

Trastuzumab in the management of gastroesophageal cancer: patient selection and perspectives

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Abstract: The addition of trastuzumab to the treatment of a subset of patients with advanced gastric and gastroesophageal junction cancers showing HER2 positivity has been shown to confer clinical benefit; however, questions remain over the optimal methods for defining and selecting such patients. This review provides an overview of current standards for assessing HER2 positivity, the evolving treatment landscape for HER2-positive gastric and esophageal cancers and the challenges and potential future directions in optimal patient selection for HER2-targeted therapy.

Keywords: HER2, trastuzumab, gastric cancer, immunohistochemistry, in situ hybridization

Introduction

Gastroesophageal (GE) cancers are among the leading causes of cancer death worldwide.¹ The outlook for metastatic disease remains bleak, with median overall survival (OS) generally not reaching more than 1 year in the majority of clinical trials.² The anti-HER2 humanized monoclonal antibody trastuzumab remains a landmark in anticancer drug discovery. It was developed jointly by Genentech and the University of California in the 1990s and gained regulatory approval for breast cancer treatment in 1998.^{3,4} It was later approved in gastric and gastroesophageal junction (GEJ) adenocarcinoma after the ToGA trial showed a survival benefit for a subset of HER2-positive patients in conjunction with first-line chemotherapy.⁵ The only approved indications remain in GE adenocarcinomas and breast cancers; however, there is growing interest in its potential utility for “molecularly triaged” subsets of other solid organ tumors that display HER2 positivity, such as lung and colorectal cancers, although such work remains exploratory.^{6,7} It is generally well tolerated, with commonly reported side effects including flu-like symptoms and mild gastrointestinal upset.⁸ A well-characterized and potentially more serious complication is cardiac dysfunction characterized by decline in ejection fraction. This is a rare and normally reversible side effect, occurring in 2%–7% of patients treated with trastuzumab alone, although the risk is increased when given in conjunction with cardiotoxic chemotherapies.⁹ A number of further HER2-targeting agents have been tested in both early- and late-stage GE cancer with generally disappointing results thus far.^{10–13} Key to the successful clinical application of HER2-directed therapy is appropriate, robust and reproducible patient selection. In the field of gastric cancer, there is some controversy over optimal immunohistochemical (IHC) and in situ hybridization (ISH) genetic analysis, and differing emphasis on the relative importance of protein expression versus gene amplification has led to divergent global standards in the definition of HER2 positivity. This review describes the

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background of HER2 targeting in gastric cancer and discusses some of the current issues and potential future directions in defining and treating HER2-positive patients.

Current treatment landscape

Treatment for advanced GE cancer remains primarily chemotherapy based; however, the recent regulatory approval of ramucirumab and a growing interest in immunotherapeutic approaches are beginning to expand non-chemotherapy treatment options. There is little consensus on optimal first-line chemotherapy, and standard regimes normally consist of a fluoropyrimidine combined with a platinum agent, with the possible addition of either an anthracycline or a taxane.^{2,14,15} Irinotecan is more commonly used as a second-line therapy; however, several studies have suggested that FOLFIRI (irinotecan with 5-fluorouracil [FU]) also has activity in the first line.^{16,17} Second-line chemotherapy with irinotecan, docetaxel and paclitaxel have all demonstrated a survival advantage over best supportive care (BSC) alone.^{18–20}

Phase III trials of ramucirumab have demonstrated significant efficacy in the second-line setting both as monotherapy and in combination with paclitaxel.^{21,22} Evaluation in the first-line setting is ongoing with the phase III RAINFALL study (NCT02314117). As with other cancer types, there is growing interest in immunotherapeutic treatment approaches, primarily focused on the use of checkpoint inhibitors. The KEYNOTE 012 study reported response rates (RRs) of 22% with pembrolizumab in a heavily pretreated population, and further trials are in progress in both first-line combination with chemotherapy (NCT02335411) and second-line comparison with paclitaxel (NCT02370498). Combination blockade of both PD-1 and VEGFR2 appeared to show a synergistic effect in preclinical models, and a phase I study is currently evaluating the combination of pembrolizumab and ramucirumab (NCT02443324).²³

HER2 in gastric and esophageal cancers

HER2 belongs to the EGFR family of transmembrane tyrosine kinase receptors. Unlike other receptors in the family, it has no known activating ligand and must heterodimerize with EGFR, HER3 or HER4 to trigger transphosphorylation and activation of downstream PI3K or MAPK signaling pathways.²⁴ When overexpressed, HER2 can homodimerize, giving rise to ligand-independent signaling.²⁵ The reported frequency of HER2 overexpression in GE cancer varies widely in the literature, ranging from

4% to >50% in some reports.^{26–29} The lowest frequency is reported in distal tumors and the highest in tumors of the GEJ.^{28,29} Rates of HER2 expression in squamous cell cancers of the upper esophagus are low, and as a result, the application of HER2-targeted therapy has generally been limited to adenocarcinomas of the lower esophagus and stomach.³⁰ An association with intestinal histology has been consistently reported, with *HER2* amplification uncommon in diffuse gastric cancers.²⁸ A recent case series of 1461 Japanese patients reported an HER2 positivity rate of 21%. Multiple logistic regression analysis identified intestinal type, hepatic metastasis and absence of peritoneal metastasis as significant independent factors related to HER2 positivity.³¹ The association between HER2 expression and prognosis in GE cancer is uncertain; however, a number of studies have now shown HER2 to be a negative prognostic factor associated with more aggressive biological behavior and higher frequencies of recurrence.^{27,32,33} A 2012 systematic review of 42 studies concluded that HER2 positivity was associated with decreased survival and adverse clinicopathological features, including early progression, serosal invasion and more advanced stage.²⁷ Such results are consistent with breast cancer, where HER2 positivity is known to be an adverse prognostic factor.³⁴

Researchers at The Cancer Genome Atlas (TCGA) recently undertook an analysis of gastric adenocarcinoma utilizing next-generation sequencing (NGS) techniques, describing four distinct subtypes Epstein–Barr virus (EBV)-positive tumors, microsatellite-unstable tumors, genomically stable tumors and tumors with chromosomal instability (CIN).³⁵ *HER2* amplification was most commonly seen in CIN tumors, with less common prevalence in the EBV-positive and genomically stable subgroups. No microsatellite-unstable tumors were *HER2* amplified, although some demonstrated *HER2* missense mutations. The CIN subtype of tumors most associated with HER2 positivity are characterized by CIN and recurrent amplification of other potentially clinically relevant receptor tyrosine kinases.³⁵ The interaction between HER2 detection and targeting with other altered tyrosine kinase signaling pathways seen in this subset of tumors is currently unclear.

Defining HER2 positivity

Standardized protocols for evaluating and defining HER2 positivity were originally developed for breast cancer, with the most widely adopted being the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) clinical practice guidelines.³⁶ Testing is through

either IHC assessment of protein expression using antibody staining or ISH assessment of gene amplification. Traditionally, the most commonly utilized method of gene amplification is fluorescent ISH (FISH), a cytogenetic technique that uses customized fluorescent probes that bind to specific DNA sequences with a high degree of sequence complementarity.³⁷ Further emerging techniques include chromogenic and silver-enhanced ISH (CISH/SISH). CISH uses a peroxidase enzyme-labeled probe for chromogenic detection by diaminobenzidine, while SISH uses the same technique with a silver-based detection system.³⁸ Because these processes do not involve fluorescent dye, a standard bright-field microscope can be used, circumventing some of the technical difficulties associated with FISH.³⁸ An additional technique now becoming more widespread is dual-color, dual-hapten, bright-field ISH (DDISH). This is an automated process that again can be evaluated by conventional microscopy. In contrast to SISH, which requires two separate slides to detect *HER2* and CEP17, DDISH uses double-stranded dual-hapten probes to detect both markers on a single slide. Concordance between FISH and DDISH *HER2* amplification results has been found to be high.³⁹ Figure 1 illustrates DDISH *HER2* positivity in a gastric cancer specimen.

In both breast and GE cancers, protein expression is categorized into IHC 0, 1+, 2+ and 3+ based upon a score incorporating both the intensity of staining and the percentage or number of cancer cells demonstrating that intensity, however, important differences between the two tumor types have led to modifications in their respective scoring systems. The membranous distribution of protein within breast cancer cells is predominantly circumferential (Figure 2), and breast tumors are defined as IHC 3+ if there is complete circumferential membrane staining in >10% of tumor cells, IHC

2+ if there is incomplete circumferential membrane staining in >10% or complete staining within <10% of cells and IHC 1+ if there is incomplete faint membrane staining.³⁶ *HER2* protein expression in GE cancer tends to spare the digestive luminal membrane, resulting in membrane staining that is not completely circumferential but is instead predominantly basolateral or lateral (Figure 3A and B).⁴⁰ Furthermore, a greater degree of intratumoral *HER2* heterogeneity has been reported in GE specimens, with the presence of variable focal areas of *HER2* positivity often seen (Figure 4).⁴¹

Given these differences, a modified IHC scoring system for gastric cancer was developed for the ToGA trial and validated on 168 specimens in a pre-ToGA study, with high concordance found between the modified IHC criteria and ISH gene amplification results.⁴² This was subsequently validated in a larger dataset and has now been widely adopted by clinicians and institutions worldwide.⁴³ The refined GE scoring system does not require complete circumferential homogeneity of staining, and the percentage of cancer cells that are required to show *HER2* expression is lower. A specimen showing weak/moderate or strong complete circumferential “or” basolateral staining is designated as 2+ or 3+, respectively. The scoring system also takes into account the more heterogeneous patterns of *HER2* positivity in GE cancer by distinguishing between surgical and biopsy specimens. When evaluation is performed on surgical samples, a cutoff point of 10% of cells in the entire specimen is necessary, whereas for endoscopic biopsy samples a single cluster of at least five positive cells is sufficient.⁴³ The sensitivity and specificity of these modified GE criteria for detecting *HER2* gene amplification were compared with the ASCO/CAP breast guidelines in a study of 1059 primary gastric adenocarcinomas, which included 123 *HER2*-amplified tumors.⁴⁴ The modified criteria

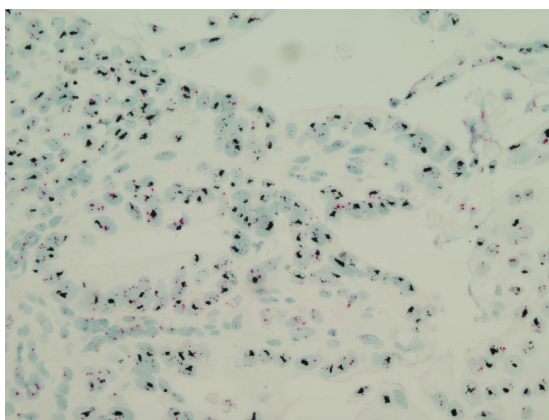


Figure 1 DDISH evaluation of *HER2* amplification in gastric cancer showing both chromosome 17 (red signals) and *HER2* probes (black signals).
Abbreviation: DDISH, dual-color, dual-hapten, bright-field in situ hybridization.

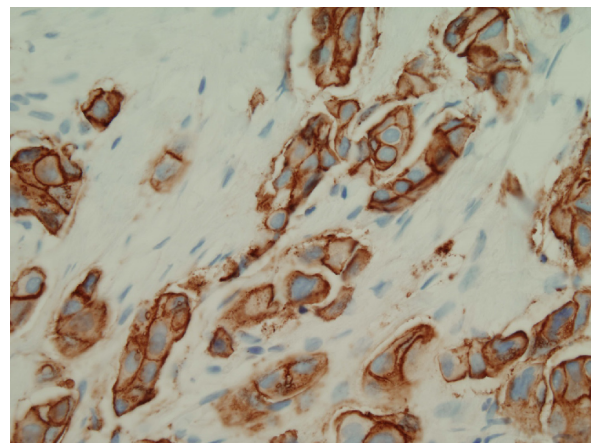


Figure 2 *HER2* staining in breast cancer illustrating circumferential membranous staining pattern.

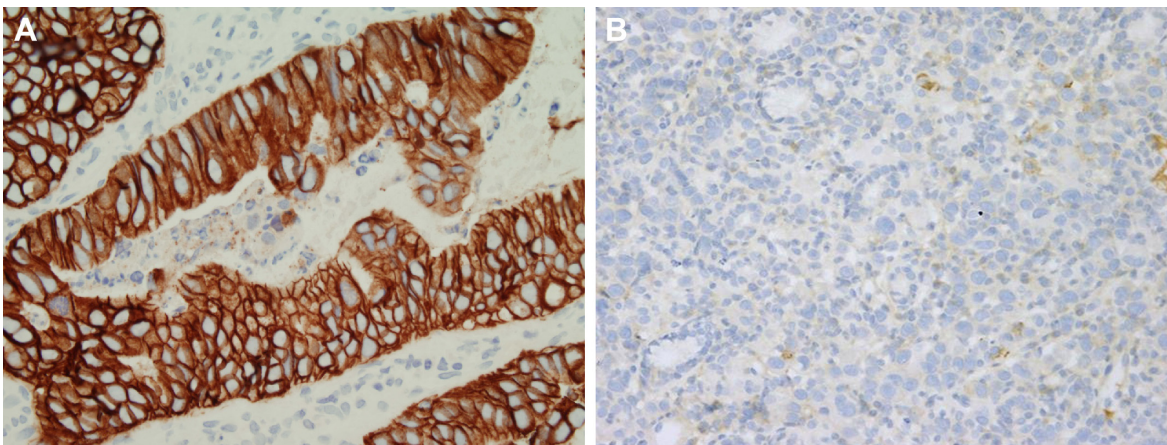


Figure 3 (A) Gastric cancer specimen showing strong staining in >10% cells (HER2 3+) illustrating predominantly basolateral staining pattern. (B) Gastric cancer specimen showing faint staining in less than 10% cells (HER2 0).

demonstrated significantly higher sensitivity for detecting gene amplification as compared with the breast criteria (66% vs 48%, respectively). Both sets of criteria demonstrated an equally high specificity for correctly identifying negative (IHC 0 and 1+) patients. Formal guidelines for HER2 scoring in gastrointestinal cancer are currently under development by a collaboration between ASCO/CAP and the American Society for Clinical Pathology (ASCP); however, they have not been published at the time of writing.⁴⁵ Comparison of differing HER2 IHC assessment criteria in breast and GE cancers is given in Table 1.

ISH results are reported as the ratio between *HER2* and the chromosome 17 enumeration probe (CEP17) within the nucleus of ~20 cancer cells. The definition of positivity in both breast and GE cancers is an *HER2*/CEP17 ratio of ≥ 2 . However, account is also taken of average *HER2* copy number per cell and ISH positivity also includes an *HER2*/CEP17 ratio of < 2 if the *HER2* copy number is ≥ 6 . ISH is equivocal if the *HER2*/CEP17 ratio is < 2 with a copy number

between 4 and 6 and ISH is negative if the *HER2*/CEP17 ratio is < 2 and copy number is < 4 .³⁶

Clinical role of HER2-directed therapies in the management of gastric and esophageal adenocarcinoma

First line

The landmark ToGA trial evaluated the combination of trastuzumab with a cisplatin/fluoropyrimidine chemotherapy doublet in patients with previously untreated, advanced HER2-positive gastric or GEJ adenocarcinoma.⁵ HER2 status was assessed using both IHC and FISH, and tumors were considered HER2 positive on the basis of either an IHC 3+ result or FISH amplification showing an *HER2*/CEP17 ratio of ≥ 2 . Among enrolled patients (n=594 of the 3807 screened), almost all tumors were FISH positive, whereas protein expression by IHC varied (47% 3+, 30% 2+ and 22% 1+ or 0). Toxicity from the combination of the antibody was minimal, and no significant cardiotoxicity was reported. The combination of trastuzumab significantly improved objective RR, progression-free survival (PFS) and OS with the primary end point of median OS improved from 11.1 to 13.8 months. A preplanned exploratory analysis according to HER2 status suggested that OS was improved in patients with high expression of HER2. Patients with the strongest expression (IHC 3+) with concomitant FISH gene amplification received the greatest benefit, with an absolute improvement in OS of >5 months (12.3 compared to 17.9 months). IHC expression also appeared to incrementally predict for benefit: trastuzumab was most effective in prolonging survival in IHC 3+ tumors, less effective in patients with IHC 2+ tumors and ineffective in those with HER2

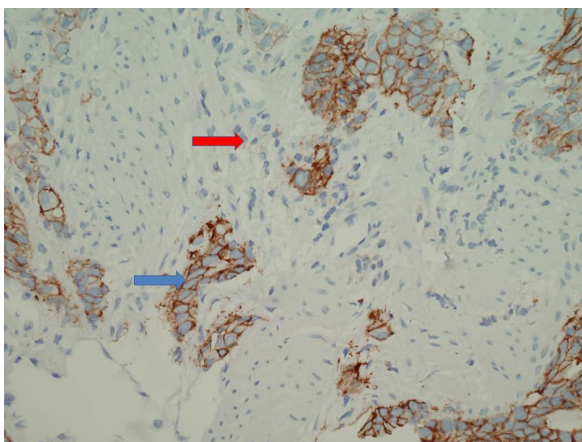


Figure 4 HER2 staining in gastric cancer illustrating tumor heterogeneity within specimen with positively (blue arrow) and negatively (red arrow) stained tumor cells.

Table 1 Comparative criteria for assessing HER2 protein expression by immunohistochemistry in breast versus GE cancer

IHC score	Breast: surgical resection or biopsy	GE: surgical resection	GE: biopsy	Assessment
3+	Uniform intense membrane staining in >10% cells	Strong complete, basolateral or lateral membrane staining in \geq 10% cells	Strong complete, basolateral or lateral membrane staining in a cluster of \geq 5 tumor cells	Positive
2+	Incomplete or weak/moderate circumferential membrane staining in >10% cells, or complete and intense circumferential membrane staining in <10% cells	Weak to moderate complete, basolateral or lateral membrane staining in \geq 10% cells	Weak to moderate complete, basolateral or lateral membrane staining in a cluster of \geq 5 tumor cells	Equivocal
1+	Faint/barely perceptible incomplete membrane staining in >10% cells	Faint membrane staining \geq 10% cells	Faint membrane staining in a cluster of \geq 5 tumor cells	Negative
0	No staining or faint/barely perceptible incomplete membrane staining in <10% cells	No staining or staining in <10% cells	No staining	Negative

Abbreviations: GE, gastroesophageal; IHC, immunohistochemical.

gene-amplified (FISH-positive) but nonprotein-expressing (IHC 0 or 1+) tumors. Some limited phase II evidence has shown that treatment with trastuzumab beyond progression with second-line chemotherapy is a safe and potentially effective treatment option which may have benefit in improving PFS; however, this approach is yet to be validated in any larger-scale clinical trials.⁴⁶

Pertuzumab is a monoclonal antibody directed at the extracellular domain (ECD) of HER2, preventing receptor dimerization. The binding site on domain II is distinct from the trastuzumab binding site on domain IV, allowing the two antibodies to be effectively delivered together.⁴⁷ The combination of pertuzumab with docetaxel and trastuzumab in the first-line treatment of HER2-positive advanced breast cancer improved median PFS by ~6 months, with little additional toxicity.⁴⁸ A phase III evaluation of pertuzumab in combination with first-line trastuzumab with cisplatin/fluoropyrimidine (the JACOB study, NCT01774786) has been completed, with results anticipated shortly.

Second line

Trastuzumab-DM1 (T-DM1) is a novel antibody–drug conjugate that combines trastuzumab with a microtubule inhibitor and is effective and well tolerated in previously treated patients with HER2-positive breast cancer.⁴⁹ The GATSBY phase III study evaluated T-DM1 compared with standard taxane therapy for patients with previously treated advanced HER2-positive gastric or GEJ cancer, with a preliminary report at the 2016 ASCO GI Cancers Symposium suggesting a lack of efficacy.¹¹ With 415 patients randomized, median OS was 8.6 months with a taxane versus 7.9 months with weekly T-DM1. Grade \geq 3 adverse events were numerically lower with T-DM1, and rates of serious adverse events and treatment discontinuations were comparable between both arms.

Lapatinib is an orally available, small-molecule tyrosine kinase inhibitor (TKI) of HER2 and EGFR. In HER2-positive

advanced breast cancer, lapatinib is licensed in combination with capecitabine in the second-line setting; however, it is now known to be inferior to single-agent T-DM1.⁴⁹ Single-agent lapatinib demonstrated only modest activity in a phase II study of unselected patients with advanced gastric cancer, but median survival was twice as long in patients with *HER2* amplification (defined as gene copy number greater than the median for the cohort) compared with those without.¹⁰ Two phase III studies of chemotherapy with lapatinib have been conducted. The first-line TRIO-013/LOGiC study failed to demonstrate a significant improvement in OS when lapatinib was combined with a capecitabine/oxaliplatin doublet, although RR and median PFS were significantly improved.¹² There was no correlation between HER2 IHC score and outcomes. In the second-line TYTAN study, lapatinib combined with paclitaxel was compared with paclitaxel alone.¹³ Again, there was no statistically significant OS benefit. A significant benefit was, however, detected in a preplanned subgroup analysis of patients with the strongest HER2 overexpression (IHC 3+) in whom median survival was almost double compared with chemotherapy alone (14.0 vs 7.6 months), while it was not significantly different in those with IHC 0–2+ tumors. Selected trials of HER2-targeted agents in the first- and second-line setting are given in Table 2.

Future directions in HER2 targeting

In early GE cancer, the phase III ST03 study (NCT00450203) has been adapted to include baseline HER2 testing and randomization of HER2-positive patients to standard perioperative chemotherapy with ECX, or the same therapy along with lapatinib. The randomized phase II INNOVATION trial (NCT02205047) opened in 2015, comparing standard perioperative chemotherapy in patients with resectable gastric cancer combined with trastuzumab or trastuzumab/pertuzumab.

Moving on from the relative disappointment of lapatinib, a number of multi-receptor-targeting TKIs are under

Table 2 Selected first- and second-line studies of HER2-targeted therapies in GE cancer

Trial	Phase	Treatment	N	ORR (%)	PFS (months)	OS (months)
First line						
ToGA ⁵	III	CX/CF	296	47	5.5	11.1
		CX/CF + trastuzumab (FISH positive/HER2 3+ subgroup)	298 (131)	35 (-)	6.7 (-)	13.8 (17.9)
LOGiC ¹²	III	CAPOX + placebo	273	40	5.4	10.5
		CAPOX + lapatinib	272	53	6.0	12.2
HERBIS-I ⁶⁶	II [#]	S1 + C + trastuzumab	53	68	7.8	16.0
Grávalos et al ⁶⁷	II [#]	C + trastuzumab	22	32	5.1	-
Second line						
GATSBY ¹¹	II/III	Taxane*	117	21	2.9	8.6
		T-DMI	228	20	2.7	7.9
TYTAN ¹³	III	Paclitaxel	129	9	4.4	8.9
		Paclitaxel + lapatinib (FISH positive/HER2 3+ subgroup)	132 (52)	27 (-)	5.4 (5.6)	11.0 (14.0)

Notes: [#]Non-randomized phase II. *Investigator's choice of either paclitaxel or docetaxel.

Abbreviations: C, cisplatin; CX, cisplatin with capecitabine; CF, cisplatin with 5-FU; CAPOX, capecitabine with oxaliplatin; GE, gastroesophageal; FISH, fluorescent in situ hybridization; 5-FU, 5-fluorouracil; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; T-DMI, trastuzumab-DMI.

investigation in the advanced disease setting. Afatinib, a multi-kinase inhibitor of EGFR, HER2 and HER4, showed signal in a phase II study involving patients with trastuzumab refractory disease.⁵⁰ An expansion cohort looking at safety and efficacy in combination with trastuzumab is currently recruiting (NCT01522768). Neratinib is a further pan-HER TKI that has shown promising results in trastuzumab refractory HER2-positive breast cancer and is being further investigated in a multi-tumor type phase II study involving solid tumors (including GE cancer) showing *HER2*, *HER3* or EGFR mutations (NCT01953926).⁵¹ Dacomitinib is an irreversible pan-HER TKI that showed a modest efficacy signal in a phase II monotherapy study; however, no phase III has followed.⁵²

A mechanism of action of increasing interest is trastuzumab's immune-mediated antibody-dependent cellular cytotoxicity via activation of antibody-binding Fc receptors.⁵³ Murine models have suggested that checkpoint-inhibitor immunotherapy could potentiate these immune-mediated cytotoxic effects, and the combination is being investigated in two clinical trials evaluating trastuzumab with pembrolizumab (NCT02318901) and margetuximab (an Fc-optimized chimeric second-generation anti-HER2 monoclonal antibody) with pembrolizumab (NCT02689284), respectively.⁵⁴

Challenges in HER2 assessment and patient selection

Tumor heterogeneity

The only standardized definition of intratumoral HER2 heterogeneity is the ASCO/CAP breast cancer guidance in which it is defined as the presence of *HER2* amplification in 5%–50%

of analyzed cells. Applying this definition to GE cancer, an HER2 heterogeneity rate of 17% has been reported.⁵⁵ However, heterogeneity in GE cancer has been variably defined, leading to large differences in described rates.^{40,42,56,57} The presence of tumor heterogeneity increases the risk of false-negative reporting from biopsy samples, and an expert panel of pathologists working with the ToGA trial has recommended six to eight viable biopsies as necessary for accurate evaluation of HER2 status.⁵⁸ At endoscopy biopsies should preferentially be taken from the lateral surrounds of the tumor as this area has been reported to be more frequently HER2 positive.⁵⁹

Most studies have reported a high concordance between HER2 status in primary tumor and metastatic sites, with a concordance rate that varies between 86% and 99%.^{60–64} The GASTHER1 study evaluated the utility of repeat endoscopic biopsy in patients whose tumors were initially found to be HER2 negative (IHC 0–2+ and ISH negative).⁶⁵ Repeat biopsy identified a “rescue” HER2 positivity rate of 8.7%, with 16 HER2-positive tumors identified out of the 183 retested. Tumors originally classified as IHC 1+ or 2+ were more than three times more likely to show HER2 positivity on repeat biopsy than IHC 0 tumors, and liver as a site of metastasis was almost six times more likely to show HER2 positivity than reassessment of other metastatic sites. Patients found to have HER2 positivity on repeat tumor sampling appeared to show similar treatment benefits with trastuzumab to those identified as HER2 positive at initial evaluation.⁶⁵ In contrast to breast cancer, re-biopsy of newly relapsed metastatic sites is not recommended in GE cancer. Data such as these, however, suggest that there may be a role for re-biopsy in carefully considered selected cases.

IHC versus gene amplification

Although chemotherapy with trastuzumab now represents the standard first-line approach for HER2-positive GE cancer, not all patients benefit, with variable RRs of ~30%–60% reported (Table 2).^{5,66,67} Thus, there are a proportion of patients who do not respond to trastuzumab therapy, despite their cancer conventionally being defined as HER2 positive. The correlation between the magnitude of *HER2* gene amplification and trastuzumab sensitivity has not been well evaluated in GE cancer, with only two exploratory reports on the topic published to date. In a 2013 article, Gomez-Martin et al⁶⁸ suggested that *HER2* gene amplification was a predictive factor for trastuzumab sensitivity. A total of 90 patients with metastatic gastric cancer treated with first-line chemotherapy plus trastuzumab were studied, and optimal *HER2*/CEP17 cutoff values for discriminating positive results in terms of response and survival were determined using receiver operating characteristic (ROC) curve analyses. An *HER2*/CEP17 ratio of 4.7 was identified as the optimal cutoff value discriminating for response. A more recent 2015 study also investigated the association between trastuzumab efficacy and *HER2* gene amplification and compared its predictability according to IHC status.⁶⁹ A total of 126 patients treated with chemotherapy plus trastuzumab were enrolled and *HER2* IHC score, *HER2*/CEP17 ratio and *HER2* gene copy number were analyzed. Patients with IHC 3+ showed significantly longer OS than patients with IHC $\leq 2+$, and an *HER2*/CEP17 ratio of 4.48 was found to be the optimal cutoff for predicting for survival (26.9 vs 14.7 months). In a subgroup analysis, the treatment outcome of patients with IHC 3+ was not influenced by the level of *HER2* gene amplification, suggesting that further gene amplification testing for this group of patients does not provide additional predictive value for clinical outcome. However, in patients with IHC $\leq 2+$, both *HER2*/CEP17 ratio and *HER2* gene copy number were informative, with an *HER2*/CEP17 ratio of >3.69 and an *HER2* gene copy number of >7.75 predicting for better outcomes. It is notable that these cutoffs are higher than the current standard FISH-positivity cutoffs of 2 and 6, respectively.

There are high levels of concordance between *HER2* gene amplification and the highest and lowest protein expression groups, with reported ISH positivity rates of 88%–100% for IHC 3+ tumors and 0%–8% for IHC 0–1+ tumors.^{44,56,70} ToGA screening data show that of those who were classified as IHC 3+, 94.9% also showed gene amplification by FISH.⁵⁶ The relationship between intermediate IHC 2+ protein expression and gene amplification, however, is less clear. Of the patients with IHC 2+ identified from

ToGA screening, 54.6% were FISH amplified. There are no existing data to suggest mechanisms other than *HER2* gene amplification to explain the overexpression of the *HER2* protein. Variable results of IHC evaluation have been reported dependent upon the IHC techniques used, and low IHC results in the presence of amplification may be caused by variability in operator-dependent IHC staining evaluation.⁷¹ The known tumor heterogeneity in GE cancer may also contribute, as areas lacking *HER2* overexpression often exhibit *HER2* amplification.⁷¹ In cases of equivocal or uncertain *HER2* expression, the use of amplification level as a continuous biomarker may potentially prove to be a more robust and rational approach for selecting patients likely to benefit from anti-*HER2* therapies.

Cardiac risk

There is a lack of consensus, evidence-based guidance for the identification, treatment and follow-up of trastuzumab-induced cardiac dysfunction, with wide variation in practice globally.⁷² Similarly, there are no published guidelines for the exclusion of patients with GE cancer for trastuzumab on the basis of excess cardiac risk, and many clinicians use the cardiac eligibility criteria from the main adjuvant trastuzumab trials in breast cancer. These were also adopted by the ToGA investigators and include congestive heart failure, baseline left ventricular ejection fraction (LVEF) $<50\%$, transmural myocardial infarction, poorly controlled hypertension ($>180/100$), angina pectoris requiring medication, clinically significant valvular heart disease and high-risk arrhythmias as contraindications to treatment.⁵ Cardiac monitoring recommendations for patients with GE cancer also generally follow those established for breast cancer, and the UK National Institute for Health and Care Excellence (NICE) guidance is representative, recommending assessment at three monthly intervals with either ECHO or multigated acquisition (MUGA) scan. If the LVEF drops by 10 percentage points or more from baseline and to below 50%, then trastuzumab treatment should be stopped, with a decision to resume based on further cardiac assessment and an informed consideration of individual risk versus benefit on a case-by-case basis.⁷³ The majority of trastuzumab-induced cardiac dysfunction occurs during the first 6 months of treatment and onset is rare during treatment beyond 6 months or after treatment cessation, thus less frequent monitoring may be justified.⁷⁴ Defining cardiac dysfunction through LVEF assessment – particularly with the use of MUGA scanning only – may lack sensitivity to detect early preclinical changes and other more subtle wall motion and valvular pathologies.⁷²

Current selection standards

Guidelines for selecting patients with GE cancer for trastuzumab treatment based upon HER2 testing are not uniform worldwide. The ToGA study led to rapid regulatory approval, with the US Food and Drug Administration (FDA) approving the drug for patients with IHC 3+ HER2 overexpression or FISH-confirmed gene amplification (with any IHC expression), in keeping with the study eligibility criteria. The European Medicines Agency (EMA), however, approved the drug in patients with IHC 3+, or IHC 2+ and FISH-amplified cancers, a subgroup that was assessed in the study only as part of an exploratory analysis. In the UK, the NICE guidelines recommend the drug for the higher threshold of HER2 IHC 3+ tumors only. The 22% of FISH-positive and IHC 0–1+ patients in ToGA did not gain significant clinical benefit. This suggests that screening for HER2 status by initial FISH testing may result in a proportion of nonresponders being exposed to both the risk and expense of trastuzumab unnecessarily. As a result, the EMA has recommended that the initial screening test for HER2 should be with IHC, followed by confirmatory FISH testing for IHC 2+ cases only.⁷⁵ Conversely, in the UK, NICE guidance mandating treatment for IHC 3+ patients only and not taking into account gene amplification is almost certainly depriving trastuzumab from a subset of patients who would benefit.

Future perspectives: circulating biomarkers

Multiple studies have addressed whether circulating HER2 protein ECD levels (c-ECD) can predict outcome with HER2-directed treatment.^{76,77} Baseline c-ECD levels do not appear to predict for response, but in a pooled analysis of seven trials of first-line trastuzumab with or without chemotherapy in patients with breast cancer, those with a 20% or greater decline in c-ECD levels over baseline had significantly higher RRs, PFS and OS compared with those with a lesser degree of decline.⁷⁷ However, a separate review of 63 studies concluded that concentrations of HER2 c-ECD are not consistently related to patient outcomes.⁷⁸ Thus, the clinical utility of assessing or following c-ECD levels during HER2-directed therapy is not established and the ASCO expert panel on tumor markers in breast cancer has recommended against its use in any clinical setting.

Digital polymerase chain reaction (PCR) has the potential to accurately quantify the concentration of nucleic acids in a sample to a much greater degree than traditional quantitative PCR by counting individual DNA molecules.⁷⁹ The procedure requires small concentrations of DNA for accurate analysis and thus is well suited to identifying genetic amplifications

in circulating tumor (ct)DNA, an area under considerable study across multiple tumor types.⁸⁰ In breast cancer, an analysis of ctDNA with digital PCR reported high accuracy in the determination of *HER2* status, with 90% concordance with tumor-derived HER2 status in an independent validation set.⁸¹ In gastric cancer, digital droplet (dd)PCR was used to evaluate *HER2* amplification in both biopsy specimens and circulating tumor (ct)DNA in 25 patients.⁸² The concordance rate between tumor analysis with ddPCR and IHC/FISH was high at 92%. The concordance rate with ctDNA was lower (62.5%); however, patients who were HER2 positive by ctDNA had significantly shorter survival compared with HER2-negative patients, suggesting a potential prognostic role. A further pilot study has evaluated the ability of ctDNA ddPCR to reflect dynamic changes in HER2 during gastric cancer treatment and follow-up.⁸³ A total of 60 patients with gastric cancer undergoing surgery were followed up with sequential analysis of ctDNA. Preoperative *HER2* ratio in ctDNA correlated well with tumor HER2 status and high plasma *HER2* ratios were identified in seven out of 13 patients at a time point of relapse after prior radical surgery. This very early data suggest some promise for the use of ddPCR *HER2* assessment as a repeatable and noninvasive approach for real-time evaluations of HER2 status during treatment; however, more work in the area is required.

Discussion

Trastuzumab has been an exemplar for biomarker-driven, targeted treatment based on individual tumor biology in GE cancer. There remain significant challenges, however, in leveraging maximum clinical benefit for patients in a resource-efficient manner. Uncertainties remain over the optimum definition of HER2 positivity and the mandating of ISH positivity in IHC 2+ equivocal cases only would seem to be more in-keeping with the patterns of clinical benefit seen in the ToGA study. It is interesting that both recently reported studies investigating the relationship between *HER2* gene amplification and clinical benefit from trastuzumab came to very similar conclusions regarding the optimal *HER2*/CEP17 ratio predicting for trastuzumab benefit, which was considerably higher than the currently mandated FISH definition of positivity.^{68,69} Further attempts to target *HER2* amplification such as with the antibody–drug conjugate T-DM1 and the small molecule inhibitor lapatinib have been disappointing. Again, however, data from lapatinib studies suggest that it may have a role in a subgroup of strongly HER2-expressing patients.^{10,13}

Heterogeneity of HER2 expression in GE cancer is a challenging but not an insurmountable problem. Endoscopic biopsies, if performed and analyzed to existing standards,

can provide reliable HER2 assessment.⁵⁸ There is relative consistency between primary GE tumors and metastatic sites, suggesting that, unlike breast cancer, routine re-biopsy of all newly relapsed metastatic sites would not be appropriate. The GASTHER1 data, however, suggest that in carefully selected cases, such as those tumors originally showing IHC 1+ or 2+ expression and where the relapsed site is in the liver, re-biopsy may be indicated to pick up discordant emergent HER2 positivity.⁶⁵

The use of circulating blood biomarkers as a measure of HER2 positivity and dynamic response to treatment is still very much at an early stage, and whether this results in meaningful clinical application has yet to be seen. Such circulating biomarkers may offer a noninvasive method of ascertaining HER2 status at baseline, while dynamic changes in HER2 expression during treatment may shed light on resistance mechanisms to HER2-targeted therapy, which are poorly understood at present. Moving forward, it is hoped that a greater understanding of the relationship between HER2 protein expression, gene amplification and blood-borne biomarkers with treatment response will allow for more individualized medical decisions regarding the application of HER2 therapies in GE cancer to be made.

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Disclosure

The authors report no conflicts of interest in this work.

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