Recent advances in novel targeted therapies for HER2-positive breast cancer

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The monoclonal antibody trastuzumab has improved the outcomes of patients with breast cancer that overexpresses the human epidermal growth factor receptor 2 (HER2). However, despite this advancement, many tumors develop resistance and novel approaches are needed. Recently, a greater understanding of cellular biology has translated into the development of novel anti-HER2 agents with varying mechanisms of action. The small molecule tyrosine kinase inhibitor lapatinib has demonstrated activity in HER2-positive metastatic breast cancer (MBC) and in the preoperative setting. Pertuzumab is a monoclonal antibody with a distinct binding site from trastuzumab, which inhibits receptor dimerization. In recent studies, the addition of pertuzumab to combination therapy has led to improvements in progression-free survival in patients with HER2-positive MBC and higher response rates in the preoperative setting. An alternative approach is the use of novel antibody–drug conjugates such as trastuzumab–emtansine, which recently demonstrated activity in MBC. Neratinib, a pan-HER tyrosine kinase inhibitor, which irreversibly inhibits HER1 and HER2, also has proven activity in MBC. A range of compounds is being developed to attempt to overcome trastuzumab resistance by targeting heat shock protein 90, a molecular chaperone required for the stabilization of cellular proteins. Furthermore, agents are being developed to inhibit the mammalian target of rapamycin, a downstream component of the PTEN/PI3K pathway, which has been implicated in trastuzumab resistance. Finally, there are emerging data indicating that combinations of anti-HER2 agents may circumvent resistance mechanisms and improve patient outcomes. In this review, recent data on these emerging agents and novel combinations for HER2-positive breast cancer are discussed. *Anti-Cancer Drugs* **23:765–776** © **2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

The human epidermal growth factor receptor 2 (HER2) belongs to the ErbB family of receptor tyrosine kinases, which normally regulate a series of cellular process including growth [1]. The extracellular domain of HER2 can adopt a fixed conformation so that it can dimerize without ligand binding, in contrast to the other family members (HER1, HER3, and HER4) [2]. Overall, in ~25% of breast cancers, there is amplification of the HER2 gene and/or overexpression of the associated protein product. In the absence of treatment, these so-called HER2-positive tumors are associated with an inferior prognosis [3–6]. Importantly, advances in translational science have led to the development of a growing array of therapies that target HER2. To date, two agents have been approved by the US Food and Drug Administration and the European Medicines Agency: the monoclonal antibody trastuzumab and the small molecule tyrosine kinase inhibitor (TKI) lapatinib. However, despite these advances, many tumors ultimately develop resistance to these agents, leading to shortened survival for patients. This review will discuss novel anti-HER2 agents, some of which are at advanced stages of clinical development and which may become components of everyday practice in the near future.

Trastuzumab

Trastuzumab is a fully humanized version of a murine HER2-targeted monoclonal antibody. Although the exact mechanisms of action have not been delineated, these are thought to include antibody-dependent cellular cytotoxicity, prevention of proliferative signaling, inhibition of cell cycle progression, prevention of HER2 cleavage and extracellular domain shedding, and antiangiogenic effects (Fig. 1) [7,8]. In early studies, trastuzumab demonstrated relatively modest single agent activity, including an overall response rate (ORR) of up to 26% in the first-line metastatic setting [9–11]. However, in the pivotal phase III randomized study of 469 women with HER2-positive metastatic breast cancer (MBC), the combination of trastuzumab and chemotherapy was associated with a higher ORR (up to 50%) [12]. Critically, the addition of trastuzumab to standard first-line anthracycline or paclitaxel chemotherapy resulted in a significant prolongation of time-to-progression (TTP, 4.6 vs. 7.4 months, $P < 0.001$) and overall survival (OS, 20.1 vs. 25.1 months, $P = 0.046$). In this study, concurrent anthracycline and trastuzumab was associated with unacceptably high rates of cardiac toxicity and this approach is generally avoided in clinical practice. Subsequently, the activity of trastuzumab (ORR 20–68%) has

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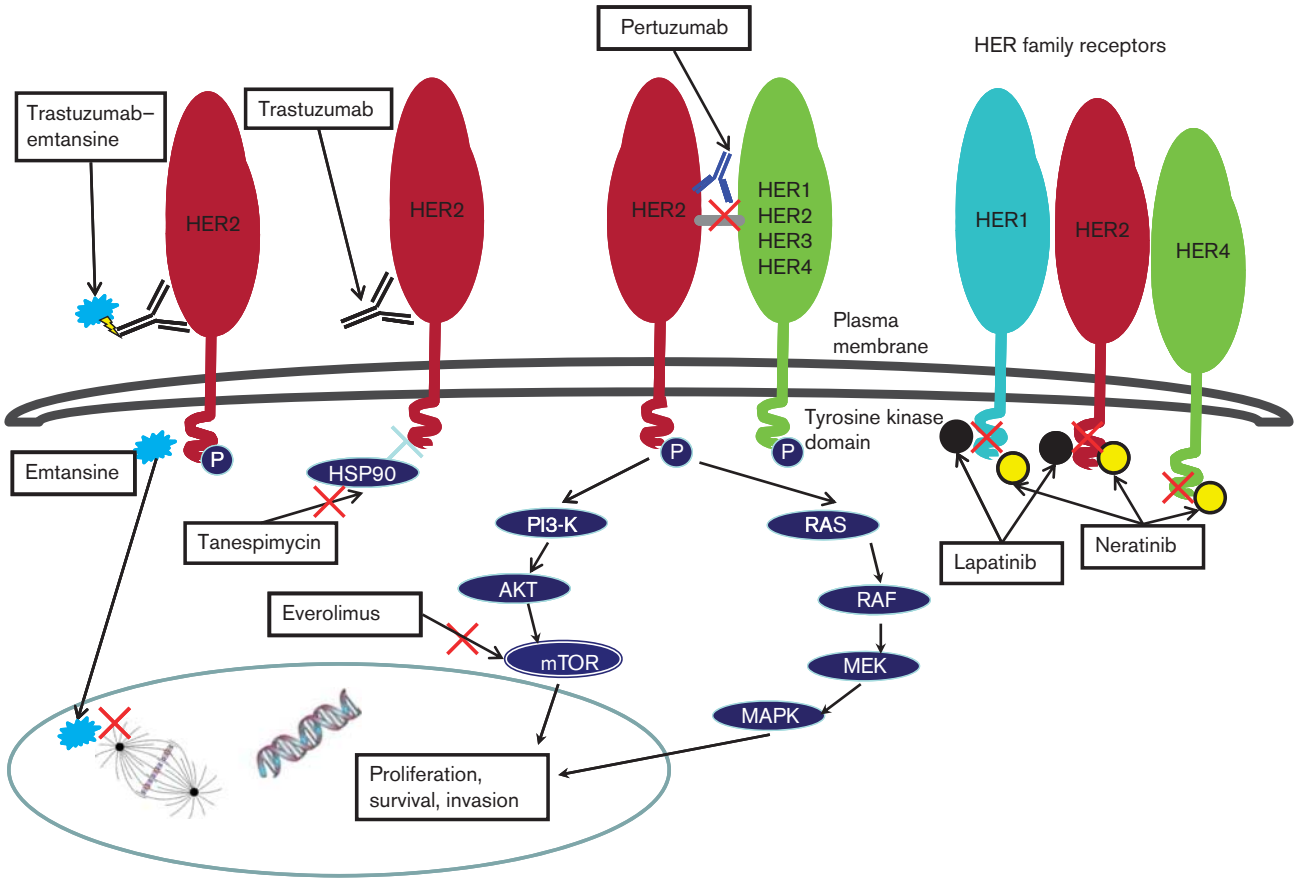
Fig. 1



Mechanism of action of selected anti-HER2 therapies. Trastuzumab is a fully humanized monoclonal antibody, which binds to the juxtamembrane domain of HER2, and through several mechanisms prevents activation along a signaling pathway involving the lipid kinase phosphoinositide 3-kinase (PI3K), Ak transforming factor (Akt), and mammalian target of rapamycin (mTOR), and ultimately prevents cell survival. In parallel, trastuzumab inhibits signaling through the rat sarcoma (RAS) enzyme, the receptor activation factor (RAF), mitogen extracellular signal kinase (MEK), and, finally, the mitogen-activated protein kinase (MAPK), which is important for driving cellular proliferation. Pertuzumab is a humanized monoclonal antibody that binds to a separate binding site (the extracellular domain II) of the HER2 receptor. Antibody binding prevents receptor dimerization between HER2 receptors and between HER2 and other family members such as HER1, HER3, and HER4. Trastuzumab–emtansine consists of trastuzumab bound by a stable thioether linkage to a derivative of maytansine, a microtubule-binding chemotherapeutic agent. In response to antibody binding the emtansine molecule is selectively released into a tumor cell, where it acts on the microtubules in the nucleus. Tanespimycin is an inhibitor of heat shock protein 90 (HSP90), which is responsible for conformational stabilization of HER2. Inhibition of HSP90 leads to proteasomal degradation of the HER2 protein. Lapatinib is an oral reversible small molecule tyrosine kinase inhibitor of both HER1 and HER2. Neratinib is an oral irreversible small molecule tyrosine kinase inhibitor of HER1, HER2, and HER4. Inhibition of these receptor tyrosine kinases has a similar inhibitory effect on cellular proliferation and growth to monoclonal antibody binding to the extracellular receptor. HER, human epidermal growth factor receptor.

been confirmed in multiple phase II studies combined with standard chemotherapeutic agents including docetaxel, capecitabine, gemcitabine, vinorelbine, and platinum agents [13–22]. Data from institutional series comparing the outcomes of women treated in the pre-trastuzumab and post-trastuzumab eras indicate that this agent has truly changed the natural history of HER2-positive breast cancer [23].

Trastuzumab in early breast cancer

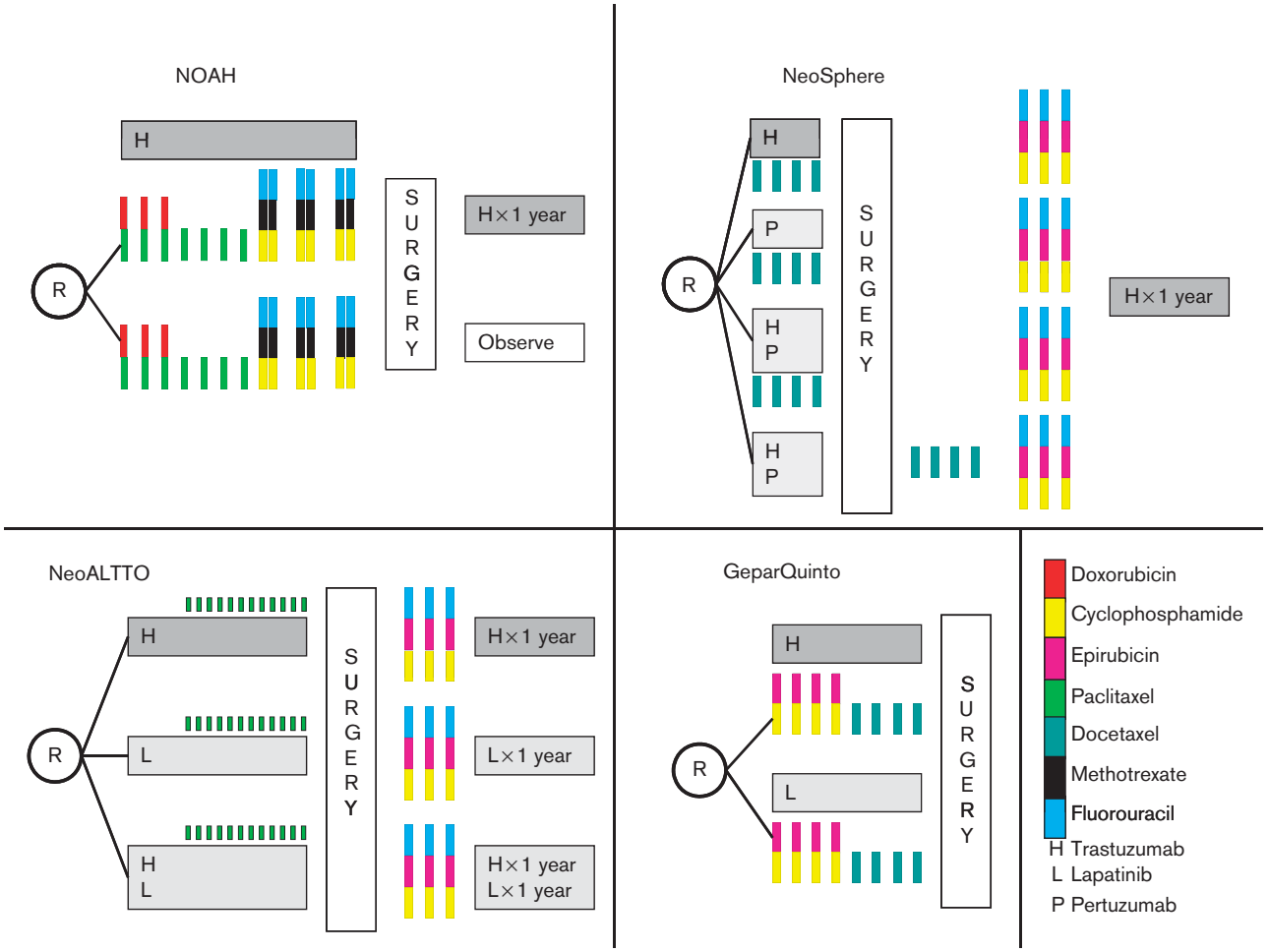
Following the proven role in MBC, several large phase III studies examined trastuzumab with chemotherapy for early breast cancer [24–27]. These studies showed consistent benefits; the addition of 1 year of trastuzumab to postoperative chemotherapy decreased the risk of recurrence by approximately half and the risk of death from breast cancer by a third [24,25,27]. Longer follow-up of these studies (up to 4 years) has shown sustained benefits [28,29]. One much smaller study showed a reduction in the risk of recurrence of similar magnitude with only 9 weeks of trastuzumab [26], whereas the results are still awaited from one of the larger studies of a comparison of 1 and 2 years of trastuzumab after chemotherapy. Therefore, the optimal duration of adjuvant trastuzumab therapy is not known but 1 year of therapy remains the standard in clinical practice, as given to the vast majority of patients in the clinical trials described. Maturing data from a study that assessed adjuvant trastuzumab in both

concurrent and sequential combinations with chemotherapy indicate that concurrent therapy (starting after the completion of an anthracycline component to minimize the risk of cardiotoxicity) is preferable [30].

Similarly, for patients who require preoperative therapy, the addition of trastuzumab to chemotherapy has been associated with approximately a doubling of response rates [31–33]. In the NeOAdjuvant Herceptin study, 228 patients with HER2-positive locally advanced breast cancer were randomized to receive sequential anthracycline–taxane-based chemotherapy alone or with trastuzumab (Fig. 2a) [33]. The addition of trastuzumab to anthracycline-taxane-based chemotherapy led to an increase in breast pathological complete response rate (pCR) from 22 to 43% ($P = 0.0007$) as well as similar benefits on including axillary tissue in the definition of pCR ($P = 0.001$) (Fig. 3a) [33]. A similar magnitude of effect was observed in the phase III GeparQuattro study, in which the combination of trastuzumab and sequential anthracycline–taxane chemotherapy (with or without capecitabine) resulted in an excellent pCR rate of 32%, whereas patients with HER2-negative disease treated with similar chemotherapy (without trastuzumab) had a pCR rate of 16% [31].

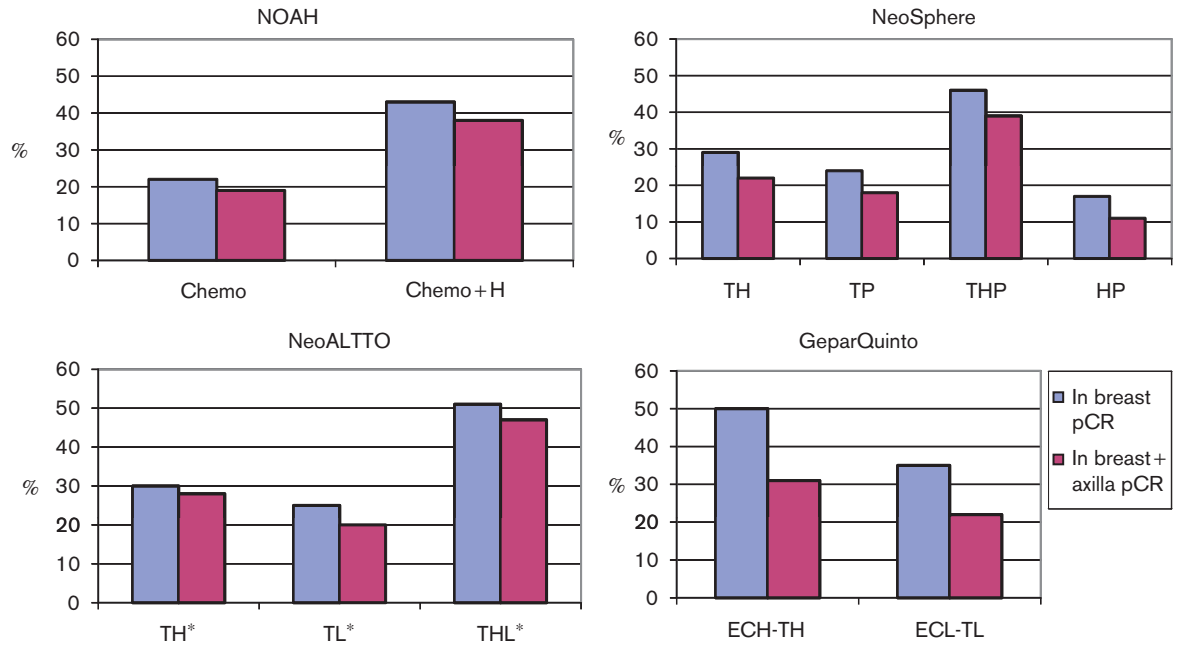
In general, trastuzumab is well tolerated, but is associated with a small but significant increase in the risk of symptomatic cardiac failure (up to ~4% at 4 years) particularly after anthracycline-based chemotherapy [28]. Asymptomatic declines in left ventricular ejection fraction (LVEF, below the lower limit of normal or by greater than 15%) are more common, requiring therapy to be interrupted or discontinued in 8–10% of patients [34]. Although the mechanism underlying this effect is poorly understood, it is thought that the expression of HER2 on cardiac myocytes is important in embryogenesis and HER2 signaling may be important for myocyte repair, particularly after prior

Fig. 2



Design of selected preoperative studies for HER2-positive MBC. NeoALTTO, Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization study; NeoSphere, Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation; NOAH, NeOAdjuvant Herceptin study; R, randomization.

Fig. 3



Pathological complete response rates in selected preoperative studies for HER2-positive MBC. C, cyclophosphamide; Chemo, chemotherapy; E, epirubicin; H, trastuzumab; L, lapatinib; NeoALTTO, Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization study; NeoSphere, Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation; NOAH, NeOAdjuvant Herceptin study; P, pertuzumab; pCR, pathological complete response; T*, paclitaxel; T, docetaxel.

anthracycline exposure [35–38]. Notably, cardiotoxicity from trastuzumab is generally less severe and more readily reversible than that seen with anthracyclines [39].

Mechanisms of resistance to trastuzumab

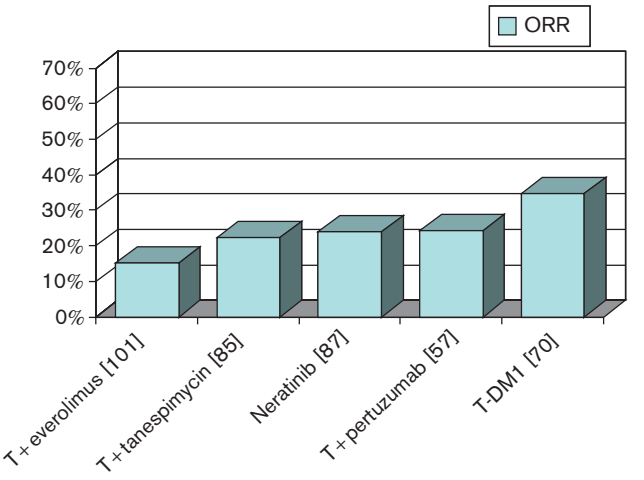
Despite significant progress, many patients treated with trastuzumab will experience disease progression. The mechanisms underlying trastuzumab resistance are incompletely understood but are thought to include physical changes preventing antibody–receptor interaction, increased expression of the membrane glycoprotein MUC4, shedding of the extracellular domain of the receptor, heterodimerization between HER2 and other HER receptors, and bypassing of HER2 signaling along the proliferative PI3K/AKT pathway by activating mutations of AKT or PI3K and/or decreased expression of PTEN [40–44]. Other potential mechanisms of resistance explored in preclinical models include transcriptional upregulation of HER2 gene expression and cyclin E amplification/overexpression [45,46]. Some of these insights have been exploited to permit the development of novel agents tailored to overcome resistance and further improve the outcomes of women with HER2-positive breast cancer.

Lapatinib

Lapatinib is an oral reversible small molecule TKI of both HER2 and the epidermal growth factor receptor (EGFR or HER1) (Fig. 1). Preclinical studies have shown that lapatinib was active in trastuzumab-resistant HER2-positive human breast cancer cells and mice xenografts, and could enhance the activity of anti-HER2 antibodies when used in combination [40,47]. Early clinical trials identified diarrhea as a dose-limiting toxicity and suggested an ORR of only 5% in pretreated patients and up to 24% in patients who had never received trastuzumab [48,49]. Two important randomized phase III studies examined the efficacy of adding lapatinib to chemotherapy. In the first of these, patients with advanced HER2-positive breast cancer who had received previous anthracycline, taxane, and trastuzumab were randomized to capecitabine (2500 mg/m^2 daily for 14 days of a 21-day cycle) or a combination of capecitabine (2000 mg/m^2 at the same schedule) and lapatinib (1250 mg daily) [50,51]. After an interim analysis demonstrated that the study had achieved its primary endpoint of TTP, lapatinib was offered to women on the control arm [50]. In an updated analysis of all 399 women enrolled, combination therapy resulted in prolongation of the median TTP from 4.3 to 6.2 months [hazard ratio (HR) 0.57, 95% confidence interval (CI), 0.43–0.77, $P < 0.001$] and improved ORR (24 vs. 14%, $P = 0.017$) [51]. However, no improvement in OS was observed and neither serum HER2 nor tumor HER1 expressions were useful as predictive biomarkers [51]. Notably, diarrhea was common (60%) in patients receiving combination therapy and was grade III in 12%. Other common toxicities included hand foot syndrome (49%), rash (27%), nausea (44%), vomiting (26%), and fatigue (18%).

In a second phase III study, patients were randomized to intravenous paclitaxel (once every 3 weeks) with lapatinib 1500 mg daily or placebo [52]. In contrast to the capecitabine study, patients with HER2-normal breast cancer were permitted to enroll, although these patients did not appear to derive any benefit. However, in the 86 patients with HER2-positive breast cancer, the addition of lapatinib was associated with a longer median TTP of 36.4 versus 25.1 weeks (HR 0.53, 95% CI 0.31–0.89, $P = 0.005$). As expected, the addition of lapatinib to paclitaxel was associated with significantly higher rates of rash, diarrhea, mucositis, and vomiting. Furthermore, there was an increased rate of fatal adverse events in the paclitaxel/lapatinib group (2.7%) compared with the paclitaxel/placebo group (0.6%), including sepsis and diarrhea. A more recent Chinese phase III study randomized a larger population of more than 400 patients with previously untreated HER2-positive breast cancer to paclitaxel with or without lapatinib and demonstrated significant improvements in ORR, progression-free survival (PFS), and OS [53].

Fig. 4



Response rates of selected agents in trastuzumab-pretreated patients in chemotherapy-free phase II studies. H, trastuzumab; ORR, overall response rate.

Pertuzumab

In addition to the approved therapies, there are a variety of other anti-HER2 agents at varying stages of clinical development. Pertuzumab is a humanized monoclonal antibody that binds to the extracellular domain II of the HER2 receptor (Fig. 1). Antibody binding prevents receptor dimerization and ligand-activated signaling with other growth factor receptors, including other HER family members such as HER1 and HER3 [54]. In preclinical studies, a combination of pertuzumab and trastuzumab had synergistic antitumor activity in a HER2-positive breast cancer xenograft model, even after progression on trastuzumab [55]. Given these findings and the distinct binding epitope, the role of pertuzumab was first investigated in patients whose disease was resistant to trastuzumab.

Unfortunately, pertuzumab monotherapy had limited activity in heavily pretreated patients with HER2-positive MBC and in patients with HER2-negative MBC [56,57]. However, a role for combination therapy was suggested from isolated cases of patients who progressed on trastuzumab, failed to respond to pertuzumab monotherapy, but then responded to the combination of trastuzumab and pertuzumab [56]. In fact, in a phase II study of 66 patients with HER2-positive MBC who had progressed on prior trastuzumab, combination antibody treatment with pertuzumab and trastuzumab (without chemotherapy) led to an ORR of 24.2% (Fig. 4) [57]. Furthermore, an additional 17 (25.8%) patients had stable disease for at least 6 months and the median PFS was 5.5 months. Combination anti-HER2 therapy was generally well tolerated and the most common adverse effect was diarrhea, which occurred in 42 (64%) patients. Furthermore, this approach did not appear to be associated with an increased risk of cardiotoxicity – no patients developed cardiac symptoms, and declines in LVEF were minimal. Although delineating the effect of individual agents is challenging in a phase II study, it is likely that HER1 inhibition from pertuzumab contributed to diarrhea, given that this is a frequent side-effect of other agents that inhibit this pathway such as lapatinib [50].

The encouraging results for combination therapy led to the launch of the CLinical Evaluation Of Pertuzumab And TRAstuzumab (CLEOPATRA) trial, which was a double-blind multicenter phase III study [58,59]. This was a study of ~800 patients from around 19 countries with untreated HER2-positive advanced breast cancer, in which patients were randomized 1:1 to docetaxel, trastuzumab, and pertuzumab versus docetaxel, trastuzumab, and placebo. Inclusion criteria included the availability of tissue for a central review of HER2 and an LVEF of at least 50%. Importantly, prior trastuzumab in the adjuvant or the neoadjuvant setting was permitted, once patients had a disease-free interval of at least 12 months from the completion of adjuvant systemic treatment. This study was powered to detect a 33% improvement in PFS (the primary endpoint), on the basis of an independent review (HR = 0.75 with a two-sided significance level of 5%) with 80% power. The secondary endpoints included OS and safety. The primary endpoint was easily achieved at the first interim analysis, presented in late 2011 [60]. The addition of pertuzumab to trastuzumab and docetaxel increased the median PFS by 6.1 months (12.4 vs. 18.5 months, HR = 0.62, 95% CI 0.51–0.75, $P < 0.001$). The combined antibody blockade also yielded a higher ORR and was associated with a trend toward improved OS, although these data are not yet mature. Of note, the combined HER2 antibody approach did not result in higher rates of symptomatic or

asymptomatic cardiac dysfunction, although it was associated with increased rates of grade III or higher febrile neutropenia (13.8 vs. 7.6%) and diarrhea (7.9 vs. 5.0%). Importantly, this study includes important quality-of-life assessments and biomarkers to attempt to identify patients most likely to benefit from this combination.

Pertuzumab has also been combined with chemotherapy in the preoperative setting for early breast cancer. In the Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation (NeoSphere) study, patients were randomized to one of four regimens consisting of various combinations of trastuzumab, pertuzumab, and chemotherapy (Fig. 2b) [61]. In three of these regimens, patients received docetaxel chemotherapy with trastuzumab (TH), pertuzumab (TP), or the combination (THP). Most importantly, the combination of two anti-HER2 therapies with chemotherapy was associated with an increased in breast pCR rate of 46% compared with trastuzumab–docetaxel (29%, $P = 0.01$) and pertuzumab–docetaxel (24%, $P = 0.003$) (Fig. 3b). Of particular interest was a chemotherapy-free arm of trastuzumab and pertuzumab (HP), which was associated with a pCR rate of 17%. Although this is clearly inferior to trastuzumab–docetaxel (Fig. 3b, $P = 0.02$), it does suggest that a subset of patients might be able to achieve satisfactory outcomes without the need for chemotherapy. Several other studies are ongoing examining pertuzumab in combination with trastuzumab and other cytotoxic agents including capecitabine and paclitaxel [62,63].

Antibody–drug conjugates

Another promising strategy for selectively targeting tumor cells involves coupling monoclonal antibodies with potent cytotoxic agents, in the form of antibody–drug conjugates (ADCs). This approach is being investigated in a variety of settings and recently led to Food and Drug Administration approval for Brentuximab vedotin, an ADC targeting refractory CD30-positive lymphoma [64,65]. For HER2-positive breast cancer, trastuzumab–emtansine (also called T-DM1) is an ADC, which utilizes a stable thioether linkage to couple trastuzumab to a derivative of maytansine, a chemotherapeutic agent with a microtubule-binding effect similar to vinca alkaloids (Fig. 1). In preclinical studies, trastuzumab–emtansine showed activity in trastuzumab-sensitive and trastuzumab-resistant models of HER2-positive breast cancer [66]. A phase I study evaluated 3-weekly dosing of trastuzumab–emtansine in patients with HER2-positive MBC, whose disease had progressed on trastuzumab [67]. Tumor responses were seen in five of nine patients at the maximally tolerated dose of 3.6 mg/kg. The dose-limiting toxicity was thrombocytopenia (grade IV in two patients), which was generally reversible. Subsequently, an alternative weekly dosing schedule was investigated and the maximally tolerated dose was 2.4 mg/kg, with an ORR of 53% (nine of 15 evaluable patients) [68].

Subsequently, single-agent trastuzumab–emtansine (3.6 mg/kg every 3 weeks) demonstrated an ORR of 25–35% (Fig. 4) in successive phase II trials in heavily pretreated patients, including some whose tumors had progressed after prior anthracycline, taxane, capecitabine, trastuzumab, and lapatinib [69,70]. In a recently reported randomized phase II study, 137 patients with advanced HER2-positive breast cancer received once every 3 week trastuzumab–emtansine (3.6 mg/kg) versus the combination of trastuzumab and docetaxel (75 or 100 mg/m^2) in the first-line setting [71]. Patients were stratified by geographical area, receipt of prior adjuvant trastuzumab, and disease-free interval. Patients were reasonably well balanced between the arms (Table 1). The study achieved its primary endpoint; the median PFS by investigator assessment increased from 9.2 (trastuzumab–docetaxel) to 14.2 months (trastuzumab–emtansine) (HR 0.59, 95% CI 0.36–0.97, $P = 0.035$). The ORR was reasonably similar in both arms of the study: 58% (95% CI 45.5–60.2) and 64.2% (95% CI 51.8–74.8%) for trastuzumab–docetaxel and trastuzumab–emtansine, respectively. However, responses appeared to be more sustained in patients receiving trastuzumab–emtansine; the median duration of response was 9.5 months (95% CI 6.6–10.6) for docetaxel–trastuzumab but has not yet been reached for trastuzumab–emtansine. Furthermore, only 46.4% patients on trastuzumab–emtansine developed grade III or higher adverse events, compared with 89.4% of patients on trastuzumab–docetaxel. As predicted from earlier studies, trastuzumab–emtansine was associated with a relatively high rate of thrombocytopenia (30.4%) but this was grade III or higher in only 8.7%. Fatigue and nausea were relatively common in both arms of the study (Table 1) but higher rates of neutropenia, alopecia, diarrhea, and peripheral edema were found with trastuzumab–docetaxel. Although trastuzumab–emtansine was generally well tolerated, elevations in aspartate transaminase (39.1%) and alanine transaminase (23.2%) were common but generally low grade. Furthermore, data from this study suggest that trastuzumab–emtansine does not appear to be associated with an increased risk of cardiac failure or asymptomatic declines in LVEF, although further data are needed from larger trials.

Overall, the results of this study suggest that improvements in PFS associated with trastuzumab–emtansine may be related to improved tolerability of this novel agent, as patients are able to continue therapy for longer. Although these results are excellent, caution is advised in the interpretation of relatively small randomized phase II studies. Notably, only 17.9 and 27.1% of patients treated with trastuzumab–emtansine and trastuzumab–docetaxel, respectively, had received prior adjuvant/neoadjuvant trastuzumab. Ongoing randomized phase III studies will hopefully more precisely define the risk–benefit ratio for trastuzumab–emtansine.

Table 1 Summary of the main findings from a randomized phase II study comparing trastuzumab–emtansine and trastuzumab–docetaxel [71]

	Trastuzumab–emtansine $N=67$ (%)	Trastuzumab–docetaxel $N=70$ (%)
Baseline characteristics		
ECOG performance status = 0	65.7	63.8
Estrogen and progesterone receptor negative	47.8	41.1
Liver or lung involvement	71.6	67.1
Disease-free interval ≤ 24 months	59.7	64.3
Prior adjuvant/neoadjuvant trastuzumab	17.9	27.1
Efficacy		
Median PFS	14.2	9.2
ORR	64.2	58
Selected adverse events (≥ grade III)		
Neutropenia	5.8	60.6
Febrile neutropenia	0	13.6
Thrombocytopenia	8.7	3.0
Elevated AST	8.7	0
Elevated ALT	8.7	0
Back pain	1.4	4.5
Fatigue	4.3	4.5
Nausea	2.9	0
Diarrhea	0	3
Peripheral edema	0	4.5
Pneumonia	5.8	0
Alopecia (all grades)	4.3	66.7

ALT, alanine transaminase; AST, aspartate transaminase; ECOG, Eastern Cooperative Oncology Group; ORR, overall response rate; PFS, progression-free survival.

Other HER2-targeted therapies

HSP90 inhibitors

Maturation and conformational stabilization of a variety of cellular proteins, including HER2, is dependent on heat shock protein 90 (HSP90), a molecular chaperone [72]. Therefore, inhibition of HSP90 is a potential therapeutic strategy for HER2-positive MBC and other cancers as this leads to instability of the 'client' proteins, leading ultimately to degradation in the proteasome (Fig. 1) [73–75]. The prototypical HSP90 inhibitor, geldanamycin, was hepatotoxic, but several derivatives have subsequently been developed [76]. One such agent is tanespimycin (17-allylamino-17-demethoxygeldanamycin), which demonstrated limited single agent activity in unselected patients with advanced solid tumors [77–83]. However, in a phase I study, the combination of tanespimycin and trastuzumab led to tumor regressions in five patients with HER2-positive MBC [84].

This led to further studies in this selected population. In one such study, 31 patients with HER2-positive MBC progressing on at least one line of trastuzumab-based therapy received this combination and the ORR was 22% (Fig. 4); six patients, all partial responses [85]. Furthermore, an additional 10 (37%) patients had stable disease as their best response and the median PFS was 6 months (95% CI 4–9 months). Although five patents withdrew from the study because of toxicity, most adverse events were low grade and included diarrhea, fatigue, nausea, and headache. This study provided proof of principle of the activity of HSP90 inhibitors in combination with anti-HER2 therapy for HER2-positive MBC. Unfortunately, further development of this particular agent has been suspended [86]. However, a range of other HSP90 inhibitors are in clinical development, which may be able to exploit this novel mechanism of action. In the future, combinations of these agents with other therapies may offer further therapeutic options to patients with a variety of solid tumors, including HER2-positive MBC.

Other small molecule inhibitors of HER2 signaling

Neratinib is a novel pan-HER TKI, which, in contrast to lapatinib, irreversibly inhibits HER1 and HER2 (Fig. 1). In a phase II study, neratinib monotherapy resulted in an ORR of 24% (Fig. 4) for patients who had received prior trastuzumab, with a median PFS of 22 weeks [87]. Similar to the experience with other anti-HER2 agents, a higher ORR of 56% and a median PFS of 40 weeks were found in patients who had never received trastuzumab. Consistent with other agents that inhibit HER1, diarrhea that occurred in 93% of the patients was the most common adverse event and was grades III–IV in 21%. Neratinib in combination with paclitaxel was evaluated in a phase Ib/II study of 38 patients treated with prior anti-HER2 therapy, of whom 26 (68%, 95% CI 51.3–82.5) patients had responses [88]. Again, diarrhea was a significant adverse effect and occurred in 91% of patients (≥ grade III in 28%). Given the myriad of other agents for HER2-positive breast cancer and significant diarrhea associated with neratinib, it is unclear how the development of this agent may proceed but ongoing studies, including a randomized trial with paclitaxel, may provide further insights.

The role of dual HER2 blockade

Recently, it has become increasingly clear that targeting of the HER2 pathway at multiple points may result in improved outcomes. In a randomized study, patients who had experienced progression on prior trastuzumab were treated with either single-agent lapatinib or combination therapy with lapatinib and trastuzumab [89]. Combination therapy resulted in a significant prolongation of PFS (8.1 vs. 12.0 weeks, HR = 0.73, 95% CI 0.57–0.93, $P = 0.008$). More recent data supporting this 'multiple hit' approach have emerged largely from neoadjuvant studies, which in drug development have the advantage of achieving an endpoint (pCR) that can be measured within a short time. As previously noted, in the NeoSphere study, the addition of pertuzumab to docetaxel–trastuzumab-based preoperative chemotherapy increased the rate of pCR from 29 to 46% [61]. In the Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization (NeoALTTO) study, patients were randomized to one of three arms evaluating various anti-HER2 strategies in combination with weekly paclitaxel in the preoperative setting: trastuzumab, lapatinib, or the combination (Fig. 2c) [90]. Consistent with data from other studies, dual anti-HER2 therapy appeared to be the most active;

patients who received trastuzumab and lapatinib with chemotherapy had the highest in breast pCR rates (51%) compared with those who received either trastuzumab (30%, $P = 0.00001$) or lapatinib (Fig. 3c). In the HER2-positive substudy of GeparQuinto, trastuzumab was compared with lapatinib in combination with chemotherapy in the preoperative setting (Fig. 2d) [91]. Consistent with other studies, the in breast pCR rate was inferior with lapatinib (35%) compared with trastuzumab (50%, $P < 0.05$; Fig. 3d).

There are several consistent findings from the collective results of these preoperative studies to date. First, when added to preoperative chemotherapy, combination anti-HER2 therapy appears to be associated with higher rates of pCR than the use of a single anti-HER2 agent [61,90]. This is supported by recent data from phase II studies in the preoperative setting presented at the American Society of Clinical Oncology Annual Meeting in 2011 [92,93]. Second, lapatinib-based regimens without trastuzumab appear to be less effective in the preoperative setting than trastuzumab-based regimens [91]. Third, data from the NeoSphere study and a recent study from the Translational Breast Cancer Research Consortium suggest that a subset of patients can achieve pCR by combining anti-HER2 agents without chemotherapy, although the long-term effects of this approach are unknown [61,94]. Notably, a significant challenge in cross-trial comparisons is the inconsistent definitions of pCR used (e.g. in breast vs. in breast and axilla, Fig. 3). Furthermore, pCR as a dichotomous variable may be a suboptimal endpoint. Various alternative endpoints have been proposed, including the concept of residual cancer burden, which is calculated on the basis of the size and extent of residual cancer deposits in the breast and lymph nodes [95]. This continuous variable can be divided into categorical minimal, moderate, and extensive residual burden groups, which have prognostic implications [95].

Finally, in these preoperative studies, the safety profile was broadly as expected for the various agents and combinations used. However, both the NeoALTTO and the GeparQuinto studies suggest that lapatinib is less well tolerated than trastuzumab, as around 35% of patients in both studies were unable to receive planned doses of the oral TKI [90,91]. Collectively, these results provide us with critical information about these novel agents for HER2-positive breast cancer. However, caution is advised in adopting pCR as a surrogate endpoint. Although patients who achieve a pCR following preoperative chemotherapy have an excellent outcome, many patients whose tumors do not fulfill the criteria for pCR achieve long-term survival. Therefore, advances in preoperative therapy can only lead to improvements in survival, if the higher pCR rate reflects increased cure of patients who would otherwise experience disease relapse. In short, the improved efficacy of these approaches can only truly be proven in a randomized phase III study in the adjuvant setting. Therefore, the results of ongoing studies, including the Adjuvant Lapatinib And/Or Trastuzumab Treatment Optimisation (ALTTO) study, are eagerly awaited.

Dual targeting of the estrogen receptor and HER2

For patients with tumors that are both estrogen receptor (ER) and HER2 positive, the combination of targeting these receptors without chemotherapy is attractive, as this approach might minimize toxicity. Furthermore, preclinical evidence suggests that cross-talk between the HER family and hormone receptor pathways is a potential mechanism for resistance to endocrine therapy [96].

In the randomized phase III Trastuzumab and Anastrozole Directed Against ER-Positive HER2-Positive Mammary Carcinoma (TAnDEM) trial, patients with ER-positive, HER2-positive MBC were treated with the aromatase inhibitor anastrozole alone or in combination with trastuzumab in the first-line setting [97].

Combination therapy resulted in a significant improvement in PFS (4.8 vs. 2.4 months, $P = 0.0016$) and ORR (20.3 vs. 6.8%, $P = 0.018$). However, no statistically significant benefit was seen in terms of OS (28.5 vs. 23.9 months, $P = 0.325$). In a similar randomized placebo-controlled phase III study, the addition of lapatinib to the aromatase inhibitor letrozole improved PFS from 3.0 to 8.2 months (HR = 0.71, 95% CI, 0.53–0.96, $P = 0.019$) in patients with HER2-positive MBC [98]. Again, no improvement in OS was observed in this study. The lack of a survival benefit from these trials is disappointing, but could have been influenced by patient cross-over and the availability of postprogression therapy with other anti-HER2 agents. Similar to many studies examining combination versus single-agent therapy, both of these studies lacked a predefined cross-over from one single agent to the other, thus limiting definitive conclusions about the combination.

Trastuzumab beyond progression

Most of the data on the activity of trastuzumab with a variety of chemotherapy regimens come from a series of phase II trials [13–22]. After progression of disease on one trastuzumab-based regimen, many investigators have tended to continue trastuzumab and change the chemotherapy partner, but evidence supporting this approach was lacking. However, this issue was addressed in a recent randomized phase III study, in which patients who progressed on trastuzumab-containing therapy were randomized to capecitabine plus trastuzumab or capecitabine alone [99]. Importantly, the continuation of trastuzumab resulted in a significant improvement in PFS from 5.6 to 8.2 months ($P = 0.03$). Although this study supports the continuation of trastuzumab, it should be noted that the trial closed early with only 156 of a planned 482 patients enrolled. Nonetheless, this study adds to the growing body of evidence from studies

examining combinations such as trastuzumab + lapatinib or trastuzumab + pertuzumab that continued inhibition of HER2 by trastuzumab is important in multiple lines of therapy [60,89].

Other combinations and approaches

As noted, possible mechanisms of resistance to trastuzumab include loss of expression of the tumor suppressor PTEN and/or activation of the PI3K/AKT-signaling proteins [100]. As the mammalian target of rapamycin (mTOR) is a downstream component of the PTEN/PI3K pathway, inhibitors of mTOR are under investigation for HER2-positive MBC (Fig. 1). In a pooled analysis of two small studies, the combination of the oral mTOR inhibitor everolimus (5 or 10 mg daily) and trastuzumab was associated with an ORR of 15% (Fig. 4) and a PFS of 4.1 months in trastuzumab-pretreated patients [101]. In a phase I study, daily everolimus in combination with weekly trastuzumab and paclitaxel yielded an ORR of 44% in heavily pretreated patients [102]. Another phase I study added trastuzumab and everolimus to vinorelbine chemotherapy and resulted in an ORR of 19.1% [103]. The combination of everolimus and trastuzumab is being assessed with paclitaxel in the first-line setting, and with vinorelbine in the second/third-line setting in ongoing large multinational phase III studies [104,105].

Another approach is the combination of anti-HER2 therapies with antiangiogenesis agents, as preclinical data demonstrate interactions between these pathways [106]. To date, a series of agents has been examined in this setting, including the monoclonal antibody bevacizumab and the TKI pazopanib, with somewhat modest results to date. Further discussion of these agents is beyond the scope of this article. Given the many emerging agents for HER2-positive breast cancer, a significant challenge is defining the optimal role of these agents and developing predictive biomarkers to guide therapeutic decisions. In the future, it is hoped that advances in translational science will continue to improve the outcome of patients with HER2-positive breast cancer.

Conclusion

The development of a range of targeted therapies has improved outcomes for patients with breast cancer that overexpresses HER2. The monoclonal antibody, trastuzumab, significantly improves survival for both early-stage and late-stage HER2-positive breast cancer. However, many patients ultimately experience disease progression. Clinical trials have demonstrated that the HER2 receptor remains an important target despite disease progression on one anti-HER2 agent. Furthermore, through translational science, an increased understanding of the mechanisms of resistance has led to the identification of new targets and novel agents with a variety of mechanisms of action. Lapatinib, a TKI, was the first agent to gain approval as a HER2-targeted therapy following progression on trastuzumab. Furthermore, recent data support an emerging role for the monoclonal antibody pertuzumab and the ADC trastuzumab–emtansine in similar settings. Additional promising agents in clinical development include HSP90 inhibitors and mTOR inhibitors, among others. Furthermore, data from recent neoadjuvant studies validate the concept of combined blockade of the HER2 pathway. Interpretation of this myriad of possibilities is a significant challenge, which centers on identifying the optimum individual agents and combinations to allow greater personalization of therapy. As such, critical research is focusing on the development of predictive biomarkers and improving our understanding of the mechanisms of resistance and tumor progression. In the future, it is hoped that clinical trials will continue to deliver on the promise of increased efficacy with novel combinations of HER2-targeted therapies, while minimizing toxicity for patients.

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Conflicts of interest

Patrick G. Morris has received honoraria from Eisai and is a consultant for Elsevier (OncologyStat.com). Conleth G. Murphy has no conflicts of interest.

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