



# Human Epidermal Growth Factor Receptor 2 (HER2) in Advanced Gastric Cancer: Current Knowledge and Future Perspectives

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## Abstract

The discovery of human epidermal growth factor receptor 2 (HER2) overexpression in 15–20% of gastric adenocarcinomas has been a key advance in the global care of this disease. Validated by the ToGA trial in the first-line setting of advanced HER2-positive (+) gastric cancer (GC), trastuzumab, an anti-HER2 monoclonal antibody (mAb), was the first therapeutic agent to significantly improve the prognosis of these patients. Since these results, many attempts have been made to improve the clinical outcomes of patients with HER2+ GC. However, all the other HER2-targeting molecules have failed to show a survival benefit in large phase III studies. The value of continuing trastuzumab after disease progression has been suggested by several retrospective studies. However, recent results of a randomized phase II trial showed no benefit from this strategy. On the other hand, novel therapeutic methods, such as immunotherapy, are emerging as new tools in the strategy of care of advanced GC, even if their benefit in the specific HER2+ population remains undetermined. Furthermore, substantial progress has been made in the understanding of the mechanisms leading to resistance to anti-HER2 therapies, and in the screening methods to detect them, thus opening new perspectives. The aim of this review was firstly to summarize the existing data on the specific strategy of care of HER2+ advanced GC, and secondly, to describe current knowledge regarding the potential mechanisms of resistance to HER2-targeting therapies. Lastly, we report the prospects for overcoming these potential obstacles, from future therapeutic strategies to new detection methods.

## 1 Introduction

Gastric cancer (GC) is the fifth most commonly diagnosed cancer and the third most leading cause of cancer-related death in the world [1]. In the majority of cases, GC is diagnosed at a metastatic or unresectable stage. For these patients, systemic treatment based on cytotoxic chemotherapy improves quality of life and survival compared to best supportive care [2]. However, despite the improvement observed during the past decades, advanced GC has a very poor prognosis, with a median overall survival (OS) of 10–12 months [3, 4]. This emphasizes the need to identify new therapeutic targets and to develop new antitumor agents to improve patient outcomes.

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### Key Points

Trastuzumab has allowed major progress in the treatment of advanced HER2-overexpressing gastric adenocarcinoma.

All other HER2-targeting molecules have failed to provide benefit in first- or second-line treatment in large randomized phase III studies, and the value of continuing trastuzumab after disease progression remains uncertain.

Our understanding of the mechanisms leading to resistance to HER2-targeting treatments has recently improved, paving the way for new therapeutic approaches.

Emerging methods for detection of circulating tumor DNA, for example, and new treatments, such as combination with immunotherapy, have shown interesting results and could become an integral part of the strategy of care of this disease.

GC is a heterogeneous disease, the initiation, growth, and dissemination of which result from different genomic alterations that can induce the activation of numerous molecular pathways. Among these molecular pathways, the identification of a subgroup of tumors that overexpress human epidermal growth factor 2 (HER2) has been a major advance in the therapeutic approach to GC. The aim of this article is first to review the existing data on the treatment of HER2+ advanced GC, then, we discuss current knowledge on the different mechanisms that result in primary or secondary resistance to anti-HER2 therapies. Lastly, we report prospects for the improvement of care for patients with HER2+ advanced GC, from new detection methods to future therapeutic strategies.

## 2 Human Epidermal Growth Factor 2 (HER2) Signaling Pathway

HER2 is a tyrosine kinase receptor, and a member of the epidermal growth factor receptor (EGFR) family [5], which is involved in numerous signaling pathways such as the PI3K/AKT/mTOR and RAS/RAF/MAP kinase pathways, which result in cellular growth, proliferation, differentiation, and migration. Many studies report overexpression or amplification of HER2 in GC. The reported proportion of GC cases with HER2 overexpression ranges from 6 to 30% [10]. In a large meta-analysis, higher HER2 expression rates were significantly correlated with intestinal-type GC according to Lauren's classification, proximal tumor sites, and well-differentiated cancers [11]. HER2 overexpression is mainly due to amplification of the *HER2* gene, though other molecular mechanisms have been reported [8, 9]. Although HER2 overexpression has been associated with a worse outcome in some studies, its role in prognosis remains uncertain in GC [6, 7].

Immunohistochemistry (IHC) and fluorescence in situ hybridization/in situ hybridization (FISH/ISH) methods have been validated in daily practice to identify HER2-overexpressing gastric tumors [12, 13]. The IHC score divides gastric tumors into three categories: negative (0+ or 1+), equivocal (2+), or positive (3+). The IHC score has several limitations, including the heterogeneous nature of HER2 expression often observed in GC (unlike breast cancer), which can lead to difficulties or errors in its interpretation. Evaluation of the IHC score in GC differs slightly from that in breast cancer, and therefore calls for an experienced pathologist.

In the case of an equivocal score of 2+, it is currently recommended that HER2 positivity be confirmed by detecting *HER2* amplification by FISH/ISH [14]. Current guidelines define FISH/ISH positivity as a ratio of HER2 signal to centromere (control) CEP17 signal  $\geq 2.0$  [14]. Two studies

have reported an optimal cut-off ratio of  $\geq 4.0$  as a predictive factor for response to trastuzumab and a prognostic factor of better survival [15, 16].

Although the results of IHC and FISH are well correlated, some studies have shown that disparities can occur [17]. These inconsistencies may be due to several factors, such as polysomy (increased copy number of the control gene signal leading to a normal signal ratio), intratumoral heterogeneity, or technical errors [18].

## 3 First-Line Treatment in HER2-Positive Advanced Gastric Cancer

### 3.1 Trastuzumab

Trastuzumab is an mAb that specifically targets HER2, and was first validated in the treatment of HER2-overexpressing breast cancer [19, 20], and also showed antitumor effects in HER2+ GC in combination with chemotherapy in preclinical studies, including one study based on a human HER2+ GC xenograft model [21, 22]. Based on these results, the ToGA phase III randomized trial compared the efficacy of chemotherapy (cisplatin and fluoropyrimidine) alone or with trastuzumab (at a dose of 8 mg/kg on the first day of first cycle, followed by 6 mg/kg every 3 weeks) in the first-line treatment of HER2-overexpressing (IHC3+ or FISH+) advanced gastric or gastroesophageal junction (GEJ) adenocarcinoma. The primary outcome was OS. Median follow-up was 18.6 months (interquartile range (IQR) 11–25) in the trastuzumab group and 17.1 months (IQR 9–25) in the placebo group. In this trial, trastuzumab with chemotherapy led to a significant increase in OS compared to chemotherapy alone (13.8 vs. 11.1 months;  $P = 0.0046$ ). Secondary endpoints including PFS, overall tumor response rate, time to progression, and duration of response were also significantly improved in the trastuzumab + chemotherapy group compared to the chemotherapy-alone group [23]. Apart from diarrhea, the frequency of grade 3 or 4 adverse events was similar between the two groups. In particular, there was no between-group difference in cardiac-related events, regardless of grade (6% in both groups). Post hoc subgroup analyses showed that patients with a high level of HER2 expression (IHC3+ or IHC2+ and FISH+) had a higher benefit from the addition of trastuzumab to chemotherapy, whereas no benefit was observed in those with a null or low level of HER2 expression (IHC 0 or 1+), even with a positive FISH. Based on these data, trastuzumab in combination with cisplatin and fluoropyrimidine has become the new standard first-line treatment for patients with advanced gastroesophageal adenocarcinoma overexpressing HER2, defined as IHC 3+ or IHC 2+/FISH+ [3, 4].

Following ToGA, two phase II studies and one retrospective study reported similar results regarding the efficacy of replacing cisplatin by oxaliplatin and/or fluorouracil by capecitabine in combination with trastuzumab [24–26]. Therefore, in many centers, oxaliplatin and fluoropyrimidine combined with trastuzumab are used in the first-line treatment of HER2-positive advanced GC, although no phase III study has evaluated oxaliplatin and capecitabine in this specific situation. In addition, Takahari et al. recently showed in a Japanese multicenter phase II study that the combination of S-1 and oxaliplatin with trastuzumab was associated with survival results comparable to those of ToGA [27].

In a phase III randomized study, Shah et al. assessed the efficacy of a high-dose regimen of trastuzumab (loading dose of 8 mg/kg followed by 10 mg/kg every 3 weeks) in combination with cisplatin and capecitabine in a cohort of 248 patients with HER2+ untreated metastatic GC. Although the serum trough concentration of trastuzumab was significantly increased in the high-dose trastuzumab group, no benefit was observed in terms of OS (primary endpoint) or PFS [28].

### 3.2 Other Anti-HER2 Agents Evaluated in First-Line Chemotherapy by Phase III Randomized Trials

All phase III randomized studies assessing anti-HER2 agents in GC are summarized in Table 1.

#### 3.2.1 Lapatinib

Lapatinib, a dual HER1 and HER2 tyrosine kinase inhibitor [29], was evaluated in the randomized LOGIC phase III study comparing capecitabine and oxaliplatin (CapeOx) alone or combined with lapatinib as first-line therapy in 487 patients with HER2 FISH-positive advanced GC [30]. In this trial, the addition of lapatinib to CapeOx was associated with improvement in PFS but not in OS, which was the primary endpoint. Note that, in this study, selection of HER2-overexpressing patients was based on FISH only, regardless of IHC status, which could explain, at least partly, the negative results, although subgroup analysis found no significant efficacy of lapatinib among IHC 2/3+ patients. Interestingly, pre-planned subgroup analyses found that in younger patients and in patients of Asian origin lapatinib was associated with a significantly increased OS, raising the question of better selection of the patients who would benefit from this drug. Moreover, 23% of patients included in this study had had prior gastrectomy removing their pylorus, which could also have negatively impacted the results by decreasing lapatinib absorption.

#### 3.2.2 Pertuzumab

Pertuzumab is a recombinant humanized mAb that binds to the dimerization domain of HER2, thereby blocking ligand-induced HER2 heterodimerization. Pertuzumab is effective in HER2+ breast cancer at both the early and the advanced stages [31, 32]. A recent phase III study (the JACOB trial) evaluated the addition of pertuzumab versus placebo to trastuzumab with chemotherapy in 780 patients with advanced HER2+ GC (defined as IHC3+ or IHC2+ and FISH+) in a first-line setting [33]. Although patients treated with pertuzumab had a 3.3-month improvement of median OS compared to those who received the placebo, the results from this study did not reach statistical significance (14.2 vs. 17.5 months; HR = 0.84,  $P = 0.056$ ). These negative results, in contradiction with those observed in breast cancer, underline the differences in HER2 biology between gastric and breast cancers and its role as an oncogenic driver. Notably, according to the authors, the heterogeneous pattern of HER2 expression observed in GC could negatively impact on pertuzumab activity.

## 4 Targeting HER2 in Second-Line Treatment

### 4.1 Anti-HER2 Agents Evaluated in Second-Line Therapy by Phase III Randomized Studies

#### 4.1.1 Lapatinib

Lapatinib was evaluated in a second-line setting in the TyTAN phase III trial, which randomized 261 patients with advanced HER2 FISH+ GC (regardless of IHC status) [34]. Note that in this trial, only 6% of patients received trastuzumab first line. The addition of lapatinib to paclitaxel was not associated with an improvement of clinical outcome. Interestingly, in a subgroup analysis of patients with baseline IHC3+ HER2-positive tumors from the TyTAN trial (39%), the addition of lapatinib to paclitaxel was associated with a significant improvement of OS (14.0 months vs. 7.6 months; HR = 0.59,  $P = 0.02$ ), whereas no difference was observed in the population of IHC2+ HER2-positive tumors. Moreover, it is interesting to note that 35% of the patients of this study were IHC0/1+ and would now be considered as HER2-negative.

#### 4.1.2 Trastuzumab Emtansine

Trastuzumab emtansine (T-DM1) is an antibody–drug conjugate of trastuzumab linked to the tubulin inhibitor emtansine. T-DM1 was recently evaluated in the randomized GATSBY phase II/III study including HER2+ GC patients (IHC3+ or IHC2+ and FISH+) who had progressed on

**Table 1** HER2-targeting therapies evaluated in phase III trials for patients with advanced HER2-positive gastric cancer

Trial (date)	Phase	Line of treatment	Chemotherapy	Inclusion HER2 status	Population origin	Trastuzumab in first line	ORR (%)	Median PFS (months)	Median OS (months)
ToGA (2010) [23]	III (N = 594)	1st	FP + cisplatin ± trastuzumab	FISH +	World	–	47 vs. 35 (P = 0.00175)	6.7 vs. 5.5 HR = 0.71 P = 0.0002	13.8 vs. 11.1 HR = 0.74 P = 0.0046
TyTAN (2014) [34]	III (N = 261)	2nd	paclitaxel ± lapatinib	FISH + (any IHC status)	Asia	6%	27 vs. 9 (P < 0.001)	4.4 vs. 5.4 HR = 0.85 P = 0.24	8.9 vs. 11 HR = 0.84 P = 0.10
TRIO/LOGiC (2016) [30]	III (N = 487)	1st	CAPOX ± lapatinib	FISH + (any IHC status)	World	–	53 vs. 39 (P = 0.003)	5.4 vs. 6.0 HR = 0.82 P = 0.04	10.5 vs. 12.2 HR = 0.91 P = 0.35
GATSBY (2017) [35]	II/III (N = 345)	2nd	taxane vs. T-DM1	IHC 3+ or IHC2+/FISH+	World	76%	20 vs. 21 (P = 0.84)	2.9 vs. 2.7 HR = 1.13 P = 0.31	8.6 vs. 7.9 HR = 1.15 P = 0.86
JACOB (2017) [33]	III (N = 780)	1st	FP + cisplatin + trastuzumab ± pertuzumab	IHC 3+ or IHC2+/FISH+	World	–	57 vs. 48	7.0 vs. 8.5 P < 0.05	14.2 vs. 17.5 HR = 0.84 P = 0.056

ORR objective response rate, HR hazard ratio, FP fluoropyrimidine, PFS progression-free survival, OS overall survival

first-line treatment. Most of the patients had received trastuzumab in first-line therapy (79% and 76% in the taxane and T-DM1 arms, respectively). Patients were randomized to receive docetaxel or T-DM1. In this study, T-DM1 was not superior to taxane in terms of PFS or OS [35]. Two factors should be noted in regard to the negative results of this trial: first, the choice of T-DM1 monotherapy might not have been the most appropriate notably in regard to its limited targeted mechanism of action, and, second, survival of patients from the control group was 2.6 months longer than expected at the time of study design. A phase I/II trial is currently ongoing to evaluate T-DM1 in combination with capecitabine in first-line treatment of HER2+ advanced GC (NCT01702558).

Beyond the previously discussed limitations that could have negatively impacted the results of these studies, the contradictory results provided by HER2-targeting drugs in a second-line setting in GC and in breast cancer seem to indicate a significant difference in changes in tumor biology during first-line HER2 blockade. Indeed, acquired resistance mechanisms specific to GC have recently been described and will be discussed further in this article. These discrepancies also underline the need for better selection of patients who would benefit from these therapies in second-line treatment.

## 4.2 Continuing Trastuzumab Beyond First-Line Progression

As presented above, trastuzumab is currently the only HER2-targeting agent that has shown efficacy in a randomized phase III study. In HER2-positive metastatic breast cancer, administration of an anti-HER2 therapy is recommended throughout disease progression, in order to continue suppression of the HER2 pathway in first-line or second-line treatment or beyond [36]. More recently, this strategy of trastuzumab beyond first-line progression has been evaluated in HER2+ GC by several studies, which have shown contradictory results (Table 2).

### 4.2.1 Retrospective and Non-Randomized Studies

Li et al. showed in a prospective observational Chinese cohort of patients with HER2+ advanced GC that the continuation of trastuzumab beyond progression in combination with chemotherapy was associated with an improvement of PFS compared to chemotherapy alone, while tumor response rate and OS were not significantly improved [37].

In a French retrospective multicenter study, Palle et al. assessed the value of maintaining or not trastuzumab in second-line treatment in 104 patients who had progressed on first-line therapy based on 5-FU plus platinum salt and trastuzumab [38]. Patients treated with trastuzumab beyond

**Table 2** Trastuzumab beyond first-line chemotherapy in advanced HER2-positive gastric cancer

Trial author (date)	Country	Phase (N patients)	Line CT	Chemotherapy	Trastu in L1	ORR (%)	Median PFS (months)	Median OS (months)
Li et al. (2016) [37]	China	Prospective (N=59)	2nd	CT ± trastuzumab	100%	9.3 vs. 3.7 ( <i>P</i> =0.62)	3.1 vs. 2.0 ( <i>P</i> =0.008)	10.5 vs. 6.5 ( <i>P</i> =0.17)
Makiyama et al. (2017) [97]	Japan	Prospective (N=85)	2nd	CT ± trastuzumab	100%	ND	ND	7.9 vs. 12.8 ( <i>P</i> =0.01)
Narita et al. (2017) [39]	Japan	Retrospective (N=46)	2nd	CT ± trastuzumab	100%	18.2 vs. 15.8 ( <i>P</i> =1.00)	4.0 vs. 2.3 ( <i>P</i> =0.14)	10.8 vs. 9.5 ( <i>P</i> =0.88)
Palle et al. (2017) [38]	France	Retrospective (N=104)	2nd	CT ± trastuzumab	100%	16.7 vs. 5.4 ( <i>P</i> =0.08)	4.4 vs. 2.3 ( <i>P</i> =0.002)	12.6 vs. 6.1 ( <i>P</i> =0.001)
Al Shamsi et al. (2017) [98]	US	Retrospective (N=43)	2nd	CT ± trastuzumab	100%	ND	5	11
Sun et al. (2011) [99]	China	Phase II randomized (N=34)	ND	FOLFIRI ± trastuzumab	ND	58.8 vs. 35.3 ( <i>P</i> <0.05)	ND	ND
Nishikawa et al. (2016) [100]	Japan	Prospective (N=29)	Chemo-refractory	IRINOTECAN + trastuzumab	ND	20.7	3.7	7.5
Kawamoto et al. (2017) [101]	Japan	Prospective (N=16)	2nd	IRINOTECAN + trastuzumab	100%	7	2.4	9.7
Zaanan et al. (2017) [102]	France	Retrospective (N=20)	2nd	FOLFIRI + trastuzumab	100%	17.6	ND	9.5
Nishikawa et al. (2016) [103]	Japan	Phase II (N=46)	Chemo-refractory	Paclitaxel + trastuzumab		37	5.1	17.1
Makiyama et al. (2018) [41]	Japan	Phase II randomized (N=91)	2nd	Paclitaxel ± trastuzumab	100%	33.3 vs. 31.6 ( <i>P</i> =1.00)	3.68 vs. 3.19 ( <i>P</i> =0.33)	10.2 vs. 9.95 ( <i>P</i> =0.20)
Horita et al. (2019) [40]	Japan	Retrospective (N=28)	2nd	Paclitaxel ± trastuzumab	100%	25 vs. 12.5 ( <i>P</i> =0.466)	5.0 vs. 2.8 ( <i>P</i> =0.244)	10.8 vs. 6.1 ( <i>P</i> =0.268)

ORR objective response rate, PFS progression-free survival, OS overall survival, CT chemotherapy

progression (37.5%) had a longer median PFS (4.4 vs. 2.3 months) and OS (12.6 vs. 6.1 months) than patients who had been treated with second-line chemotherapy alone (PFS: hazard ratio (HR)=0.51; 95% confidence interval (CI) [0.33–0.79]; OS: HR=0.44; 95% CI [0.27–0.73]).

Lastly, a multicenter retrospective Japanese study in 46 patients suggested that continuation of trastuzumab significantly improved PFS in selected subgroups of patients with tumors exhibiting an HER2 IHC expression score of 3+ (HR=0.41;  $P=0.04$ ), intestinal-type histology (HR=0.32;  $P<0.01$ ), and a first-line PFS > 6 months (HR=0.44;  $P=0.04$ ) [39]. This impact of first-line response duration on survival benefit of trastuzumab beyond progression was also suggested in another Japanese retrospective study comparing tumor response and survival in 28 patients who received paclitaxel alone ( $n=8$ ) or with trastuzumab ( $n=20$ ) in second-line treatment of advanced HER2+ GC. No difference was observed in terms of objective response rate (ORR), PFS, or OS. However, OS and PFS were significantly improved in the subgroup of patients with a first-line PFS > 6 months [40].

This might emphasize the importance of better selection of patients who may benefit from trastuzumab beyond progression. However, given the small number of patients included in the above-cited studies and their retrospective/non-randomized, nature, these results should be considered with caution.

#### 4.2.2 Randomized Studies

At the 2018 American Society of Clinical Oncology (ASCO) congress, Makiyama et al. presented the first results of a Japanese randomized phase II study that assessed the benefit of trastuzumab beyond progression [41]. In this study, 91 patients with advanced HER2+ gastroesophageal adenocarcinoma that had progressed on first-line treatment with fluoropyrimidine, cisplatin, and trastuzumab were randomized to receive either weekly paclitaxel alone or in combination with trastuzumab. The primary outcome was PFS. In this study, unlike the retrospective studies cited above, continuation of trastuzumab in combination with paclitaxel did not significantly improve PFS (3.19 vs. 3.68 months; HR=0.91; 95% CI, [0.67–1.22];  $P=0.33$ ) or OS (9.95 vs. 10.2 months; HR=1.23; 95% CI, [0.75–1.99];  $P=0.2$ ). In a subgroup analysis, patients who had a longer period (> 30 days) without trastuzumab prior to randomization (representing 31 patients), PFS (primary endpoint) was significantly longer in the paclitaxel + trastuzumab arm than in the paclitaxel alone arm (4.68 months vs. 2.98 months; HR=0.45 [0.21–0.96];  $P=0.033$ ) [41]. This could suggest a phenomenon of re-sensitization to trastuzumab after a treatment-free interval, possibly due to a decrease of pressure selection on HER2+ clones.

Furthermore, in this study, HER-2 status was reassessed at first progression in 16 patients. Interestingly, the authors observed that for 11 out of the 16 patients (69%), the HER2 positivity was lost, once more raising the question of the need to select patients who could benefit from maintaining trastuzumab beyond progression. The complete results of this study have not yet been published to our knowledge.

Given the contradictory results of the studies cited above, new trials testing this approach and considering the “plasticity” of HER2 status during treatment may still be relevant.

## 5 Mechanism of Primary Resistance to Anti-HER2 Agents

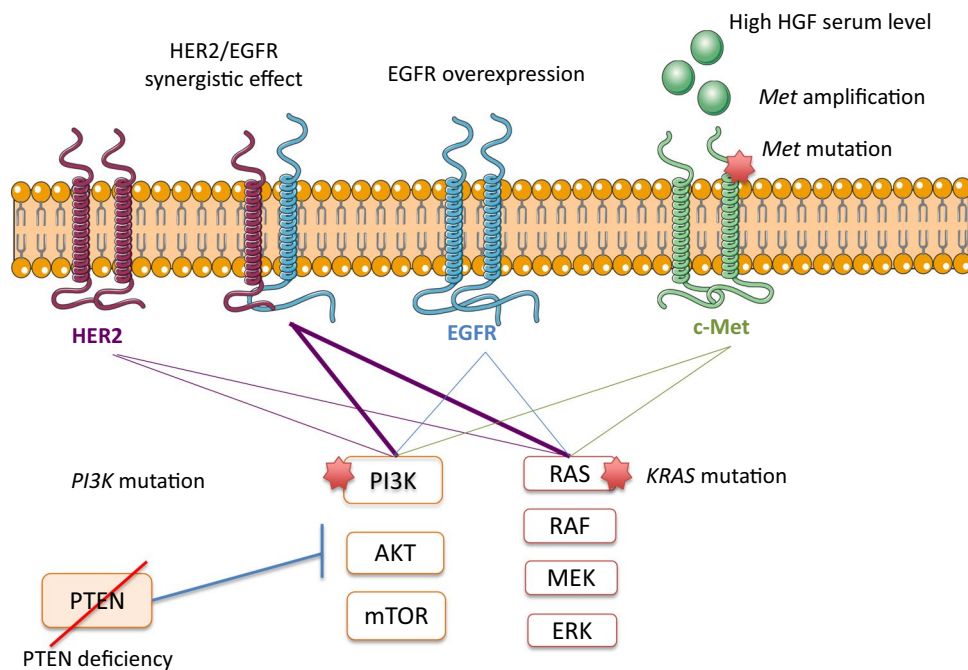
### 5.1 HER2 Heterogeneity

Unlike breast cancer, HER2 overexpression and/or amplification by GC can be heterogeneous (from 26 to 79% in IHC) [42, 43], which could affect response to anti-HER2 therapies such as trastuzumab. In two Japanese studies of patients with HER2+ metastatic or unresectable GC receiving trastuzumab-containing first-line therapy, HER2 heterogeneity (defined on endoscopic biopsy specimens) was an independent factor of worse prognosis [42, 44]. Similarly, in a cohort of 28 patients with HER2+ GC who had gastrectomy prior to receiving trastuzumab, heterogeneous expression of HER2 (defined on surgical specimens) was associated with a significantly worse outcome in a multivariate analysis, compared to homogeneous expression [45]. However, in a Chinese study including 48 patients, this correlation between HER2 heterogeneity and response to trastuzumab was not found [46]. Moreover, it is important to note that unlike breast cancer, HER2 heterogeneity in GC is not clearly defined, with different cut-off values between studies. Guidelines concerning the assessment of HER2 heterogeneity in IHC and/or FISH in GC are therefore needed.

### 5.2 Co-Existing Oncogenic Alterations

Oncogenic alterations can co-exist in *HER2*-amplified tumors. Indeed, several genomic alterations such as point mutations or amplifications have been described, leading to activation of tyrosine kinase receptors other than HER2 or downstream signaling pathways. In their study of point mutations and amplifications in the exome of 62 *HER2*-amplified GC samples, Kim et al. found that more than half of the *HER2*-amplified gastric tumors showed additional oncogenic alterations that could potentially hamper the growth-inhibitory effect of HER2-targeting drugs.

Oncogenic alterations associated with HER2 amplification/overexpression in GC are summarized in Fig. 1.



**Fig. 1** Oncogenic alterations associated with HER2 overexpression/amplification in gastric cancer conferring primary resistance to trastuzumab. Primary resistance to trastuzumab has mainly been attributed to oncogenic alterations that can co-occur in *HER2*-amplified tumors [48]. PTEN deficiency and/or PI3K-activating mutations, resulting in activation of AKT/mTOR signaling, have been described in vitro and in vivo in HER2-overexpressing tumors and in *HER2* amplified GC cell lines conferring primary resistance to trastuzumab [47, 48].

### 5.2.1 PTEN and PI3K

Phosphatase and tensin homolog (PTEN) deficiency or *PI3K* mutations can lead to a constitutive activation of the phosphatidylinositol 3 kinase (PI3K) pathway. In a Japanese study, Deguchi et al. evaluated the co-occurrence of HER2 overexpression and PTEN loss or *PI3K*-mutations in 264 surgically removed gastric adenocarcinomas [47]. In this study, PTEN loss was observed in 34.5% of HER2-overexpressing tumors. In the same work, the authors evaluated PTEN expression in 23 patients receiving trastuzumab as first-line treatment. While a clinical response was observed in 50% of PTEN-positive tumors, none of the tumors with PTEN loss responded to first-line treatment with trastuzumab, therefore suggesting that PTEN loss could be associated with trastuzumab resistance. In this study, PI3K activating mutations were rarer (5.6%), whereas Kim et al. showed that it could confer resistance to HER2-targeting drugs in *HER2*-amplified cell line in vitro models [48].

Hyperactivation of the HGF/MET pathway, induced by *MET* amplification, *MET* mutations and elevated serum HGF levels, could also impact negatively on tumor response to trastuzumab [48, 49]. Lastly, EGFR overexpression has been found in a large proportion of *HER2*-amplified GC cases and could have a synergistic action on the HER signaling pathway and its transforming effect, leading to resistance to HER2-targeting therapies [48, 50]

### 5.2.2 HGF/MET

Hyperactivation of the hepatocyte growth factor (HGF)/mesenchymal epithelial transition factor (MET) pathway could also be involved in primary resistance to trastuzumab. In a study conducted by Takahashi et al. on 46 patients with metastatic GC treated with trastuzumab, high serum HGF was predictive of poor response [49]. In another study, amplification of MET was found in some HER2-amplified GC samples, and conferred resistance to HER2-targeting drugs in HER2-amplified cell-line in vitro models [48].

To our knowledge, no study has evaluated the feasibility and efficacy of combined HER2 and cMet/HGF targeting treatment.

### 5.2.3 EGFR

In a genomic study based notably on TCGA data, including 62 *HER2*-amplified untreated GC cases, Kim et al. found co-amplification of *EGFR* in 7.8% of cases. In a separate cohort of 12 *HER2*-amplified GC cases, co-existing EGFR overexpression was observed in 58.3% of cases [48]. However, in this small series no data were given regarding the

concordance between *EGFR* amplification and EGFR expression in the context of GC. More recently, the synergistic transforming effects of EGFR and HER2 in GC were studied by Wang et al. using specific small interfering RNAs (siRNAs) to inhibit mRNA and protein expression of target genes [50]. In this study, EGFR-specific siRNA and EGFR/HER2 siRNAs inhibited cell growth, whereas HER2-specific siRNA had no effect. Resistance to HER2-siRNA was linked to compensatory activation of EGFR.

These preclinical data suggest that a dual anti-HER2 anti-EGFR could be beneficial in tumors showing both EGFR and HER2 overexpression.

Emphasizing the importance of concomitant oncogenic alterations in primary resistance to trastuzumab, Pietrantonio et al. proposed a panel of genomic alterations including *EGFR/MET/KRAS/PI3K/PTEN* mutations and *EGFR/MET/KRAS* amplifications that was significantly associated with a poorer response to trastuzumab in a prospective

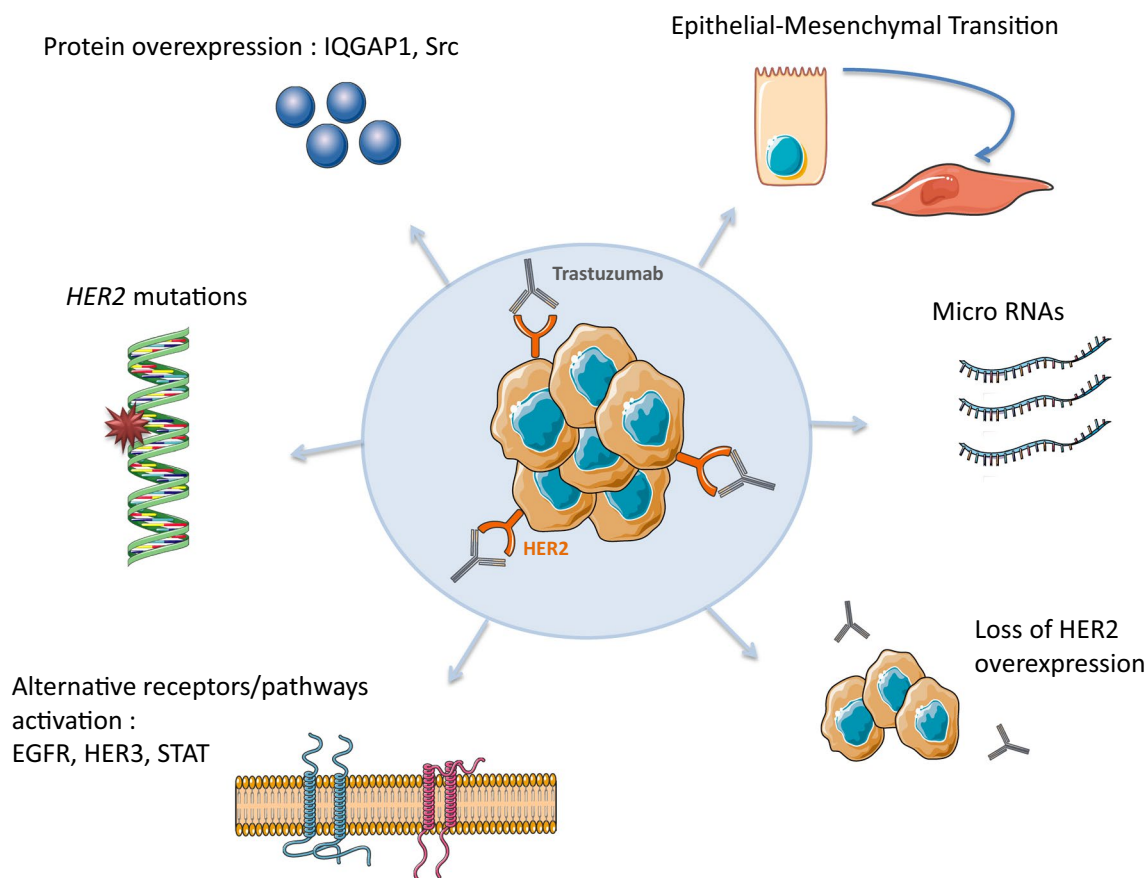
case-control study including 37 patients with previously untreated HER2+ metastatic GC [51].

## 6 Mechanism of Secondary Resistance to Anti-HER2 Agents

Mechanisms of acquired resistance to trastuzumab are summarized in Fig. 2.

### 6.1 Acquired *HER2* Mutations

*HER2* de novo mutations have been described in vitro, using HER2 overexpressing GC-derived cell lines treated with trastuzumab. These mutations were located in the portion of the protein involved in the regulation of kinase activity and affected the conformation of the HER2 receptor, which could result in the maintenance of its active form [52].



**Fig. 2** Mechanisms of acquired resistance to trastuzumab. In treatment with trastuzumab, several molecular mechanisms have been described in vitro and/or in vivo, resulting in secondary resistance to trastuzumab and other anti-HER2 treatments: *HER2* de novo mutations [52], compensatory pathway activation such as activation of the PI3K/Akt pathway through HER3 signaling [52, 56], protein overexpression such as Src, involved in crosstalk between growth signaling

pathways [54, 55], and IQGAP1, involved in HER2 phosphorylation [52], regulation by micro RNAs [57], increased capacities of epithelial and mesenchymal transition [59, 60]. Moreover, a loss of HER2 positivity and/or overexpression has been observed in several clinical studies and could occur in up to two-thirds of patients receiving trastuzumab in first-line treatment [61–63]



## 6.2 Activation of Alternative Pathways

In a study by Sampera et al. conducted in vitro on HER2+ GC cell lines, acquired resistance to trastuzumab was associated with persistent activation of the MAPK/ERK and PI3K/mTOR pathways mediated by Src, a non-receptor tyrosine kinase involved in signaling and crosstalk between growth-promoting pathways [53]. The role of Src in trastuzumab-acquired resistance was also suggested in several preclinical studies conducted in vitro and in vivo in HER2-overexpressing breast cancer [54, 55].

In the same study, GC cell lines with secondary resistance to trastuzumab showed increased mRNA expression of other EGFR family receptors (*EGFR*, *ERBB3*, and *ERBB4*) as well as EGFR family ligands such as epidermal growth factor (*EGF*) and amphiregulin (*AREG*) compared with the trastuzumab-sensitive parental cells. Moreover, in the same study, in a mouse model of trastuzumab-resistant derived xenograft, triple inhibition of EGFR, HER2, and human epidermal receptor 3 (HER3) could overcome acquired resistance to trastuzumab. Lastly, in another study conducted in vitro, HER3 overexpression was observed in GC cell lines with secondary resistance to trastuzumab [52]. Consistently with what is observed in GC, several preclinical studies of HER2+ breast cancer suggested that overexpression of HER3 could be induced by HER2 blockade and might play an important role in trastuzumab-acquired resistance [56].

## 6.3 Protein Overexpression

IQGAP1 is a scaffold protein that governs HER2 expression, phosphorylation, and signaling. It has recently been shown in vitro that trastuzumab-resistant GC cell lines express an increased level of IQGAP1, and that this resistance could be reversed by blocking IQGAP1 production with the use of siRNAs [52].

## 6.4 Micro RNAs

Micro RNAs (miRNAs) are a group of small non-coding RNAs that regulate cellular biological processes by regulating target genes at the post-transcriptional level. Several miRNAs target HER2 signaling pathway components (such as PI3K, PTEN, AKT) or HER2 compensatory receptors (such as HER3) and may be involved in primary and acquired resistance to anti-HER2 therapies [57].

## 6.5 Epithelial to Mesenchymal Transition

Epithelial to mesenchymal transition (EMT) in epithelial cells results in the loss of polarity and cell–cell adhesion capacities and increases their migratory and invasive properties. This process has been described as one of the promoters

of tumor initiation and metastatic dissemination in cancer and in particular in GC [58]. Several studies suggest that EMT could be involved in acquired resistance to HER2 therapies. Using HER2-positive GC cell lines treated with trastuzumab, Jiaolong et al. showed that cells that acquired resistance to trastuzumab showed a mesenchymal phenotype, increased migration, and invasive capacities as well as higher levels of mesenchymal markers and lower levels of epithelial markers, compared to the parental cells [59]. Another team showed that EMT observed in trastuzumab-resistant HER2+ cell lines was dependent on the TGFβ/ZEB/miR-200 axis and that overexpression of miR-200c alleviated the resistance of trastuzumab and inhibited invasion and migration in vitro [60].

## 6.6 Loss of HER2 Positivity

Recently, several authors have addressed the question of a possible loss of HER2 overexpression acquired during anti-HER2-containing therapy. In these studies, which used paired biopsies at baseline and after first progression, a loss of HER2 positivity (IHC < 3+ and absence of FISH amplification) and/or loss of HER2 overexpression (scoring down from 2+/3+ to 1+/0+ independently of FISH status) was observed in 28.6–69% of patients [61–63]. This loss of HER2 positivity could occur more frequently in tumors with an initial IHC score of 2+ [63].

However, if loss of HER2 overexpression could theoretically lead to progression, the clinical role in progression is not really known, and such loss of HER2 expression has also been described in two case reports of patients responding to trastuzumab-based chemotherapy [61, 64].

These results indicate that dynamic changes of HER2 status may potentially be induced by chemotherapy with trastuzumab and suggest that reassessment of HER2 status after progression could help to identify patients who may benefit from HER2 inhibitor maintenance or rechallenge.

## 7 New Screening Methods for HER2 Status and Therapeutic Impact

### 7.1 Circulating Tumor Cells

Tumor cells can be isolated in the blood stream of patients harboring numerous types of cancers including GC. In GC, circulating tumor cells (CTC) could have a prognostic impact and might become a noninvasive tool to monitor disease progression throughout treatment [65, 66]. The molecular characterization of CTC is challenging, notably because of their low levels in the blood. In a recent study using FISH, *HER2* amplification detected in CTC was strongly concordant with tissue amplification in patient-matched samples

[67]. Moreover, in a study using a combination of immunofluorescence and FISH, 13 out of 50 patients with GC identified as HER2- based on tissue assessment (IHC 0 or 1+ or 2+ and FISH-) showed *HER2* amplification in CTC, and preliminary clinical data showed that such patients could benefit from trastuzumab like patients with HER2+ tumors (IHC3+ or IHC2+ and FISH+) [68].

## 7.2 Circulating Tumor DNA

Circulating tumor DNA (ctDNA) carries tumor-related genetic alterations released by apoptotic and necrotic tumor cells and can be detected in the bloodstream as well as in other body fluids [69]. In early studies of ctDNA in GC, the concordance rate of HER2 amplification from the DNA samples and tumor tissues was around 60% [70]. However, more recent studies have shown a higher concordance (between 75 and 90%) in HER2 detection between tumor tissues and plasma, with more modern techniques such as digital droplet PCR (ddPCR) and next-generation sequencing (NGS) suggesting that ctDNA could be used as an alternative method to screen targeted HER2- populations [71–74]. Differences between plasma and tumor in HER2 status could also be partially explained by the high heterogeneity of HER2 expression in gastric tumor cells. More data are needed to confirm the validity of ctDNA as a tool for HER2 screening, notably with current innovative methods of detection.

In addition, a study suggested that ctDNA could be a predictive marker for treatment efficacy in the first-line setting. Indeed, Wang et al. showed in a cohort of 56 patients with advanced GC given oxaliplatin-based first-line treatment, with or without trastuzumab, that the patients with plasma *HER2* amplification who were treated with trastuzumab had a significantly higher response rate than those without amplification ( $P=0.032$ ) [72]. In another study using NGS, *HER2* amplification detected in ctDNA at baseline was not associated with the outcome of HER2-targeting treatment. However, in the same study based on 23 patients, *HER2* amplification in ctDNA adjusted for *HER2* gene copy number and combined with tissue *HER2* amplification was associated with a better prognosis ( $P=0.004$ ), suggesting that ctDNA could be a complementary tool to predict response to treatment [74]. Furthermore, in the Wang et al. study, the role of plasma *HER2* amplification in dynamic monitoring was investigated in 20 patients treated with trastuzumab. As compared to *HER2* copy number at baseline, most patients had a decrease in *HER2* copy number in the case of survival benefit afforded by trastuzumab [72]. These results tend to indicate that *HER2* ctDNA dynamic evaluation could be a surrogate marker of treatment efficacy, although this needs to be confirmed by further studies.

However, to our knowledge, no data have been published regarding the reliability of ctDNA as a predictive marker of

response to anti-HER2 therapies in the specific setting of progression on first-line treatment.

## 7.3 HER2 Extracellular Domain

The extracellular domain (ECD) of HER2 can be measured in serum. In a study that included 133 cases of metastatic GC, there was a significant relationship between serum concentrations of HER2 ECD and tissue levels of HER2 protein, with an area under the curve for serum HER2 ECD of 0.77 (95% CI 0.68–0.86) [75]. Furthermore, several studies have evaluated changes in HER2 ECD level during HER2-targeting treatment in breast cancer, suggesting that it might predict response to HER2-targeted therapies [76, 77].

## 7.4 <sup>89</sup>Zr-Trastuzumab

<sup>89</sup>Zr-Trastuzumab is a novel imaging agent that combines trastuzumab radiolabeled with zirconium-89. A preclinical study using xenografts showed that <sup>89</sup>Zr-trastuzumab positron emission tomography (PET) could identify and delineate HER2+ tumors and measure the pharmacodynamic effect of anti-HER2 therapies [78]. More recently, <sup>89</sup>Zr-trastuzumab was assessed in a small cohort of ten patients with metastatic HER2+ GC and revealed the primary tumor site as well as known metastatic sites, with high-quality images and an acceptable safety profile, raising the possibility of noninvasive evaluation of variations of HER2 overexpression at both primary and metastatic sites [79].

## 8 Therapeutic Perspectives

### 8.1 Monoclonal Antibodies

Margetuximab is an Fc-modified chimeric anti-HER2 mAb that binds with high affinity to CD16A, an important receptor for antibody-dependent cell-mediated cytotoxicity. A phase I study has demonstrated its tolerability and promising antitumor activity [80]. One phase Ib/II study is currently evaluating the combination of margetuximab and pembrolizumab (an anti-PD1 mAb) in patients with refractory HER2-positive advanced gastric and gastroesophageal junction (GEJ) cancer (NCT02689284).

Trastuzumab deruxtecan (DS-8201a) is an antibody–drug conjugate that combines trastuzumab with a topoisomerase I inhibitor by a cleavable peptide linker [81]. Based on the results of a multicenter phase II study [82], trastuzumab deruxtecan was recently approved by the US Food and Drug Administration for the treatment of metastatic or unresectable HER2+ breast cancer, after two or more anti-HER2 agent-containing lines of treatment.

In GC, trastuzumab deruxtecan was recently evaluated in a dose-expansion/phase I trial conducted in the USA and Japan. In this study, 44 patients with HER2-positive (IHC 3+ or IHC 2+ and FISH-positive) advanced GC or GEJ carcinoma received at least one dose of trastuzumab deruxtecan. Patients had received a median of three previous lines. All patients had been previously treated with trastuzumab. The most frequent grade 3 or worse events were anemia, low platelet count, and low neutrophil count. The median follow-up was 5.5 months [2.8–13.1]. Nineteen patients (43.2%; 95% CI [28.3–59.0]) achieved an objective response and 35 (79.5%; 95% CI [64.7–90.2]) achieved disease control [83]. An international phase II trial is currently ongoing to further investigate these promising results (NCT04014075).

ZW25 is a novel bispecific antibody that targets two domains of HER2: ECD2 (pertuzumab binding site) and ECD4 (trastuzumab binding site). A phase I study presented at ASCO 2018 showed promising antitumor activity and an acceptable safety profile in patients with pretreated advanced HER2+ cancers, including GC and GEJ cancers [84]. A phase II trial is currently ongoing to evaluate the efficacy of ZW25 in combination with chemotherapy in first-line treatment of HER2+ unresectable or metastatic GC and GEJ cancers (NCT02892123).

## 8.2 Tyrosine Kinase Inhibitors (TKI)

Pozotinib is an irreversible pan-HER TKI that targets HER2, EGFR, and HER4. It has been evaluated in a prospective, multicenter, open-label, phase I/II study. Thirty-seven patients with advanced HER2+ GC who had received one prior line of chemotherapy (regardless of trastuzumab exposure) were enrolled to receive pozotinib in combination with paclitaxel and trastuzumab. Median PFS and OS were 13.0 weeks (95% CI [9.8–21.9]) and 29.5 weeks (95% CI [17.9–59.2]), respectively [85].

Dacomitinib is an irreversible panHER inhibitor that has shown preclinical antitumor activity in HER2+ GC. In a multicenter Korean phase II trial, 27 patients with advanced HER2+ GC, who had received from one to more than three prior chemotherapy regimens, were treated with dacomitinib monotherapy. Median PFS was 2.1 months (95% CI [2.3–3.4]) and median OS was 7.1 months (95% CI [4.4–9.8]). Note that only seven patients had received prior anti-HER2 therapy [86]. To our knowledge, no phase III trial is currently ongoing to further confirm these results.

Afatinib is an irreversible inhibitor of HER1, HER2, and HER3 that has shown efficacy in HER2-overexpressing GC in preclinical studies [87]. Afatinib is currently being evaluated in two ongoing phase II studies in combination with paclitaxel in second-line treatment of HER2+ advanced gastric adenocarcinoma, after progression during chemotherapy and trastuzumab (NCT02501603; NCT01522768).

## 8.3 Immune Checkpoint Inhibitors

Monoclonal antibodies targeting immune checkpoints such as programmed death-1 (PD-1), programmed death-ligand 1 (PD-L1), or cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) have shown significant benefit in various cancers by restoring an efficient antitumor immune response [88].

The efficacy of immunotherapy in GC has been assessed in four randomized phase III studies showing contradictory results. In the ATTRACTION-2 trial, patients with advanced, refractory GC treated by nivolumab (an anti-PD1 mAb) had a significantly improved OS compared to patients receiving placebo (5.26 vs. 4.14 months; HR = 0.63; 95% CI [0.51–0.78];  $P < 0.0001$ ) [89]. On the other hand, the JAVELIN 300 (avelumab, anti-PD-L1) and KEYNOTE-061 (pembrolizumab, anti-PD1) randomized phase III trials did not show significant survival benefits in third- and second-line chemotherapy, respectively [90, 91]. More recently, the KEYNOTE-062 randomized trial showed that addition of pembrolizumab to first-line chemotherapy was not associated with improvement of survival [92]. Based on these results, more studies are needed to improve selection of GC patients who may benefit from immune checkpoint inhibitors.

Data on the efficacy of immunotherapy in the specific context of HER2+ GC are scarce. In the ATTRACTION-2 trial, HER2 status was not collected at inclusion. However, an exploratory subgroup analysis later assessed the efficacy of nivolumab in the population of patients with HER2+ tumors, considering the prior use of trastuzumab as a surrogate for HER2 status. The median OS was significantly longer for patients receiving nivolumab, in the group of patients previously treated with trastuzumab and assumed to be HER2+ (8.3 months vs. 3.1 months; HR = 0.38; 95% CI [0.22–0.66];  $P = 0.0006$ ), and in those who did not receive trastuzumab and were assumed to be HER2- (4.8 months vs. 4.2 months; HR = 0.71 95% CI [0.57–0.88];  $P = 0.0022$ ) [93]. Even though one must be very cautious interpreting these exploratory data, these results may indicate that the benefit of immunotherapy might be greater in patients with HER2+ tumors compared to HER2-. Interestingly, trastuzumab stimulates HER2-specific T cell responses [94] and increases tumor PD-L1 expression [95], and anti-PD-1 antibody can help enhance the T-cell-specific immunity of trastuzumab. Moreover, oxaliplatin can further enhance T cells by activating dendritic cells. At the ASCO meeting in 2019, Janjigian et al. reported the results of a phase II study including 37 patients with HER2+ advanced GC treated in first-line chemotherapy by CAPOX and trastuzumab in combination with pembrolizumab. The ORR and 6-month PFS rate were 88.6% and 74%, respectively [96]. In order to confirm these preliminary efficacy results, the phase III KEYNOTE-811 trial comparing chemotherapy + trastuzumab

with or without pembrolizumab in first-line treatment is currently ongoing (NCT03615326).

## 9 Conclusions

Since the ToGA trial, several anti-HER2 agents have been evaluated in randomized studies, but none of them have improved survival significantly enough to justify registration. Despite promising retrospective data on the strategy of maintaining trastuzumab beyond progression, the first results from a randomized phase II trial have failed to show a benefit of the addition of trastuzumab to paclitaxel after progression. These results emphasize the need for a better understanding of mechanisms leading to secondary resistance to anti-HER2. Notably, a loss of HER2 overexpression on trastuzumab treatment has been described in several studies and might occur in up to two-thirds of patients. Thus, it appears that the selection of patients who would benefit from the continuation of anti-HER2 therapy beyond progression will be the determinant factor in this approach. Although clinical and histological selection criteria have been suggested, more precise and noninvasive methods are necessary. In this context, the emergence of liquid biopsies containing plasma circulating DNA has been increasingly reported to yield genomic or epigenomic information about tumors. Regarding HER2 status, ctDNA seems to have a high concordance with HER2 amplification in tumor tissues and could serve as a surrogate marker to monitor the efficacy of anti-HER2 targeted agents. Furthermore, new therapeutic agents and combination therapies have recently emerged. In particular, novel antibody–drug conjugates and the combination of anti-HER2 mAb with immunotherapy could yield interesting results.

## Compliance with Ethical Standards

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