Published in final edited form as:

Cancer Treat Rev. 2013 May ; 39(3): . doi:10.1016/j.ctrv.2012.04.008.

Current approaches and future directions in the treatment of HER2-positive breast cancer

Sara Hurvitz, Yufang Hu, Neil O'Brien, and Richard S. Finn
University of California at Los Angeles, Los Angeles, California, USA

Abstract

Human epidermal growth factor receptor 2 (HER2), a member of the ErbB family of transmembrane receptor tyrosine kinases, is amplified in 20–30% of invasive breast cancers. HER2 amplification is associated with metastasis and reduced survival. Two HER2-directed therapies have been approved by the United States Food and Drug Administration for the treatment of HER2-overexpressing breast cancer: trastuzumab, a humanized monoclonal antibody against the extracellular portion of HER2; and lapatinib, a dual HER2- and epidermal growth factor receptor-specific tyrosine kinase inhibitor. Despite the improvement in overall survival with the addition of HER2-targeted agents to chemotherapy, many patients do not benefit from these agents because of inherent resistance. In addition, many patients who achieve an initial response eventually acquire drug resistance. Currently, several mechanisms of resistance have been described, including mutations in other signaling pathways, expression of a truncated form of HER2, receptor crosstalk, and autophagy. There are several approaches under study to target these pathways of resistance, including blocking PI3 kinase and mammalian target of rapamycin signaling, blocking neoangiogenesis and the vascular endothelial growth factor axis, using monoclonal antibody targeting of the HER2 dimerization site, and using HER2 monoclonal antibody-drug conjugates. Here we will review the current scientific rationale for these agents and how combinations of these agents may yield additive or synergistic effects and lead to improved outcomes for patients with HER2-amplified breast cancer.

Keywords

breast cancer; HER2; resistance; lapatinib; tyrosine kinase inhibitors; trastuzumab

Correspondence to: Sara Hurvitz; University of California at Los Angeles; 10945 Le Conte Avenue, PVUB Suite 3360; Los Angeles, CA 90095 USA. Tel.: +1 310-829-5471; Fax: +1 310-829-6192; shurvitz@mednet.ucla.edu.

Coauthor Contact Information

Yufang Hu, PhD, University of California at Los Angeles, 0945 Le Conte Avenue, PVUB Suite 3360, Los Angeles, CA 90095 USA, Tel.: +1 310-829-5471, Fax: +1 310-829-6192, yhu2007@gmail.com

Neil O'Brien, PhD, UCLA Med-Hemat & Onc, BOX 951724, 5-535 MRL, Los Angeles, CA 90095-1724, Phone: 310-825-9918, Fax 310-586-6380, nobrien@mednet.ucla.edu

Richard S. Finn, MD, University of California at Los Angeles, 2825 Santa Monica Blvd, Suite 200, Santa Monica CA 90404, Phone: 310-586-2091, Fax: 310-586-6380, RFinn@mednet.ucla.edu

Conflict of Interest Statement

See separate Conflict of Interest Statement file.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Breast cancer (BC) remains one of the leading causes of cancer-related death worldwide.[1] Although chemotherapy has improved outcomes for patients, the marginal benefits achieved with cytotoxic agents seem to have reached a plateau. Fortunately, technological advances have enabled characterization of the molecular subtypes[2, 3] of BC and this in turn has facilitated the development of molecularly targeted therapeutics for this disease. One subtype that has been identified is distinguished by amplification of the gene encoding the human epidermal growth factor receptor 2 (HER2). This subtype accounts for approximately 20–30% of invasive BCs and is associated with reduced disease-free survival (DFS), increased risk of metastases and shorter overall survival (OS).[4, 5] HER2 is a member of the ErbB family of receptor tyrosine kinases (RTKs), which include HER1 (epidermal growth factor receptor [EGFR]), HER3, and HER4. HER2-mediated signal transduction is believed to depend largely on heterodimerization with other family members.[6] Trastuzumab is a humanized monoclonal antibody targeted against the extracellular portion of HER2. This is the first HER2-targeted agent to be approved by the United States Food and Drug Administration (FDA) for the treatment of both early stage and metastatic HER2-overexpressing (HER2+) BC.[7, 8] Subsequently, lapatinib, an orally bioavailable small molecule dual HER2- and EGFR/HER1-specific tyrosine kinase inhibitor (TKI), received FDA approval in combination with capecitabine for patients with advanced HER2+ BC.[9]

Although HER2-targeted therapies have had a significant impact on patient outcomes, resistance to these agents is common. In clinical trials, 74% of patients with HER2+ metastatic BC (MBC) did not have a tumor response to first-line trastuzumab monotherapy[10] and 50% did not respond to trastuzumab in combination with chemotherapy.[7] These examples illustrate the problem that inherent (*de novo*) resistance to HER2-targeted agents poses for effective treatment of HER2+ BC. Moreover, only approximately one-quarter of patients with HER2+ MBC who were previously treated with trastuzumab achieved a response with lapatinib plus capecitabine.[9] These limitations have led to efforts to better understand the molecular determinants of resistance to these agents in order to better select patients who are most likely to benefit from specific therapies, and to develop new agents that can overcome resistance. This review article will focus on the HER2 signaling pathway, proposed mechanisms of resistance to HER2-targeted therapies, and current approaches to overcoming resistance to HER2-targeted therapies in BC.

HER2 signaling in BC

HER2 signaling is initiated by receptor homodimerization or heterodimerization with ligand-bound HER1, HER3, and HER4. Unlike its family members, no known ligand for HER2 has been identified, and signaling diversity is achieved by its dimerization partner.[6] Dimerization leads to activation of the receptor TK domain, autophosphorylation, and ultimately activation of several downstream pathways, including the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway and the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway (Figure 1).[11, 12] Aberrant activation of the PI3K/Akt/mTOR pathway has been implicated in the development and progression of multiple malignancies.[13] Subsequent activation of mTOR, a cytoplasmic serine/threonine kinase, results in the phosphorylation of eukaryotic initiation factor 4E-binding protein 1 (4EBP1) and p70S6 kinase (p70S6K), two regulators of protein synthesis. Consequently, mTOR activation leads to the differential expression of genes involved in cell growth and survival, cellular metabolism, and angiogenesis.[14] Specifically, in BC specimens, overexpression of HER2 has been associated with activation of the PI3K/Akt/mTOR pathway and hyperactivation of this pathway has been associated with poor prognosis.[15, 16]

HER2 also has been shown to activate NF- κ B-dependent transcription of genes involved in cell growth and proliferation via the PI3K/Akt pathway[17] and to increase expression of survivin, an inhibitor of apoptosis.[18] In addition, crosstalk between HER2 and other growth-promoting receptors affects HER2-mediated signal transduction and provides potential mechanisms for trastuzumab resistance. Insulin-like growth factor-1 receptor (IGF-1R) can activate both the PI3K/Akt pathway and the MAPK pathway,[17] and results from a cellular model suggest that HER2 heterodimerized with IGF-1R can be activated by IGF-1, despite the presence of trastuzumab.[19] Furthermore, HER2 gene amplification in primary breast tumors is associated with increased levels of angiogenesis.[20] A study of tumor lysates from 611 unselected women with BC demonstrated detectable VEGF expression in a significantly larger proportion of HER2-overexpressing tumors compared with HER2-nonoverexpressing tumors, and concentrations of VEGF were significantly higher in HER2-overexpressing tumors compared with HER2-nonoverexpressing tumors.[21]

Mechanism of action of HER2-targeted agents

It is hypothesized that binding of trastuzumab to the extracellular domain of the HER2 receptor reduces signaling through the PI3K/Akt and Ras/Raf/MEK/MAPK pathways. This leads to the upregulation of p27 through activation of protein synthesis and promotion of protein stability.[22] Upregulation of p27 inhibits cyclin D kinase 2 and thereby induces cell cycle arrest in G1.[22] Trastuzumab-induced upregulation of p27 has also been shown to inhibit DNA repair after damage from chemotherapy[23, 24] or radiation.[25]

In addition to the regulation of p27, a number of mechanisms have been implicated in trastuzumab-mediated antitumor activity.[11, 26, 27] These include antiangiogenic effects which appear to be the result of decreased secretion of angiogenic factors such as VEGF and transforming growth factor (TGF)- β [28] as well as blockage of the proteolytic cleavage of the HER2 extracellular domain. Another possible mechanism of action of trastuzumab is the induction of antibody-dependent cellular cytotoxicity (ADCC).[29] Data from human BC xenografts in mice[30] and from 2 small clinical studies in patients with BC[31, 32] have suggested a dominant role for ADCC through immune cell/Fc receptor (FcR) binding. These studies suggested that patients with an FcR genotype that yields a stronger binding between FcR and the immune cell is associated with a better outcome from trastuzumab. However, recent analysis of genomic DNA samples from a large cohort of patients (N=1286) with HER2-amplified early stage BC and a separate smaller cohort of patients (N=53) with HER2-positive MBC found no significant correlation between FcR genotype and DFS or progression-free survival (PFS).[33]

Lapatinib, a reversible, small molecule TKI has been shown preclinically to cause cell cycle arrest and to promote apoptosis by blocking cell signaling pathways that are activated by HER2 and EGFR, including the PI3K/Akt/mTOR pathway and the Ras/Raf/MAPK pathway.[34]

Resistance to trastuzumab and lapatinib

Numerous mechanisms have been proposed that may mediate *de novo* and acquired resistance to trastuzumab and lapatinib.[7, 9, 10, 35] Some of these are thought to be common to both agents, whereas others are unique to each.

Mechanisms implicated for both agents

Resistance to HER2-targeted therapies may be related to loss/deregulation of phosphatase and tensin homolog (PTEN). PTEN is a negative regulator of PI3K; therefore, loss of PTEN

enables continued Akt activation. One study reported PTEN loss in 48% of breast tumors analyzed and associated loss of PTEN with an increased risk of disease-related death, node-positive status, and estrogen receptor-(ER) negative status.[36] In addition, patients with PTEN-deficient BC had significantly lower response rates to trastuzumab-based therapy compared with those with normal PTEN.[37]

A recent study using a systems biology approach was conducted to assess resistance factors to anti-RTK therapy in tumor biopsy samples and identified quantitative PTEN protein expression as the main determinant of resistance to anti-HER2 therapy.[38] A large-scale RNA interference genetic screening of a HER2-overexpressing BC cell line identified the *PTEN* tumor suppressor gene as the only gene (of 8000 genes tested) whose suppression led to trastuzumab resistance.[39] A biomarker analysis of patients with HER2+ tumors demonstrated that tumors with *PTEN* loss were more likely to be resistant to trastuzumab and were associated with shorter survival times.[40] Although results of small *in vitro* and clinical studies had suggested that loss of PTEN was not involved in resistance to lapatinib, [41–43] a genome-wide, loss-of-function screen of a HER2-overexpressing breast cancer cell line carried out to determine mediators of lapatinib resistance also found that only shRNA suppression of the *PTEN* gene resulted in resistance to lapatinib.[44] In this study, when *PTEN* knockdown cells were treated with lapatinib or trastuzumab, only limited growth inhibition was observed, confirming that PTEN expression is required for sensitivity to these agents.[44]

Resistance to anti-HER2 therapy also has been associated with activating mutations in genes coding for proteins of the PI3K/Akt/mTOR pathway. Mutations in *PIK3CA*, the gene that encodes the catalytic subunit of PI3K, are present in approximately 30% of primary BCs. [41, 45] A comparison of primary tumors and corresponding asynchronous metastatic breast tumors carried out to determine potential changes in *PIK3CA* mutations during disease progression revealed that the frequency of mutations was higher in tumor metastases than in primary tumors (50% vs 38%, respectively). Consequently, acquisition of *PIK3CA* mutations during disease progression may reflect an increased activation of the PI3K pathway.[46] Introduction of mutant *PIK3CA* into HER2-overexpressing BC cells conferred resistance to growth inhibition by trastuzumab and lapatinib.[44] Likewise, in BC cell lines with HER2 gene amplification, the presence of *PIK3CA* gain-of-function mutations was associated with resistance to trastuzumab[43] and to a HER2-targeted TKI.[47] In addition, trastuzumab and the HER2-TKI moderately inhibited the phosphorylation of Akt and S6K in cell lines containing wild type *PIK3CA*, whereas no inhibition of Akt and S6K phosphorylation was observed in cell lines containing *PIK3CA* gain-of-function mutations. [47] *PIK3CA* gain-of-function mutations did not influence the sensitivity of HER2 gene-amplified cells to a PI3K inhibitor (LY294002), suggesting that HER2 amplification is associated with signaling dependence of the cells on the PI3K pathway.[47] As shown in Figure 2, PFS following trastuzumab-based treatment was significantly shorter in patients with an overactivated PI3K pathway, attributable to PTEN loss or *PIK3CA* mutations, than it was in patients without PTEN loss or *PIK3CA* mutations ($p = 0.007$).[39] In a study that used tissue microarrays representing over 5000 patient tumor samples, the presence of *PIK3CA* mutations in HER2-positive breast tumors was associated with significantly reduced DFS; however, in contrast to the data described above, *PIK3CA* mutations were not found to be predictive of resistance to trastuzumab.[45]

The HER2 receptor lacks a functional ligand binding domain and thus relies on heterodimerization with other RTKs to transduce downstream signaling. Studies have shown the HER2/HER3 heterodimer to be the most potent driving force behind the proliferation of HER2-amplified cells.[48, 49] Thus, increased activation of HER3 may play a role in resistance to HER2-targeted therapy. Studies have shown that trastuzumab does not inhibit

ligand dependent HER2-HER3 heterodimerization[50] and increased levels of HER-family activating ligands have been detected in trastuzumab resistant cells.[51, 52] Furthermore, targeting HER2 with either mAbs or TKIs has been shown to reactivate HER3 via an AKT-mediated feedback loop.[53–55]

A recent report by Zhang *et al*[56] proposes an intriguing role of the cytoplasmic TK SRC during the development of trastuzumab resistance. The authors demonstrated that hyperactivation of SRC is a common event after loss of PTEN, overexpression of EGFR, or overexpression of IGF-1R in PI3K-dependent or -independent manners[56]. Thus, SRC activation appears to be a common downstream event of various trastuzumab resistance pathways. There is also preclinical evidence to suggest a role for SRC in lapatinib resistance.[57] Cell lines conditioned to acquire lapatinib resistance showed elevated levels of phosphorylated SRC and the combination of lapatinib and SRC inhibition restored sensitivity in both *in vitro* and *in vivo* models of resistance.

Mechanisms unique to trastuzumab (antibody) resistance

One potential mechanism of trastuzumab-specific resistance is the overexpression of truncated HER2 (p95HER2). p95HER2 is a membrane-bound molecule that has constitutive kinase activity but lacks the HER2 extracellular domain (ECD), thereby eliminating the trastuzumab binding site. Overexpression of p95HER2 is associated with reduced DFS in women with HER2-positive BC.[58] In laboratory models, BC cells engineered to express p95HER2 were resistant to the effects of trastuzumab but retained sensitivity to lapatinib.[59] Early small studies suggested that p95HER2 expression may correlate with response to trastuzumab.[59, 60] However, a recent pooled analysis that included 323 patients reported no correlation between baseline serum ECD levels nor change in serum ECD levels and response to trastuzumab.[61] ECD levels also appear to have no predictive value for response to lapatinib.[62]

Elevated expression of EphA2, a member of the Eph family of RTKs that regulates cellular processes involved in growth, survival, migration and angiogenesis, has been reported in a variety of cancers.[63] Increased expression of EphA2 has been implicated in *de novo* trastuzumab resistance. In a screen of samples from a large cohort of patients with HER2-positive BC, EphA2 expression was inversely correlated with OS and DFS. Cells engineered to overexpress EphA2 showed enhanced proliferation compared with the parent cells and did not respond to trastuzumab, while inhibition of EphA2 with an antibody against human EphA2 restored sensitivity to trastuzumab.[64] When trastuzumab-resistant cells were used to establish xenografts in athymic nude mice, the resulting tumors did not respond to trastuzumab, responded moderately to the anti-EphA2 antibody, and responded markedly to the combination of trastuzumab and the anti-EphA2 antibody.[64]

IGF-1R is a membrane-bound RTK that has been shown in several experimental systems to mediate acquired resistance to trastuzumab.[19, 65] A biomarker study showed that patients with HER2-positive BC whose tumors were positive for IGF-1R were significantly less likely to have a tumor response with trastuzumab and vinorelbine than patients with tumors that were negative for IGF-1R.[66] Increased signaling through IGF-1R in HER2+ cells can overcome trastuzumab-induced growth inhibition.[65]

Acquired resistance to trastuzumab has also been shown to be mediated by increased signaling through other alternative pathways. For example, in a xenograft model of trastuzumab-resistant BC, resistance was associated with increased expression of phosphorylated EGFR and EGFR/HER2 heterodimers and with increased levels of transcripts for EGFR, TGF β , heparin-binding EGF, and heregulin. The resistant clones retained HER2 overexpression and trastuzumab binding and were sensitive to the growth-

inhibitory effects of lapatinib.[51] Other mechanisms implicated in the development of trastuzumab resistance include masking of HER2 via increased expression of the membrane-associated mucin MUC4,[67] downregulation of the cyclin-dependent kinase inhibitor p27, [68] and expression of EGFR ligands such as TGF- β . [11]

Autophagy is another potential mechanism of acquired trastuzumab resistance. Autophagy is a catabolic survival mechanism wherein vesicles called autophagosomes form within the cell, engulf regions of cytoplasm and organelles, and fuse with lysosomes to break down the contents for use in subsequent cellular processes.[69] In preclinical BC models, HER2-overexpressing cells rendered resistant to trastuzumab via chronic trastuzumab exposure showed upregulation of autophagic activity. In addition, trastuzumab sensitivity was restored when formation of the preautophagosomal structure was inhibited with 3-methyladenine and when small interfering RNA (siRNA) to LC3, an autophagosome marker, was used to block autophagosome formation.[69]

Mechanisms unique to lapatinib resistance

Studies using lapatinib-resistant cell-lines have shown that lapatinib inhibits the phosphorylation of HER1, HER2, and HER3 in both sensitive and resistant cells (HER4 was not detectable in these cells), but phosphorylation of downstream targets was inhibited only in sensitive cells.[70] Increased expression of AXL, a membrane-bound RTK with transforming ability, was identified as a mechanism of acquired resistance; both inhibition of AXL with the multikinase inhibitor GSK1363089 and downregulation of AXL with an AXL-targeted siRNA restored sensitivity to lapatinib. Lapatinib-resistant cells were also resistant to the growth inhibitory effects of trastuzumab, and AXL inhibition and downregulation restored trastuzumab sensitivity.[70]

In another cell-based model, enhanced ER signaling was shown to be responsible for the development of resistance to lapatinib. Lapatinib-induced inhibition of activated Akt enabled derepression of the transcription factor FOXO3a, which promotes ER signaling.[71] Knockdown of ER synthesis with siRNA reduced survivin expression in these cells, suggesting that in resistant cells, cell survival switches from dependence on HER2 signaling to dependence on ER signaling.[71]

Approaches to overcome resistance to trastuzumab and lapatinib in HER2+ BC

Continued targeting of HER2 in the face of trastuzumab-resistance

Studies evaluating patients with HER2+ MBC whose disease has progressed on or after trastuzumab-based therapy suggest that at least some tumors that display resistance to specific HER2-targeted therapies still depend on HER2-mediated signaling. One study that evaluated the combination of trastuzumab and capecitabine in patients whose disease had progressed on trastuzumab showed that the objective response rate (ORR) and median time to progression (TTP) was significantly better for patients who received trastuzumab with capecitabine compared with women who received single-agent capecitabine.[72] Another study evaluated the combination of lapatinib and trastuzumab in women whose disease had progressed on trastuzumab and showed that continuing trastuzumab led to a significantly longer OS compared with lapatinib alone (60.7 weeks vs. 41.4 weeks, respectively).[73] These findings underscore the importance of continuing HER2-targeted therapy when treating HER2-driven breast cancer and also highlights the need for additional targeted agents to improve outcomes for women with HER2+ BC.

Combining lapatinib and trastuzumab to improve outcomes

Preclinical data suggest that the combination of lapatinib and trastuzumab is synergistic compared with either agent alone.[34] Phase III clinical trial evidence discussed above also indicates that the combination of lapatinib and trastuzumab is superior to lapatinib alone for MBC.[35] A phase III, randomized, open-label, neoadjuvant study evaluated lapatinib, trastuzumab, and their combination with paclitaxel in HER2+ BC patients (NeoALTTO). [74] The pathological complete response (pCR) rate for the combination treatment (51.3%) was significantly higher than that of the trastuzumab alone (29.5%) or lapatinib alone (24.7%) arms.[74] The addition of lapatinib did lead to increased, albeit manageable levels of toxicity. DFS and OS data from this ongoing study will be reported in the future.

Targeting downstream pathways

Identification of mechanisms of trastuzumab and lapatinib resistance provides information for the rational development of new targeted therapies to be evaluated in combination with anti-HER2 treatments. One novel therapeutic target is the PI3K/Akt/mTOR pathway. Preclinical evidence supporting the role of the PI3K/Akt/mTOR pathway in breast cancer comes from experiments with a transgenic mouse model of HER2-overexpressing breast cancer, in which treatment with the mTOR inhibitor rapamycin blocked multiple stages of tumor progression.[75]

Combined targeting of hormone receptors and HER2

The hormone receptors (HR) estrogen receptor (ER) and/or progesterone receptor (PgR) are coexpressed with HER2 in approximately 50% of BC.[76, 77] Although patients with HR-positive, HER2+ BC derive benefit from trastuzumab,[77–79] some evidence suggests that antihormonal therapy is less effective in these patients compared with those with HER2–/HR+ disease.[80–82] Cross-talk between HER2 and ER pathways has been shown to lead to resistance to endocrine therapy; however, trastuzumab in combination with HR blockade may restore sensitivity of these tumors to hormonal manipulation.[83–85] Two large randomized studies evaluated whether dual blockade of the HER2 and HR pathways is more effective than blocking one pathway in patients with tumors coexpressing these receptors. The TAnDEM trial was a randomized, phase III study of a hormonal agent and trastuzumab without chemotherapy to treat HER2/HR+ MBC.[86] Patients in the study were divided into two arms: one was treated with trastuzumab plus anastrozole and the other with anastrozole alone. Patients in the trastuzumab plus anastrozole arm experienced improvements in median PFS compared with those in the anastrozole-alone arm (4.8 vs. 2.4 months), with no statistically significant difference in overall survival. However, adverse and serious adverse events associated with the combination therapy were more pronounced.[86] A phase III trial by Johnston *et al.* evaluated the effect of combining lapatinib to letrozole as the first-line treatment of HR+ MBC. In the subgroup of patients with HR+ and HER2+ disease, there was a significant improvement in the median PFS (8.2 months for dual blockade vs. 3.0 months in control arm). In contrast, patients with HR+ and HER2– status did not show improvement from the combination treatment. AEs were more frequent but manageable in the treatment arm.[87, 88]

mTOR inhibition

Given the preclinical activity of mTOR inhibitors in models of HER2-overexpressing cancer and the role of PI3K pathway mutations in anti-HER2 therapy resistance, combination therapy with an mTOR inhibitor and an anti-HER2 agent represents a rational choice for investigation. In a mouse model of HER2-overexpressing BC, inhibition of mTOR with rapamycin showed synergy with trastuzumab in achieving tumor regression.[89] Everolimus, an orally administered mTOR inhibitor currently approved by the FDA for the

treatment of metastatic renal cell carcinoma, restored sensitivity to trastuzumab when combined with chemotherapy in a HER2-overexpressing and *PTEN*-deficient BC cell line. [90] Everolimus also provided clinical benefit when combined with trastuzumab and chemotherapy in patients with heavily pretreated HER2-overexpressing BC in phase I trials. [91, 92] In two phase Ib multicenter dose-escalation studies of patients with HER2-overexpressing MBC pretreated with trastuzumab, everolimus demonstrated an ORR of 44% and disease control for 6 months in 74% of 27 evaluable patients when combined with paclitaxel and trastuzumab, [91] as well as an ORR of 19% and a disease control rate of 83% when combined with vinorelbine and trastuzumab, in 47 evaluable patients. [92] A phase I/II study evaluating the combination of trastuzumab and everolimus without chemotherapy in 47 patients with trastuzumab-resistant HER2+ MBC demonstrated an ORR of 15%, clinical benefit rate of 34% and PFS of 4.1 months. [93]

Currently, a phase III randomized, double-blind, placebo-controlled trial (BOLERO-1; ClinicalTrials.gov Identifier NCT00876395) is ongoing to assess the value of adding everolimus to weekly paclitaxel and trastuzumab in the first-line HER2+ MBC setting. BOLERO-3 is a phase III randomized, double-blind, placebo-controlled trial (NCT01007942) that is recruiting participants to evaluate the efficacy of vinorelbine and trastuzumab with or without everolimus in women with locally advanced or metastatic HER2-positive BC resistant to trastuzumab and previously treated with a taxane. Other MTOR inhibitors are also being clinically evaluated. Ongoing phase II studies are evaluating the clinical activity of ridaforolimus (formerly deforolimus) plus trastuzumab in patients with HER2-positive, trastuzumab-refractory, MBC (NCT00736970) and of sirolimus (rapamycin) plus trastuzumab in patients with HER2-positive MBC (NCT00411788).

PI3K inhibition

NVP-BEZ235, an investigational dual PI3K/mTOR inhibitor, inhibited the proliferation of trastuzumab-resistant BC cells carrying activating PI3K mutations both *in vitro* and in BC xenografts in nude mice. [94] NVP-BEZ235 also inhibited the growth of lapatinib-resistant BC cells *in vitro*. [70] When studied in combination regimens, NVP-BEZ235 has shown additive effects with trastuzumab and additive effects with lapatinib in a BC cell line with *PTEN* knockdown. [44] This agent is currently being tested in a phase I clinical trial including patients with BC (NCT00620594).

VEGF inhibition

HER2 induction of VEGF expression likely plays a role in the pathogenesis of HER2-amplified BC. [21] In a study of HER2-overexpressing BC cells, VEGF expression was induced, while in cells with functionally inactivated HER2, VEGF induction was decreased. Treatment of HER2-overexpressing cells with heregulin 1, the ligand for HER3 and HER4, led to HER2 activation and enhanced VEGF secretion. Conditioned media from heregulin 1-treated cells stimulated the proliferation of endothelial cells, and antibody blocking experiments identified the active factor as VEGF. [95] In human BC xenografts transplanted into severe combined immunodeficient mice, trastuzumab-resistant clones demonstrated elevated VEGF expression, and sensitivity to trastuzumab was restored upon treatment of mice harboring resistant tumors with bevacizumab, a monoclonal antibody against VEGF that is approved by the US FDA for the first-line treatment of HER2-negative MBC in combination with paclitaxel. [96] Therefore, the combination of a VEGF inhibitor with a HER2 inhibitor merited clinical investigation. Results of a phase II, open-label, nonrandomized trial in 50 women 18 to 75 years of age who received trastuzumab and bevacizumab as first-line therapy for HER2-overexpressing MBC showed that this combination achieved an ORR of 48%. [97] In the 24 responding patients, the median duration of response was 10.9 months. The median OS was 43.8 months. Tolerability was

acceptable, with hypertension reported as the most common treatment-related adverse event. A phase III, randomized, open-label, adjuvant trial (BETH; NCT00625898) to determine the value of adding bevacizumab to chemotherapy plus trastuzumab in resected node-positive or high-risk node-negative HER2-positive BC has completed enrollment with results pending. Another phase III study (AVEREL; NCT00391092) enrolled 424 patients to evaluate the combination of trastuzumab and docetaxel with or without bevacizumab as first-line therapy for HER2-positive metastatic MBC. The first results of AVEREL were presented in December 2011[98] and showed that according to investigator assessment PFS was longer for patients receiving bevacizumab, although it did not reach statistical significance (13.7 months vs. 16.5 months, HR 0.82, 95% CI 0.65–1.02, $p=0.0775$). PFS assessed by independent reviewer that was stratified and censored for non-protocol therapy showed a significant difference between the arms (13.9 months vs. 16.8 months, HR 0.72, 95% CI 0.54–0.94, $p=0.0162$).[98]

Targeting the extracellular domain of HER2: pertuzumab

Pertuzumab, a monoclonal antibody against an epitope on the extracellular domain of HER2 that is distinct from that which trastuzumab binds, prevents receptor homodimerization and heterodimerization within the HER-family as well as with IGF-1R.[19, 99] Thus, pertuzumab not only prevents HER2-mediated signaling, but also may help to reverse trastuzumab resistance mediated by receptor crosstalk. *In vitro*, combination trastuzumab and pertuzumab synergistically inhibited the survival of a HER2-overexpressing BC cell line.[100] A phase II study (NCT00301899) showed that the combination of pertuzumab and trastuzumab was clinically active in patients with HER2-positive MBC who had progressed on trastuzumab.[101] A phase III study (CLEOPATRA; NCT00567190), randomly assigned 808 patients to trastuzumab plus docetaxel with or without pertuzumab for the first line treatment of HER2+ MBC.[102] Patients who received pertuzumab had a significantly improved PFS (12.4 months for control arm vs. 18.5 months pertuzumab arm, HR 0.62; 95% CI, 0.51–0.75; $p<0.001$). Patients who received pertuzumab also had a strong trend for improved survival. It is interesting to note that approximately 90% of patients enrolled in this study were trastuzumab-naïve. NeoSphere (NCT00545688) is a phase II, randomized, open-label study evaluating pertuzumab in combination with trastuzumab in neoadjuvant settings for HER2+ BC patients.[103] Patients were assigned to trastuzumab plus docetaxel (arm A), trastuzumab plus docetaxel plus pertuzumab (arm B), pertuzumab plus trastuzumab (arm C), or pertuzumab plus docetaxel (arm D). Although toxicity was lower in the arm without docetaxel, pCR rates were lower. Patients assigned to the combination of docetaxel plus trastuzumab plus pertuzumab achieved a pCR rate of 45.8%, which was significantly improved compared to 29.0% for that of trastuzumab plus docetaxel ($p=0.0141$). pCR was achieved in 16.8% of patients in the trastuzumab plus pertuzumab arm and 24.0% in the pertuzumab plus docetaxel arm.[103] Lower pCR rates were seen in each arm for patients with hormone receptor (ER and/or PR) positive BC (pCR rates for ER/PR– vs. ER and/or PR+: arm A, 20% vs 37%; arm B, 26% vs. 63%; arm C, 6% vs. 27%; arm D 17% vs. 30%). The striking pCR rate (63%) seen in the triple combination arm in the hormone receptor–negative subset of patients may suggest that dual targeting of HER2 in combination with chemotherapy may be particularly effective in this disease subtype.

Targeted delivery of cytotoxic chemotherapy to tumor cells: trastuzumab-MCC-DM1 (T-DM1)

T-DM1 is an antibody-drug conjugate containing the antimicrotubule agent emtansine stably linked to trastuzumab. T-DM1 selectively targets the chemotherapy to HER2-expressing cells. A phase I study and two single-arm phase II studies evaluating T-DM1 in heavily pretreated HER2+ MBC have shown this drug to be well tolerated and to have promising activity.[104–106]

T-DM1 was evaluated against trastuzumab + docetaxel in a phase II study TDM4450g (NCT00679341) for HER2+ MBC patients who had not received prior chemotherapy for their metastatic disease.[107] The primary end point of the study was PFS. Although the objective response rates were similar for the two treatment arms (58% docetaxel/trastuzumab vs 64% T-DM1), PFS was significantly improved in patients who received T-DM1 (14.2 months vs 9.2 months, HR 0.594, $p=0.0353$). Moreover, T-DM1 was better tolerated with 89% of patients having grade 3 toxicity in the control arm compared to 46% of patients in T-DM1 arm.

A phase III randomized, open-label, active-controlled trial (EMILIA; NCT00829166), is recruiting patients with trastuzumab-resistant HER2-positive MBC to compare the safety and efficacy of T-DM1 to that of the combination of capecitabine and lapatinib. Another phase III randomized study (MARIANNE; NCT 01120184) is comparing T-DM1 alone or in combination with pertuzumab to trastuzumab plus a taxane in the first-line HER2+ MBC setting.

Vaccines

Studies have also evaluated whether stimulating the immune system to recognize HER2+ BC improves outcomes for patients. HER2 peptide vaccine, E75, derived from the extracellular domain of HER2 was capable of inducing a cytotoxic T lymphocyte-mediated immune response against tumor cells. Preclinical and phase I and II clinical trials have been conducted on this peptide vaccine. These data showed potentials of disease prevention.[108] In a separate study by Disis *et al.*, the safety and immunogenicity of trastuzumab and an HER2 peptide vaccine combination therapy was evaluated. Twenty-two patients with late-stage HER2+ BC were enrolled in the study. This therapy was well tolerated by patients and boosted patients' immunity in a sustained pattern.[109]

Metformin

Metformin is an antidiabetic biguanide agent that is associated with a reduced risk of developing BC.[110] One action of metformin is to activate adenosine monophosphate-activated protein kinase, which promotes the inhibition of mTOR.[111] Forced expression of HER2 in human BC cells significantly enhanced metformin-induced growth inhibition,[111] and administration of exogenous metformin to cells naturally overexpressing HER2 downregulated HER2 expression in a dose- and time-dependent fashion via inhibition of p70S6K1, suggesting a potential benefit of metformin in HER2-positive BC.[111] There is also evidence to suggest that the combination of trastuzumab and metformin may synergistically eliminate elevated stem cell populations detected in cells lines with acquired trastuzumab resistance.[112]

Other agents

In addition to the agents listed above, other agents currently in early clinical development aiming to improve the efficacy of trastuzumab or to overcome trastuzumab resistance in patients with HER2-overexpressing advanced BC include the irreversible pan-ErbB inhibitor neratinib, and the irreversible TKI BIBW 2992 (afatinib), both of which showed substantial clinical activity and were well tolerated in phase II trials of patients with advanced HER2-positive BC refractory to trastuzumab.[113, 114] A phase II study (NCT00431067) of BIBW2992 for HER2+ MBC patients who had failed trastuzumab therapy was initiated for a group of 41 patients.[114] Of the 34 evaluable patients 4 had PR and 15 had SD. The toxicity was manageable, with skin rash and diarrhea being the most commonly observed AE. Ongoing phase III trials are investigating BIBW 2992 plus vinorelbine (NCT01125566) and neratinib plus paclitaxel (NCT00915018) in patients with advanced HER2-positive BC. Additional avenues of investigation include the hsp90 inhibitor tanespimycin,⁸⁶ and

vaccines containing the extra- or intracellular domain of HER2.[17] Table 1 provides an overview of novel targeted therapies in development.

Key features of active clinical trials listed in the National Cancer Institute's clinical trials database for patients with HER2-overexpressing BC that progressed on or after prior HER2-targeted therapy are summarized in Table 2.

Conclusions

HER2 overexpression is associated with poor prognosis in patients with BC. Targeting HER2 with a monoclonal antibody or a TKI has demonstrated efficacy; however, *de novo* and acquired resistance remain significant challenges to the successful treatment of the majority of patients with HER2-positive MBC. Identification of molecular mechanisms of drug action and resistance is fueling the design of new targeted approaches to treatment. Numerous clinical trials are now in progress to determine whether findings from in vitro and preclinical studies will translate into valuable new regimens to improve the prognosis for patients with HER2-overexpressing BC.

Acknowledgments

Supported by funds from Novartis Pharmaceuticals Corporation and the Marni Levine Memorial Research Award. We thank Stephanie Leinbach, PhD, and Amy Zannikos, PharmD, of Scientific Connexions, Newtown, PA, for providing background research and editorial assistance and Matthew Grzywacz, PhD, of ApotheCom, Yardley, PA, for providing additional technical assistance. The writing and final approval of the manuscript for submission were solely the responsibility of the authors.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011; 61(2):69–90. [PubMed: 21296855]
2. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, et al. Molecular portraits of human breast tumours. *Nature.* 2000; 406(6797):747–752. [PubMed: 10963602]
3. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A.* 2001; 98(19):10869–10874. [PubMed: 11553815]
4. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science.* 1987; 235(4785):177–182. [PubMed: 3798106]
5. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science.* 1989; 244(4905):707–712. [PubMed: 2470152]
6. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol.* 2001; 2(2):127–137. [PubMed: 11252954]
7. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med.* 2001; 344(11):783–792. [PubMed: 11248153]
8. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med.* 2005; 353(16):1673–1684. [PubMed: 16236738]
9. Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, et al. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med.* 2006; 355(26):2733–2743. [PubMed: 17192538]

10. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, Slamon DJ, Murphy M, Novotny WF, Burchmore M, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol*. 2002; 20(3):719–726. [PubMed: 11821453]
11. Valabrega G, Montemurro F, Aglietta M. Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. *Ann Oncol*. 2007; 18(6):977–984. [PubMed: 17229773]
12. Bender LM, Nahta R. Her2 cross talk and therapeutic resistance in breast cancer. *Front Biosci*. 2008; 13:3906–3912. [PubMed: 18508484]
13. Wong KK, Engelman JA, Cantley LC. Targeting the PI3K signaling pathway in cancer. *Curr Opin Genet Dev*. 2010; 20(1):87–90. [PubMed: 20006486]
14. Albanell J, Dalmases A, Rovira A, Rojo F. mTOR signalling in human cancer. *Clin Transl Oncol*. 2007; 9(8):484–493. [PubMed: 17720651]
15. Zhou X, Tan M, Stone Hawthorne V, Klos KS, Lan KH, Yang Y, Yang W, Smith TL, Shi D, Yu D. Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers. *Clin Cancer Res*. 2004; 10(20):6779–6788. [PubMed: 15501954]
16. Rojo F, Najera L, Lirola J, Jimenez J, Guzman M, Sabadell MD, Baselga J, Ramon y Cajal S. 4E-binding protein 1, a cell signaling hallmark in breast cancer that correlates with pathologic grade and prognosis. *Clin Cancer Res*. 2007; 13(1):81–89. [PubMed: 17200342]
17. Bedard PL, de Azambuja E, Cardoso F. Beyond trastuzumab. overcoming resistance to targeted HER-2 therapy in breast cancer. *Curr Cancer Drug Targets*. 2009; 9(2):148–162. [PubMed: 19275756]
18. Siddiqua A, Long LM, Li L, Marciniak RA, Kazhdan I. Expression of HER-2 in MCF-7 breast cancer cells modulates anti-apoptotic proteins Survivin and Bcl-2 via the extracellular signal-related kinase (ERK) and phosphoinositide-3 kinase (PI3K) signalling pathways. *BMC Cancer*. 2008; 8:129. [PubMed: 18454859]
19. Nahta R, Yuan LX, Zhang B, Kobayashi R, Esteva FJ. Insulin-like growth factor-I receptor/human epidermal growth factor receptor 2 heterodimerization contributes to trastuzumab resistance of breast cancer cells. *Cancer Res*. 2005; 65(23):11118–11128. [PubMed: 16322262]
20. Blackwell KL, Dewhirst MW, Liotcheva V, Snyder S, Broadwater G, Bentley R, Lal A, Riggins G, Anderson S, Vredenburgh J, et al. HER-2 gene amplification correlates with higher levels of angiogenesis and lower levels of hypoxia in primary breast tumors. *Clin Cancer Res*. 2004; 10(12 Pt 1):4083–4088. [PubMed: 15217943]
21. Konecny GE, Meng YG, Untch M, Wang HJ, Bauerfeind I, Epstein M, Stieber P, Vernes JM, Gutierrez J, Hong K, et al. Association between HER-2/neu and vascular endothelial growth factor expression predicts clinical outcome in primary breast cancer patients. *Clin Cancer Res*. 2004; 10(5):1706–1716. [PubMed: 15014023]
22. Le XF, Pruefer F, Bast RC Jr. HER2-targeting antibodies modulate the cyclin-dependent kinase inhibitor p27Kip1 via multiple signaling pathways. *Cell Cycle*. 2005; 4(1):87–95. [PubMed: 15611642]
23. Pietras RJ, Fendly BM, Chazin VR, Pegram MD, Howell SB, Slamon DJ. Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene*. 1994; 9(7):1829–1838. [PubMed: 7911565]
24. Pietras RJ, Pegram MD, Finn RS, Maneval DA, Slamon DJ. Remission of human breast cancer xenografts on therapy with humanized monoclonal antibody to HER-2 receptor and DNA-reactive drugs. *Oncogene*. 1998; 17(17):2235–2249. [PubMed: 9811454]
25. Pietras RJ, Poen JC, Gallardo D, Wongvipat PN, Lee HJ, Slamon DJ. Monoclonal antibody to HER-2/neureceptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells overexpressing this oncogene. *Cancer Res*. 1999; 59(6):1347–1355. [PubMed: 10096569]
26. Pohlmann PR, Mayer IA, Mernaugh R. Resistance to Trastuzumab in Breast Cancer. *Clin Cancer Res*. 2009; 15(24):7479–7491. [PubMed: 20008848]

27. Kruser TJ, Wheeler DL. Mechanisms of resistance to HER family targeting antibodies. *Exp Cell Res.* 2010; 316(7):1083–1100. [PubMed: 20064507]
28. Izumi Y, Xu L, di Tomaso E, Fukumura D, Jain RK. Tumour biology. herceptin acts as an anti-angiogenic cocktail. *Nature.* 2002; 416(6878):279–280. [PubMed: 11907566]
29. Spector NL, Blackwell KL. Understanding the mechanisms behind trastuzumab therapy for human epidermal growth factor receptor 2-positive breast cancer. *J Clin Oncol.* 2009; 27(34):5838–5847. [PubMed: 19884552]
30. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med.* 2000; 6(4):443–446. [PubMed: 10742152]
31. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, Laccabue D, Zerbini A, Camisa R, Bisagni G, et al. Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol.* 2008; 26(11):1789–1796. [PubMed: 18347005]
32. Tamura KSC, Koizumi F, Kouno T, Katsumata N, Kinoshita T, Aogi K, Nishio K, Ando M, Fujiwara Y. Correlation of Fc R IIa-H131R and IIIa-V158F polymorphisms and clinical outcome of trastuzumab in both neoadjuvant and metastatic setting in patients with HER-2 positive breast cancer. *J Clin Oncol.* 2009; 27(15s):abst 1100.
33. Hurvitz SABD, Stern HM, et al. Analysis of Fcg receptor IIA & IIIA polymorphisms: correlation with outcome in trastuzumab-treated HER2/Neu amplified early and metastatic breast cancer patients. *Proc SABCS.* 2009; (Abs 64)
34. Konecny GE, Pegram MD, Venkatesan N, Finn R, Yang G, Rahmeh M, Untch M, Rusnak DW, Spehar G, Mullin RJ, et al. Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. *Cancer Res.* 2006; 66(3): 1630–1639. [PubMed: 16452222]
35. Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J, et al. Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. *J Clin Oncol.* 2010; 28(7):1124–1130. [PubMed: 20124187]
36. Depowski PL, Rosenthal SI, Ross JS. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. *Mod Pathol.* 2001; 14(7):672–676. [PubMed: 11454999]
37. Nagata Y, Lan KH, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell.* 2004; 6(2):117–127. [PubMed: 15324695]
38. Faratian D, Goltsov A, Lebedeva G, Sorokin A, Moodie S, Mullen P, Kay C, Um IH, Langdon S, Goryanin I, et al. Systems biology reveals new strategies for personalizing cancer medicine and confirms the role of PTEN in resistance to trastuzumab. *Cancer Res.* 2009; 69(16):6713–6720. [PubMed: 19638581]
39. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez-Angulo AM, Stemke-Hale K, Hauptmann M, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell.* 2007; 12(4):395–402. [PubMed: 17936563]
40. Esteva FJ, Guo H, Zhang S, Santa-Maria C, Stone S, Lanchbury JS, Sahin AA, Hortobagyi GN, Yu D. PTEN, PIK3CA, p-AKT, and p-p70S6K status. association with trastuzumab response and survival in patients with HER2-positive metastatic breast cancer. *Am J Pathol.* 2010; 177(4):1647–1656. [PubMed: 20813970]
41. Johnston S, Trudeau M, Kaufman B, Boussen H, Blackwell K, LoRusso P, Lombardi DP, Ben Ahmed S, Citrin DL, DeSilvio ML, et al. Phase II study of predictive biomarker profiles for response targeting human epidermal growth factor receptor 2 (HER-2) in advanced inflammatory breast cancer with lapatinib monotherapy. *J Clin Oncol.* 2008; 26(7):1066–1072. [PubMed: 18212337]
42. Xia W, Husain I, Liu L, Bacus S, Saini S, Spohn J, Pry K, Westlund R, Stein SH, Spector NL. Lapatinib antitumor activity is not dependent upon phosphatase and tensin homologue deleted on chromosome 10 in ErbB2-overexpressing breast cancers. *Cancer Res.* 2007; 67(3):1170–1175. [PubMed: 17283152]

43. O'Brien NA, Browne BC, Chow L, Wang Y, Ginther C, Arboleda J, Duffy MJ, Crown J, O'Donovan N, Slamon DJ. Activated phosphoinositide 3-kinase/AKT signaling confers resistance to trastuzumab but not lapatinib. *Mol Cancer Ther.* 2010; 9(6):1489–1502. [PubMed: 20501798]
44. Eichhorn PJ, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, Beijersbergen RL, Valero V, Seoane J, Bernards R, et al. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. *Cancer Res.* 2008; 68(22):9221–9230. [PubMed: 19010894]
45. Gardner HBT, Nuciforo P, et al. PI3 kinase pathway analysis in tissue microarrays using laser capture microdissection and immunohistochemistry. *Proc SABCS.* 2009:Abs 4043.
46. Dupont JLA, Knoop A, et al. PIK3CA mutations can be acquired during tumor progression in breast cancer. *Proc SABCS.* 2009:Abs 6023.
47. Kataoka Y, Mukohara T, Shimada H, Saijo N, Hirai M, Minami H. Association between gain-of-function mutations in PIK3CA and resistance to HER2-targeted agents in HER2-amplified breast cancer cell lines. *Ann Oncol.* 2010; 21(2):255–262. [PubMed: 19633047]
48. Lee-Hoeflich ST, Crocker L, Yao E, Pham T, Munroe X, Hoeflich KP, Sliwkowski MX, Stern HM. A central role for HER3 in HER2-amplified breast cancer: implications for targeted therapy. *Cancer Res.* 2008; 68(14):5878–5887. [PubMed: 18632642]
49. Holbro T, Beerli RR, Maurer F, Koziczak M, Barbas CF 3rd, Hynes NE. The ErbB2/ErbB3 heterodimer functions as an oncogenic unit: ErbB2 requires ErbB3 to drive breast tumor cell proliferation. *Proc Natl Acad Sci U S A.* 2003; 100(15):8933–8938. [PubMed: 12853564]
50. Junttila TT, Akita RW, Parsons K, Fields C, Lewis Phillips GD, Friedman LS, Sampath D, Sliwkowski MX. Ligand-independent HER2/HER3/PI3K complex is disrupted by trastuzumab and is effectively inhibited by the PI3K inhibitor GDC-0941. *Cancer Cell.* 2009; 15(5):429–440. [PubMed: 19411071]
51. Ritter CA, Perez-Torres M, Rinehart C, Guix M, Dugger T, Engelman JA, Arteaga CL. Human breast cancer cells selected for resistance to trastuzumab in vivo overexpress epidermal growth factor receptor and ErbB ligands and remain dependent on the ErbB receptor network. *Clin Cancer Res.* 2007; 13(16):4909–4919. [PubMed: 17699871]
52. Valabrega G, Montemurro F, Sarotto I, Petrelli A, Rubini P, Tacchetti C, Aglietta M, Comoglio PM, Giordano S. TGF α expression impairs Trastuzumab-induced HER2 downregulation. *Oncogene.* 2005; 24(18):3002–3010. [PubMed: 15735715]
53. Serra V, Scaltriti M, Prudkin L, Eichhorn PJ, Ibrahim YH, Chandarlapaty S, Markman B, Rodriguez O, Guzman M, Rodriguez S, et al. PI3K inhibition results in enhanced HER signaling and acquired ERK dependency in HER2-overexpressing breast cancer. *Oncogene.* 2011; 30(22):2547–2557. [PubMed: 21278786]
54. Gijzen M, King P, Perera T, Parker PJ, Harris AL, Larijani B, Kong A. HER2 phosphorylation is maintained by a PKB negative feedback loop in response to anti-HER2 herceptin in breast cancer. *PLoS Biol.* 2010; 8(12):e1000563. [PubMed: 21203579]
55. Sergina NV, Rausch M, Wang D, Blair J, Hann B, Shokat KM, Moasser MM. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. *Nature.* 2007; 445(7126):437–441. [PubMed: 17206155]
56. Zhang S, Huang WC, Li P, Guo H, Poh SB, Brady SW, Xiong Y, Tseng LM, Li SH, Ding Z, et al. Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat Med.* 2011; 17(4):461–469. [PubMed: 21399647]
57. Rexer BN, Ham AJ, Rinehart C, Hill S, de Matos Granja-Ingram N, Gonzalez-Angulo AM, Mills GB, Dave B, Chang JC, Liebler DC, et al. Phosphoproteomic mass spectrometry profiling links Src family kinases to escape from HER2 tyrosine kinase inhibition. *Oncogene.* 2011
58. Saez R, Molina MA, Ramsey EE, Rojo F, Keenan EJ, Albanell J, Lluch A, Garcia-Conde J, Baselga J, Clinton GM. p95HER-2 predicts worse outcome in patients with HER-2-positive breast cancer. *Clin Cancer Res.* 2006; 12(2):424–431. [PubMed: 16428482]
59. Scaltriti M, Rojo F, Ocana A, Anido J, Guzman M, Cortes J, Di Cosimo S, Matias-Guiu X, Ramon y Cajal S, Arribas J, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst.* 2007; 99(8):628–638. [PubMed: 17440164]

60. Kostler WJ, Schwab B, Singer CF, Neumann R, Rucklinger E, Brodowicz T, Tomek S, Niedermayr M, Hejna M, Steger GG, et al. Monitoring of serum Her-2/neu predicts response and progression-free survival to trastuzumab-based treatment in patients with metastatic breast cancer. *Clin Cancer Res.* 2004; 10(5):1618–1624. [PubMed: 15014012]
61. Lennon S, Barton C, Banken L, Gianni L, Marty M, Baselga J, Leyland-Jones B. Utility of serum HER2 extracellular domain assessment in clinical decision making: pooled analysis of four trials of trastuzumab in metastatic breast cancer. *J Clin Oncol.* 2009; 27(10):1685–1693. [PubMed: 19255335]
62. Finn RS, Gagnon R, Di Leo A, Press MF, Arbushites M, Koehler M. Prognostic and predictive value of HER2 extracellular domain in metastatic breast cancer treated with lapatinib and paclitaxel in a randomized phase III study. *J Clin Oncol.* 2009; 27(33):5552–5558. [PubMed: 19858400]
63. Walker-Daniels J, Hess AR, Hendrix MJ, Kinch MS. Differential regulation of EphA2 in normal and malignant cells. *Am J Pathol.* 2003; 162(4):1037–1042. [PubMed: 12651595]
64. Zhuang G, Brantley-Sieders DM, Vaught D, Yu J, Xie L, Wells S, Jackson D, Muraoka-Cook R, Arteaga C, Chen J. Elevation of receptor tyrosine kinase EphA2 mediates resistance to trastuzumab therapy. *Cancer Res.* 2010; 70(1):299–308. [PubMed: 20028874]
65. Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J Natl Cancer Inst.* 2001; 93(24):1852–1857. [PubMed: 11752009]
66. Harris LN, You F, Schnitt SJ, Witkiewicz A, Lu X, Sgroi D, Ryan PD, Come SE, Burstein HJ, Lesnikowski BA, et al. Predictors of resistance to preoperative trastuzumab and vinorelbine for HER2-positive early breast cancer. *Clin Cancer Res.* 2007; 13(4):1198–1207. [PubMed: 17317830]
67. Nagy P, Friedlander E, Tanner M, Kapanen AI, Carraway KL, Isola J, Jovin TM. Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Res.* 2005; 65(2):473–482. [PubMed: 15695389]
68. Nahta R, Takahashi T, Ueno NT, Hung MC, Esteva FJ. P27(kip1) down-regulation is associated with trastuzumab resistance in breast cancer cells. *Cancer Res.* 2004; 64(11):3981–3986. [PubMed: 15173011]
69. Vazquez-Martin A, Oliveras-Ferraro C, Menendez JA. Autophagy facilitates the development of breast cancer resistance to the anti-HER2 monoclonal antibody trastuzumab. *PLoS One.* 2009; 4(7):e6251. [PubMed: 19606230]
70. Liu L, Greger J, Shi H, Liu Y, Greshock J, Annan R, Halsey W, Sathe GM, Martin AM, Gilmer TM. Novel mechanism of lapatinib resistance in HER2-positive breast tumor cells: activation of AXL. *Cancer Res.* 2009; 69(17):6871–6878. [PubMed: 19671800]
71. Xia W, Bacus S, Hegde P, Husain I, Strum J, Liu L, Paulazzo G, Lyass L, Trusk P, Hill J, et al. A model of acquired autoresistance to a potent ErbB2 tyrosine kinase inhibitor and a therapeutic strategy to prevent its onset in breast cancer. *Proc Natl Acad Sci U S A.* 2006; 103(20):7795–7800. [PubMed: 16682622]
72. von Minckwitz G, du Bois A, Schmidt M, Maass N, Cufer T, de Jongh FE, Maartense E, Zielinski C, Kaufmann M, Bauer W, et al. Trastuzumab beyond progression in human epidermal growth factor receptor 2-positive advanced breast cancer: a german breast group 26/breast international group 03–05 study. *J Clin Oncol.* 2009; 27(12):1999–2006. [PubMed: 19289619]
73. Blackwell KLBH, Sledge GW, Stein S, Ellis C, Casey M, Baselga J, O'Shaughnessy. Updated Survival Analysis of a Randomized Study of Lapatinib Alone or in Combination with Trastuzumab in Women with HER2-Positive Metastatic Breast Cancer Progressing on Trastuzumab Therapy. [Abstract 61]. *Cancer Res.* 2009; 69(24 Suppl)
74. Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, de Azambuja E, Aura C, Gomez H, Dinh P, Fauria K, Van Dooren V, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet.* 2012; 379(9816):633–640. [PubMed: 22257673]
75. Mosley JD, Poirier JT, Seachrist DD, Landis MD, Keri RA. Rapamycin inhibits multiple stages of c-Neu/ErbB2 induced tumor progression in a transgenic mouse model of HER2-positive breast cancer. *Mol Cancer Ther.* 2007; 6(8):2188–2197. [PubMed: 17699716]

76. Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE Jr, Martino S, Mamounas EP, Kaufman PA, Wolmark N. Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. *J Clin Oncol*. 2011; 29(25):3366–3373. [PubMed: 21768458]
77. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, Mackey J, Glaspy J, Chan A, Pawlicki M, et al. Adjuvant trastuzumab in HER2-positive breast cancer. *N Engl J Med*. 2011; 365(14):1273–1283. [PubMed: 21991949]
78. Untch M, Gelber RD, Jackisch C, Procter M, Baselga J, Bell R, Cameron D, Bari M, Smith I, Leyland-Jones B, et al. Estimating the magnitude of trastuzumab effects within patient subgroups in the HERA trial. *Ann Oncol*. 2008; 19(6):1090–1096. [PubMed: 18296421]
79. Brufsky A, Lembersky B, Schiffman K, Lieberman G, Paton VE. Hormone receptor status does not affect the clinical benefit of trastuzumab therapy for patients with metastatic breast cancer. *Clin Breast Cancer*. 2005; 6(3):247–252. [PubMed: 16137436]
80. Dowsett M, Allred C, Knox J, Quinn E, Salter J, Wale C, Cuzick J, Houghton J, Williams N, Mallon E, et al. Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial. *J Clin Oncol*. 2008; 26(7):1059–1065. [PubMed: 18227529]
81. De Laurentiis M, Arpino G, Massarelli E, Ruggiero A, Carlomagno C, Ciardiello F, Tortora G, D'Agostino D, Caputo F, Cancellio G, et al. A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. *Clin Cancer Res*. 2005; 11(13):4741–4748. [PubMed: 16000569]
82. Lipton A, Ali SM, Leitzel K, Demers L, Chinchilli V, Engle L, Harvey HA, Brady C, Nalin CM, Dugan M, et al. Elevated serum Her-2/neu level predicts decreased response to hormone therapy in metastatic breast cancer. *J Clin Oncol*. 2002; 20(6):1467–1472. [PubMed: 11896093]
83. Osborne CK, Shou J, Massarweh S, Schiff R. Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. *Clin Cancer Res*. 2005; 11(2 Pt 2):865s–870s. [PubMed: 15701879]
84. Shou J, Massarweh S, Osborne CK, Wakeling AE, Ali S, Weiss H, Schiff R. Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. *J Natl Cancer Inst*. 2004; 96(12):926–935. [PubMed: 15199112]
85. Witters L, Engle L, Lipton A. Restoration of estrogen responsiveness by blocking the HER-2/neu pathway. *Oncol Rep*. 2002; 9(6):1163–1166. [PubMed: 12375012]
86. Kaufman B, Mackey JR, Clemens MR, Bapsy PP, Vaid A, Wardley A, Tjulandin S, Jahn M, Lehle M, Feyereislova A, et al. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. *J Clin Oncol*. 2009; 27(33):5529–5537. [PubMed: 19786670]
87. Johnston S, Pippen J Jr, Pivot X, Lichinitser M, Sadeghi S, Dieras V, Gomez HL, Romieu G, Manikhas A, Kennedy MJ, et al. Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-positive metastatic breast cancer. *J Clin Oncol*. 2009; 27(33):5538–5546. [PubMed: 19786658]
88. Schwartzberg LS, Franco SX, Florance A, O'Rourke L, Maltzman J, Johnston S. Lapatinib plus letrozole as first-line therapy for HER-2+ hormone receptor-positive metastatic breast cancer. *Oncologist*. 2010; 15(2):122–129. [PubMed: 20156908]
89. Miller TW, Forbes JT, Shah C, Wyatt SK, Manning HC, Olivares MG, Sanchez V, Dugger TC, de Matos Granja N, Narasanna A, et al. Inhibition of mammalian target of rapamycin is required for optimal antitumor effect of HER2 inhibitors against HER2-overexpressing cancer cells. *Clin Cancer Res*. 2009; 15(23):7266–7276. [PubMed: 19934303]
90. Lu CH, Wyszomierski SL, Tseng LM, Sun MH, Lan KH, Neal CL, Mills GB, Hortobagyi GN, Esteva FJ, Yu D. Preclinical testing of clinically applicable strategies for overcoming trastuzumab resistance caused by PTEN deficiency. *Clin Cancer Res*. 2007; 13(19):5883–5888. [PubMed: 17908983]

91. Andre F, Campone M, O'Regan R, Manlius C, Massacesi C, Sahmoud T, Mukhopadhyay P, Soria JC, Naughton M, Hurvitz SA. Phase I study of everolimus plus weekly paclitaxel and trastuzumab in patients with metastatic breast cancer pretreated with trastuzumab. *J Clin Oncol.* 2010; 28(34): 5110–5115. [PubMed: 20975068]
92. Jerusalem G, Fasolo A, Dieras V, Cardoso F, Bergh J, Vittori L, Zhang Y, Massacesi C, Sahmoud T, Gianni L. Phase I trial of oral mTOR inhibitor everolimus in combination with trastuzumab and vinorelbine in pre-treated patients with HER2-overexpressing metastatic breast cancer. *Breast Cancer Res Treat.* 2011; 125(2):447–455. [PubMed: 21107682]
93. Morrow PK, Wulf GM, Ensor J, Booser DJ, Moore JA, Flores PR, Xiong Y, Zhang S, Krop IE, Winer EP, et al. Phase I/II Study of Trastuzumab in Combination With Everolimus (RAD001) in Patients With HER2-Overexpressing Metastatic Breast Cancer Who Progressed on Trastuzumab-Based Therapy. *J Clin Oncol.* 2011
94. Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, Botero ML, Llouch E, Atzori F, Di Cosimo S, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res.* 2008; 68(19): 8022–8030. [PubMed: 18829560]
95. Yen L, You XL, Al Moustafa AE, Batist G, Hynes NE, Mader S, Meloche S, Alaoui-Jamali MA. Heregulin selectively upregulates vascular endothelial growth factor secretion in cancer cells and stimulates angiogenesis. *Oncogene.* 2000; 19(31):3460–3469. [PubMed: 10918604]
96. du Manoir JM, Francia G, Man S, Mossoba M, Medin JA, Vilorio-Petit A, Hicklin DJ, Emmenegger U, Kerbel RS. Strategies for delaying or treating in vivo acquired resistance to trastuzumab in human breast cancer xenografts. *Clin Cancer Res.* 2006; 12(3 Pt 1):904–916. [PubMed: 16467105]
97. Hurvitz SAPM, Lin LS. Final results of a phase 2 trial evaluating trastuzumab and bevacizumab as first-line treatment of HER2-amplified advanced breast cancer. *Proc SABCS.* 2009; Abs 6094.
98. Gianni LRG, Lichinitser M, Serrano S, Mansutti M, Pivot X, Smirnova I, Moliterni A, Andre F, Chan A, Lipatov O, Chan S, Wardley A, Greil R, Provencher L, Moore N, Prot S, Semiglazov V. First results of AVEREL, a randomized phase III trial to evaluate bevacizumab (BEV) in combination with trastuzumab (H) + docetaxel (DOC) as first-line therapy for HER2-positive locally recurrent/metastatic breast cancer (LR/mBC). *Cancer Res.* 2011; 71(24):S4–8.
99. Whenham N, D'Hondt V, Piccart MJ. HER2-positive breast cancer: from trastuzumab to innovative anti-HER2 strategies. *Clin Breast Cancer.* 2008; 8(1):38–49. [PubMed: 18501058]
100. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies trastuzumab and pertuzumab synergistically inhibit the survival of breast cancer cells. *Cancer Res.* 2004; 64(7):2343–2346. [PubMed: 15059883]
101. Baselga J, Gelmon KA, Verma S, Wardley A, Conte P, Miles D, Bianchi G, Cortes J, McNally VA, Ross GA, et al. Phase II trial of pertuzumab and trastuzumab in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer that progressed during prior trastuzumab therapy. *J Clin Oncol.* 2010; 28(7):1138–1144. [PubMed: 20124182]
102. Baselga J, Cortes J, Kim SB, Im SA, Hegg R, Im YH, Roman L, Pedrini JL, Pienkowski T, Knott A, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *N Engl J Med.* 2012; 366(2):109–119. [PubMed: 22149875]
103. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, Lluch A, Staroslawska E, de la Haba-Rodriguez J, Im SA, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. *Lancet Oncol.* 2012; 13(1):25–32. [PubMed: 22153890]
104. Krop IE, Beeram M, Modi S, Jones SF, Holden SN, Yu W, Girish S, Tibbitts J, Yi JH, Sliwkowski MX, et al. Phase I study of trastuzumab-DM1, an HER2 antibody-drug conjugate, given every 3 weeks to patients with HER2-positive metastatic breast cancer. *J Clin Oncol.* 2010; 28(16):2698–2704. [PubMed: 20421541]
105. Burris HA 3rd, Rugo HS, Vukelja SJ, Vogel CL, Borson RA, Limentani S, Tan-Chiu E, Krop IE, Michaelson RA, Girish S, et al. Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer after prior HER2-directed therapy. *J Clin Oncol.* 2011; 29(4):398–405. [PubMed: 21172893]

106. Krop, IE.; LoRusso, P.; Miller, KD., et al. A phase II study of trastuzumab-DM1 (T-DM1), a novel HER2 antibody-drug conjugate, in patients with HER2+ metastatic breast cancer who were previously treated with an anthracycline, a taxane, capecitabine, lapatinib, and trastuzumab. Poster presented at: Annual San Antonio Breast Cancer Symposium; San Antonio, Texas. 2009. p. Abstract 710
107. Hurvitz SDL, Kocsis J, Gianni L, Lu J, Vinholes J, Song C, Tong B, Chu YW, Perez EA. Trastuzumab emtansine (T-DM1) vs trastuzumab plus docetaxel (H+T) in previously-untreated HER2-positive metastatic breast cancer (MBC): primary results of a randomized, multicenter, open-label phase II study (TDM4450g/B021976. *Eur J Cancer*. 2011; 47(S330):Abstract 5001.
108. Mittendorf EA, Holmes JP, Ponniah S, Peoples GE. The E75 HER2/neu peptide vaccine. *Cancer Immunol Immunother*. 2008; 57(10):1511–1521. [PubMed: 18536917]
109. Disis ML, Wallace DR, Gooley TA, Dang Y, Slota M, Lu H, Coveler AL, Childs JS, Higgins DM, Fintak PA, et al. Concurrent trastuzumab and HER2/neu-specific vaccination in patients with metastatic breast cancer. *J Clin Oncol*. 2009; 27(28):4685–4692. [PubMed: 19720923]
110. Bodmer M, Meier C, Krahenbuhl S, Jick SS, Meier CR. Long-term metformin use is associated with decreased risk of breast cancer. *Diabetes Care*. 2010; 33(6):1304–1308. [PubMed: 20299480]
111. Vazquez-Martin A, Oliveras-Ferraro C, Menendez JA. The antidiabetic drug metformin suppresses HER2 (erbB-2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells. *Cell Cycle*. 2009; 8(1):88–96. [PubMed: 19106626]
112. Vazquez-Martin A, Oliveras-Ferraro C, Del Barco S, Martin-Castillo B, Menendez JA. The anti-diabetic drug metformin suppresses self-renewal and proliferation of trastuzumab-resistant tumor-initiating breast cancer stem cells. *Breast Cancer Res Treat*. 2011; 126(2):355–364. [PubMed: 20458531]
113. Burstein HJ, Sun Y, Dirix LY, Jiang Z, Paridaens R, Tan AR, Awada A, Ranade A, Jiao S, Schwartz G, et al. Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. *J Clin Oncol*. 2010; 28(8):1301–1307. [PubMed: 20142587]
114. Hickish TWD, Lin N, Carey L, Houston S, Mendelson D, Solca F, Uttenreuther-Fischer M, Jones H, Winer E. Use of BIBW 2992, a Novel Irreversible EGFR/HER1 and HER2 Tyrosine Kinase Inhibitor To Treat Patients with HER2-Positive Metastatic Breast Cancer after Failure of Treatment with Trastuzumab. *Proc SABCs*. 2009:Abs 5060.

Figure 1.

Human epidermal growth factor receptor 2 (HER2) signal transduction pathways. Homodimerization and heterodimerization of HER2 leads to TK activation and downstream signaling via the PI3K/Akt/mammalian target of rapamycin pathway and the Ras/Raf/mitogen-activated protein kinase pathway to stimulate processes involved in cell survival and proliferation.¹⁴

Adapted from Valabrega G, Montemurro F, Aglietta M. Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. *Ann Oncol.* 2007;18:977–984, by permission of Oxford University Press.

Figure 2.

Kaplan-Meier analysis of time to progression in patients with activated PI3K pathways (yes; orange curve; $n = 24$) and nonactivated PI3K pathways (no; blue curve; $n = 29$) shows that PI3K pathway activation is associated with significantly shorter time to progression after trastuzumab-based therapy ($p = 0.007$).⁴⁰

Reprinted from Berns K, Horlings HM, Hennessy BT, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell* 2007;12:395–402. Copyright 2007, with permission from Elsevier.

Table 1

Novel targeted agents in development for HER2-overexpressing advanced breast cancer²⁰

Agent	Class	Mechanism of action	Line(s) of therapy investigated
Gefitinib	TKI	Inhibition of EGFR/TK	1st line, + trastuzumab and docetaxel
Erlotinib	TKI	Inhibition of EGFR/TK	1st line, + trastuzumab
BIBW 2992	TKI	Inhibition of EGFR and HER2 TK	2nd line, + trastuzumab; 2nd line, + vinorelbine
Neratinib	TKI	pan-ErbB inhibition	2nd line, + temsirolimus
HK1-272	TKI	Inhibition of EGFR and HER2, 3, 4 TK	1st or 2nd line; 2nd line, + paclitaxel
Temsirolimus	TKI	Inhibition of mTOR	2nd line, + neratinib
Everolimus	TKI	Inhibition of mTOR	2nd line, + trastuzumab; + trastuzumab and vinorelbine; 2nd or 3rd line, + paclitaxel and trastuzumab; neoadjuvant, + trastuzumab and paclitaxel; 2nd or 3rd line, + paclitaxel and cisplatin
Sirolimus	TKI	Inhibition of mTOR	2nd line, + trastuzumab
Deforolimus	TKI	Inhibition of mTOR	2nd line, + trastuzumab
Lonafarnib	TKI	Inhibition of Ras/farnesyltransferase	1st line, + trastuzumab and paclitaxel
Sunitinib	TKI	Inhibition of VEGFR/TK	1st line + trastuzumab and paclitaxel; 1st line, + trastuzumab and docetaxel
Pazopanib	TKI	Inhibition of VEGFR, c-kit, PDGFR/TK	2nd line, + lapatinib
Tanespimycin	Heat shock protein inhibitor	Inhibition of HSP90	2nd line, + trastuzumab
Alvespimycin	Heat shock protein inhibitor	Inhibition of HSP90	1st line; 2nd line, + trastuzumab +/- paclitaxel
Bortezomib	Proteasome inhibitor	Inhibition of the 26S proteasome	1st line, + trastuzumab
Cetuximab	Receptor antibody	Binding to EGFR	1st line, + trastuzumab
Pertuzumab	Receptor antibody	Inhibition of HER2 dimerization	1st line, + trastuzumab and docetaxel
Trastuzumab- MCC-DM1	Receptor antibody-toxin conjugate	Binding to HER2, toxin delivery	1st line; 2nd line; 2nd line, + paclitaxel and pertuzumab
Erumaxomab	Receptor antibody	HER2 and CD3	2nd line
INCB7839	Sheddase inhibitor	Inhibition of ADAM10 and ADAM17	1st line
HER2/neu vaccine	Immunotherapy	Inhibition of HER2	2nd line, after response to a 1st line therapy
MVA-BN-HER2	Immunotherapy	Inhibition of HER2 + tetanus toxoid	Any

Adapted with permission from Bedard PL, de Azambuja E, Cardoso F. Curr Cancer Drug Targets 2009;9:148-62.

EGFR, epidermal growth factor receptor; HER2 indicates human epidermal growth factor receptor 2; HSP, heat shock protein. mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; TK, tyrosine kinase; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

Table 2
National Cancer Institute–listed active clinical trials in patients who progressed on prior anti-HER2 therapy

ClinicalTrials.gov identifier/study name	Mechanism of action	Trial design	Prior anti-HER2 therapy	Estimated enrollment	Treatment arm(s)	Primary end point/ outcome measure	Estimated primary completion date
NCT01007942/BOLERO-3	mTOR inhibitor	Phase III, randomized, double-blind, placebo- controlled	Trastuzumab	572	1 Everolimus + vinorelbine + trastuzumab 2 Placebo + vinorelbine + trastuzumab	PFS	12/12
NCT00964704	Anti-VEGF	Phase II, nonrandomized, single-arm, open-label	Trastuzumab	50	Single arm: Trastuzumab Bevacizumab Capecitabine Docetaxel	PFS	6/14
NCT00391092/AVEL	Anti-VEGF	Phase III, randomized, open- label	No	410	1 bevacizumab + trastuzumab + docetaxel 2 bevacizumab + trastuzumab	PFS	Not reported
NCT00398567	EGFR/HER2 TKI	Phase II, single-arm, open- label	No	45	Neratinib + trastuzumab	Safety, tumor response	12/08
NCT00915018	EGFR/HER2 TKI	Phase III, randomized, active control, open- label	No	1200	1 Neratinib + Paclitaxel 2 Trastuzumab + Paclitaxel	PFS	8/12
NCT00788333	Antihyperglyce mic	Phase I/II, single-arm, open- label	Trastuzumab, other HER2- targeted agents allowed	48	Single arm: BMS-754807 + trastuzumab	MTD Phase II dose	7/12
NCT00684983	Anti-IGF-1R	Phase II, randomized	Trastuzumab, no lapatinib	154	1 Capecitabine + lapatinib 2 Cixutumumab + capecitabine + lapatinib	PFS	8/09
NCT00829166/EMILIA	Trastuzumab-toxin conjugate	Phase III, randomized, open- label	Trastuzumab, no lapatinib	580	1 Trastuzumab-MCC-DM1 2 Lapatinib + capecitabine	AEs PFS	8/13
NCT00951665	HER2 dimerization inhibitor; trastuzumab-toxin conjugate	Phase Ib, single-arm, open- label	Trastuzumab, no T-DM1, no pertuzumab	27	Single arm: Paclitaxel + pertuzumab + trastuzumab-MCC- DM1	AEs, SAEs DLTs, PK	Not reported
NCT01026142	HER2 dimerization inhibitor	Phase II, randomized, open- label	Trastuzumab (in previous regimen), no pertuzumab	450	1 Capecitabine + trastuzumab 2 Pertuzumab + capecitabine + trastuzumab	PFS	12/15

ClinicalTrials.gov identifier/study name	Mechanism of action	Trial design	Prior anti-HER2 therapy	Estimated enrollment	Treatment arm(s)	Primary end point/ outcome measure	Estimated primary completion date
NCT00943670	HER2 dimerization inhibitor	Phase II, single-arm, open-label	Trastuzumab, no T-DM1, no pertuzumab	50	Single arm: Pertuzumab + trastuzumab-MCC-DM1	Baseline-adjusted QTcF	8/11
NCT00567190/CLEOPATRA	HER2 dimerization inhibitor	Phase III, randomized, double-blind, placebo-controlled	No	800	<div><div>1</div>docetaxel + pertuzumab + trastuzumab</div> <div><div>2</div>docetaxel + placebo + trastuzumab</div>	PFS	March 2012

AE: adverse event; DLT: dose-limiting toxicity; EGFR: epidermal growth factor receptor; HER2: human epidermal growth factor receptor 2; IGF-1R: insulin-like growth factor-1 receptor; MTD: maximum tolerated dose; mTOR: mammalian target of rapamycin; PFS: progression-free survival; PK: pharmacokinetics; QTcF: corrected QT interval; SAE: serious adverse event; TKI: tyrosine kinase inhibitor; VEGF: vascular endothelial growth factor.