms, improve quality of life, increase tumor response rate, and prolong progression free and overall survival while balancing the toxicities of therapy. The most common type of GC is adenocarcinoma, which demonstrates morphological, biological, and clinical heterogeneity. A plethora of genomic alterations and the activation of numerous molecular pathways including human epidermal growth receptor 2 (HER2), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor-2 (FGFR2), mesenchymal epidermal transforming factor receptor (MET), and the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) are responsible for the complex heterogeneity of GC. Efforts to validate the therapeutic effects of inhibiting some of these aberrantly expressed pathways have failed to lead to a clinically meaningful outcome apart from the overexpression/amplification of the HER2 gene, inhibition of which has had a significant impact on clinical practice. The only available biomarkers to guide the effective treatment of patients with advanced GC are HER2 overexpression, MSI/PD-L1 status, and FGFR alterations. Various anti-HER2 agents have been evaluated after the success of the ToGA trial, but none led to a significant enough clinical improvement to be considered a viable alternative for HER2-targeted therapy in advanced GC until the global Keynote-811 trial, which added pembrolizumab to trastuzumab in combination with chemotherapy. This combination demonstrated a survival advantage for the first time in the 11 years since ToGA. Trastuzumab deruxtecan (T-DXd) was also found to be effective in patients who had already received >2 previous lines of treatment. Despite these promising avenues, the optimal management of HER-2 positive GC still requires further development.

Keywords: gastric cancer, HER-2, trastuzumab, targeted therapy

Introduction

Gastric cancer (GC) is the fifth most common cancer worldwide.[1] GC has a very heterogeneous morphologic, biologic and clinical nature. The most common type of GC is adenocarcinoma. Of the several morphology-based classification systems that have been proposed, the World Health Organization (WHO) (papillary, tubular, mucinous, and poorly cohesive (PCC-NOS)/signet ring)[2] the Lauren (intestinal, diffuse, and mixed) classifications are the most commonly preferred.[3] However, conventional morphology-based classification systems are unable to reflect the molecular heterogeneity of GC and are therefore not a sufficient guide for molecularly targeted treatments based on precision

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oncology results. A special molecular/genetic classification system can better define the genetic landscape of GC, and may be helpful to further understand, prevent, and treat GC based on the guidance of predictive biomarkers. To this end, a panel of GC cell lines was sequenced by a group of investigators and two major intrinsic subgroups of GC were identified.[4] These subtypes were similar to the Lauren's intestinal and diffuse subtypes, so they were named genomic intestinal (G-INT) and genomic diffuse (G-DIF). The characteristics of these intrinsic subgroups reflect the diverse biologic behavior of GC, and their association with patient survival and treatment benefits are shown in Table 1.[4,5]

Following the advent of more comprehensive genomic assays, a new molecular classification system for the molecular and genomic basis of GC was proposed in 2014 by The Cancer Genome Atlas (TCGA) Consortium. This system classified GC into four major genomic subtypes: tumors positive for Epstein-Barr virus (EBV), microsatellite unstable tumors (MSI), genomically stable (GS), and tumors with chromosomal instability (CIN).[6] For practical reason, more recently the Asian Cancer Research Group (ACRG) specifically divided GC into four subtypes: MSI, microsatellite stable (MSS)/epithelial–mesenchymal transition (EMT), MSS/tumor protein 53 (TP53) active, and MSS/TP53 inactive.[7] There are some differences between the two classification systems. The TCGA study evaluated 295 primary GCs from Europe and the United States, while the ACRG system examined 300 primary GCs from a single center in South Korea.[8] Next-generation sequencing (NGS) technology was used in the TCGA molecular classification, while immunohistochemistry (IHC) was used in the ACRG molecular classification to reduce costs and make it more practical.[8] ACGR subtypes had significant survival differences, unlike the TCGA subtypes. The clinical correlations of each of the four subtypes of GC as classified by the TCGA and the ACGR are shown in Table 2.[5,8,9]

Table 1 Intrinsic Gastric Cancer (GC) Subgroups Based on Gene Expression Pattern

gene expression analysis of 37 GC cell lines two major intrinsic subgroups were identified validated in primary tumors from 521 patients (from Singapore, Australia and South Korea) the intrinsic genomic subtypes have 64% similarity to Lauren's classification	
G-INT	**G-DIF**
Genes related to carbohydrate and protein metabolism (FUT2) and cell adhesion (LGALS4, CDH17) up-regulated	Cell proliferation (AURKB) and fatty acid metabolism (ELOVL5) functional annotations were enriched
G-INT tumors had superior overall survival compared with patients with G-DIF tumors	Worse survival outcomes
G-INT cell lines were significantly more sensitive to 5-FU and oxaliplatin in vitro More resistant to cisplatin	G-DIF cell lines were more sensitive to cisplatin

Abbreviations: G-INT, genomic intestinal; G-DIF, genomic diffuse.

Infectious agents, including the bacterium Helicobacter pylori and the Epstein-Barr virus (EBV), play an important role in GC. Infection has gradually been recognized as a major driver of inflammation-induced tumorigenesis and is thus a preventable cause of GC. Gastroesophageal reflux disease (GERD) is also considered an important risk factor for upper GC. Major processes implicated by frequently mutated genes associated with inflammation and cancer in three organs (stomach, colon, and liver) were extracted from molecular profiling databases by Guo et al, who established a multiscale model of the long-term evolutionary dynamics that lead from inflammation to tumorigenesis.[10] The authors suggested that their findings provided a method for quantifying cancer risk and for the discovery of pathways driving inflammation-induced tumorigenesis, which could be used in the early detection and prevention of GC and the development of new treatment regimes. Zhang et al performed a single-cell transcriptomic study on gastric antral biopsies from patients with a variety of premalignant gastric lesions (ie chronic atrophic gastritis and intestinal metaplasia) and early gastric cancer (EGC).[11] EGC is a lesion that is confined to the mucosa and submucosa regardless of lymph node involvement, and has a survival rate of >90% at 5 years. The authors proposed that since some genes were not expressed in premalignant lesions but were prominently expressed in early and advanced gastric cancer cells, they can be used as cancer cell-specific molecular markers and precisely correlate with the earliest stages of GC tumorigenesis.

In most cases, GC is diagnosed at the metastatic or unresectable stage. Despite the latest improvements in GC treatment options, advanced GC remains one of the hardest to cure cancers and has a poor prognosis, with a median overall survival (OS) of 10–12 months and a 5-year OS of approximately 5–20%.[12] There is therefore an unmet need for further efforts to palliate disease-related symptoms, improve patient quality of life, increase response rate and prolong progression free and overall survival while balancing the toxicities of therapy.

Table 2 Comparison of Each Four Subtypes of Gastric Cancer (GC) According to TCGA or ACGR

TCGA Subtypes	ACRG Subtypes
Tumor samples from 295 treatment-naive primary GC patients	mRNA expression level analyzed in 300 tumor samples
Whole-exome sequencing, copy number analysis, messenger ribonucleic acid sequencing, microRNA sequencing, DNA methylation profiling and reverse-phase protein array analyses were done	Molecular subtypes have distinct prognostic significance
EBV (+) GC This subgroup represents 9% of all tumors and located more frequently in the corpus and fundus Has significant CIMP phenotype Highest rate (80%) of *PIK3CA* mutations, *Increased rate of ARID1A* and *BCOR* mutations, Over-expressions of *PD-L1/2, JAK2*, and *ERBB2* CDKN2A silencing	**MSS/TP53+ GC** 26% of the total samples frequent mutations in *APC, ARID1A*, *KRAS, PIK3CA*, and *SMAD4* EBV infection is more frequent intermediate prognosis
Microsatellite instability (MSI) GC Constitutes 22% of total samples associated with *Helicobacter pylori* (HP) infection and intestinal metaplasia silencing of MLH1 gene, resulting in genomic instability TP53, KRAS, PIK3CA, EGFR, ERBB2 and ERBB3, ARID1A mutations can be seen a high rate of PD-L1expression a predilection for antral location diagnosed at older ages (median 72 years) more frequently seen in females (56%)	**MSI-high GC** 23% of total samples intestinal histology (> 60% Lauren intestinal type) diagnosed at early stages (>50%) *ARID1A* (44.2%), the PI3K-PTEN-mTOR pathway (42%), *KRAS* (23.3%), and *ALK* (16.3%) mutations **the best overall prognosis** (can be diagnosed at early stages) and lowest frequency of recurrence (22%)
Chromosomal instability (CIN) GC Represents 50% of the total samples Located at gastroesophageal junction or cardia (65%) Most commonly has intestinal histology Activation of receptor tyrosine kinases-Ras (RTK/RAS) pathway Amplifications of the genes *ERBB2, KRAS/NRAS, EGFR, ERBB3, FGFR2*, *MET* and genes encoding cell cycle mediators, such as cyclins E1, D1 (*CCNE1, CCND1*) and *CDK6* High frequency of *TP53* mutations (73%)	**MSS/TP53- GC** 36% of the total samples the highest frequency of *TP53* mutations (60%), frequent RHOA mutations *ERBB2, EGFR, CCNE1, CCND1, MDM2, ROBO2, GATA6* and *MYC* enriched
Genomically stable (GS) GC 20% of the tumours young patients (median 59 years) the diffuse histological variant is common cell adhesion and angiogenesis related pathways up-regulated: E-cadherin (*CDH1*) (with the highest percentage) rare TP53 mutations Ras homolog family member A (*RHOA*) recurrent mutations CLDN18-ARHGAP fusions	**MSS/EMT GC** %15 of the tumours diagnosed at younger age diffuse type histology (> 80%) and signet ring cell carcinomas a lower number of mutation events **the worst prognosis** and the highest recurrence frequency (63%) diagnosed at advanced stages (III/IV) loss of CDH1 expression is common frequent peritoneal seeding (64.1%)

Abbreviations: TCGA, the cancer genome atlas; ACGR, asian cancer research group; CIMP, CpG island methylator phenotype; PIK3CA, phosphatidylinositol 3-kinase; ARID1A, AT-rich interactive domain-containing protein 1A; BCOR, B-cell lymphoma 6 corepressor; ERBB2, Erb-B2 receptor tyrosine kinase 2; PD-L1/2, programmed death ligand-1/2; JAK2, Janus associated kinase 2; MSI, microsatellite instability; CIN, chromosomal instability; CDK6, cell division protein kinase 6.

Recent genome-scale sequencing studies have identified a plethora of genomic alterations and the activation of numerous molecular pathways in GC, including Human Epidermal Growth Receptor 2 (HER2), epidermal growth factor receptor (EGFR), fibroblast growth factor receptor-2 (FGFR2), mesenchymal epidermal transforming factor receptor (MET), the phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR), microsatellite instability (MSI), which are responsible for the complex heterogeneity of this disease. However, it is challenging to distinguish driver mutations from passenger mutations. Furthermore, hypermutated tumors (ie MSI or POLE mutant) more commonly include passenger mutations due to their inherent genetic instability.[13] At this point, it is important to determine if the detected mutations are directly druggable oncogenic targets. Efforts to validate the therapeutic effects of the inhibition of some altered pathways have failed to yield a clinically meaningful outcome except for the identification of the overexpression/amplification of HER2, from which HER2 inhibition has significantly impacted clinical practice. Ultimately, the biomarkers that are available to guide the effective treatment of patients with advanced GC are HER2 overexpression, MSI/PD-L1 status and FGFR alterations.

HER2 (also known as erythroblastosis oncogene B2, ERBB2) is a proto-oncogene that encodes the transmembrane receptor-like HER2 protein. Its abnormally induced tyrosine kinase activity initiates signaling pathways that lead to cell proliferation, differentiation, and vascular and lymphatic angiogenesis. The reported rates of HER2 amplification in patients with GC range from 7 to 34%.[14–16] HER2 positivity also varies by tumor location (GEJ>gastric body), histologic subtype (intestinal>diffuse) and tumor grade (moderately differentiated>poorly differentiated).[17] HER2 overexpression is associated with the CIN subgroup according to the TCGA classification and with the MSS/TP53 inactive subtype according to the ACRG categorization. Both molecular subgroups demonstrate widespread genomic instability, which could explain the significant number of copy variations in major oncogenic drivers such as HER2. Although HER2 overexpression was shown to be associated with a poor prognosis in the most current and comprehensive meta-analysis to investigate the correlation between clinicopathologic characteristics and the prognostic significance of HER2 expression in GC patients,[18] the overall body of the literature regarding the prognostic significance of HER2 status in GC remains unsettled.[19–21] Factors responsible for these inconsistencies include the use of different IHC staining methods, the lack of a uniform criteria for defining HER2 positivity, the potential influence of confounders in clinical studies (ie patient selection bias leading to unique study group characteristics compared with the general population or an insufficient observation period for calculating survival outcome) and the heterogeneous inherent characteristics of HER2 positive GC that may represent a specific intrinsic subtype that harbors unique genetic alterations. The design criteria of these studies are heterogeneous, and it should be kept in mind that most HER2-positive GC patients did not receive anti-HER2-directed treatment before the ToGA trial. The overall prognostic role of HER2 may also depend on tumor stage. The literature should therefore be interpreted with consideration of potential bias and the variation criteria for determining HER2 status.

The Fundamental Role of Trastuzumab in Advanced GC

A panel of murine monoclonal antibodies (mAb) capable of specifically inhibiting HER2-positive cell lines has been developed, of which muMAb 4D5 was the most potent. A humanized form of muMAb 4D5, trastuzumab has three times stronger binding affinity for HER2 than its parent and is a therapeutic mAb that targets the extracellular domain (ECD) of HER2.[22] Breast cancer xenograft experiments were the first to show that trastuzumab has anti-tumor activity. Several subsequent pivotal clinical trials revealed the tremendous impact of trastuzumab on the clinical management of HER2 positive breast cancer, and trastuzumab is now the standard of care at all treatment steps for these patients. After the identification of HER2 amplification in GC, the clinical efficacy of trastuzumab was evaluated in the treatment of advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma in the global, Phase 3 TOGA trial. Enrolled patients were randomized to receive either trastuzumab plus chemotherapy (fluoropyrimidine plus cisplatin) or chemotherapy alone as first-line therapy. The OS benefit of adding trastuzumab to frontline chemotherapy has been shown in patients with HER2-positive advanced GC. Post hoc exploratory analyses of the TOGA trial demonstrated a 4.2-month improvement in median OS with trastuzumab in patients who expressed high HER2 levels (2+) on IHC 2+ and fluorescence in situ hybridization (FISH) amplified HER2/CEP17 $\geq$ 2 or IHC 3+ (hazard ratio (HR) = 0.65) compared with those with a low expression of HER2 protein and an IHC of 0 or 1+ despite FISH positivity.[23] Given the

results of TOGA, assessment of tumor HER2 overexpression using IHC and FISH or some other in situ hybridization (ISH) method is recommended for all patients with inoperable locally advanced, recurrent, or metastatic gastric adenocarcinomas, and trastuzumab-containing regimens are now a standard option for the first-line treatment of patients with tumors that express HER2.

GC is a disease of aging, and there are several important and potentially life-threatening drawbacks to combination chemotherapy in the elderly population. In a meta-analysis including available data from 41 studies, HER2 positivity was not correlated with age.[18] Prior literature on this relationship is controversial.[24–26] Several early phase and retrospective studies evaluated the efficacy and tolerability of adding single-agent chemotherapy (ie lower dose capecitabine, cisplatin, or S-1) to trastuzumab in elderly patients with HER2-positive advanced gastric cancer (AGC). The results of these studies were comparable to the ToGA trial endpoints in terms of overall survival and response rates.[27–29] While a consensus has yet to be achieved, the general body of the literature suggests that trastuzumab enhances the antitumor activity of chemotherapy, and that the combination of single-agent chemotherapy and trastuzumab is a logical and safe option for elderly patients with HER2-positive AGC.

Failures of HER2-Targeted Therapy in Advanced GC

The Phase III HELOISE randomized study compared high dose (HD) trastuzumab plus chemotherapy with standard-of-care trastuzumab plus chemotherapy as first-line management of HER2-positive metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma to investigate if HD trastuzumab increases the serum trough concentration of trastuzumab and improves OS.[30] Although the serum trough concentration of trastuzumab was significantly increased in the HD trastuzumab group, no improvement in OS or progression-free survival (PFS) was observed.

Lapatinib, a dual EGFR and HER2 tyrosine kinase inhibitor, is approved as a second-line therapy for HER2-positive breast cancer. However, in the phase 3 LOGiC trial, the addition of lapatinib to capecitabine plus oxaliplatin as first-line therapy for HER2-positive advanced or metastatic esophageal, gastric or GEJ adenocarcinoma failed to achieve its primary OS endpoint despite a significant improvement in PFS and overall response rate (ORR).[31] Possible explanations for these negative results include the selection of patients with HER2 overexpression based on FISH alone that 23% of patients had a prior gastrectomy, which could reduce lapatinib absorption, and the lack of antibody-dependent, cell-mediated cytotoxicity in the absence of trastuzumab.

Pertuzumab, is a recombinant humanized mAb that inhibits HER2 heterodimerization with HER3. The HER2/HER3 signaling dimer has been shown to be the most potent.[32] Combining trastuzumab with pertuzumab is an effective strategy for patients with HER2+ breast cancer. Preclinical studies noted improved anti-tumor activity with dual HER2-blockade (pertuzumab and trastuzumab) compared with pertuzumab monotherapy in a xenograft model of HER2-positive GC.[33]

The JACOB trial evaluated the effectiveness of pertuzumab in combination with trastuzumab plus chemotherapy in patients with metastatic gastric or GEJ cancer. Although both PFS and ORR favored the pertuzumab-containing arm, no statistically significant difference was found in OS.[34] Given the effectiveness of trastuzumab in the treatment of breast cancer, the benefits of continuing anti-HER2 therapy after progression while on trastuzumab have been studied in patients with HER2-positive AGC, with heterogeneous results.[35–37] In the Phase 2 T-ACT trial, patients with advanced HER2+ gastroesophageal adenocarcinoma (GEA) were randomized to receive weekly paclitaxel alone or in combination with trastuzumab as second-line therapy.[38] Trastuzumab in combination with paclitaxel beyond progression failed to improve PFS (primary end point), and no beneficial biomarkers were identified. Tumor biopsy samples were tested for HER2 status both prior to enrollment and after disease progression in first-line trastuzumab-containing therapy, and it was found that HER2 amplification was lost in 11 of 16 patients (69%). Additionally, in a subgroup analysis of this study, patients with longer trastuzumab-free intervals (> 30 days) had a trend towards improved ORR, PFS, and OS in the paclitaxel plus trastuzumab group compared with paclitaxel alone.

Due to the unmet need for second-line treatment options in patients progressing on or after trastuzumab-based treatment, the benefits of adding HER2-targeted therapy as second-line therapy for advanced HER2-positive GC have been the subject of multiple clinical trials. Trastuzumab emtansine (T-DM1) was the first antibody–drug conjugate (ADC) to be composed of trastuzumab linked to emtansine, a tubulin inhibitor. Emtansine released into HER2-positive tumor cells causes mitotic arrest and apoptosis. T-DM1 has a role in the management of patients with both advanced and early stage HER2-positive breast cancer. The adaptive GATSBY phase II/III study assessed the role of

T-DM1 as second-line therapy for GC.[39] HER2+ GC patients (IHC3+ or IHC2+ and FISH+) received either docetaxel or T-DM1. Trastuzumab was administered in 79% and 76% of the patients who received taxane and T-DM1, respectively, as part of first-line therapy. This trial did not favor T-DM1 compared with taxane.

In the phase 3 TyTAN trial, lapatinib was used as second-line therapy for HER2-positive AGC in the Asian population. Although a minority (6%) of patients had previously received trastuzumab-containing therapy and the ORR was significantly higher with lapatinib plus paclitaxel versus paclitaxel alone, no OS or PFS benefit was derived from lapatinib treatment.[40]

When the results of these two studies are interpreted together, it appears that continuing anti-HER2 treatments once disease progression is noted based on initial HER2 status is not advised. However, the negative results of these works may be due to the temporal heterogeneity in HER2 expression being ignored. Pivotal clinical trials on HER2-positive GC are shown in Table 4.

Mechanisms of Resistance to HER2-Targeted Therapies

Repetitive failures after TOGA, unlike the response seen in breast cancer, highlight the distinct HER2 biology in GC. Unlike breast cancer, HER2 overexpression in gastric/GEJ adenocarcinomas tends to be more heterogeneous, both with respect to morphology and the immunoreactivity of tumor cells to antibodies detecting HER2.[41,42] GCs are gland-forming, mucin-producing carcinomas that demonstrate incomplete basolateral or lateral staining patterns.[42] Intra-tumor heterogeneity of HER2 expression occurs in 5 to 50% of cases depending on different definitions, leading to difficulties in assessing HER2 status in GCs.[43] Table 3 summarizes a comparison of HER2 scoring systems for breast and gastric cancer according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) 2013 guidelines and the scoring systems provided by Hoffman et al and Rüschoff et al.[44–47]

Discordance between FISH/IHC and next-generation sequencing (NGS) on intra-tumoral heterogeneity was recently reported. Four of 50 patients whose HER2 status was positive with FISH or IHC were shown to be negative for HER2 amplification by NGS and progressed rapidly on trastuzumab therapy.[48] In addition to intra-tumoral heterogeneity, which can even be seen in the same tumor gland, discordant HER2 status between primary and metastatic disease is common in GC.

Several oncogenic alterations, such as phosphatase and tensin homolog (PTEN) deficiency, PI3K mutations, hyperactivation of the hepatocyte growth factor (HGF)/mesenchymal epithelial transition factor (MET) pathway, co-existing EGFR overexpression, and MET/KRAS amplifications can potentially reduce the growth-inhibitory effect of HER2-targeting drugs.[49] This might also explain the variability in the overall response rates (between 32% and 68%) of patients treated with trastuzumab as first-line therapy even when trastuzumab-based treatment is the standard of care.[50–52] This implies that not all patients benefit from trastuzumab, even in the presence of HER2 expression. To this end, Gomez et al showed that the level of HER2 gene amplification significantly predicted the sensitivity of the tumor to trastuzumab therapy and the OS of AGCs treated with trastuzumab-based chemotherapy.[53] The authors emphasized the importance of utilizing quantitative variables such as HER2 amplification ratio that can be objectively measured, rather than subjective factors such as IHC scores, which are not uniformly measured. On the other hand, tumors determined to be IHC 3+ may not benefit from further information on HER2 gene amplification to reach a clinical decision on the use of trastuzumab-based treatment.[52]

Increased mRNA expression of EGFR, ERBB3, and ERBB4 were shown in a xenograft mouse model of trastuzumab-resistant GC.[54] Data indicated that HER3 plays a role in tumor resistance to HER2 inhibitors and contributes to the proliferation of HER2-amplified cells through the activation of the PI3K-AKT pathway.[55]

HER2 status may be changed after trastuzumab-based treatment, leading to a reduced response to anti-HER2 treatment. Several studies have addressed the acquired loss of HER2 overexpression during therapy with anti-HER2 containing agents.[56–58] It was found that the loss of HER2 positivity is more frequent in tumors with an initial IHC score of 2+.[58] Further, Janjigian et al reported in their NGS analysis of tumor tissues from 44 patients after trastuzumab treatment that HER2 amplification was lost in 14% of samples in addition to other secondary variable alterations.[48]

Micro RNAs (miRNAs) are a class of non-coding RNAs that play an important role in the regulation of target genes at the post-transcriptional level and may therefore play a crucial role in regulating cancer biology. Transfection of a miRNA-125a precursor significantly enhanced trastuzumab inhibition of gastric cancer cell growth. There is growing evidence that several miRNAs regulate trastuzumab resistance through HER2 signaling

Table 3 Summary of Characteristics of HER2 Scoring in Gastric and Breast Cancer

		Breast Cancer	Gastric Cancer
Immunohistochemistry Scoring		The first screening method for HER 2 evaluation high concordance with FISH. Widespread method, Easy, cheap, and quick.	
HER2 expression in the neoplastic cells		Membranous and predominantly circumferential	Basolateral or lateral, incomplete, (usually not circular IHC staining)
Intratumoral heterogeneity		Not frequent	Commonly seen, focal or patchy positivity Possible association between between Helicobacter pylori bacterium and HER2 intratumoral heterogeneity
HER-2 IHC Scoring			
IHC Score 0:	Her 2 overexpression negative by IHC	No reactivity or membranous reactivity in less than10% of cells	No immunostaining
IHC Score 1+:	Her 2 overexpression negative by IHC	Faint / visible membranous reactivity in more 10% of cells at 40X magnification/ detected in only one part of the membrane	Weak immunostaining in less than 30% of tumor cells
IHC Score 2+	Her 2 overexpression equivocal by IHC (Equivocal HER2 expression by IHC to be confirmed by FISH)	Weak to moderate complete or basolateral membranous reactivity in ≥10% of tumour cells (visible at 10–20X magnification)	Complete membranous staining, either uniform or weak in ≥10% of cells
IHC Score 3+	Her 2 overexpression positive	Strong, complete basolateral or lateral membranous reactivity in ≥10% of tumour cells (visible at 2.5–5X magnification)	Uniform intense membranous staining in ≥30% of cells
FISH analysis	Objective and accurate gold standard method higher cost, need more time, equipment and specialist fluorescent/silver/chromogenic/dual-color dual-hapten in situ hybridization can be used		
HER2 gene copies: *CEP17* gene copies ratio for HER2 overexpression		HER2/CEP 17 ratio ≥2 (2.2); positive or the mean HER2 copy number was ≥6	HER2/CEP 17 ratio ≥2; positive
Frequency		15–25%	4.4–53.4%
Anatomic location of the tumour		No correlation	More frequent at gastric cardia and gastro–esophageal junction adenocarcinoma and intestinal subtype
Prognostic significance		Unfavourable	Favorable/not fully established

Abbreviations: IHC, immunohistochemistry; CEP 17, chromosome 17 centromere.

pathway components and HER2 compensatory receptors, and that serum-based miRNA signature can effectively distinguish patients with HER2-positive advanced cancers who are sensitive to trastuzumab from those who are resistant.[59–61]

Novel Anti-HER2 Strategies to Overcome Trastuzumab Resistance and Future Perspectives

New therapeutic agents and combination therapies beyond trastuzumab for the management of HER2-positive GC have

Table 4 Pivotal Clinical Trials of HER2-Positive GC

Trial	Phase	Agent	Line of Therapy	Region	ORR (%)	Median PFS	Median OS
ToGA	3 (N = 594)	Trastuzumab	I st	Global	47 vs 35 P = 0.0017	6.7 vs 5.5 HR = 0.71 P = 0.0004	13.8 vs 11.1 HR = 0.74 P = 0.0046
HELOISE	3 (N = 248)	HD Trastuzumab	1st	Global	56,9 vs 58.9 P= 0.76	5.6 vs 5.7 HR = 1.04 P = 0.8222	10.6 vs 12.5 HR = 1.24 P = 0.2401
LOGIC	3 (N = 487)	Lapatinib	1st	Global	53 vs 39 P = 0.0031	6.0 vs 5.4 HR = 0.82 P = 0.081	12.2 vs 10.5 HR = 0.91 P = 0.35
JACOB	3 (N = 780)	Pertuzumab	1st	Global	56.7 vs 48.9 P = 0.026	8.5 vs 7.0 P = 0.0001	17.5 vs 14.2 HR = 0.84 P = 0.057
T-ACT	2 (N = 89)	Paclitaxel plus trastuzumab	2nd	Japan	33.3 vs 32 P = 1.00	3.7 vs 3.2 HR = 0.91 P = 0.33	10.2 vs 10 HR = 1.23, P = 0.20
GATSBY	2/3 (N = 345)	TDM-1	2nd	Global	20.6 vs 19.6 P = 0.84	2.7 vs 2.9 HR = 1.13 P = 0.31	7.9 vs 8.6 HR = 1.15 P = 0.86
TyTAN	3 (N =261)	Lapatinib	2nd	Asia	27 vs 9 P < 0.001	5.4 vs 4.4 HR = 0.85 P = 0.244	11 vs 8.9 HR = 0.84 P = 0.10

recently been studied. Pan-HER inhibitors may exert a better antitumor effect than HER2 blockade alone by blocking the activation of all HER family receptors, including heterodimers. Combining precision therapies may also overcome anti-HER2 treatment resistance in GC. Nam et al demonstrated the effectiveness of dacomitinib (an irreversible pan-HER tyrosine kinase inhibitor) in the inhibition of EGFR/HER2, HER2/HER3, and HER3/HER4 heterodimer formation, as well as HER3 with p85 in a large panel of gastric cancer cell lines and noted synergy between dacomitinib and trastuzumab, IGF1R inhibitors, ERK1/2 inhibitors, and PI3K/mTOR inhibitors.[55] A series of pan-HER TKIs, such as afatinib, dacomitinib, neratinib, and pyrotinib, have been tested for treatment effects against HER2-positive GC[62,63] (SUMMIT, NCT01953926; NCT02500199).

Tucatinib (ONT 380), an oral HER2-targeted TKI with increased selectivity for HER2 compared with earlier-generation HER2-targeted TKIs, such as lapatinib and neratinib, was approved by the Food and Drug Administration (FDA) based on data from the pivotal HER2CLIMB trial for the treatment of patients with advanced HER2-positive breast cancer, including those with brain metastases, in combination with trastuzumab and capecitabine after at least one round of anti-HER2 treatment.[64,65] Data showed improved response rates and central nervous system PFS (HR = 0.32; 95% CI, 0.22–0.48; P <0.0001) in patients with HER2-positive brain metastatic breast cancer in the first year of therapy.[66] Tucatinib demonstrated substantial antitumor activity in HER2-amplified esophageal and gastric cancers in preclinical studies. Hence, a Phase 1b dose escalation study of tucatinib in combination with trastuzumab and oxaliplatin-based chemotherapy for HER2+ gastrointestinal cancers, and a randomized, double-blind, placebo-controlled, phase 2/3 study of tucatinib in combination with trastuzumab, ramucirumab, and paclitaxel in patients with previously treated advanced HER2+ gastric or GEJ adenocarcinoma are ongoing (NCT04430738, NCT04499924, respectively,).

Until the DESTINY-Gastric01 study of trastuzumab deruxtecan, there were no other globally established

HER2-directed agents for the treatment of GC that progressed after trastuzumab. Trastuzumab deruxtecan (T-DXd) (DS-8201a) is a novel anti-HER2 antibody–drug conjugate (ADC) that combines trastuzumab with a topoisomerase I inhibitor. In the international Phase II DESTINY-Gastric01 trial, patients with HER2-positive advanced gastric or gastroesophageal junction adenocarcinoma were randomly assigned T-DXd or the physician's choice of chemotherapy in a 2:1 ratio as third-line or later therapy.[67]

A significantly higher number of objective responses, a longer median duration of response (DOR) and prolonged OS were observed with T-DXd compared with chemotherapy. The confirmed ORR was 43% in the T-DXd group and 12% in the comparison group. Lower HER2 levels (IHC score of 2+ with negative results on ISH or an IHC score of 1+) seemed to correlate with a lower response rate (The confirmed ORR was 26.3% in the IHC score of 2+ with negative results on ISH and 9.5% in the IHC score of 1+).[68,69] This persistent but decreasing response might be due to the internalization of T-DXd by HER2-positive cells, the release of DXd into the cytoplasm of these cells and the transfer of the released DXd into adjacent HER2-negative cells (bystander effect).[70,71] The high drug-to-antibody ratio of T-DXd and the membrane permeability of its payload may also make this ADC less dependent on a high level of HER2 expression. Following this study, the FDA approved T-DXd for use in advanced gastric/GEJ cancers after failure with a trastuzumab-containing regimen.

Interstitial lung disease and myelosuppression, which were mostly treated with dose reduction and interruption, were the only notable adverse events attributed to T-DXd. Interstitial lung disease developed in 10% of the patients in the T-DXd group, and 3 out of 12 cases were grade 3–4 adverse effects. Patients receiving T-DXd therefore require vigilant surveillance of pulmonary symptoms to avoid lethal lung toxicity.

Other HER2-targeted ADCs beyond T-DXd were evaluated in early clinical trials. In a phase II study, RC48-ADC, a novel ADC comprised of a humanized anti-HER2 IgG1, a valine-citrulline linker and a microtubule inhibitor, MMAE, provided a clinically meaningful response and survival benefit in heavily pretreated patients with HER2-overexpressing (IHC 2+ or 3+) gastric or GEJ cancers.[72] The investigator-assessed confirmed ORR of the 127 included patients was 18.1% (95% CI: 11.8%, 25.9%), and the median overall survival was 7.6 months (95% CI: 6.6–9.2), with an acceptable safety profile. Phase 1 studies are ongoing to evaluate the safety, tolerability, and activity of Trastuzumab duocarmazine (known as SYD985), XMT-1522 (TAK-522), ARX788, ZW49, and other novel HER2-targeting ADCs in patients with advanced gastric tumors with variable HER2 expression levels.

ZW25 (Zanidatamab), a novel IgG1 bispecific antibody, targets two non-overlapping epitopes of HER2, in what is known as biparatopic binding, which results in dual HER2 signal blockade and enhanced receptor downregulation compared with trastuzumab. In the Phase I basket trial that was presented at the American Society for Clinical Oncology (ASCO) in 2018, single-agent ZW25 had a 56% disease control (DCR) and 44% ORR rate in patients with HER2-positive gastroesophageal cancer that progressed on trastuzumab.[73] Zanidatamab was well tolerated and toxicities were manageable. The third part of the trial evaluating the efficacy of ZW25 in combination with chemotherapy in the first-line treatment of HER2+ advanced GC and GEJ cancers is currently ongoing (NCT02892123). Zanidatamab was well tolerated and the toxicities were manageable. The third part of the trial evaluating the efficacy of ZW25 in combination with chemotherapy in the first-line treatment of HER2+ advanced GC and GEJ cancers is currently ongoing (NCT02892123).

Margetuximab is a novel investigational antibody derived from 4D5, the parent antibody of trastuzumab, that is designed to alter fragment crystallizable region (Fc) binding affinities. Fc engineering of margetuximab yielded increased affinity for the activating Fcγ receptor (FcγR) CD16A (FcγRIIIa) and decreased affinity for the inhibitory FcγR CD32B (FcγRIIb), which led to enhanced antibody-dependent cellular cytotoxicity (ADCC) against HER2-positive tumor cells, even at low HER2 expression levels.[74] The aim of margetuximab was also to potentiate innate and adaptive immunity and upregulate tumor PD-L1 expression levels. Phase 1 study of margetuximab (MGAH22) found that single-agent margetuximab was well tolerated, with promising activity in heavily pretreated patients with HER2-expressing tumors.[75]

Data on the impact of immunotherapy on HER2+ GC continue to emerge. Trastuzumab has been found to stimulate HER2-specific T cell response and increase tumor PD-L1 expression.[76,77] Potential synergistic anti-tumor activity has been noted when anti-HER2 therapeutic approaches are combined with anti-PD-1 antibodies. In CP-MGAH22-05, a single-arm, multicenter, phase Ib/II study, the combination

of margetuximab and pembrolizumab, a selective humanized monoclonal immunoglobulin G4-Kappa antibody that binds to PD-1 and provokes an antitumor immune response, was administered to patients with advanced HER2+ gastric and gastroesophageal cancers who had received at least one previous treatment with trastuzumab plus chemotherapy.[78] ORR was 18.48% (95% CI, 11.5–27.93), DCR was 53% (95% CI, 43–64), median PFS by investigator assessment was 2.73 months (95% CI, 1.61–4.34), and median OS was 12.48 months (95% CI, 9.07–14.09). These results suggest that the combination of margetuximab and pembrolizumab might be an effective treatment option for GC or GEA while avoiding the toxicity of chemotherapeutic agents. Clinical outcomes were more pronounced in patients with HER2 IHC 3+, PD-L1-positive and HER2-amplified tumors assessed by circulating tumour DNA (ctDNA) analysis. This trial also suggests that HER2 amplification by ctDNA could be used to reassess HER2 status without the need to obtain a post-progression biopsy.

Due to the activity and safety profile observed in the CP-MGAH22-05 trial, the phase 2–3 MAHOGANY study is evaluating margetuximab in combination with checkpoint inhibitors (retifanlimab-Anti PD1 mAb, tebotelimab-bispecific anti-PD1, and anti-lymphocyte activation gene 3 (LAG-3) mAb) with or without chemotherapy as first-line therapy for patients with HER2-positive advanced GEA (NCT04082364).[79]

In the phase 1b/2 PANTHERA trial (NCT02901301), first-line triple therapy with pembrolizumab, trastuzumab and chemotherapy showed promising efficacy in the treatment of advanced HER2 amplified gastric cancer regardless of PD-L1 status.[80] The ORR was 77%, and patients with HER2 amplification per NGS (≥ 4 copy number) had a statistically significantly longer median PFS than those without HER2 amplification (median PFS, 22.0 months vs 7.7 months; P =0.03). The same trend was shown in patients with altered RTK/RAS pathways compared with wild-type RTK/RAS (median PFS, 13.8 months vs 4.9 months; P = 0.001).

In another trial of the combination of pembrolizumab, trastuzumab and chemotherapy (oxaliplatin or cisplatin) in patients with HER-2-positive advanced EGA, the ORR was 91% (32 of 35 patients; six CRs).[81] PD-L1 expression and tumor mutation burden did not correlate with PFS, and no association was found between depth or duration of response and degree of DNA copy number alterations or the number of predicted strong clonal neoantigens. It was emphasized that none of the treated patients had tumors that were positive for high microsatellite instability or Epstein-Barr virus, which are metastatic EGC subsets with a greater likelihood of response to anti-PD-L1 therapy. In this study, the success of combination therapy was repeatedly demonstrated regardless of PD-L1 expression.[81,82] The researchers speculated that the induction of antibody-dependent cell-mediated cytotoxicity by trastuzumab improves the anti-tumor immune response by enhancing the presentation of tumor antigens. Future studies are needed to investigate the mechanism behind the synergistic benefits of combining pembrolizumab with trastuzumab and chemotherapy. The available evidence has suggested that pembrolizumab combined with trastuzumab and chemotherapy is a promising treatment option in patients with HER2-positive disease, and that correlative biomarkers found in the early-phase studies need to be validated by ongoing trials. The randomized, double-blind, placebo-controlled, global Phase III KEYNOTE-811 study to evaluate the efficacy and safety of pembrolizumab or placebo in combination with trastuzumab and chemotherapy as first-line treatment for patients with advanced HER2-positive GC or GEJ adenocarcinoma (NCT03615326) is still ongoing.[83] The first interim results of the KEYNOTE-811 trial were presented at the 2021 ASCO Annual Meeting. Patients were included regardless of PD-L1 status, although 88% and 85% of those in the pembrolizumab and placebo (trastuzumab plus chemotherapy alone) arms at the interim timepoint, respectively, had a PD-L1 combined positive score ≥ 1. The ORR of the pembrolizumab arm was 74.4%, compared with 51.9% for the placebo arm (p = 0.00006). The addition of pembrolizumab also led to deeper responses, with 11% of patients in the pembrolizumab arm achieving a complete response compared with 3% in the placebo arm. In the interim safety analysis, although immune-mediated adverse events, particularly pneumonitis and colitis, were more common in the pembrolizumab group, the absence of new adverse events associated with combination treatment was highlighted. Adding pembrolizumab to the standard of care demonstrated a survival advantage for the first time since TOGA 11 years earlier. The practice-changing findings of the KEYNOTE-811 trial led the US FDA to grant accelerated approval of pembrolizumab in combination with trastuzumab and fluoropyrimidine and platinum-based chemotherapy as first-line therapy for patients with HER2-positive advanced GEA. KEYNOTE-811 is still recruiting, and overall survival and progression-free survival, which are the final primary endpoints of the study, are pending.

The multicenter phase II INTEGA trial was also designed to assess the efficacy, safety and tolerability of

ipilimumab or 5-FU/folinic acid and oxaliplatin (FOLFOX) in combination with nivolumab and trastuzumab as first-line therapy for patients with advanced HER2-positive EGC (NCT03409848). This ongoing study aims to evaluate the therapy options for advanced HER2-positive GC in the first-line setting, including a chemotherapy-free experimental arm. In addition, the ability of immune profiling via liquid biopsy to identify predictive biomarkers to tailor treatment prior to initiation and before the second dose of nivolumab is under investigation.[84]

Based on previous data from studies that suggested a high overall response rate and manageable toxicities with T-DXd, the Phase 1b/2 multi-center, open-label DESTINY-Gastric03 study is underway to investigate the safety, tolerability, pharmacokinetics, immunogenicity, and preliminary anti-tumor activity of trastuzumab deruxtecan alone and in combination with chemotherapy and/or durvalumab in HER2-positive advanced/metastatic gastric/GEJ adenocarcinoma patients (NCT04379596).[85]

Due to the highly immunogenic nature of HER2 tumors, combining anti-HER2 therapies with immune checkpoint blockade is a high potential approach. An open-label, two cohort phase 1B/2 study was designed to evaluate ZW25 plus chemotherapy with/without tislelizumab, an investigational anti-PD-1 antibody, as first-line therapy in patients with HER2-positive metastatic breast cancer (cohort 1) or advanced gastric/gastroesophageal junction adenocarcinoma (cohort 2).[86] Safety, tolerability profile, and objective response rate are the primary endpoints of the study, which is ongoing. Novel HER2-directed strategies are summarized in Table 5.

Table 5 Novel HER2-Directed Strategies

Strategy	Selected Agents/Trial
Antibody–drug conjugates	♣ Trastuzumab deruxtecan (DS-8201a) ♣ RC48-ADC (NCT03556345)
Monoclonal antibodies (with augmented ADCC)	♣ Margetuximab
Bispecific antibodies	♣ Tucatinib ♣ Neratinib (+ trastuzumab or cetuximab): (NCT03457896)
Immunotherapy combinations	♣ KEYNOTE-811 (NCT03615326) (Pembrolizumab or placebo + trastuzumab +chemotherapy) ♣ MAHOGANY (NCT04082364) (Margetuximab ± PD-1 inhibitor ± chemotherapy ± dual checkpoint inhibitor) ♣ INTEGA (NCT03409848) (Ipilimumab or FOLFOX + nivolumab + trastuzumab) ♣ DESTINY-Gastric03 (NCT04379596) (Trastuzumab deruxtecan ± chemotherapy ±durvalumab) ♣ NCT04276493 (ZW25 + chemotherapy ±tislelizumab)

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; PD-1, programmed death-1; ZW25, zanidatamab.

Conclusion

The only available biomarkers able to guide the effective treatment of advanced GC are HER2 overexpression, MSI/PD-L1 status, and FGFR alterations. Various anti-HER2 agents have been evaluated after the success of the ToGA trial, but these failed to improve clinical outcomes significantly enough to merit the establishment of new options for HER2-targeted therapy in AGC. The recent practice-changing first interim findings of the KEYNOTE-811 trial led to the accelerated approval of pembrolizumab in combination with trastuzumab plus chemotherapy for patients with HER2-positive advanced gastric or GEJ cancer by the FDA, which is now a first-line therapy for GC. The combination of anti-HER2 mAb with immunotherapy appears to be a reasonable strategy for overcoming the immune insensitivity of patients with AGC. T-DXd was also found to be effective in patients who had already received ≥2 previous lines of treatment. As we know that assessing HER2 status in patients with GC is challenging due to their multiple heterogeneities, HER2 amplification level may represent a predictive biomarker for selecting patients who can benefit the most from HER2 targeted therapies, and ctDNA might serve as a more precise and non-invasive method for improving clinical outcomes. It is important to confirm over time that HER2 is still positive due to the loss of HER2 expression that can occur after anti-HER2-based treatment. Tumor NGS, ctDNA, and other biomarkers may clarify the population that can derive the most clinical benefit from anti-HER2 agents. It is expected that the optimal management of HER-2 positive GC will continue to evolve following further investigation.

Disclosure

The authors reported no conflicts of interest for this work.

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