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REVIEW ARTICLE

Human epidermal growth factor receptor 2 (HER2) in advanced gastric cancer: where do we stand?

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Abstract

Gastric cancer is one of the most common malignancy worldwide. In unresectable or metastatic disease, the prognosis is poor and in generally less than a year. HER2 expression remains an important biomarker to lead the addition of trastuzumab to first-line systemic chemotherapy in unresectable or metastatic gastroesophageal adenocarcinoma. To date, a major issue is represented by resistance to trastuzumab developed during treatment, considering the not improved outcomes in this molecular subtype of gastroesophageal adenocarcinoma to other HER2 target strategies. In this review, we summarize the available data on the mechanisms underlying primary and secondary resistance to HER2-targeted therapy and current challenges in the treatment of HER2-positive advanced gastric cancer refractory to trastuzumab. Furthermore, we describe the prognostic value of new non-invasive screening methods, under development novel agents (e.g., HER2 antibody-drug conjugates and bispecific antibodies) and strategies with antitumor activity in early studies.

Keywords HER2 · Trastuzumab · Pertuzumab

Introduction

Gastric cancer (GC) is the fifth most common cancer worldwide [1]. According to 2018 estimates, GC caused more than 7,00,000 deaths annually, representing the third leading cause of cancer death, mainly due to the advance stage present at the diagnosis [2–5]. For these patients with advanced, unresectable disease, systemic chemotherapy is the gold standard in the first-line treatment [6, 7]. Despite the improvement observed during the past decades and the steady decline in the incidence of mortality, the prognosis of advanced GC is very poor with a median overall survival (OS) of 10–12 months [8, 9]. The unavailability of accurate diagnostic test for early detection and the absence of valuable prognostic factors, are critical limitations for diagnostic and therapeutic process in the GC treatment. The Cancer Genome Atlas classification identifies four molecular subtypes of GC; the identification of a subgroup of cancer that overexpress human epidermal growth factor 2 (HER2) has allowed major improvements in the treatment [2, 10]. Herein, we report the status of HER2-positive advanced GC treatment, highlighting the mechanisms potentially linked to anti-HER2 drugs resistance. Moreover, new detection methods for HER2 status and the future therapeutic strategies are discussed.

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The epidermal growth factor 2 (HER2) pathway

The family of human epidermal growth factor receptor (HER) have been implicated in the development of different tumours and comprises four members: HER 1 (ErbB1), HER 2 (ErbB2), HER 3 (ErbB3) and HER 4 (ErbB4). where ErbB refers to the *Erb-b* gene responsible for avian erythroblastosis [11]. The protein structures of all HER receptors are composed by an intracellular domain with tyrosine kinase properties, a transmembrane lipophilic domain and a cysteine-rich extracellular ligand-binding domain [12]; they regulate cell differentiation, growth and survival for the development of embryo and adult tissues [13] (Fig. 1). HER receptor family exist as monomers on the cell surface and, once the ligand has bound to the extracellular domain, dimerization of HER proteins took place followed by the transphosphorylation of intracellular domains [13].

In detail, HER2 receptor, whose gene is located on the human chromosome 17 (17q12) and also known as *ErbB2*, *p185* or *neu*, is a 185 kD (1255 amino acid) transmembrane glycoprotein [14, 15]. As aforementioned, its inappropriate activation, mainly due to overexpression via *HER2* gene amplification and other secondary genetic mechanisms [16, 17], is correlated with the development of different malignancies such as gastric, ovarian, breast, pancreatic, colorectal and endometrial cancer [13, 18–20]. HER2 has no specific single

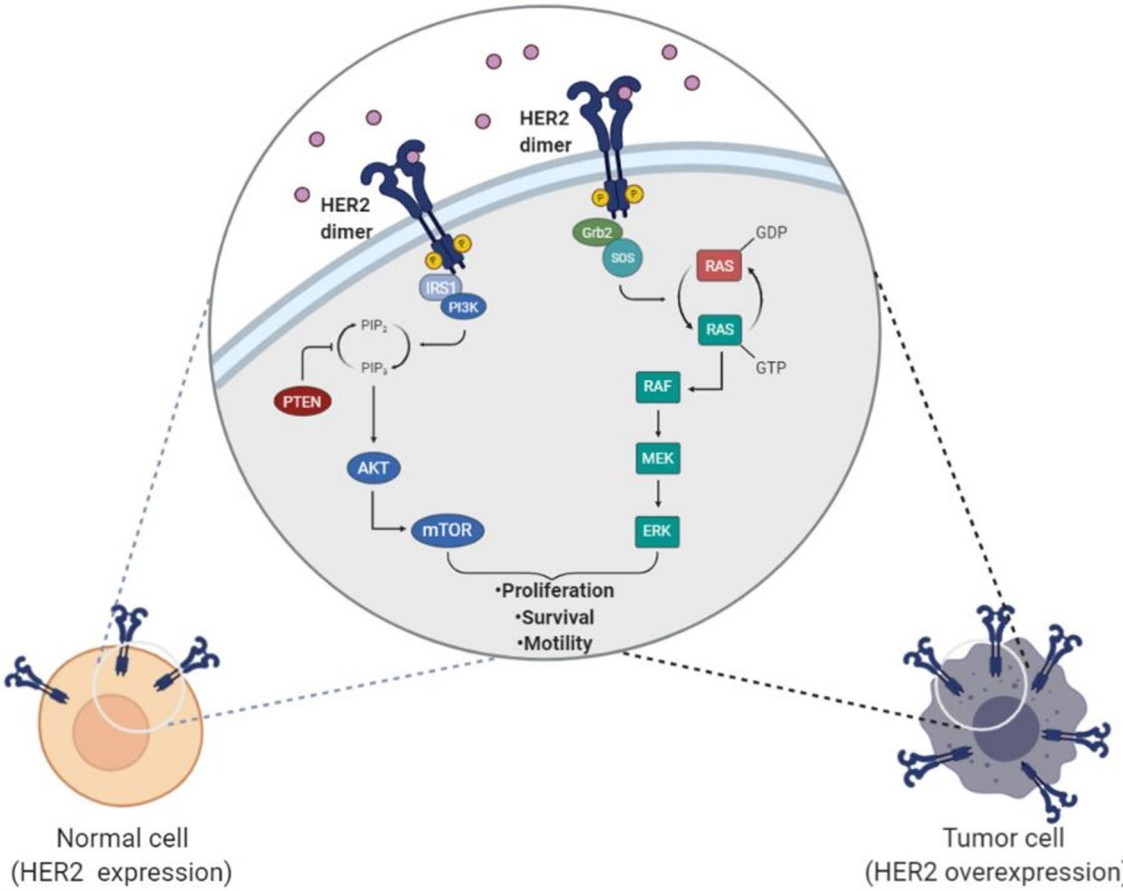

Fig. 1 HER2 activation. Activation of the HER2 receptor triggers a number of downstream signaling steps through cytoplasm and nucleus, culminating with increased cell growth, survival, and motility. Activation of PI3K/Akt pathway is one of the most studied processes involved with HER2 activation. PI3K converts phosphatidylinositol (4,5)-bisphosphate (PIP2) into phosphatidylinositol (3,4,5)- triphosphate (PIP3). PIP3 acts as a docking site for pleckstrin homology (PH)-containing proteins, such as Akt that becoming phosphorylated Akt activates mTOR and other intracellular pathways resulting in cell proliferation, invasion, and survival. PIP3 in turn is dephosphorylated back to PIP2 by PTEN. PTEN is therefore a negative regulator of PI3K/Akt signaling and functions as a tumor suppressor. The RAS/Raf/ MAPK signaling cascade is also triggered by HER2 activation. Growth Factor Receptor bound Protein 2 (GRB2) binds to the guanine nucleotide exchange factor Son of Sevenless (SOS) that becomes activate and removes guanosine diphosphate (GDP) from inactive RAS. Free RAS can then bind guanosine-5′-triphosphate (GTP) and become active. RAS/GTP binds efficiently to Raf-1 (MAP3K), which becomes activated. Raf-1 can then activate MEK that phosphorylates and activates the extracellular signal-regulated kinase ERK resulting in cell cycle control, differentiation, and migration. Figure created with Biorender.com

activating ligand and it has been speculated that its constitutively activated state might occur following the heterodimerization with other family members (HER1 and/or HER3) [21]. HER2 activation leads to the autophosphorylation of tyrosine residues within the cytoplasmic domains and the activation of different signalling pathways, primarily protein kinase C (PKC), phosphatidylinositol-4,5 bisphosphate 3-kinase (PI3K) and the mitogen-activated protein kinase (MAPK) resulting in in cell survival, angiogenesis, and metastasis [13, 18]. Heterodimers containing HER2 provide a more robust signal than stand-alone homodimers or when coupled with other family members, with a significant higher ligand-binding affinity [22]. For instance, the PI3K/Akt downstream pathway, which is considered the leader regulator of cell growth, is generated by HER2–HER3 heterodimer [21, 22]. HER2 dimerization leads to cell-cycle progression also supporting the fast processing of cell-cycle inhibitor p27 [21]. Lastly, HER2 can be triggered via the dimerization with other membrane receptors including the insulin growth factor-1 (IGF1) [23].

HER2 amplification/over-expression

Amplification or over-expression of HER2 has demonstrated an crucial role in the development and progression of certain aggressive cancers (e.g., breast and GC) [24–26]. Recently, the protein has become a therapy biomarker and target for patient with GC, in which HER2 overexpression ranges from 6 to 30% of cases [2].

HER2 status assessment is performed on a biopsy tissue sample from the tumor using immunohistochemistry (IHC) that measures the amount of HER2 protein expressed by cancer cells reported on a scale from 0 to 3+ (negative 0− 1+, equivocal 2+ or positive 3+) and fluorescence in situ hybridization (FISH) that evaluate the number of HER2 gene copies and a dichotomous result are reported (negative or positive) FISH is currently recommended in the case of equivocal HER2 score. The correlation between IHC and FISH is elevated, although intra-tumoral heterogeneity, technical errors or other genetic factors, can lead to inconsistent results [27, 28].

HER2-positive tumors usually have a higher tumor grade growing and spreading more rapidly than cancers with a normal expression of HER2. HER2 overexpression was found to be a negative prognostic factor in GC in some studies, even though its role remains still uncertain [29–32].

Anti-HER2 agents in first-line positive advanced gastric cancer

Trastuzumab

Trastuzumab is a humanized monoclonal antibody targeting HER2, that exerts its antitumor-mediated response causing the internalization and downregulation of HER2 and inhibiting cancer cell proliferation [33, 34]. First, it was approved for the treatment of HER2 positive breast cancer; subsequently, its use was authorized for advanced GC, based on the result of the ToGA trial [35, 36]. In this phase III trial, patients with advanced GC or gastroesophageal junction adenocarcinoma with HER2 overexpression (ICH3+ or FISH+) were randomly assigned to receive first-line chemotherapy (cisplatin and fluoropyrimidine) with or without trastuzumab. Trastuzumab-containing regimen yielded an increased median overall survival (OS), (13.8 vs 11.1 months; $p = 0.0046$), median progression-free survival (PFS) (6.7 vs 5.5 months $p = 0.0017$) and overall response rate (ORR) (47 vs 35%, $p = 0.0017$). The safety profile was similar in the two groups, and no differences were observed in cardiac-related events. *Post hoc* analysis showed null or lower benefit from adding trastuzumab to chemotherapy in patients with IHC 0 or 1+ and FISH+, compared to patients with ICH2 or 3+ and FISH+ [2, 36, 37]. Henceforth, similar efficacy was also assessed by combining trastuzumab with other chemotherapeutic regimens. In particular, three phase II studies evaluated the combination of trastuzumab with capecitabine and oxaliplatin reporting a median OS of 13.8–21.0 months, a median PFS of 7.1–9.8 months, and an ORR of 46.7–67.3% [38–40]. In addition, a meta-analysis proved that capecitabine or 5FU can be replaced by S−1 and cisplatin by oxaliplatin [38, 40–43]. Intriguingly, trastuzumab seems to elicit T cell response; therefore, its association with immune checkpoint inhibitors has earned attraction in the last few years [44–46].

Lapatinib

Lapatinib is a dual tyrosine kinase inhibitor which affects both HER2 and epidermal growth factor receptor (EGFR) [47]. It was evaluated in a phase III trial (TRIO-013/LOGiC) for patients with HER2-positive GC in combination with capecitabine and oxaliplatin failing to achieve its primary endpoint of OS against chemotherapy alone (12.2 and 10.5 months, respectively) [48].

Pertuzumab

Pertuzumab is a recombinant humanized monoclonal antibody that inhibits the dimerization of HER2 with other HER receptors, which prevents signaling promotion and, thus, cell growth and proliferation. The phase III JACOB trial examined the effect of the addition of pertuzumab to trastuzumab and chemotherapy for patients with HER2-positive advanced GC [49]. No significant improvement regarding its primary endpoint of OS was observed (14.2 vs 17.5 months, HR 0.84, 95% C.I. 0.71–1.00, $p = 0.057$), despite the benefit in terms of PFS (8.5 vs 7.0 months, HR 0.73, 95% C.I. 0.86,

p =0.0001). These data support the intrinsic differences in the HER2 biology between breast and GC cells in driving disease progression, probably due to the heterogeneous pattern observed in GC [50, 51] (Table 1).

Anti-HER2 agents in second line advance positive gastric cancer

Lapatinib

The survival benefit of lapatinib combined with paclitaxel in second-line setting to test its efficacy in restoring trastuzumab sensitivity was evaluated in the phase III trial TyTAN. [52, 53] No OS benefit, PFS or time to progression improvement was recorded for lapatinib in the whole cohort. In a subgroup analysis of data from patients with baseline IHC3+ tumors, OS (14 vs 7.6 months; HR 0.59, 95% C.I. 0.37–0.93, $p = 0.02$) and PFS (5.6 vs 4.2 months; HR 0.54 95% C.I. 0.33–0.90, $p = 0.010$) were significantly higher in lapatinib arm [53]. These results suggest that lapatinib would be beneficial in patients with HER2 IHC 3+ GC, but this approach is not recommended in clinical practice. Noteworthy, in this study, 35% of the patients were IHC0/1+ and, to date, this would be considered as HER2 negative [53].

Trastuzumab emtansine

Trastuzumab emtansine (T-DM1) is a monoclonal antibody conjugate of trastuzumab linked to the tubulin inhibitor emtansine. Based on promising results about the antitumor activity of T-DM1 in HER2-positive GC cells, even in trastuzumab-resistant tumors [54], the phase II/III trial (GASBY) compared T-DM1 with the physician's choice in the second-line setting. No improvements in terms of OS and PFS with the experimental arm were observed [55]. Recently, a phase I/II trial evaluated T-DM1 plus capecitabine in first-line treatment of HER2-positive advanced GC was stopped for treatment failure (NCT01702558). Among the reasons for study failure, the evaluation of HER2 status through archival tissue specimens should be primarily considered. The loss of HER2 expression is common among patients with HER2-positive GC receiving HER2-targeted therapies and might explain the failed response to T-DM1. Interestingly, several acquired resistance processes have been recently explored and will be further described below.

Trastuzumab beyond progression

Trastuzumab is currently the only anti-HER2 agent capable of demonstrating efficacy in a randomized phase III study. In patients with HER2-positive breast cancer, the use of trastuzumab beyond progression is an established treatment strategy that has been shown to prolong survival outcomes [56]. Recently, this approach has also been proposed in HER2-positive advanced GC by several studies; however, the results are still conflicting. Particularly, in the phase II randomized clinical trial conducted by the West Japan Oncology Group (WJOG7112G/T-ACT), trastuzumab plus paclitaxel showed no benefit over paclitaxel alone in GC patients resistant to first-line trastuzumab-based therapy [57–61].

In a retrospective analysis, maintenance with trastuzumab in the second-line setting after first-line trastuzumab-based therapy, recorded a longer median PFS (4.4 vs 2.3 months) and OS (12.6 vs 6.1 months) compared with chemotherapy alone [57, 58].

Conversely, other studies demonstrated no benefit of trastuzumab beyond progression, despite an increased PFS (4.6 vs 2.9 months) in patients not exposed to anti-HER2 agents for a period longer than 30 days [59–61] (Table 2).

Based on these results, a resensitization to trastuzumab after a treatment-free interval, could reduce selective pressure on HER2-positive clones [62]. In this landscape, the identification of patients who would benefit from the continuation of HER2 blockage beyond progression could be determinant for this strategy.

Table 1 Anti-HER2 agents in first-line positive advanced gastric cancer

Trial/author (year), ref	Phase	Chemotherapy	ORR (%)	Median PFS (months)	Median OS (months)
TOGA (2010) [36]	III	Cisplatin + fluoropyrimidine ± trastuzumab	47 vs 35 ($p = 0.001$)	6.7 vs 5.5 ($p < 0.001$)	13.8 vs 11.1 ($p = 0.004$)
Kurokawa et al. (2014) [41]	II	S-1 + cisplatin ± trastuzumab	68	7.8	16
TRIO-013/LOGiC (2016) [48]	III	Capecitabine + oxaliplatin ± lapatinib	53 vs 39 ($p = 0.003$)	6.0 vs 5.4 ($p = 0.04$)	12.2 vs 10.5 ($p = 0.35$)
Shah et al. (2017) [146]	III	Cisplatin + capecitabine + trastuzumab (SoC or HD)	73 vs 70	5.7 vs 5.6 ($p = 0.822$)	12.5 vs 10.6 ($p = 0.240$)
JACOB (2018) [49]	III	Fluoropyrimidine + cisplatin + trastuzumab ± pertuzumab	57 vs 48	8.5 vs 7.0 ($p < 0.05$)	17.5 vs 14.2 ($p = 0.056$)

SoC standard of care, *HD* higher dose, *ORR* objective response, *PFS* progression free survival, *OS* overall survival

Table 2 Anti-HER2 agents in second line advance positive gastric cancer

Trial/Author (year), ref	Phase	Chemotherapy	Trastuzumab in 1st line	ORR (%)	Median PFS (months)	Median OS (months)
TyTAN (2014) [53]	III	Paclitaxel ± lapatinib	6%	27 vs 9 ($p < 0.001$)	5.4 vs 4.4 ($p = 0.24$)	11vs 8.9 ($p = 0.10$)
Li et al. (2016) [59]	Prospective	CT ± trastuzumab	100%	9.3 vs 3.7 ($p = 0.62$)	3.1 vs 2.0 ($p = 0.008$)	10.5 vs 6.5 ($p = 0.17$)
GASBY (2017) [55]	II/III	Taxane vs T-DM1	76%	20 vs 21 ($p = 0.84$)	2.9 vs 2.7 ($p = 0.31$)	8.6 vs 7.9 ($p = 0.86$)
Palle et al. (2017) [57]	Retrospective	CT + Trastuzumab	100%	16.7 vs 5.4 ($p = 0.08$)	4.4 vs 2.3 ($p = 0.002$)	12.6 vs 6.1 ($p = 0.001$)
Narita et al. (2017) [58]	Retrospective	CT ± trastuzumab	100%	18.2 vs 15.8 ($p = 1.00$)	4.0 vs 2.3 ($p = 0.14$)	10.8 vs 9.5 ($p = 0.88$)
Makiyama et al. (2018)[61]	II	Paclitaxel ± trastuzumab	100%	33.3 vs 31.6 ($p = 1.00$)	3.68 vs 3.1 ($p = 0.33$)	10.2 vs 9.9 ($p = 0.20$)

T-DM1 Trastuzumab emtansine, *CT* chemotherapy, *ORR* objective response, *PFS* progression free survival, *OS* overall survival

Therapeutic perspective in HER2-positive gastric cancer

ZW25

ZW25 is a bispecific antibody that simultaneously binds to two HER2 epitopes: ECD4, the trastuzumab-binding domain, and ECD2, the pertuzumab-binding domain [63]. Preclinical studies suggested that ZW25 has strong antitumor activity at a range of HER2 expression levels and may more effectively silence HER2 signaling than trastuzumab or pertuzumab and stimulates the immune system. It was tested in a phase I basket trial showing encouraging efficacy in pretreated patients with HER2-positive gastroesophageal cancer. ORR and disease control rate was 44 and 56%, respectively [64]. Adverse events (AEs) were all grade 1–2 except for one patient who experienced reversible grade 3 hypophosphatemia, arthralgia, and fatigue. Based on these results, ZW25 has been granted a fast-track designation by the Food and Drug Administration (FDA) for the treatment of patients with HER2-overexpressing gastroesophageal adenocarcinoma to be used in combination with standard of care chemotherapy. Currently, a trial to assess the safety, tolerability and preliminary antitumor activity of ZW25 in combination with tislelizumab (a humanized monoclonal antibody directed against PD-1) and chemotherapy in patients with HER2-positive gastric/gastroesophageal junction adenocarcinoma is ongoing (NCT04276493).

Margetuximab

Margetuximab is a next-generation Fc-modified anti-HER2 monoclonal antibody that binds with elevated affinity CD16A, an Fc receptor important for antibody dependent cell-mediated cytotoxicity (ADCC) against tumor cells [65]. A phase I study, including 20 patients with GC, evaluated the toxicity profile, maximum tolerated dose, pharmacokinetics features, and antitumor activity of margetuximab in patients with HER2-overexpressing carcinomas [66].

Over half [45/66 (68%)] of patients received at least one prior anti-HER2 therapy in the metastatic setting. Common toxicities were primarily ≤ grade 2; grade 3/4 AEs attributed to margetuximab were infrequent and included: increased lipase, blood amylase, blood alkaline phosphatase lymphocyte decreased, and infusion-related reaction (IRR), including cytokine release syndrome [66].

Despite the heavily pre-treated population, margetuximab demonstrated evidence of clinical activity with an ORR of 12%. The effect of single-agent margetuximab in the population studied suggests potential for antitumor activity after progression or following other anti-HER2 regimens. Moreover, in a single-arm phase Ib/II trial, margetuximab has been used in combination to pembrolizumab in HER2-positive advanced GC patients, as second-line therapy [67]. The preliminary results have demonstrated acceptable toxicities with serious AEs (grade ≥3) in about 9% of patients, including autoimmune hepatitis. The response rate was ~ 20% in the 92 evaluable patients. Noteworthy, all responses have been reported in patients with HER2 ICH 3+ and the responses were more frequent PD-L1-positive tumors. Preliminary data of median PFS of 3 months and median OS of 13 months were reported [68].

The ongoing phase II/III randomized open-label trial (MAHOGANY) for treatment of patients with HER2-positive GC was designed to determine the efficacy of margetuximab combined with an anti-PD-1 monoclonal antibody, INCMGA00012 (also known as MGA012) (Cohort A) and margetuximab combined with INCMGA00012 or MGD013 (anti-PD-1/anti-LAG-3 dual-affinity re-targeting protein) and chemotherapy compared to trastuzumab combined with chemotherapy (Cohort B) [69].

Trastuzumab deruxtecan

Trastuzumab deruxtecan (T-DXd) is an antibody-drug conjugate composed of an anti-HER2 antibody, a cleavable tetrapeptide-based linker, and a cytotoxic topoisomerase I inhibitor [70]. The FDA approved as fast-track designation, trastuzumab deruxtecan for female patients with HER2-positive, unresectable and/or metastatic breast cancer [71, 72]. In two phase I trials, trastuzumab deruxtecan was administered in pretreated GC patients, recording ORR about 43%. Serious treatment AEs (mainly grade ≥3 myelosuppression) were reported in the 25% of patients [73–75]. A randomized phase II trial (DESTINY-Gastric01) evaluated efficacy and safety of trastuzumab deruxtecan in advanced HER2-positive GC [76]. Patients who progressed during treatment (two or more previous regimens including trastuzumab) have been enrolled to receive trastuzumab deruxtecan or the physician's choice recording an ORR of 51.3 and 14.3% in two groups, respectively. The safety profile was generally manageable, and the common AEs were hematologic or gastrointestinal. Based on DESTINY-Gastric01 trial, FDA has recently approved Trastuzumab deruxtecan for the treatment of adult patients with HER2-positive locally advanced or metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma who have received a prior trastuzumab-based regimen

Trastuzumab deruxtecan is effective not only against tumor cells positive for HER2 protein but also, in the presence of HER2-positive cells, against those negative for such expression [77]. This "bystander killing effect" seems due to the internalization of trastuzumab deruxtecan by HER2-positive cells, the release of DXd into the cytoplasm and the subsequent transfer of DXd into adjacent HER2-negative cells [78]. Indeed, trastuzumab deruxtecan has proven effectiveness against tumors that express HER2 but are negative for HER2 amplifications [77]. Moreover, the efficacy of trastuzumab deruxtecan is independent to the absence or presence of other gene alterations that can active alternative pathways, suggesting the possibility of overcoming trastuzumab resistance [79]. Therefore, the action of trastuzumab deruxtecan could be significant in cases with absent homogeneity of HER2 amplification and expression.

A phase Ib/II trial (DESTINY-Gastric03) is testing safety and efficacy of trastuzumab deruxtecan alone or in combination with chemotherapy and/or immunotherapy in HER2-positive advanced or metastatic gastric/gastroesophageal junction adenocarcinoma patients (NCT004379596).

Pan-HER tyrosine-kinase inhibitors

Considering the suggested antitumor efficacy of pan-HER blockade , several pan HER2-targeted tyrosine-kinase inhibitors (TKIs) have been evaluated in clinical trials for GC treatment [80].

Afatinib is an oral kinase inhibitor that irreversibly blocks EGFR, HER2, HER3, and HER4 [81]. It is being investigated for breast cancer as well as other EGFR/HER2-driven cancers, including HER2 positive gastrointestinal tumors [82]. It was evaluated in a phase II study in patients with esophagogastric cancer previously treated with trastuzumab, providing a moderate therapeutic benefit with an ORR of 10% [83]. A phase II study with afatinib and paclitaxel in patients with HER2-positive GC progressed to trastuzumab and chemotherapy is currently ongoing (NCT02501603; NCT01522768).

Poziotinib is another TKI that binds irreversibly to the active site of the tyrosine kinase domain and blocks signal transduction by EGFR, HER2, and HER4. It has been tested in a phase I/II study, in which patients with HER2-positive advanced GC, previously treated with one line of chemotherapy regardless trastuzumab exposure, were enrolled to receive poziotinib with trastuzumab and paclitaxel. A median PFS of 13 weeks and a median OS of 29.5 weeks was reported [84].

Dacomitinib, an irreversible pan-HER inhibitor, has been evaluated in monotherapy, in a multicenter phase II study showing anti-tumoral activity in HER2-positive GC. Patients enrolled had received from one to more than three prior chemotherapy regimens and a median PFS and OS of 2.1 and 7.1 months has been reached, respectively [85].

Immune checkpoint inhibitors

Blocking immune checkpoint, especially programmed cell death-1 (PD-1) and its ligand (PD L1 or B7-H1), has proven efficacy in several solid cancers, and seems to become a potential option in gastric cancer treatment [86]. The efficacy of immunotherapy in GC has been tested in several studies, but few data on the efficacy in the specific setting of HER2-positive GC have been published.

The combination of pembrolizumab plus trastuzumab, fluoropyrimidines and oxaliplatin has been investigated as first-line therapy in a small phase II cohort of patients in HER2-positive advanced GC. A median PFS of 11.3 months, with 67% 6 months of PFS and an ORR of 87% has been observed [45].

The phase III KEYNOTE-811 trial to confirm these results, comparing chemotherapy plus trastuzumab with or without pembrolizumab, is currently ongoing (NCT03615326). Moreover, the phase 2 trial (INTEGA) is assessing the efficacy of two experimental first-line treatment strategies in advanced or metastatic esophagogastric adenocarcinoma: chemo-free immunotherapy with trastuzumab, nivolumab and ipilimumab or addition of nivolumab

to the standard regimen (FOLFOX chemotherapy and trastuzumab) (NCT03409848).

Moreover, an exploratory subgroup analysis of HER2-positive patients of phase III ATTRACTION-2 trial, assessed a significant longer OS in patients receiving nivolumab with history of trastuzumab use vs placebo (8.3 vs 3.1 months; HR: 0.38, 95% C.I. 0.22–0.66, p= 0.0006) [87].

Several clinical trials are evaluating the safety and efficacy of different immune checkpoint inhibitors in patients with advanced G/GEJ cancer. The combination of anti-HER2 and immunotherapeutic agents seems to be a promising strategy [88–92].

These encouraging results have raised again the interest in developing and assessing strategies acting on HER2 in GC. Indeed, a decade after the publication of the TOGA trial, no other study had confirmed the validity of the strategy either in the first or in the second line. These innovative molecules in the GC treatment seem to have the potential of synergistic effect on innate and acquired immunity, combining immunotherapy with an engineered intervention aimed at optimizing precision medicine (Table 3).

Table 3 Ongoing phase II and III trials targeting HER2+ gastric and gastroesophageal junction (GEJ) cancer

Trials/ID	Status	Treatments	Phase	Patients	Line of therapy
NCT02378389	Unknown	Pyrotinib ± Docetaxel	I	HER2+ advanced gastric cancer	1st
NCT02205463	Withdrawn	KD019	I	HER2 overexpressed or amplified metastatic or unresectable gastric or GEJ adenocarcinoma	1st
NCT03330561	Recruiting	PRS-343 (HER2/41BB Bispecific)	I	HER2+ advanced or metastatic solid tumors	≥2nd
NCT03650348	Active, not recruiting	PRS-343 + Atezolizumab	I	Previously treated HER2+ advanced or metastatic solid tumors.	≥2nd
NCT04464967	Not yet recruiting	SNK01 + trastuzumab or cetuximab	I/II	Advanced/metastatic HER2 or EGFR expressing cancers	1st
NCT02501603	Recruiting	Afatinib + paclitaxel	II	Previously treated HER2+ gastric cancer	2nd
NCT01402401	Terminated	AUY922 + trastuzumab	II	HER2+ advanced gastric cancer	2nd
NCT01522768	Active, not recruiting	Afatinib + paclitaxel	II	Previously treated HER2+ esophagogastric cancer	>1st
NCT04276493	Recruiting	ZW25 + chemotherapy ± tislelizumab	I/II	HER2+ gastric or GEJ adenocarcinoma	1st
NCT02689284	Active, not recruiting	Margetuximab + pembrolizumab	I/II	Relapsed/refractory HER2+ advanced gastric or GEJ adenocarcinoma	>1st
DESTINY-Gastric01/ NCT03329690	Active, not recruiting	Trastuzumab deruxtecan *vs* irinotecan or paclitaxel	II	HER2+ advanced/metastatic gastric or GEJ adenocarcinoma	>2nd
DESTINY-Gastic03/ NCT04379596	Recruiting	Trastuzumab deruxtecan ± chemotherapy and/or durvalumab	II	HER2+ advanced/metastatic gastric or GEJ adenocarcinoma	Part 1: >1st Part 2: 1st
INTEGA/NCT03409848	Active, not recruiting	Trastuzumab + nivolumab + ipilimumab or trastuzumab + nivolumab + FOLFOX	II	HER 2+ locally advanced gastric or GEJ adenocarcinoma	1st
MAHOGANY/NCT04082364	Recruiting	Margetuximab ± PDL-1 inhibitor ± chemotherapy ± dual checkpoint inhibitor	II/III	HER2+ gastric cancer or GEJ cancer	1st
KEYNOTE-811/ NCT03615326	Recruiting	Pembrolizumab/placebo + trastuzumab + chemotherapy	III	HER2+ advanced gastric or GEJ adenocarcinoma	1st

HER Human Epidermal Growth Factor Receptor 2, *GEJ* gastroesophageal junction, *PDL-1* Programmed Death-Ligand 1, *EGFR* Epidermal Growth Factor

Mechanism of resistance to anti-HER2 agents

HER2 positivity accounts for around 15% of GC, although its expression is quite heterogeneous compared with breast cancer (from 26 to 79% in IHC), which could negatively affect response to anti-HER2 targeted therapy [37, 93, 94] (Fig. 2a). Indeed, although the inhibition of HER2 has dramatically influenced the overall outcomes of HER2-positive GC patients, over 75% of them develop disease progression within 12 months. Of note, several potential mechanisms may give rise to primary and secondary resistance to HER2 blockage in breast cancer: impaired access of trastuzumab to HER2 by expression of an extracellular domain-truncated form of HER2 or overexpression of MUC4; activation of downstream signaling pathways (PI3K/AKT, MAPK, MEK and mTOR); loss of downstream controllers (PTEN and p27); alternative signaling from the insulin-like growth factor-1 receptor, or mesenchymal–epithelial transition (MET). However, all molecular mechanisms underlying resistance to HER2-targeted therapy in GC are not yet fully characterized, although some of them are shared with breast cancer.

Tumor heterogeneity—primary resistance

GC is a highly heterogeneous malignancy with a complex genomic landscape of molecular alterations. In breast cancer cells, the membranous distribution of the antibody is generally circumferential and complete staining for HER2 is required for the tumor to be classified as HER2 positive, whereas in GC, it is prevalently basolateral and incomplete,

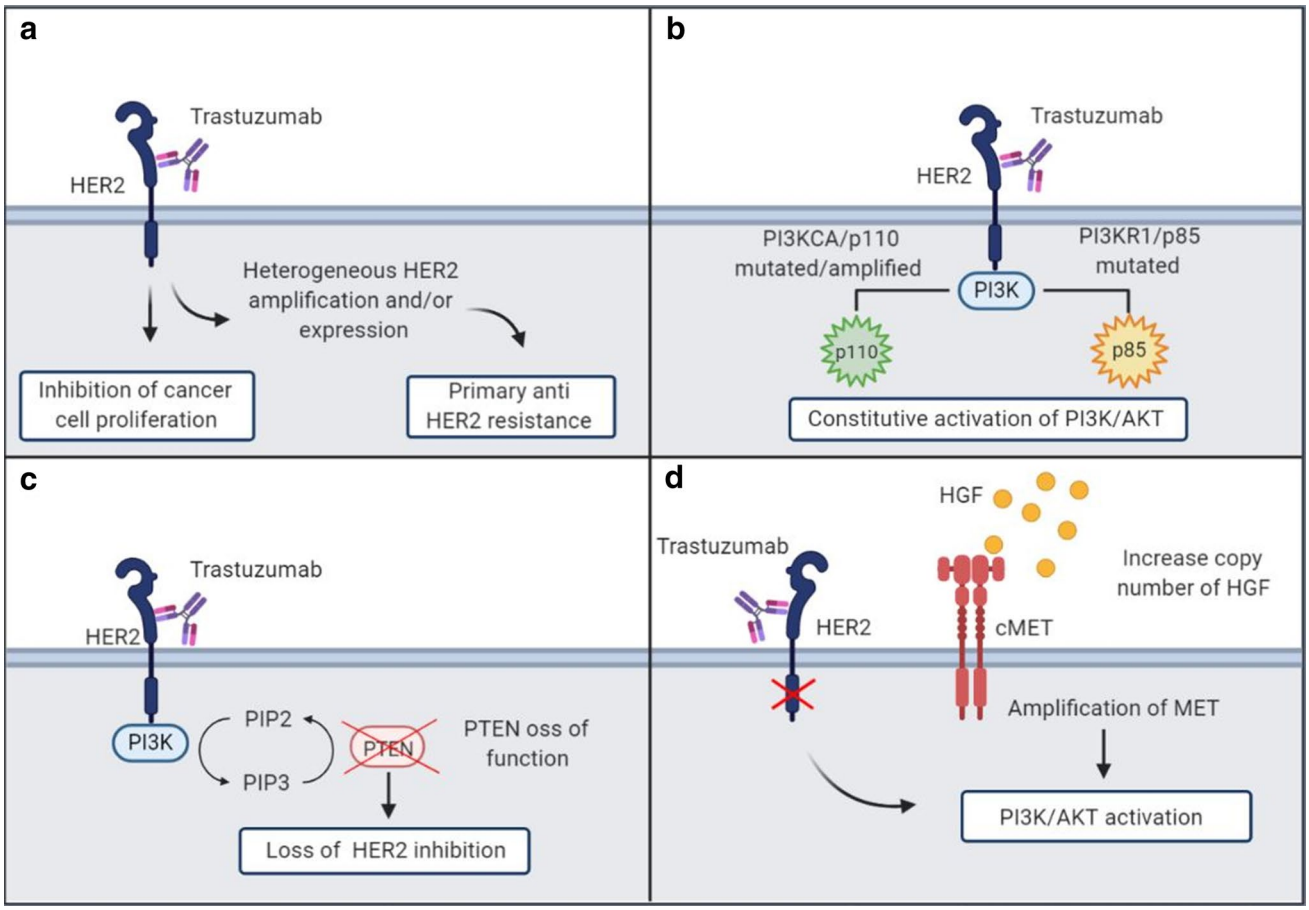

Fig. 2 General mechanisms of resistance to trastuzumab: presence of upregulation of HER2 downstream signaling pathways. **a** HER2 expression and/or amplification is highly heterogeneous in GC and this heterogeneity may negatively affect the response to HER2 blockage strategies leading to primary resistance. **b** Genomic aberrations in the PI3K pathway produce constitutive activation of the pathway, which will signal downstream to the nucleus regardless of trastuzumab binding to HER2. This is the case with activating mutations of PIK3R1 and PIK3CA, encoding genes for PI3K p85α and p110α, respectively. **c** PTEN is a tumor suppressor. Trastuzumab binding stabilizes and activates PTEN and consequently down-regulates the PI3K/Akt signaling pathway. When PTEN function is lost, PI3K remains constitutively active regardless of binding of trastuzumab to HER2 causing unresponsiveness to trastuzumab treatment. **d** Trastuzumab-induced growth inhibition in HER2-overexpressing cells can be compensated for by increased of other pathways, resulting in resistance to trastuzumab. c-Met is frequently co-expressed with HER2 in cell lines and its amplification or an increase of its ligand (HGF), contribute to trastuzumab resistance through sustained Akt activation. Figure created with Biorender.com

associated with intra-tumoral heterogeneity which provides a possible explanation for the differences in efficacy of the same HER2-targeted therapies between patients with GC and those with breast cancer. Notably, HER2 expression is highly discrepant between primary and metastatic disease [95, 96]. In the GASTHER1 study, 5.7% initial HER2-negative patients on primary tumor resulted HER2-positive on metastatic sites, reaching 17% discordance for liver lesions [97].

Co-existing oncogenic alterations—primary resistance

Oncogenic alterations such as point mutations or amplification have been reported, leading to the activation of downstream pathways and hampering the inhibitory effect of HER2-directed agents [66]. Those genetic alterations may be primed as negative predictors of trastuzumab benefit and, potentially, exploited as therapeutic co-targets. PI3KCA-activating mutations (Fig. 2b) and/or PTEN loss (Fig. 2c) may cause constitutive activation of the AKT–mTOR pathway, and the constitutive/aberrant activation of this signaling cascade may result in an inefficiency HER2 inhibition [98–100]. Deguchi et al. analyzed the occurrence of HER2 expression and PI3K mutation or PTEN loss in 264 GC patients, reporting 34.5% of HER2-postive patients with PTEN loss. No responses were observed in patients with PTEN loss treated with trastuzumab [98].

Hyperactivation of the hepatocyte growth factor (HGF) or the amplification of MET may be involved in the primary resistance in GC (Fig. 2d) [101–103]. Takahashi et al. showed that high-serum HGF was associated with poor response during HER2 blockage [98]. Furthermore, HER2 and HER1 may co-amplify in about 7% of GCs, according to the TCGA database report [104]. Preclinical studies showed that those cases may be resistant to upfront trastuzumab, while dual inhibitors might be beneficial in obtaining a more complete growth inhibition.

A multicenter, prospective, case–control study including 37 HER2-positive GC patients tested the negative predictive impact of a panel (AMNESIA) evaluating *EGFR/MET/KRAS/PI3K/PTEN* mutations and *EGFR/MET/KRAS* amplifications [105]. AMNESIA panel alterations were significantly more frequent in resistant (11 out of 20; 55%) as compared to sensitive (0% of 17) patients ($p < 0.001$). Furthermore, GC patients without panel alterations had a significantly longer median PFS (5.2 vs 2.6 months, HR: 0.34, 95% CI: 0.07–0.48, $p = 0.001$) and OS (16.1 vs 7.6 months, HR: 0.38, 95% CI: 0.09–0.75, $p = 0.015$) compared to the GC patients with panel positive tumors. The predictive accuracy of AMNESIA panel and HER2 IHC was 76 and 65%, respectively, while the predictive accuracy of the combined evaluation of AMNESIA panel and HER2 IHC was 84%. However, the retrospective nature of the study, the lack of a control arm (without trastuzumab), the elevated cost of the molecular screening, and the impossibility of detecting rare mutations may limit the current application in the clinical practice. A more extended, prospectively validated database called AMNESIA Global is currently ongoing.

Activation of alternative pathways—acquired resistance

HER2 acquired mutations src-induced activation of the MAP/ERK downstream pathway, NRF2 expression, FGFR amplification or HER3 overexpression may all contribute to the development of secondary resistance to trastuzumab [106–110].

Combination regimens involving HER2-targeted agents and those targeting IGF1R, PI3K, SRC and other proteins might overcome resistance to already approved HER2-targeted agents.

Loss of HER2 positivity—acquired resistance

Several authors have addressed the issue of the loss of the target during the upfront treatment, showing that a substantial proportion of patients may have a complete decline in surface HER2 expression after trastuzumab exposure [111]. The disappearance of the target is particularly frequent in the HER2-positive tumors classified as IHC2+ and FISH+ that may justify the insufficient HER2-inhibitor activity. Re-evaluation of HER2 status at disease recurrence or disease progression is needed to determine the appropriate use of HER2-targeted therapies, although is not considered mandatory in clinical practice.

Micro RNAs—acquired resistance

Upregulation of several microRNAs may regulate genes involved in the HER2 signaling pathway or HER3 at the post-transcriptional level and may be involved in acquired resistance to HER2-targeted therapies [112, 113].

Epithelial-to-mesenchymal transition—acquired resistance

Preclinical evidence suggest that MET may be involved in HER2-inhibition secondary resistance [114, 115]. Particularly, Jialong et al. showed a mesenchymal phenotype, increased migration, and invasive capacities in HER2-positive GC cell lines.

New screening techniques

The development of newer and performing diagnostic, prognostic and disease monitoring tools are crucial for the improvement of the clinical outcome of GC patients [4]. Tissue samples were the main sources for evaluating

tumor-associated genetic alterations in these patients, but the invasive nature and the inability to reflect tumor heterogeneity are crucial limitations [36].

Other methods, such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), extracellular domain (ECD) of HER2 and new imaging agents [i.e., zirconium 89 (89Zr) trastuzumab PET], have demonstrated to have prognostic value in several types of cancer, including GC, or used as non-invasive tool to monitor disease progression throughout treatment [116–119].

A prognostic role for the enumeration of CTC in numerous cancers, including GC has been highlighted [120–122]. CTCs may represent the genetic compositions of both primary and metastatic tumors, and the real-time assessment of prognostic and therapeutic biomarkers on CTCs may be useful to improve the clinical outcomes and effecting on targeted cancer therapy [116, 117]. Indeed, Nevis et al., have demonstrated that the HER2 amplification in CTCs, evaluated using FISH, was strongly concordant with tissue amplification, considering their application as a potential alternative to tumor biopsy [123].

Nonetheless, this accordance was not confirmed in another study where13 out to 50 patients with GC considered HER2 negative, had a benefit to trastuzumab therapy similar to patients with GC HER2-positive tumor in preliminary clinical data [124].

Circulating tumor DNA is tumor-derived fragmented DNA in the bloodstream not associated with cells [125]. Originating from primary tumor cells, CTCs and/or distant metastasis, ctDNA give a broad cross-section of the disease offering information on methylation status, genetic alterations as mutations, amplifications, rearrangements, copy number variation (CNV) [126]. ctDNA represents only a small percentage of the cell-free circulating DNA (cfDNA), which is increased considerably in late-stage disease [127]. However, ctDNA can be detected in the plasma of cancer patients even in the early stages of disease [128, 129]. Circulating tumor DNA analysis refined the liquid biopsy to identify tumor molecular traces circulating and may give deeper insight into the cancer heterogeneity, early biomarker detection, therapeutic target detection, real-time evaluation of treatment response and possible resistance and prognosis.

Initially, a concordance rate of HER2 expression between the ctDNA and tumor tissue around 60% has been observed [130], which became up to 90% with the use of modern techniques (i.e., digital droplet PCR, next-generation sequencing) [131, 132]. The difference between plasma and tumor in HER2 amplification could be explained at least in part, by the high heterogeneity of HER2 expression in GC cells but, if confirmed the validity of ctDNA, it could be used as alternative HER2 screening method. Moreover, recent data suggest that ctDNA could be a complementary tool to predict response to anti-HER2 treatment and that their dynamic evaluation could be a surrogate marker of treatment efficacy, although there is still reliable data and further confirmations are needed [132–134].

The extracellular domain of HER2, quantifiable in the serum, seems to be another valid alternative to tissue biopsy. A significant relationship between serum concentrations of HER2 ECD and tissue levels of HER2 protein was found in patients with GC [135]. Moreover, HER2 ECD level could become a predictive marker of response to anti-HER2 therapies as has been suggested by several studies in breast cancer [136, 137].

Discordant HER2 expression within a single cancer or between different sites of cancer can introduce sampling error and confound treatment decision-making. First tried in breast cancer and more recently in GC, positron emission tomography (PET), using the radiolabeled zirconium 89 [89Zr] trastuzumab, has shown several advantages over biopsy-based methods as it can noninvasively assess variation in level of HER2 in both the primary tumor and all sites of metastases simultaneously [138]. [89Zr] trastuzumab PET directly assesses the availability of HER2 to be bound to trastuzumab; thus, it is potentially a more reliable predictor of response to trastuzumab therapy. In addition, [89Zr] trastuzumab PET has the ability to assess intra-patient heterogeneity of HER2 tumor expression and considering its not invasiveness [89Zr] trastuzumab PET can be repeated during therapy to assess response [138–140].

Recently, the organoid technology has been emerged to overcome the issues related to the widespread histological and molecular heterogenicity that characterize GC as well as other tumors as well as the limitations related to cancer cell lines and patient-derived xenografts (PDX), as reviewed in [141].

Briefly, long-term organoid cultures can be established from several primary tumors (e.g., stomach, breast, colon cancer tissues) and collection of patient-derived tumors are generated and biobanked [141]. These organoid models were used to investigate molecular features in different subtypes and patient treatment response. For instance, a library with more than 100 breast cancers (primary and metastatic tumors) organoid lines has been already built as well as a patient-derived GC organoid library with numerous histological and genetic subtypes [142, 143].

Moreover, the development of single-cell profiling, that allow to study tumors (and the related complex microenvironment) at the resolution of individual cells, could have a profound impact on clinical decisions [144]. Indeed, single-cell sequencing can reveal clonal repopulation, factors related to acquired drug resistance and dynamic changes in tumor-associated microenvironment. To note, Wang et al. have demonstrated that the use of high-throughput single-cell profiling allows to individuate target specific cell population (i.e., intra-tumoral immunosuppressive myeloid cell),

targetable by tyrosine kinase inhibitor, that restores the vulnerability to checkpoint blockade immunotherapy, overcoming resistance [145].

Conclusions

Unfortunately, a large portion of patients with GC are initially diagnosed with unresectable or metastatic disease and systemic treatments have led to modest improvement in overall survival when compared to BSC alone. The identification of tumor with HER2 overexpression in metastatic CG patients remains significant to improve treatment outcomes. However, to enable progress beyond currently approved therapies in this molecular subset will require composite testing strategies to properly identify tumoral heterogeneity underlying trastuzumab resistance. Several clinical trials evaluating novel anti-HER2 approaches are ongoing and the introduction of new screening methods, such as circulating tumor cells, circulating tumor DNA, extracellular domain of HER2 and new imaging agents, could deeply improve their therapeutic impact in GC.

Declarations

Conflict of interest The authors declare no conflict of interest.

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