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Perspectives of HER2-targeting in gastric and esophageal cancer

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Abstract

Introduction—The blockade of HER2 signaling has significantly improved the outlook for esophagogastric cancer patients. However, targeting HER2 still remains challenging due to complex biology of this receptor in gastric and esophageal cancers.

Areas covered—Here, we review complex HER2 biology, current methods of HER2 testing and tumor heterogeneity of gastroesophageal cancer. Ongoing and completed clinical research data are discussed.

Expert Opinion—HER2 overexpression is a validated target in gastroesophageal cancer, with therapeutic implications resulting in prolonged survival when inhibited in the front-line setting. With standardized HER2 testing in gastro-esophageal cancer, the ongoing trials are testing newer agents and combinations including combination of anti-HER2 antibodies with immunotherapy. Clonal heterogeneity and emergence of resistance will challenge our approach to treating these patients beyond the frontline settings.

Keywords

HER2; ErbB2; trastuzumab; gastric cancer; esophageal cancer

1. Introduction

The erythroblastic leukemia viral oncogene homolog 2 (ErbB2, also known as HER2) belongs to the family of epidermal growth factor (EGF) receptor tyrosine kinases. HER2, as other members of the EGF family, forms homo- and heterodimers with other EGF family receptors such as EGFR and ErbB3. As opposed to epidermal growth factor receptor (EGFR), the kinase activity of HER2 is independent of the ligand binding, and in fact, no ligands have been established¹. The HER2 signaling in conjunction with its co-receptor

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Declaration of Interest

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ErbB3 and the ErbB3 ligand, neuregulin, plays a fundamental role in the normal physiology of adrenal gland, liver, and distinct cell layers of the dermis and brain (lateral ventricles, peripheral ganglia)². Through discovery of cardiotoxicity as of the main side-effect of HER2-blocking antibody, the role of HER2 signaling in cardiomyocyte maintenance became known and further confirmed experimentally through conditional knockout in mice³. Crystal structure studies⁴ of HER2 in complex with pertuzumab bound to the dimerization domain II demonstrated structural basis for the propensity of HER2 towards heterodimerization vs. formation of homodimers⁵. Formation of dimeric complexes brings together the kinase domains of the two partner molecules allowing for cross-phosphorylation and activation of downstream signaling⁶. Using HER2-specific antibodies as molecular probes, strategies were designed to disrupt HER2 signaling via receptor depletion from the cell surface and interference with its heterodimerization as the two principal mechanisms⁷. The two FDA-approved HER2 antibodies, trastuzumab and pertuzumab, act synergistically by attacking these two mechanisms of HER2 signaling⁸. High expression of HER2 protein as the result of gene amplification drives oncogenic signaling in adenocarcinomas of various organs and tissues of origin including esophagogastric, breast, ovarian, pancreatic, colorectal, uterine and other types (Figure 1, TCGA Datasets, cbiportal.org).

2. Mechanistic considerations of HER2 targeting

HER2 and its partners

As a cell surface receptor, HER2 has no known ligands and, interestingly, requires formation of heterodimers with ErbB3 or EGFR for full catalytic activity^{1,4,5}. Consistent with its constitutively active conformation, HER2 lacks ligand-binding activity and its signaling function is engaged by its ligand-bound heterodimeric partners⁹. The EGFR and ErbB3 ligands¹⁰ stabilize this interaction to activate the canonical mitogen-activated protein kinase and phosphatidylinositol-3 kinase signaling cascades. Unlike the other members of the EGF family, the extracellular domain of HER2 does not pivot between active and inactive conformations and constitutively exists in an activated conformation^{5,11}. A variety of human cancers use HER2 as their “oncogenic driver” via amplification of the gene locus on chromosome 17 to increase the HER2 protein expression. The increased membrane expression and activity of HER2 heterodimers is further enhanced via efficient HER2 recycling which provides sustained activation of downstream signaling following stimulation with EGF or neuregulin in comparison with cells with low HER2 expression¹². Combination of antibodies against HER2 has been proposed as the mechanism to force HER2 internalization and antagonize its recycling through formation of large protein “lattices”¹³.

Activated intracellular domain of HER2

An unusual feature of the EGF family or receptors is the proteolytic cleavage by ADAM-family extracellular proteases at the juxtamembrane sites leading to the formation of truncated proteins containing mostly intracellular domains (ICD). The ICD p95-HER2 retains the kinase domain but lacks the binding site for trastuzumab and can participate in dimerization with other EGF family receptors and activate downstream signaling pathways^{14,15}. Various studies have shown increased p95-HER2 expression in tumors with trastuzumab resistance and associated with worse clinical outcome^{16,17}. The problem of

emergence of such intracellular fragments of p95-HER2 is not only their continuous signaling activity within the endosomal compartment, but also the ability of the ICD proteins to activate gene transcription and interact with transcriptional factors such as STAT3¹⁸. Trastuzumab binds to the extracellular juxtamembrane domain of HER2 receptor to the three loop regions of HER2 (residues 557–561 (loop 1), 570–573 (loop 2) and 593–603 (loop 3))¹¹. This binding introduces steric hindrance for HER2 heterodimerization and blocks proteolytic cleavage of the extracellular domain of HER2, resulting in diminished levels of p95-HER2^{14,19}. The truncated form of HER2 has been shown to induce cellular invasion and increase kinase activity. Expression of the truncated form of p95-HER2 has been found more commonly in nodal metastases compared to primary tumors suggesting its pathological association with metastatic process²⁰.

HER2 addiction

Despite HER2 copy number and high expression predicting the benefit of trastuzumab in clinical trials in breast²¹ and esophagogastric²² cancers, only a minority of tumors respond to HER2 blockade with trastuzumab monotherapy. In the earlier breast cancer trials, the response rates in patients with HER2 overexpression by immunohistochemistry (IHC) at 3+ were remarkably higher at 35% compared to none in patients with tumors assessed as 2+²³. This and many subsequent observations highlight the importance of objectively defining what constitutes the oncogenic addiction to HER2 in cancer^{24,25}. Despite the statistical complexities of how to draw the line between the “HER2-addicted” cancers vs. non-addicted, one biological principal seems to hold true: the higher the copy number of amplified HER2 and HER2 expression, the more likely is the benefit of rapid withdrawal of oncogenic signaling from a dominant oncogenic molecule²⁶. As an example, in patients with operable HER2-amplified breast cancer receiving neoadjuvant lapatinib plus trastuzumab therapy in NeoALTTO trial²⁷, the benefit of HER2 blockade was directly proportionate to the level of quantified HER2 expression. These clinical observations are supported by experimental models where HER2 knockdown induced apoptosis in cell culture, or alternatively tumor regression *in vivo*, only in cancer models with high HER2^{28–30}. Engineered models of HER2-driven transformation using tetracycline inducible systems also have confirmed that HER2 induced tumors require HER2 for continued tumorigenic growth and survival. This has been demonstrated in a HER2-transformed NIH3T3 tumor model wherein tumors regress upon withdrawal of the HER2 oncogene^{31,32}.

Immune response and HER2 targeting

Early *in vivo* tumor xenograft studies showed inhibition of growth of breast cancer cells after exposure to 4D5, an antibody to the HER2 receptor³³. This antibody had significant immunogenicity, and was thus humanized to create the anti-HER2 monoclonal antibody trastuzumab³⁴. Preclinical activity of trastuzumab was found to include antibody-dependent cell-mediated cytotoxicity (ADCC) as well as inhibition of growth stimulation³⁵. Targeting cancer cells with antibodies has been demonstrated to actively engage the host immune system through the engagement of the Fc antibody receptors as was demonstrated in FcR knockouts³⁶. However, these effects proved to be difficult to document in the clinic. Most compelling evidence has been inferred from the analyses of Fc receptor 2B (FCGR2B, variant I232T) polymorphisms in a large N9831 Alliance trial so that the carriers of I/I allele

derived greater benefit from trastuzumab ($P < 0.001$) compared with the 232T carriers who did not ($P = 0.81$)³⁷. These associations are not confirmed in another large study³⁸ of over 900 patients in which the high affinity Fc receptors FCGR3A and FCGR2A did not show any effect on survival. On the other hand, trastuzumab may induce active immune responses against intracellular and extracellular domains of HER2 which in a small series of cases of breast cancer patients was associated with better PFS (HR=0.5) and OS (HR=0.7)³⁹.

Complexity of esophageal and gastric cancer genome

The complex genetic landscape of esophageal and gastric (EG) cancers has been extensively characterized in the recent massive genetic profiling effort by several research groups and The Cancer Genome Atlas (TCGA) consortium^{40,41}. Patterns of DNA mutations, DNA methylation, RNA expression and copy number alterations have been used to define four major clusters unified mechanistically by the recurrent patterns of alterations. Interestingly, a relatively smaller (<10%) subset of gastric cancers are associated with chronic integration of the Epstein-Barr virus genome and are likely caused by chronic viral persistence which is concurrent with *PIK3CA* mutations, DNA hypermethylation, and amplification of *JAK2*, *PD-L1* and *PD-L2*. A subset of microsatellite instability (MSI) -high tumors carry a characteristic signature of extremely high mutational load and largely lacking chromosomal gains or losses. The diffuse subset of gastric cancer demonstrates frequent mutations affecting components of the focal adhesion complex, *RHOA*, *CDH1*, *CTNNA1*, *CTNNA2* and *CTNNB1*, which may contribute to loss of cell adhesion in this subtype of gastric cancer^{40–42}. Amplifications involving multiple oncogenic kinases, including *EGFR*, *HER2*, *ErbB3*, *FGFR2*, *MET* are most prevalent in the chromosome instability (CIN, roughly 1/3 of all cases) subtype of gastric cancer⁴¹. It is also worth noting that in several cases included in the TCGA datasets of esophageal and gastric cancer (cbioportal.org, Figure 1) the chromosomal amplifications involve several of these oncogenes in one tumor specimen. It remains unclear whether these concurrent amplifications seen in as many as 6% of all profiled gastric cancers (TCGA provisional dataset, n=441), or in nearly one third (26 out of 90) of the CIN subtype are occurring in independent cancer clones within the same tumor, or less likely, coincide in a single cell. Given the multifocal origin of gastroesophageal cancers⁴³, the emergence of resistant clones following suppression of HER2 with inhibitors should not be surprising. Genetic surveillance in such cases indeed revealed that the resistant clones were relying on other upregulated oncogenic receptor tyrosine kinases such as *MET*, *EGFR*⁴⁴.

2. HER2 targeting in gastroesophageal cancer

Methods of detection of HER2 in gastric and esophageal cancers

The incidence of HER2 amplification in gastric cancer varies depending on the methods used and the heterogeneity of gastroesophageal tumors in each reported series. In the ToGA trial evaluating front-line trastuzumab with chemotherapy in advanced gastric cancer, the prevalence of HER2 amplification/positivity was the highest among carcinomas with intestinal histology (31.8%) compared to the diffuse type (6.1%), and was lower in metastatic sites (16.7%) compared to the stomach (21.4%) or gastroesophageal junction (32.2%). The threshold for clinically relevant HER2 positivity in this trial⁴⁵ was based on

previously validated criteria for gastric cancer⁴⁶: *in situ* hybridization (ISH) ratio relative to the chromosome 17 centromere probe of 2.0 or greater, or immunohistochemistry (IHC) 3+ with basolateral pattern of staining. In clinical practice, tumors with IHC staining of 0 or 1+ are considered negative for HER2, while those with IHC scores of 2+ require further testing with *in situ* hybridization for confirmation⁴⁷. Those with IHC scores of 3+ do not require further testing and are considered positive. However, microarray analysis using *in situ* hybridization and 2 IHC techniques on a large pool of 1040 primary tumors and matched 720 nodal metastases has recently called into question the accuracy of IHC in assessing HER2 overexpression⁴⁸: amplification was not associated with protein overexpression in about 10% of cases depending on the IHC method. Furthermore, as in breast cancer, there is a discordance of IHC staining and ISH positivity between the primary focus of carcinoma and the metastatic sites can be seen in up to 25% of cases⁴⁹. Therefore, sampling of carcinoma tissue from the primary and multiple metastatic sites may be a reasonable consideration^{49,50}.

Recently, the American Society for Clinical Oncology and the College of American Pathologists issued a very detailed and specific guideline for HER2 testing and clinical decision making in gastroesophageal adenocarcinoma⁵¹. The panel summarized the existing technical and biological difficulties in assessing the HER2 status. In scoring gastric cancer HER2 by IHC, the panel recommended the use of Ruschhoff-Hofmann method⁴⁶. The discrepancies in HER2 scores are attributed to a different pattern of immunoreactivity (basolateral membranous) in glandular cells and a higher rate of tumor heterogeneity in gastric cancer compared with breast cancer⁴⁶. With emergence of next generation sequencing based on large panels of genes, copy number can be inferred from the proportionate number of reads corresponding to the targeted genomic locus. These sequencing platforms are being used for screening of genomic loci copy number alterations. In application to HER2, the NGS determination would still require confirmatory testing with validated assays⁵².

Given the operator dependence of IHC scoring and the lack of wide-spread adoption of NGS, we believe that adopting the molecularly-defined classification of gastroesophageal carcinomas^{40,41} discussed above will better stratify these heterogeneous tumors into therapy-relevant groups. Again, the existence of multiple tumor clones (as illustrated in Figure 1) complicates further the genetic analyses of gastroesophageal carcinomas leading the expert panel to recommend assessment of multiple tissue samples when available⁵¹.

3. Targeting HER2: Antibodies, Drug positivity conjugates, and tyrosine kinase inhibitors in clinical practice

Monoclonal antibodies

Trastuzumab was the first therapeutic antibody developed for the treatment of HER2-positive carcinomas. Initial testing of trastuzumab in patients with refractory HER2-positive breast cancer demonstrated responses both, as single-agent, and twice as many in combination with cisplatin chemotherapy (11.6% and 24.3%, respectively)⁵³. Additional phase II studies established a standard dosing schedule while several large randomized phase

III clinical trials combining trastuzumab with chemotherapy in metastatic and early stage breast cancer demonstrated improved response rates, progression- or disease-free survival, and overall survival^{21,54,55}.

Given the success in breast cancer in both the metastatic and adjuvant settings, evaluation of trastuzumab in gastric and esophageal cancers was inevitable. The randomized phase III ToGA trial evaluated trastuzumab in combination with standard platinum/fluoropyrimidine combination versus platinum/fluoropyrimidine chemotherapy alone in treatment-naïve HER2+ metastatic HER2 positive GE junction (GEJ) or gastric adenocarcinoma. Patients were considered eligible for enrollment if they had either HER2 overexpression by IHC or gene amplification by ISH. The addition of trastuzumab to chemotherapy improved median OS (13.8 months with the combination vs. 11.1 months for chemotherapy alone, hazard ratio [HR] 0.74; $p=0.0046$)²². An exploratory analysis limited to patients with classic higher HER2 positivity (IHC 3+ or IHC2+ and ISH ratio > 2.0) demonstrated an even greater survival advantage (16.0 vs. 11.8 months; HR 0.65). In about 50% of cases, the post hoc analysis showed significant variability in HER2 staining (less or equal to 30 % stained cells), however this did not affect treatment outcome, suggesting the importance of these specific IHC criteria for the screening of HER2-positivity in esophagogastric cancers⁴⁵. Trastuzumab has also been evaluated in the adjuvant setting after trimodality therapy for non-metastatic esophageal cancer in RTOG 1010, and results of this study are eagerly awaited (Table 1). Another study, the TRIGGER trial, is investigating the use of neo-adjuvant trastuzumab with chemotherapy in resectable gastric cancer with extensive nodal involvement⁵⁶. This phase II clinical trial, which is recruiting in Japan, is comparing cisplatin, S-1, and trastuzumab to cisplatin and S-1 alone in the neoadjuvant setting followed by 1 year of adjuvant therapy.

Trastuzumab beyond progression

The clinical utility of continuing trastuzumab beyond progression is still a research question that deserves further study. A small study conducted in China in patients with advanced HER2-positive gastric cancer showed slightly better survival on trastuzumab beyond progression (3 months vs. 2 months without trastuzumab)⁵⁷. Additionally, the control arm of a recently reported phase II study of paclitaxel and trastuzumab with or without a novel bispecific HER2/HER3-targeted antibody demonstrated a survival of greater than one year (56.1 weeks) in a second-line setting in which the majority (80%) of patients had received prior trastuzumab⁵⁸. At this time, larger studies of trastuzumab beyond progression in trastuzumab-pretreated patients are needed before this strategy can be recommended as standard of care. At the 2017 ASCO Gastrointestinal Cancers Symposium, Makiyama et al. (Abstract 93) presented data of observational cohort study KSCC1105 conducted in Japan to investigate the clinical outcomes associated of trastuzumab continued beyond progression in combination with chemotherapy. Of 85 evaluable patients, those who continued on trastuzumab with second line chemotherapy had better OS (12.8 months) compared to 7.9 months without trastuzumab (HR=0.50, $p=0.01$). Concordant results were also presented from a multicenter retrospective AGEO study by Palle et al. (2017 ASCO Gastrointestinal Cancers Symposium, Abstract 94) in which 104 patients with HER2 + advanced gastric or gastro-esophageal junction adenocarcinoma received trastuzumab after progression on the frontline regimen including trastuzumab. The 39 patients who continued on trastuzumab had

significantly better OS of 12.6 months compared to no trastuzumab (n=65, OS=6.1 months, HR=0.47, p=0.001). In total, these results deserve prospective randomized validation.

Pertuzumab is a monoclonal antibody specific to domain II of HER2 designed to block HER2 dimerization⁵⁹. The early clinical testing of pertuzumab showed very limited activity as a single agent⁵⁹. However, pertuzumab in combination with trastuzumab produced synergistic effects in patients with HER2-amplified breast cancer who have previously progressed on trastuzumab⁶⁰. Pertuzumab has been combined with trastuzumab in the CLEOPATRA trial for front-line therapy in metastatic breast cancer⁸ and in two neoadjuvant trials combining it with trastuzumab and chemotherapy^{61,62}. Response rates and survival were all improved with the addition of pertuzumab to standard trastuzumab-based chemotherapy. Based on these data, the combination of pertuzumab, trastuzumab and chemotherapy is now being tested in HER2-positive GEJ and gastric cancers in the ongoing INNOVATION (NCT02205047) trial evaluating this therapy in the neoadjuvant setting and recently completed JACOB (NCT01774786) trial evaluating this therapy in treatment-naïve metastatic disease.

Bispecific antibodies

Fusion of two recombinant antibodies into bispecific antibodies allows dual targeting and functionality⁶³. One such bispecific antibody to HER2 and CD16 was highly active in inducing ADCC against HE2-positive cells *in vitro*⁶⁴. This unfortunately proved highly toxic in a phase I clinical trial and the drug was not pursued further⁶⁵. MM-111 is a bispecific antibody that targets both ErbB2 and ErbB3. Binding of MM-111 to ErbB3 blocks heregulin binding and inhibits heregulin-activated ErbB3 signaling⁶⁶. The molecule was studied in combination with paclitaxel and trastuzumab in the second-line setting, with a comparator arm of paclitaxel and trastuzumab alone. Despite promising preclinical evidence⁶⁶, the triplet combination was associated with shorter OS or PFS in a phase II trial reported at ASCO 2016⁵⁸. Further investigation on why this combination failed to improve outcomes is warranted.

Antibody-drug conjugates

Trastuzumab emtastine (T-DM1) is an antibody-drug conjugate in which a microtubule-inhibitory agent is chemically linked with trastuzumab. This allows drug delivery directly to HER2 expressing cancer cells while limiting exposure to normal tissue⁶⁷. Such approach has been successfully tested in patients with HER2-positive metastatic or recurrent locally advanced breast cancer in the EMILIA trial where T-DM1 improved PFS and OS⁶⁸. Similar results were not seen in the recently reported GATSBY trial⁶⁹, a phase II/III study that compared second line T-DM1 to docetaxel in previously treated HER2 positive locally advanced or metastatic gastric and GEJ adenocarcinoma. This randomized study found no difference in OS or PFS between the two arms. NCT01641939, a phase II clinical trial comparing docetaxel to two dose levels of T-DM1 in HER2-positive advanced gastric cancer, has completed accrual and results are pending. Another trial, NCT01702558, a phase II trial that uses the combination of T-DM1 and capecitabine in HER2-positive metastatic breast cancer or HER2-positive locally advanced or metastatic gastric cancer, is ongoing

(Table 1). Targeting chemotherapy containing liposomes to HER2 with antibodies has shown preclinical efficacy in HER2-dependent breast and gastric cancer models⁷⁰.

Small molecule Kinase inhibitors

Small molecule tyrosine kinase inhibitors have been thus far disappointing in advanced HER2-positive gastric and esophageal. Lapatinib, a HER2 kinase and a weak EGFR kinase inhibitor, has been studied in SWOG S0413 phase II trial⁷¹ as a first-line single agent therapy for HER2-positive gastric adenocarcinoma. The trial failed to reach its pre-defined end-point for clinical benefit, showing an overall response rate of only 11% with a 4.8 month median overall survival. A subsequent phase II trial in Europe compared lapatinib to lapatinib plus capecitabine in the second line setting for patients with HER2 amplified gastroesophageal cancer⁷². Of 37 enrolled patients, only 2 achieved an objective response and the study was closed prematurely for futility. The phase III TRIO-013/LOGiC trial⁷³ evaluated capecitabine and oxaliplatin with or without lapatinib in patients with HER2 amplified gastric cancer and failed to show a survival benefit (12.2 vs. 10.5 months, HR 0.91; $p=0.3492$), although progression-free survival and response rate were significantly higher in the lapatinib arm. Interestingly, subgroup analysis suggested a benefit for younger patients and those of Asian descent. Finally, TyTAN, a phase III trial⁷⁴ utilizing paclitaxel with or without lapatinib in the second line setting for patients with HER2 positive gastric cancer also showed no survival benefit (HR 0.84; $p=0.1044$). The results in gastric cancer are contrasted by the data in breast cancer where lapatinib in combination with capecitabine improved time to progression with a hazard ratio of 0.49 in trastuzumab-resistant metastatic or locally advanced HER2-positive breast cancer⁷⁵. More recently, an exploratory phase II biomarker-embedded trial combined lapatinib and capecitabine as first-line treatment of advanced gastric and gastroesophageal junction adenocarcinoma independent of HER2 expression⁷⁶. The combination was well tolerated with a median OS of 6.3 months. No changes in gene expression were observed. Interestingly, an association between response rate and elevated baseline HER-3 mRNA expression was found. The authors concluded that further investigation of this association is warranted.

Additional agents are currently being evaluated in this space. Afatinib in combination with trastuzumab appears to be promising in heavily pretreated HER2+ patients, with a disease control rate of 42% at 4 months with single agent afatinib⁷⁷ and the combination cohort ongoing. 1E11, a novel humanized monoclonal antibody that targets HER2, has been shown to have synergistic activity with trastuzumab in HER2 overexpressing gastric cancer cell lines⁷⁸. Recently, a new anti-HER2 antibody with optimized Fc-receptor binding affinity has entered phase II of clinical testing in combination with anti-PD1 antibody pembrolizumab in advanced metastatic gastric and GEJ adenocarcinoma (NCT02689284, *clinicaltrials.gov*).

4. Outstanding Questions: Resistance, Treatment Opportunities, and Future Directions

Despite promising early data for HER2-targeted therapies, studies of HER2-directed therapies have been overall disappointing. This may be in part due to the heterogeneity of gastroesophageal cancers, which have less uniform expression of HER2 on the cellular

membrane than breast cancer cells. Discordance in HER2 status between primary tumors and metastatic lesions may also confound the efficacy of HER2-directed agents⁷⁹. Additionally, levels of HER2 expression as currently defined may be suboptimal for identifying trastuzumab responders. Two retrospective studies have suggested that the HER2:CEP17 ratio associated with treatment response is greater than 4.0, much higher than the currently utilized ratio of 2.0 as defined by the ToGA trial^{80,81}. Exploratory analyses of the ToGA trial support this as well, with the greatest benefit to trastuzumab-based therapy seen in those whose tumors have high expression of HER2 by IHC²².

A second consideration in understanding the failure of alternative HER2-directed agents such as lapatinib may be the toxicity of the agents evaluated as well as the activity of the agent itself. Lapatinib and capecitabine have overlapping toxicities including gastrointestinal effects, and this may have limited the amount of therapy that lapatinib-treated patients received⁷³. Similar concerns arise when lapatinib is combined with paclitaxel in the TyTAN trial⁷⁴, where the lapatinib arm had a higher toxicity-related discontinuation rate that may have impacted efficacy. Data from the second-line TyTAN trial also suggest that prior gastrectomy may influence the pharmacokinetics of lapatinib, resulting in subtherapeutic levels of the drug⁷⁴. In addition, studies of lapatinib and trastuzumab in breast cancer have demonstrated the superiority of trastuzumab in the metastatic and adjuvant setting^{82,83}. Thus, lapatinib may not be the most effective HER2-targeting small molecule tyrosine kinase inhibitor in this disease setting due to both drug delivery and efficacy.

The failure of second-line therapies in multiple studies raises questions about why these agents were not successful in gastroesophageal cancer despite striking benefits in breast cancer. This question is most prominent in the GATSBY trial of TDM-1⁶⁹. This agent has had remarkable benefit in pretreated breast cancer patients, yet in the gastroesophageal cancer population, no demonstrable benefit was noted and the experimental arm was numerically slightly inferior. HER2 expression can change at the time of disease progression in approximately 1/3 of patients who have previously received trastuzumab-based therapy^{84,85}. Similar results were seen in the second-line trial of MM-111 plus trastuzumab-based chemotherapy, where a lower rate of HER2 amplification was seen in pre-treatment biopsies than in archived tumor specimens⁸⁶. Additionally, the development of resistance to trastuzumab-based therapy was associated with additional genomic alterations that most commonly affected cell cycling or PI3 kinase signaling⁴⁴. Further work on the mechanisms of resistance to front-line HER2-targeted therapies is needed to elucidate the optimal population in which to test future therapies, and it is likely that confirmation of persistent HER2-positivity will be necessary before initiating HER2-targeted therapy in refractory patients.

Some lessons can be learned from our previous experience in breast and gastroesophageal cancers: for instance, the thresholds for positivity may be reconsidered and the inherent resistance mechanisms discussed above should be investigated in the defining, perhaps, a smaller subset of likely responders. In early stage breast cancer, the NeoALLTO trial^{27,68,87} in early stage breast cancer of lapatinib vs. trastuzumab, vs. both, combined with chemotherapy, found all regimens to be equivalent in terms of overall survival and pathologic complete response. Nonetheless, the trial provided valuable tissue material for

correlative studies which identified the expression of HER2 protein and mRNA as the most significant predictor of pathological complete response^{27,68}, potentially identifying a predictive of the clinical benefit biomarker correlating with the degree of tumors' oncogenic addiction to HER2. It is also very important to consider the mechanistic data obtained earlier in the HER2-positive models showing the antitumor effect only if the therapy was blocking over 90% of HER2 signaling activity⁸⁸. Earlier work from the Moasser laboratory identified ErbB3 as one of the escape mechanisms by which increased ErbB3 phosphorylation made cell lines refractory to HER2 TKI due to a shift in its phosphorylation-dephosphorylation equilibrium. As the result, these compensatory changes maintained PI3 kinase pathway activity leading to AKT-dependent cancer cell survival⁸⁹. From this perspective, to inactivate highly redundant signaling of HER2 and its partnering EGFR family co-receptors would require blockade of dimerization with pertuzumab, or dual antibody-TKI HER2 blockade. Recent studies showed indeed a better efficacy of combination of afatinib, a pan-EGFR irreversible kinase inhibitor, with cetuximab in non-small cell lung cancer with resistance T790M mutation and HER2 amplification in some cases⁹⁰. Dual, or "vertical", blockade of the HER2 signaling axis that also precludes compensatory signaling loops through EGFR and ErbB3 have been effective in the preclinical models⁹¹.

Given the promise of immunotherapy with anti-PD1/PD1L checkpoint inhibitors in the gastroesophageal cancers, studies combining novel HER2-targeted agents with immunotherapeutics are ongoing. Margetuximab, an Fc-optimized antibody targeting HER2, is currently being tested in combination with pembrolizumab in the second-line setting (Table 1). Additional novel HER2-targeted agents including ADXS31-164, an attenuated strain of *Listeria* expressing an MHC class I epitopes of the HER2 oncogene as a fusion protein with a non-hemolytic fragment of listeriolysin O⁹², are currently in phase 1 testing and may hold promise for this disease.

5. Conclusion

With current front-line trastuzumab-based therapies, the prognosis of advanced HER2-positive gastric cancer is improving and currently rivals or surpasses that of HER2-negative disease^{93,94}. However, additional work is necessary to optimize the selection of patients for therapy, define mechanisms of resistance, and develop effective targeted therapies for refractory disease. As clonal heterogeneity and changing protein expression is a known phenomenon in this disease, repeat tumor testing to identify changes in HER2 expression at times of disease progression is worthwhile. The definition of "HER2-positive" specific to gastroesophageal cancers may also need to be refined, perhaps allowing us to improve effectiveness by limiting targeted therapies to those tumors truly "addicted" to the HER2 signaling pathway. Combination therapy with other known effective targeted strategies, including dual HER2 blockade and co-targeting of angiogenic or immunomodulatory pathways may also be more effective than our current single-target plus chemotherapy strategy. We await with great anticipation the results of current ongoing studies and look forward to the potential direction that new non-cytotoxic combination regimens may lead.

6. Expert Opinion

Testing of HER2 expression should be performed in all patients with advanced or incurable gastro-esophageal adenocarcinoma, and trastuzumab should be part of the front-line treatment regimen in combination with fluoropyrimidine/platinum. Tumors with an IHC score of 3+ or a score of 2+ with an ISH ratio of 2.0 or greater are considered HER2 positive and thus candidates for trastuzumab-based therapy. Alternative methods for assessing HER2 status include next generation sequencing, although the therapeutic significance of a HER2 mutations in gastro-esophageal adenocarcinoma is unknown at this time. Current data do not support the use of lapatinib in first- or second-line therapy, nor does TDM-1 have any role in second-line therapy. At present time, the role for the addition of pertuzumab to trastuzumab-based therapies remains to be defined, although studies evaluating this strategy are eagerly awaited. The role of trastuzumab in the neoadjuvant and adjuvant setting is being determined in recently completed large randomized controlled trials.

Given the possibility of loss of HER2 expression in up to a quarter of patients after treatment with trastuzumab, retesting of HER2 expression is warranted if trastuzumab is to be considered after progression on the first-line chemotherapy. Evolving evidence from retrospective observational studies of trastuzumab beyond progression will need to be prospectively validated given the potential survival advantage when compared to chemotherapy alone. Until these studies are completed, the current guidelines⁹⁵ recommend switching to a non-HER2 targeted cytotoxic chemotherapy alone or ramucirumab and paclitaxel combination due to the established survival advantage of this approach⁹⁶. Should continuation of trastuzumab beyond progression be warranted, monitoring of cardiac tolerance of prolonged trastuzumab exposure is necessary.

Newer agents and combinations are being actively investigated in gastro-esophageal cancer including immunotherapy with checkpoint inhibitors such as nivolumab, and by this token, combined targeting of PD-1 and HER2 is rational. Ongoing studies of pembrolizumab in combination with the new Fc-receptor optimized anti-HER2 antibody margetuximab will provide insight into whether this strategy is worth pursuing. While the results seen with bispecific antibody MM-111, antibody-drug conjugates, or HER2 kinase inhibitors are disappointing, the search for novel ways to deregulate the oncogenic HER2 is ongoing. In the meantime, results of recently completed trials such as JACOB and INNOVATION will provide insight into whether already available agents, currently approved for use in breast cancer, can find a role in gastroesophageal cancer.

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Spivak-Kroizman T, Rotin D, Pinchasi D, et al. Heterodimerization of c-erbB2 with different epidermal growth factor receptor mutants elicits stimulatory or inhibitory responses. *The Journal of biological chemistry*. 1992; 267:8056–63. [PubMed: 1349015]
2. Orr-Urtreger A, Trakhtenbrot L, Ben-Levy R, et al. Neural expression and chromosomal mapping of Neu differentiation factor to 8p12–p21. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90:1867–71. [PubMed: 8095334]
3. Crone SA, Zhao YY, Fan L, et al. ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nature medicine*. 2002; 8:459–65.
4. Franklin MC, Carey KD, Vajdos FF, et al. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer cell*. 2004; 5:317–28. [PubMed: 15093539]
5. Garrett TP, McKern NM, Lou M, et al. The crystal structure of a truncated ErbB2 ectodomain reveals an active conformation, poised to interact with other ErbB receptors. *Molecular cell*. 2003; 11:495–505. [PubMed: 12620236]
6. Zhang X, Gureasko J, Shen K, et al. An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell*. 2006; 125:1137–49. [PubMed: 16777603]
7. Klapper LN, Vaisman N, Hurwitz E, et al. A subclass of tumor-inhibitory monoclonal antibodies to ErbB-2/HER2 blocks crosstalk with growth factor receptors. *Oncogene*. 1997; 14:2099–109. [PubMed: 9160890]
8. Swain SM, Baselga J, Kim SB, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *The New England journal of medicine*. 2015; 372:724–34. [PubMed: 25693012]
9. Sliwkowski MX. Ready to partner. *Nature structural biology*. 2003; 10:158–9. [PubMed: 12605220]
10. Sierke SL, Cheng K, Kim HH, et al. Biochemical characterization of the protein tyrosine kinase homology domain of the ErbB3 (HER3) receptor protein. *The Biochemical journal*. 1997; 322(Pt 3):757–63. [PubMed: 9148746]
11. Cho HS, Mason K, Ramyar KX, et al. Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab. *Nature*. 2003; 421:756–60. [PubMed: 12610629]
12. Karunakaran D, Tzahar E, Beerli RR, et al. ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer. *The EMBO journal*. 1996; 15:254–64. [PubMed: 8617201]
13. Ben-Kasus T, Schechter B, Lavi S, et al. Persistent elimination of ErbB-2/HER2-overexpressing tumors using combinations of monoclonal antibodies: relevance of receptor endocytosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:3294–9. [PubMed: 19218427]
14. Liu PC, Liu X, Li Y, et al. Identification of ADAM10 as a major source of HER2 ectodomain shedase activity in HER2 overexpressing breast cancer cells. *Cancer biology & therapy*. 2006; 5:657–64. [PubMed: 16627989]
15. Zhou BB, Peyton M, He B, et al. Targeting ADAM-mediated ligand cleavage to inhibit HER3 and EGFR pathways in non-small cell lung cancer. *Cancer cell*. 2006; 10:39–50. [PubMed: 16843264]
16. Arribas J, Baselga J, Pedersen K, et al. p95HER2 and breast cancer. *Cancer research*. 2011; 71:1515–9. [PubMed: 21343397]
17. Chandralapaty S, Scaltriti M, Angelini P, et al. Inhibitors of HSP90 block p95-HER2 signaling in Trastuzumab-resistant tumors and suppress their growth. *Oncogene*. 2010; 29:325–34. [PubMed: 19855434]
18. Cordo Russo RI, Beguelin W, Diaz Flaue MC, et al. Targeting ErbB-2 nuclear localization and function inhibits breast cancer growth and overcomes trastuzumab resistance. *Oncogene*. 2015; 34:3413–28. [PubMed: 25174405]

19. Molina MA, Codony-Servat J, Albanell J, et al. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer research*. 2001; 61:4744–9. [PubMed: 11406546]
20. Molina MA, Saez R, Ramsey EE, et al. NH(2)-terminal truncated HER-2 protein but not full-length receptor is associated with nodal metastasis in human breast cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2002; 8:347–53. [PubMed: 11839648]
21. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *The New England journal of medicine*. 2001; 344:783–92. [PubMed: 11248153]
- 22•. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet*. 2010; 376:687–97. This randomized controlled trial of trastuzumab in combination with chemotherapy versus chemotherapy alone in untreated advanced gastric cancer demonstrated a significant survival advantage with the addition of trastuzumab, and forms the basis of our current standard of care. [PubMed: 20728210]
23. Vogel CL, Cobleigh MA, Tripathy D, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2002; 20:719–26. [PubMed: 11821453]
24. Bean GR, Ganesan YT, Dong Y, et al. PUMA and BIM are required for oncogene inactivation-induced apoptosis. *Science signaling*. 2013; 6:ra20. [PubMed: 23532334]
25. Faber AC, Corcoran RB, Ebi H, et al. BIM expression in treatment-naïve cancers predicts responsiveness to kinase inhibitors. *Cancer discovery*. 2011; 1:352–65. [PubMed: 22145099]
26. Metzger-Filho O, Procter M, de Azambuja E, et al. Magnitude of trastuzumab benefit in patients with HER2-positive, invasive lobular breast carcinoma: results from the HERA trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013; 31:1954–60. [PubMed: 23589556]
27. Scaltriti M, Nuciforo P, Bradbury I, et al. High HER2 expression correlates with response to the combination of lapatinib and trastuzumab. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2015; 21:569–76. [PubMed: 25467182]
28. Colomer R, Lupu R, Bacus SS, et al. erbB-2 antisense oligonucleotides inhibit the proliferation of breast carcinoma cells with erbB-2 oncogene amplification. *British journal of cancer*. 1994; 70:819–25. [PubMed: 7947086]
29. Roh H, Pippin J, Drebin JA. Down-regulation of HER2/neu expression induces apoptosis in human cancer cells that overexpress HER2/neu. *Cancer research*. 2000; 60:560–5. [PubMed: 10676637]
30. Faltus T, Yuan J, Zimmer B, et al. Silencing of the HER2/neu gene by siRNA inhibits proliferation and induces apoptosis in HER2/neu-overexpressing breast cancer cells. *Neoplasia*. 2004; 6:786–95. [PubMed: 15720805]
31. Baasner S, von Melchner H, Klenner T, et al. Reversible tumorigenesis in mice by conditional expression of the HER2/c-erbB2 receptor tyrosine kinase. *Oncogene*. 1996; 13:901–11. [PubMed: 8806679]
32. Schiffer IB, Gebhard S, Heimerdinger CK, et al. Switching off HER-2/neu in a tetracycline-controlled mouse tumor model leads to apoptosis and tumor-size-dependent remission. *Cancer research*. 2003; 63:7221–31. [PubMed: 14612517]
33. Fendly BM, Winget M, Hudziak RM, et al. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. *Cancer research*. 1990; 50:1550–8. [PubMed: 1689212]
34. Carter P, Presta L, Gorman CM, et al. Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proceedings of the National Academy of Sciences of the United States of America*. 1992; 89:4285–9. [PubMed: 1350088]
35. Sliwkowski MX, Lofgren JA, Lewis GD, et al. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Seminars in oncology*. 1999; 26:60–70.

36. Clynes RA, Towers TL, Presta LG, et al. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nature medicine*. 2000; 6:443–6.
37. Norton N, Olson RM, Pegram M, et al. Association studies of Fcγ receptor polymorphisms with outcome in HER2+ breast cancer patients treated with trastuzumab in NCCTG (Alliance) Trial N9831. *Cancer immunology research*. 2014; 2:962–9. [PubMed: 24989892]
38. Hurvitz SA, Betting DJ, Stern HM, et al. Analysis of Fcγ receptor IIIa and IIa polymorphisms: lack of correlation with outcome in trastuzumab-treated breast cancer patients. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2012; 18:3478–86. [PubMed: 22504044]
39. Knutson KL, Clynes R, Shreeder B, et al. Improved Survival of HER2+ Breast Cancer Patients Treated with Trastuzumab and Chemotherapy Is Associated with Host Antibody Immunity against the HER2 Intracellular Domain. *Cancer research*. 2016; 76:3702–10. [PubMed: 27197192]
40. Wang K, Yuen ST, Xu J, et al. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nature genetics*. 2014; 46:573–82. [PubMed: 24816253]
41. Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014; 513:202–9. This study defines four subtypes of gastric cancer through molecular characterization. [PubMed: 25079317]
42. Kakiuchi M, Nishizawa T, Ueda H, et al. Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. *Nature genetics*. 2014; 46:583–7. [PubMed: 24816255]
43. Chung YJ, Choi JR, Park SW, et al. Evidence for two modes of allelic loss: multifocal analysis on both early and advanced gastric carcinomas. *Virchows Archiv : an international journal of pathology*. 2001; 438:31–8. [PubMed: 11213833]
44. Janjigian YY, Sanchez-Vega F, Tuvy Y, et al. Emergence of RTK/RAS/PI3K pathway alterations in trastuzumab-refractory HER2-positive esophagogastric (EG) tumors. *ASCO Meeting Abstracts*. 2016; 34:11608.
45. Van Cutsem E, Bang YJ, Feng-Yi F, et al. HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. *Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association*. 2015; 18:476–84.
46. Hofmann M, Stoss O, Shi D, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology*. 2008; 52:797–805. [PubMed: 18422971]
47. Wolff AC1HM, Hicks DG, Dowsett M, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Journal of Clinical Oncology*. 2013; 31(31):3997–4013. [PubMed: 24101045]
48. Cappellesso R1FM, Hanspeter E2, Bornschein J, et al. HER2 status in gastroesophageal cancer: a tissue microarray study of 1040 cases. *Human Pathology*. 2015; 46(5):665–72. [PubMed: 25800719]
49. Qiu Z, Sun W, Zhou C, et al. HER2 expression variability between primary gastric cancers and corresponding lymph node metastases. *Hepato-gastroenterology*. 2015; 62:231–3. [PubMed: 25911901]
50. Ye P1ZM, Fan S1, Zhang T. Intra-Tumoral Heterogeneity of HER2, FGFR2, cMET and ATM in Gastric Cancer: Optimizing Personalized Healthcare through Innovative Pathological and Statistical Analysis. *PLoS One*. 2015; 10(11):e0143207. [PubMed: 26587992]
51. Bartley AN, Washington MK, Colasacco C, et al. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and the American Society of Clinical Oncology. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2017; 35:446–64. [PubMed: 28129524]
52. Lih CJ, Si H, Das B, et al. Certified DNA Reference Materials to Compare HER2 Gene Amplification Measurements Using Next-Generation Sequencing Methods. *The Journal of molecular diagnostics : JMD*. 2016; 18:753–61. [PubMed: 27455875]
53. Pegram MD, Lipton A, Hayes DF, et al. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients

with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1998; 16:2659–71. [PubMed: 9704716]

54. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *The New England journal of medicine*. 2005; 353:1659–72. [PubMed: 16236737]
55. Slamon D, Eiermann W, Robert N, et al. Adjuvant trastuzumab in HER2-positive breast cancer. *The New England journal of medicine*. 2011; 365:1273–83. [PubMed: 21991949]
56. Kataoka K1TM, Mizusawa J1, Machida N, et al. A randomized Phase II trial of systemic chemotherapy with and without trastuzumab followed by surgery in HER2-positive advanced gastric or esophagogastric junction adenocarcinoma with extensive lymph node metastasis: Japan Clinical Oncology Group study JCOG1301 (Trigger Study). *Japanese Journal of Clinical Oncology*. 2015; (11):1082–6. [PubMed: 26355164]
57. Li Q, Jiang H, Li H, et al. Efficacy of trastuzumab beyond progression in HER2 positive advanced gastric cancer: a multicenter prospective observational cohort study. *Oncotarget*. 2016
58. Denlinger, CSMADW., Sym, S., Bendell, J., Park, S., Arkenau7, H., Bekai-Saab, T., Kudla, A., McDonagh, CF., Czibere, A., Chibaudel, B., Press, MF., Yoon, H., Hecht, JR., Cunningham, D., Bang, Y. Abstract #4043 Randomized Phase 2 Study of Paclitaxel (PTX), Trastuzumab (T) with or without MM-111 in HER2 Expressing Gastroesophageal Cancers (GEC). ASCO; 2016.
59. Gianni L, Llado A, Bianchi G, et al. Open-label, phase II, multicenter, randomized study of the efficacy and safety of two dose levels of Pertuzumab, a human epidermal growth factor receptor 2 dimerization inhibitor, in patients with human epidermal growth factor receptor 2-negative metastatic breast cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010; 28:1131–7. [PubMed: 20124183]
60. Baselga J, Swain SM. CLEOPATRA: a phase III evaluation of pertuzumab and trastuzumab for HER2-positive metastatic breast cancer. *Clinical breast cancer*. 2010; 10:489–91. [PubMed: 21147694]
61. Gianni L, Pienkowski T, Im YH, et al. 5-year analysis of neoadjuvant pertuzumab and trastuzumab in patients with locally advanced, inflammatory, or early-stage HER2-positive breast cancer (NeoSphere): a multicentre, open-label, phase 2 randomised trial. *The lancet oncology*. 2016; 17:791–800. [PubMed: 27179402]
62. Schneeweiss A, Chia S, Hickish T, et al. Pertuzumab plus trastuzumab in combination with standard neoadjuvant anthracycline-containing and anthracycline-free chemotherapy regimens in patients with HER2-positive early breast cancer: a randomized phase II cardiac safety study (TRYPHAENA). *Annals of oncology : official journal of the European Society for Medical Oncology/ESMO*. 2013; 24:2278–84.
63. Kontermann RE, Brinkmann U. Bispecific antibodies. *Drug discovery today*. 2015; 20:838–47. [PubMed: 25728220]
64. McCall AM, Adams GP, Amoroso AR, et al. Isolation and characterization of an anti-CD16 single-chain Fv fragment and construction of an anti-HER2/neu/anti-CD16 bispecific scFv that triggers CD16-dependent tumor cytotoxicity. *Molecular immunology*. 1999; 36:433–45. [PubMed: 10449096]
65. Borghaei H, Alpaugh RK, Bernardo P, et al. Induction of adaptive Anti-HER2/neu immune responses in a Phase 1B/2 trial of 2B1 bispecific murine monoclonal antibody in metastatic breast cancer (E3194): a trial coordinated by the Eastern Cooperative Oncology Group. *Journal of immunotherapy*. 2007; 30:455–67. [PubMed: 17457220]
66. McDonagh CF, Huhlov A, Harms BD, et al. Antitumor activity of a novel bispecific antibody that targets the ErbB2/ErbB3 oncogenic unit and inhibits heregulin-induced activation of ErbB3. *Molecular cancer therapeutics*. 2012; 11:582–93. [PubMed: 22248472]
67. Isakoff SBJ. Trastuzumab-DM1: building a chemotherapy-free road in the treatment of human epidermal growth factor receptor 2-positive breast cancer. *Journal of Clinical Oncology*. 2011; 29(4):351–4. [PubMed: 21172881]
68. Verma S, Miles D, Gianni L, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *The New England journal of medicine*. 2012; 367:1783–91. [PubMed: 23020162]

69. Kang Y-K, Shah MA, Ohtsu A, et al. A randomized, open-label, multicenter, adaptive phase 2/3 study of trastuzumab emtansine (T-DM1) versus a taxane (TAX) in patients (pts) with previously treated HER2-positive locally advanced or metastatic gastric/gastroesophageal junction adenocarcinoma (LA/MGC/GEJC). ASCO Meeting Abstracts. 2016; 34:5. Evaluation of TDM-1 in second-line therapy for gastric cancer, demonstrating no benefit over taxanes.
70. Espelin CW, Leonard SC, Geretti E, et al. Dual HER2 Targeting with Trastuzumab and Liposomal-Encapsulated Doxorubicin (MM-302) Demonstrates Synergistic Antitumor Activity in Breast and Gastric Cancer. *Cancer research*. 2016; 76:1517–27. [PubMed: 26759238]
71. Iqbal S, Goldman B, Fenoglio-Preiser CM, et al. Southwest Oncology Group study S0413: a phase II trial of lapatinib (GW572016) as first-line therapy in patients with advanced or metastatic gastric cancer. *Annals of oncology : official journal of the European Society for Medical Oncology/ ESMO*. 2011; 22:2610–5.
72. Lorenzen S1RKJ, Haag GM3, Pohl M, et al. Lapatinib versus lapatinib plus capecitabine as second-line treatment in human epidermal growth factor receptor 2-amplified metastatic gastro-oesophageal cancer: a randomised phase II trial of the Arbeitsgemeinschaft Internistische Onkologie. *European Journal of Cancer*. 2015; 51(5):569–76. [PubMed: 25694417]
73. Hecht JR, Bang YJ, Qin SK, et al. Lapatinib in Combination With Capecitabine Plus Oxaliplatin in Human Epidermal Growth Factor Receptor 2-Positive Advanced or Metastatic Gastric, Esophageal, or Gastroesophageal Adenocarcinoma: TRIO-013/LOGiC--A Randomized Phase III Trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2016; 34:443–51. Evaluation of lapatinib in combination with fluoropyrimidine and platinum; demonstrating no advantage with the addition of lapatinib. [PubMed: 26628478]
74. Satoh T, Xu RH, Chung HC, et al. Lapatinib plus paclitaxel versus paclitaxel alone in the second-line treatment of HER2-amplified advanced gastric cancer in Asian populations: TyTAN--a randomized, phase III study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2014; 32:2039–49. [PubMed: 24868024]
75. Geyer CE, Forster J, Lindquist D, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *The New England journal of medicine*. 2006; 355:2733–43. [PubMed: 17192538]
76. LaBonte MJ1YD, Zhang W2, Wilson PM, et al. A Phase II Biomarker-Embedded Study of Lapatinib plus Capecitabine as First-line Therapy in Patients with Advanced or Metastatic Gastric Cancer. *Molecular Cancer Therapy*. 2016; 15(9):2251–8.
77. Yelena Yuriy Janjigian, GYK., Ilson, David H., Boyar, Michelle S., Capanu, Marinela, Chou, Joanne F., Kelsen, David Paul, Imtiaz, Tooba, Berger, Michael F., Vakiani, Efsevia. A phase II study of afatinib in patients (pts) with metastatic human epidermal growth factor receptor (HER2)-positive trastuzumab refractory esophagogastric (EG) cancer. ASCO; 2015; Chicago IL. 2015.
78. Ko BK1LS, Lee YH2, Hwang IS, et al. Combination of novel HER2-targeting antibody 1E11 with trastuzumab shows synergistic antitumor activity in HER2-positive gastric cancer. *Molecular Oncology*. 2015; 9(2):398–408. [PubMed: 25306393]
79. Gumusay O, Benekli M, Ekinci O, et al. Discordances in HER2 status between primary gastric cancer and corresponding metastatic sites. *Japanese journal of clinical oncology*. 2015; 45:416–21. [PubMed: 25732909]
80. Gomez-Martin C, Plaza JC, Pazo-Cid R, et al. Level of HER2 gene amplification predicts response and overall survival in HER2-positive advanced gastric cancer treated with trastuzumab. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2013; 31:4445–52. [PubMed: 24127447]
81. Ock CY, Lee KW, Kim JW, et al. Optimal Patient Selection for Trastuzumab Treatment in HER2-Positive Advanced Gastric Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2015; 21:2520–9. [PubMed: 25712681]
82. Gelmon KA, Boyle FM, Kaufman B, et al. Lapatinib or Trastuzumab Plus Taxane Therapy for Human Epidermal Growth Factor Receptor 2-Positive Advanced Breast Cancer: Final Results of NCIC CTG MA. 31. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2015; 33:1574–83. [PubMed: 25779558]
83. Piccart-Gebhart, MJ., Holmes, AP., Baselga, J., et al. First results from the phase III ALTTO trial (BIG 2–06; NCCTG [Alliance] N063D) comparing one year of anti-HER2 therapy with lapatinib alone (L), trastuzumab alone (T), their sequence (T>L), or their combination (T+L) in the

- adjuvant treatment of HER2-positive early breast cancer (EBC). ASCO Meeting Abstracts; 2014. p. LBA4
84. Pietrantonio F, Caporale M, Morano F, et al. HER2 loss in HER2-positive gastric or gastroesophageal cancer after trastuzumab therapy: Implication for further clinical research. *International journal of cancer Journal international du cancer*. 2016; 139:2859–64. [PubMed: 27578417]
 85. Janjigian YY, Riches JC, Ku GY, et al. Loss of human epidermal growth factor receptor 2 (HER2) expression in HER2-overexpressing esophagogastric (EG) tumors treated with trastuzumab. ASCO Meeting Abstracts. 2015; 33:63. Single institution series demonstrating loss of HER2 expression after treatment with trastuzumab in a subset of patients.
 86. Denlinger CS, Alsina Maqueda M, Watkins DJ, et al. Randomized phase 2 study of paclitaxel (PTX), trastuzumab (T) with or without MM-111 in HER2 expressing gastroesophageal cancers (GEC). ASCO Meeting Abstracts. 2016; 34:4043.
 87. Fumagalli D, Venet D, Ignatiadis M, et al. RNA Sequencing to Predict Response to Neoadjuvant Anti-HER2 Therapy: A Secondary Analysis of the NeoALTTO Randomized Clinical Trial. *JAMA oncology*. 2016
 88. Sergina NV, Rausch M, Wang D, et al. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature*. 2007; 445:437–41. [PubMed: 17206155]
 89. Sergina NVRM, Wang D, et al. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature*. 2007; 445:437–41. [PubMed: 17206155]
 90. Subramaniam DHA, Hwang J, Deeken J. Irreversible multitargeted ErbB family inhibitors for therapy of lung and breast cancer. *Current Cancer Drug Targets*. 2014; 14(9):775–93.
 91. Zhang X1PK, Bose R, Jura N, et al. Inhibition of the EGF receptor by binding of MIG6 to an activating kinase domain interface. *Nature*. 2007; 450(7170):741–4. [PubMed: 18046415]
 92. Shahabi V, Seavey MM, Maciag PC, et al. Development of a live and highly attenuated *Listeria monocytogenes*-based vaccine for the treatment of Her2/neu-overexpressing cancers in human. *Cancer gene therapy*. 2011; 18:53–62. [PubMed: 20725099]
 93. Shitara K, Yatabe Y, Matsuo K, et al. Prognosis of patients with advanced gastric cancer by HER2 status and trastuzumab treatment. *Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association*. 2013; 16:261–7.
 94. Qiu MZ, Li Q, Wang ZQ, et al. HER2-positive patients receiving trastuzumab treatment have a comparable prognosis with HER2-negative advanced gastric cancer patients: a prospective cohort observation. *International journal of cancer*. 2014; 134:2468–77. [PubMed: 24155030]
 95. Ajani JA, D'Amico TA, Almhanna K, et al. Gastric Cancer, Version 3. 2016, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network : JNCCN*. 2016; 14:1286–312. [PubMed: 27697982]
 96. Wilke H, Muro K, Van Cutsem E, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *The lancet oncology*. 2014; 15:1224–35. [PubMed: 25240821]

Figure 1.

Incidence of HER2 gene mutations among human cancers in The Cancer Genome Atlas data. **(A)** Percent of HER2 gene amplification. **(B)** HER2 point mutations. **(C)** Incidence of amplification of selected receptor tyrosine kinases in esophageal and gastric cancers. Concurrent amplifications are indicated by numbers next to lines connecting genes. Percent of amplified cases are shown below each gene. Source: *cbiportal.org*

Table 1

Ongoing phase I–III clinical trials in HER2 positive gastroesophageal cancer

Trial name	Phase	Agent	Stage	Line of therapy	Status
NCT02205047(INNOVATION)	II	Trastuzumab + pertuzumab + chemotherapy	Early stage, resectable gastric	Neoadjuvant	Recruiting
NCT01774786 (JACOB)	III	pertuzumab or placebo + Trastuzumab + 5FU + cisplatin	Locally advanced, metastatic gastric	First-line	Completed, awaiting results
NCT01641939	II/III	TDM1 vs docetaxel	Locally advanced, metastatic gastric	Second- or greater line	Completed, awaiting results
NCT01702558	I/II	I: TDM1 + capecitabine; II: TDM1 + capecitabine vs TDM1 alone	Locally advanced, metastatic gastric	Second- or greater line	Active, not recruiting
NCT02015169	II	Lapatinib + XELOX	Resectable with liver-only metastasis gastric	Neoadjuvant	Active, not recruiting
NCT02726399	II	Trastuzumab + ramucirumab + capecitabine or cisplatin	Metastatic gastric	First-line	Recruiting
JCOG1301 (TRIGGER)	II	Cisplatin+S1 vs Cisplatin+S1+Trastuzumab	Locally advanced, resectable	Neoadjuvant	Recruiting
NCT02689284	Ib/II	Magetuximab + pembrolizumab	Metastatic gastric	Second- or greater line	Recruiting
RTOG 1010	III	Radiation, carboplatin, and paclitaxel with or without trastuzumab, followed by surgery	Early stage resectable esophageal	Neoadjuvant	Completed, awaiting results