Published in final edited form as:

Crit Rev Oncog. 2012 ; 17(1): 1–16.

Intrinsic and Acquired Resistance to HER2-Targeted Therapies in HER2 Gene-Amplified Breast Cancer: Mechanisms and Clinical Implications

Brent N. Rexer and Carlos L. Arteaga*

Departments of Medicine and Cancer Biology; Breast Cancer Research Program, Vanderbilt-Ingram Cancer Center; Vanderbilt University School of Medicine, Nashville, TN

Abstract

Approximately 25% of human breast cancers overexpress the HER2 (ErbB2) proto-oncogene, which confers a more aggressive tumor phenotype and associates with a poor prognosis in patients with this disease. Two approved therapies targeting HER2, the monoclonal antibody trastuzumab and the tyrosine kinase inhibitor lapatinib, are clinically active against this type of breast cancer. However, a significant fraction of patients with HER2+ breast cancer treated with these agents eventually relapse or develop progressive disease. This suggests that tumors acquire or possess intrinsic mechanisms of resistance that allow escape from HER2 inhibition. This review focuses on mechanisms of intrinsic and/or acquired resistance to HER2-targeted therapies that have been identified in preclinical and clinical studies. These mechanisms involve alterations to HER2 itself, coexpression or acquisition of bypass signaling through other receptor or intracellular signaling pathways, defects in mechanisms of cell cycle regulation or apoptosis, and host factors that may modulate drug response. Emerging clinical evidence already suggests that combinations of therapies targeting HER2 as well as these resistance pathways will be effective in overcoming or preventing resistance.

Keywords

breast cancer; HER2; trastuzumab; lapatinib; therapeutic resistance

I. INTRODUCTION

HER2 is a member of the ErbB family of receptor tyrosine kinases (RTKs), which includes EGFR (ErbB1), HER3 (ErbB3), and HER4 (ErbB4). Binding of ligands to the extracellular domain of EGFR, HER3, or HER4 (HER2 has no known ligand) induces homo- and heterodimerization of receptors followed by phosphorylation of several tyrosines in the receptors' C-termini. In turn, these residues serve as docking sites for a number of src homology (SH)2-containing adaptors and signal transducers that mediate the transforming effects of this receptor network.¹ Of the ErbB family members, HER2 is the preferred dimerization partner and possesses the most potent kinase catalytic activity capable of amplifying signaling through ErbB co-receptors. Heterodimers of HER2 with kinase-inactive HER3 are the most transforming of the ErbB network. They potently activate the

phosphatidylinositol 3-kinase (PI3K)-Akt survival pathway via HER2-mediated phosphorylation of HER3, which, in turn, recruits the p85 regulatory subunit of PI3K.²⁻⁶

Amplification of HER2 occurs in approximately 25% of human breast cancers. Prior to the development of HER2-targeted therapies, HER2 over-expression was associated with poor patient outcome.^{7,8} There are two FDA-approved therapies that target HER2 in breast cancer: (1) trastuzumab, a monoclonal antibody directed against the extracellular domain of HER2, and (2) lapatinib, a small molecule ATP-competitive reversible kinase inhibitor of HER2 and EGFR. These therapies have shown significant clinical benefit, including a 50% reduction in the risk of death after treatment with trastuzumab or a similar improvement in disease-free survival with lapatinib after progression on trastuzumab.⁹⁻¹³ However, most patients with advanced HER2 gene-amplified breast cancer eventually relapse after treatment, suggesting that tumors acquire or intrinsically possess mechanisms for escape from HER2 inhibition. Clinical efficacy of HER2-directed therapies appears to be limited to breast cancers that overexpress the HER2 protein as determined by immunohistochemistry (IHC), or exhibit gene amplification of HER2 as determined by fluorescence in situ hybridization (FISH).¹⁴ Thus, HER2 overexpression as defined by these two assays is currently the only biomarker predictive of response to HER2-targeted therapy.

Trastuzumab is a humanized IgG₁ that recognizes an epitope in the extracellular domain of HER2. The therapeutic effect of the antibody is likely due to a number of effects, including antibody-mediated immunomodulatory activities as well as disruption of HER2 function and downstream signaling. Both the innate and adaptive immune responses are required for the therapeutic effect of trastuzumab when tested in mouse models.^{15,16} Trastuzumab also inhibits tumor cell growth via induction of G1 cell cycle arrest associated with induction of the CDK inhibitor p27^{KIP1}.¹⁷⁻²⁰ Binding of trastuzumab to the juxtamembrane region of HER2 disrupts ligand-independent HER2-HER3 heterodimerization.²¹ This results in partial inhibition of PI3K-Akt signaling, a major effector of HER2-mediated tumor progression.²²

Lapatinib is an ATP-competitive reversible tyrosine kinase inhibitor (TKI) of HER2 and EGFR. In HER2+ breast cancer, lapatinib blocks HER2 phosphorylation, resulting in inhibition of PI3K-Akt and MAPK downstream of the HER2 receptor. Blockade of these pathways inhibits cell viability in vitro and xenograft growth in vivo.^{23,24} In patients with metastatic HER2+ breast cancer, treatment with lapatinib induces variable levels of signaling inhibition and apoptosis.²⁵ In a recently reported neoadjuvant trial in patients with newly diagnosed HER2+ breast tumors, the major effect of single-agent lapatinib was inhibition of cell proliferation without a detectable induction of tumor cell apoptosis.²⁶ Conversely, treatment with trastuzumab, which has less profound effects on signaling *in vitro* than does lapatinib, was shown to induce apoptosis in primary HER2+ tumors,²² suggesting the antibody effect may involve a host-antibody interaction not detectable in tumor cell autonomous experiments *in vitro*.

In patients with metastatic disease, the therapeutic action of trastuzumab and lapatinib tends to be short lived. Even though some patients treated in the adjuvant setting will likely be cured of their cancer, it is anticipated that a fraction will eventually recur. This suggests that tumors harbor *de novo* or acquired mechanisms of resistance to therapeutic inhibitors of HER2. We will discuss potential mechanisms of drug resistance that have been identified in preclinical models and, in some cases, confirmed in patient tumors. These occur at three levels: first, mechanisms intrinsic to the target itself; second, resistance that involves parallel bypass signaling pathways to overcome HER2 inhibition; and third, resistance that arises from defects in the apoptosis pathway in tumor cells or in extrinsic host factors that participate in drug action (Fig. 1).

II. INTRINSIC HER2 ALTERATIONS

A mechanism of resistance to inhibitors of HER2 is mutation of the target itself, resulting in alteration of drug binding. This mechanism is exemplified by the acquired so-called “gatekeeper” kinase domain mutations observed in patients with lung cancer treated with EGFR TKIs and patients with CML and gastrointestinal stromal tumors treated with imatinib.^{27–32} For HER2, this would also include mutations in the juxtamembrane region that contains the binding epitope of trastuzumab. Anido et al. described p95-HER2, a truncated form of HER2 lacking the antibody binding region, which arises from alternate transcription initiation sites in HER2.³³ This form of HER2 retains kinase activity and is susceptible to inhibition by lapatinib but not trastuzumab.³⁴ Patients with metastatic breast cancer harboring cytosolic expression of p95-HER2 exhibit a very low response rate to treatment with trastuzumab and chemotherapy compared to those patients without p95-HER2 in their tumors. Conversely, tumors with p95-HER2 are still susceptible to kinase inhibition with a TKI, as was suggested by a similar response rate to capecitabine and lapatinib observed in patients with breast cancer with and without p95-HER2.³⁵ A recent study reported a nuclear localized truncated form of HER2, also 95 kDa in size, which retains phosphorylation and nuclear localization upon treatment with lapatinib.³⁶ The frequency and clinical significance of this finding are unknown at this time.

A splice variant that eliminates exon 16 in the extracellular domain of the HER2 receptor has also been identified in HER2+ breast cancers and cell lines.^{37,38} Cell lines expressing this D16 HER2 isoform are resistant to trastuzumab.^{38,39} This variant does not eliminate the trastuzumab epitope on HER2, but does appear to stabilize HER2 homodimers and may potentially prevent their disruption upon binding by the antibody.³⁸ In addition, the D16 isoform was found to interact directly with the Src tyrosine kinase, and treatment with the Src inhibitor dasatinib overcame the resistance to trastuzumab conferred by the alternative splicing variant.³⁹ A role for Src kinases in HER2 inhibitor resistance will be further discussed below.

Point mutations or small insertions in the HER2 gene have been identified in other cancers. A small number (2%–4%) of non-small-cell lung cancers (NSCLC), as well as gastric, colorectal, and head and neck cancers, have been found to have alterations in the HER2 gene.^{40–46} These include primarily amino acid substitutions or insertions localized in the kinase domain. An insertion in exon 20, originally identified in NSCLC, was able to confer resistance to lapatinib and trastuzumab when expressed in breast cancer cell lines.⁴⁷ HER2 mutations have been reported in a small number of human breast cancers but in the absence of HER2 gene amplification.⁴¹ To our knowledge, HER2 mutations in HER2-overexpressing breast tumors have not been reported to date. One possible reason is that these mutations may comprise only a portion of the amplified HER2 alleles and, therefore, exist below the limits of sensitivity of traditional DNA sequencing methods. Nonetheless, using a next-generation sequencing approach with higher sensitivity that was measured to detect a variant frequency as low as 0.08%, no mutations in HER2+ breast cell lines or tumors were detected.⁴⁸ Another possibility is that these mutations may be selected for or acquired only after the selective pressure of anti-HER2 treatment. If so, they are likely to be detected in tumors that are progressing after primary HER2-targeted therapy.

Finally, unique for the case of trastuzumab is the possibility of abrogating drug binding to the target by coexpression of another protein that binds to the drug target. For example, mucin-4 (MUC4), a membrane-associated glycoprotein, when overexpressed can co-localize with HER2 and mask the binding site for trastuzumab.^{49,50} A cleaved form of another mucin family member, MUC1*, was also found to be overexpressed in a cell line selected for trastuzumab resistance.⁵¹ While this cleaved MUC1 isoform has been shown to associate

with HER2, and the interaction is enhanced by ligands,⁵² other studies have shown that MUC1* can homodimerize and induce signaling for survival and proliferation on its own.^{53,54} Figure 1A illustrates several of these alterations in HER2 that are thought to play a role in resistance.

III. ACTIVATION OF COMPENSATORY SIGNAL TRANSDUCTION PATHWAYS

A. Receptor Tyrosine Kinases (RTKs)

Signaling through other RTKs can transactivate HER2 and amplify signal transduction downstream, thus bypassing the inhibitory effect of lapatinib or trastuzumab. HER2 is known to heterodimerize with other ErbB family members, and signaling initiated by ligands of EGFR, HER3, or HER4 can rescue the antiproliferative effects of trastuzumab.^{55,56} In a model of acquired resistance to trastuzumab in breast cancer xenografts, our laboratory reported increased expression of EGFR and HER3 ligands, resulting in activation of EGFR and HER3 as well as increased EGFR/HER2 heterodimers in trastuzumab-resistant cells (Fig. 1B).⁴ This is consistent with data showing that trastuzumab is unable to block ligand-induced heterodimerization of HER2.⁵⁷ Another mechanism for increased availability of ErbB ligands associated with trastuzumab resistance is activation of TGF β receptors that, in turn, activate the sheddase TACE/ADAM17 to release more TGF α , amphiregulin, and heregulin. This increase in ligand activates HER3. A gene signature of TGF β activity, derived from gene expression profiling after expression of a constitutively active mutant of the TGF β type I receptor (ALK5), correlates with resistance to trastuzumab and poor clinical outcome in patients.⁵⁸ Of note, these models of trastuzumab resistance appear to remain dependent on HER2 signaling, as treatment with lapatinib to block HER2 kinase activity directly, or with pertuzumab, an antibody that blocks HER2 heterodimerization, restores trastuzumab action.^{4,57,59,60}

Cross-talk with receptors outside of the ErbB family has also been implicated as a means to bypass trastuzumab action (Fig. 1D). Overexpression of the IGF-1 receptor or an increase in levels of IGF-1R/HER2 heterodimers can potentially activate PI3K-Akt signaling and confer resistance to trastuzumab.^{61,62} IGF-1R has been found to complex with both HER2 and HER3 in a heterotrimeric complex.⁶³ Inhibition of IGF-1R with a neutralizing antibody or a small molecule TKI, or targeting the HER2 kinase with lapatinib, was found to overcome IGF-1R-mediated resistance to trastuzumab.^{62,64,65} Recently, targeting the IGF-1R axis with metformin was also shown to overcome resistance to the antibody.⁶⁶ In a neo-adjuvant trial of chemotherapy plus trastuzumab, a high level of IGF-1R expression measured by IHC correlated with a poor clinical response.⁶⁷ In another study, however, IGF-1R levels in pre-treatment biopsies did not correlate with response to trastuzumab or to clinical outcome.⁶⁸ One explanation is that the IGF-1R expression is selected for after acquired resistance and, therefore, the pre-treatment levels of IGF-1R are not predictive. Indeed, in some studies, increased IGF-1R or IGF-1R/HER2 complexes were observed only after development of acquired resistance to trastuzumab.^{62,63,66}

The RTK MET has also been implicated in trastuzumab resistance. MET has been shown to interact with HER3 to engage PI3K signaling downstream of EGFR in gefitinib-resistant NSCLC.⁶⁹ Upon treatment with trastuzumab, HER2+ breast cancers up-regulate MET levels. HGF-induced signaling through MET has been shown to abrogate the action of trastuzumab. Further, overexpression of MET and its ligand, HGF, were reported in a cohort of HER2+ patients who did not respond to chemotherapy plus trastuzumab.⁷⁰

Overexpression of the EphA2 receptor was found to predict reduced disease-free and overall survival in a cohort of patients with HER2+ breast cancer. When overexpressed in cell lines,

EphA2 conferred resistance to trastuzumab. Moreover, cell lines with acquired trastuzumab resistance showed increased activation of EphA2, and treatment with an EphA2 neutralizing antibody restored trastuzumab sensitivity.⁷¹ Most recently, the erythropoietin receptor was found to be co-expressed in cell lines and primary tumors that overexpress HER2. In these cell lines, concurrent treatment with recombinant human erythropoietin conferred trastuzumab resistance. This was mediated through signaling through Jak and Src, leading to inactivation of the lipid phosphatase PTEN. Finally, in patients with HER2+ metastatic breast cancer, the concurrent administration of erythropoietin and trastuzumab correlated with a shorter progression-free and overall survival compared to patients not receiving erythropoietin.⁷²

Several groups have shown an increase in HER3 transcription and HER3 phosphorylation after short-term treatment with lapatinib.^{73–75} This response follows the inhibition of AKT and AKT-mediated phosphorylation of FoxO followed by translocation of FoxO to the nucleus and derepression of FoxO-mediated HER3 transcription. Although not necessarily a mechanism of resistance, it is of interest that inhibition of HER3 with genetic or pharmacological means greatly enhances the action of lapatinib. Finally, in a model of acquired resistance to lapatinib, the RTK AXL was up-regulated in the resistant cells. AXL potentially engaged p85 to activate PI3K and bypass the effects of either lapatinib or trastuzumab. A multikinase inhibitor with activity against AXL restored sensitivity to HER2 antagonists.⁷⁶

B. Intracellular Kinases

Mutations in components of the PI3K/AKT pathway are the most frequent tumor somatic alterations in breast cancer, altogether occurring in >30% of invasive tumors. These include gain of function mutations in PIK3CA, the gene encoding the p110 α catalytic subunit of PI3K, AKT1, or PIK3R1 (p85 α), amplification of PIK3CA or AKT2, loss of PTEN, the lipid phosphatase that dephosphorylates PIP3 and negatively regulates PI3K, and loss of the tumor suppressor INPP4B.^{77–83} It is generally accepted that the antitumor activity of HER2 inhibitors depends on inhibition of PI3K-AKT downstream of HER2.^{6,21} Thus, it is not surprising that activating mutations in the PI3K pathway itself can confer resistance to HER2 inhibitors.

Using a large-scale siRNA genetic screen, Berns et al. identified PTEN as the only gene whose knockdown conferred trastuzumab resistance. Further, induced overexpression of gain-of-function PIK3CA mutants similarly conferred trastuzumab resistance. Finally, patients with “hot spot” PIK3CA mutations and undetectable or low PTEN measured by IHC, exhibited a poorer outcome after treatment with chemotherapy and trastuzumab compared to patients without those alterations.⁸⁴ An earlier study had also shown that loss of PTEN correlates with a lower response to trastuzumab in patients.⁸⁵ More recently, Esteva et al. found that PI3K pathway activation, defined as PTEN loss and/or PIK3CA mutation, was associated with a poor response to trastuzumab as well as a poorer overall survival.⁸⁶ In addition, human breast cancer cell lines containing endogenous mutations in PIK3CA are intrinsically resistant to trastuzumab.^{6,21,87,88} Human breast cancer cell lines in which PIK3CA mutations are ectopically expressed exhibit an attenuated response to lapatinib.⁸⁹ On the other hand, loss of PTEN has not been consistently associated with resistance to lapatinib.⁹⁰ The centrality of the PI3K pathway in *de novo* and acquired drug resistance is further underscored by the effects of inhibitors of PI3K/AKT in resistant cells. For example, combinations of trastuzumab with the PI3K inhibitor XL147, or trastuzumab or lapatinib with the dual PI3K-mTOR inhibitor BEZ235, inhibit growth of PIK3CA mutant xenografts resistant to anti-HER2 therapies.^{89,91,92} In a model of trastuzumab resistance caused by PTEN loss, targeting mTOR or AKT was able to at least partially overcome resistance.⁹³ Evidence is also emerging from the clinic that targeting the PI3K axis in

addition to HER2 may be a strategy to overcome resistance. For example, in a phase II study, the combination of the TORC1 inhibitor everolimus with trastuzumab and chemotherapy showed a partial response rate of 19% and a clinical benefit rate of 81% in patients with HER2+ metastatic breast cancer that had previously shown progression on trastuzumab plus taxanes.⁹⁴ Some of these alterations in the PI3K/ATK pathway are diagrammed in Fig. 1C.

Using a mass spectrometry approach to identify aberrant tyrosine phosphorylation in HER2+ breast cancer cells with acquired resistance to lapatinib, we found up-regulation of Src family kinase (SFK) activity, particularly Yes, in several of the resistant cell lines. Resistance was associated with recovery of PI3K-AKT signaling despite continued inhibition of HER2 with lapatinib. Combination of lapatinib and a SFK inhibitor partially blocked PI3K activation in the resistant cells and restored sensitivity to lapatinib in BT474 xenografts.⁹⁵ There also appears to be a role for Src activity in resistance to trastuzumab. In trastuzumab-resistant HER2+ cells, PTEN was no longer capable of dephosphorylating Src. Addition of Src inhibitor to trastuzumab overcame trastuzumab resistance.⁹⁶ Src activity is also involved in the resistance conferred by the D16 HER2 isoform, where combination of trastuzumab with Src inhibitor was able to restore trastuzumab action.³⁹ Src kinase mediates at least part of the resistance conferred by activation of the erythropoietin receptor. EpoR activates Src via Jak2 and Src associates with HER2, where it is proposed to phosphorylate and inhibit PTEN. The inhibition of PTEN, in turn, up-regulates PI3K, providing a viable mechanism for attenuation of trastuzumab action.⁷² Finally, the activation of EphA2 seen after chronic trastuzumab treatment and onset of trastuzumab resistance is also mediated through Src.⁷¹

IV. DEFECTS IN APOPTOSIS AND CELL CYCLE CONTROL

In “oncogene-addicted” tumors, such as HER2+ breast cancer, intracellular survival and proliferation signals are under the dominant control of a single oncogene. In these tumors, targeting that oncogene product is expected to not only arrest proliferation but also induce apoptosis. Therefore, it is not surprising that alterations in the normal apoptotic machinery are emerging as important causes of resistance to oncogene-targeted therapies. In a recent report, levels of the pro-apoptotic BH3-only Bcl2 family member BIM were shown to be predictive of response to targeted therapy, not only in HER2+ breast cancer, but also in NSCLC harboring EGFR mutations and other cancers with mutations in the PIK3CA gene. In this study, although lapatinib inhibited HER2 and downstream signaling in all HER2+ breast cancer cell lines, only the cell lines with high basal levels of BIM underwent apoptosis.⁹⁷ This suggests that BIM levels might be a biomarker predictive of a likelihood of response to a TKI in an oncogene-addicted cancer. Another group reported similar results in HER2 gene-amplified breast cell lines with and without activating mutations. Lapatinib-induced apoptosis was associated with BIM up-regulation and depended on basal levels of BIM expression. In PIK3CA mutant cells, however, both the growth inhibitory effect and induction of BIM following treatment with lapatinib were blunted.⁹⁸

Survivin is a member of the inhibitor of apoptosis (IAP) protein family, which inhibits the activity of caspases, important effectors of programmed cell death. Modulation of survivin levels has been shown to be a point of convergence of several pathways that bypass the action of HER2 inhibitors. HER2+ breast cancer cells with acquired resistance to lapatinib up-regulated ERa, which, in turn, induced FoxO3a-mediated transcription of survivin. High levels of survivin allowed for escape of these cells from lapatinib.⁹⁹ Elevated levels of survivin and the antiapoptotic Mcl-1 proteins were found in trastuzumab-resistant cells; treatment with a broad-spectrum kinase inhibitor that reduces levels of survivin and Mcl-1 inhibited the growth and survival of the cells.¹⁰⁰ Knockdown of survivin followed by

trastuzumab restored sensitivity to the HER2 antibody.¹⁰¹ Furthermore, survivin expression is regulated by PI3K signaling in HER2-amplified breast cancer cells. Inhibition of HER2-PI3K reduces survivin expression simultaneous with drug-induced apoptosis in HER2-overexpressing breast cancer cells.^{98,102}

Altered control of progression through the cell cycle in response to HER2 inhibition also plays a role in resistance. Cell lines made resistant to trastuzumab by chronic exposure showed focal amplification of cyclin E. In a cohort of patients with HER2+ breast cancers treated with trastuzumab, amplification of cyclin E was associated with lower response to trastuzumab. Finally, CDK2 inhibitors reduced growth of trastuzumab-resistant xenografts.¹⁰³ Alternatively, down-regulation of the Cdk inhibitor p27^{KIP1} and a resulting increase in Cdk activity is also associated with trastuzumab resistance.¹⁰⁴ Modulation of levels of p27^{KIP1} appears to be a common endpoint for several of the resistance pathways noted above, including signaling from IGF-1R and MET.^{61,62,70} These data are in agreement with earlier results in which treatment with trastuzumab in antibody-sensitive cells resulted in an increase in p27 levels and nuclear localization.⁶

V. HOST FACTORS REQUIRED FOR TRASTUZUMAB ACTION

The resistance mechanisms to HER2 inhibition described thus far have been tumor cell autonomous. However, there is strong evidence to support that trastuzumab exerts its antitumor effect via the engagement of host immune effectors and subsequent antibody-dependent, cell-mediated cytotoxicity (ADCC) in addition to HER2 down-regulation and inhibition of post-receptor signaling. Thus, host factors that affect this immunomodulatory function can also contribute to trastuzumab resistance. In mice lacking FcγRIII and, thus, deficient in NK cells and macrophages capable of binding the Fc region of trastuzumab, the therapeutic effect of trastuzumab was markedly diminished.¹⁶ Consistent with this important preclinical experiment, polymorphisms in the gene encoding FcγRIII in humans are associated with response to trastuzumab. Notably, PBMCs from patients with polymorphisms associated with an improved outcome after trastuzumab induce a stronger trastuzumab-mediated ADCC in vitro.¹⁰⁵ Moreover, an inability of host (patient) cells to mediate a sufficiently strong ADCC response may contribute to de novo resistance, as leukocytes from those patients who did derive benefit from trastuzumab show a higher level of in vitro ADCC activity than those who did not.¹⁰⁶ A follow-up study found that the quantity and lytic efficiency of CD16(+) lymphocytes are the major factors to affect the level of ADCC induction by trastuzumab, which, in turn, correlated with tumor response.¹⁰⁷

VI. OVERCOMING RESISTANCE WITH COMBINATIONS OF ANTI-HER2 THERAPIES

The majority of patients with HER2+ metastatic breast cancer progress after an initial clinical response of variable duration. Several lines of clinical data suggest that HER2+ breast cancers continue to depend on HER2 at the time of progression. For example, the HER2 TKI lapatinib is effective in patients who progress on trastuzumab.⁹ Furthermore, continuation of trastuzumab in combination with chemotherapy after progression on trastuzumab is associated with a better progression-free survival compared to chemotherapy alone.¹⁰⁸ A post-hoc analysis of this last study showed that patients receiving anti-HER2 treatment even as third-line therapy had a superior progression-free survival compared to those who did not continue HER2-directed therapy.¹⁰⁹ Like lapatinib, the dual EGFR/HER2 TKI neratinib has shown clinical activity in patients with HER2+ metastatic breast cancer who progress on trastuzumab.¹¹⁰ Moreover, the combination of lapatinib plus trastuzumab was more effective than lapatinib alone following progression on trastuzumab.¹¹¹ More recently, the NeoALTTO study compared trastuzumab, lapatinib, and both inhibitors, in

combination with paclitaxel for 12 weeks prior to breast surgery. The combination arm exhibited a higher rate of pathologic complete response (51.3%) than either the trastuzumab (29.5%) or lapatinib (24.7%) arms.¹¹²

Other combination strategies that exploit this continued dependence on HER2 at the time of acquired resistance are in clinical development. The antibody-toxin fusion trastuzumab-DM1 consists of trastuzumab conjugated via a non-cleavable linker to a derivative of maytansine, an inhibitor of microtubule polymerization. T-DM1 has shown high tolerability and remarkable clinical activity in phase I-II trials. T-DM1 induces a 25% clinical response rate in heavily pretreated patients with HER2-overexpressing metastatic breast cancer who had progressed on trastuzumab, lapatinib, taxanes, and anthracyclines.^{113,114} Even though it is used at lower doses and frequency than trastuzumab, T-DM1 can still inhibit signaling and engage an ADCC response in lapatinib-resistant xenografts.¹¹⁵ Targeting HER2 with trastuzumab in combination with pertuzumab, an antibody that binds to an epitope in the extracellular heterodimerization domain of HER2, is also an effective strategy. Treatment with pertuzumab in combination with trastuzumab, but not either alone, was effective in trastuzumab-resistant xenografts and in patients who had previously progressed on trastuzumab.^{116,117} It is proposed that both antibodies are required to completely disable ligand-induced and ligand-independent HER2-HER3 heterodimers and, therefore, maximally inhibit PI3K signaling.²¹ In the recent neoadjuvant NeoSphere trial, patients with newly diagnosed HER2+ breast cancer treated with chemotherapy plus trastuzumab and pertuzumab in combination achieved a pathologic complete response rate of 45.8%, significantly higher than the 29% rate in the chemotherapy plus trastuzumab arm.¹¹⁸ In Table 1, we summarize several combinations of anti-HER2 targeted therapies and their rationale.

VII. CONCLUSIONS

Several mechanisms of resistance to both trastuzumab and lapatinib have been identified in preclinical studies. Few of these have been prospectively validated in the clinic. However, there is enough evidence to suggest that some of them do limit the effectiveness of HER2-directed therapy, particularly when these therapies are used as single agents. Emerging clinical data suggest the concept that combinations of therapies targeting the HER2 signaling network at multiple points early in the natural history of HER2+ breast cancer can abrogate drug resistance. What remains a challenge is determining the resistance mechanism(s) in a particular patient so that potential therapies can be individualized. This will require commitment to rebiopsy and molecular analysis of recurrent tumors after progression on primary HER2-targeted therapy. Alternatively, the increasing use of pre-operative therapy should provide a clinical research platform where combinations of anti-HER2 agents can be compared using pathological complete response in the breast as a clinical endpoint predictive of long-term outcome. Another benefit of a pre-operative platform is that residual tumor tissue is available at the time of surgery. These “drug resistant” residual cancers may well reflect the molecular profile of drug-resistant micrometastases and can be interrogated with open-ended molecular approaches to identify biomarkers and/or effectors of resistance to anti-HER2 therapies.

Acknowledgments

Supported in part by Department of Defense BC087465 Post-Doctoral Fellowship, ASCO Young Investigator Award, NCI K08 grant CA143153, and Vanderbilt Personalized Medicine Core grant P30 GM092386 (BNR); and NCI R01 grant CA80195, ACS Clinical Research Professorship Grant CRP-07-234, the Lee Jeans Translational Breast Cancer Research Program, Breast Cancer Specialized Program of Research Excellence (SPORE) P50 CA98131, and Vanderbilt-Ingram Cancer Center Support Grant P30 CA68485 (CLA).

Abbreviations

RTK	receptor tyrosine kinase
SH2	Src homology 2
PI3K	phosphatidylinositol 3-kinase
FDA	Food and Drug Administration
ATP	adenosine triphosphate
IHC	immunohistochemistry
FISH	fluorescence in situ hybridization
CDK	cyclin dependent kinase
TKI	tyrosine kinase inhibitor
kDa	kilodalton
NSCLC	non-small-cell lung cancer
PIP3	phosphatidylinositol (3,4,5)-trisphosphate
SFK	Src family kinase
IAP	inhibitor of apoptosis
ADCC	antibody-dependent cell-mediated cytotoxicity
PBMC	peripheral blood mononuclear cells
T-DM1	trastuzumab emtansine (trastuzumab conjugated derivative of maytansine 1)

REFERENCES

1. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Bio.* 2001 Mar; 2(2):127–137. [PubMed: 11252954]
2. Alimandi M, Romano A, Curia MC, Muraro R, Fedi P, Aaronson SA, Di Fiore PP, Kraus MH. Cooperative signaling of ErbB3 and ErbB2 in neoplastic transformation and human mammary carcinomas. *Oncogene.* 1995 Jun 04; 10(9):1813–1821. [PubMed: 7538656]
3. 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 Jul 15; 68(14):5878–5887. [PubMed: 18632642]
4. 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 Aug 15; 13(16):4909–4919. [PubMed: 17699871]
5. Wallasch C, Weiss FU, Niederfellner G, Jallal B, Issing W, Ullrich A. Heregulin-dependent regulation of HER2/neu oncogenic signaling by heterodimerization with HER3. *EMBO J.* 1995 Sep 01; 14(17):4267–4275. [PubMed: 7556068]
6. Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL. Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. *Cancer Res.* 2002 Jul 15; 62(14):4132–4141. [PubMed: 12124352]
7. 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 Jan 9; 235(4785):177–182. [PubMed: 3798106]
8. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science.* 1989 May 12; 244(4905):707–712. [PubMed: 2470152]

9. Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, Skarlos D, Campone M, Davidson N, Berger M, Oliva C, Rubin SD, Stein S, Cameron D. Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med*. 2006 Dec 28; 355(26):2733–2743. [PubMed: 17192538]
10. Joensuu H, Kellokumpu-Lehtinen P-L, Bono P, Alanko T, Kataja V, Asola R, Utriainen T, Kokko R, Hemminki A, Tarkkanen M, Turpeenniemi-Hujanen T, Jyrkkö S, Flander M, Helle L, Ingalsuo S, Johansson K, Jääskeläinen A-S, Pajunen M, Rauhalä M, Kaleva-Kerola J, Salminen T, Leinonen M, Elomaa I, Isola J, Investigators FS. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N Engl J Med*. 2006 Feb 23; 354(8):809–820. [PubMed: 16495393]
11. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CH, Steger G, Huang C-S, Andersson M, Inbar M, Lichinitser M, Láng I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter TM, Rüschoff J, Suto T, Grotzer V, Ward C, Straehle C, McFadden E, Dolci MS, Gelber RD. Team HAHTS. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med*. 2005 Oct 20; 353(16):1659–1672. [PubMed: 16236737]
12. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med*. 2005 Oct 20; 353(16):1673–1684. [PubMed: 16236738]
13. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med*. 2001 Mar 15; 344(11):783–792. [PubMed: 11248153]
14. Press MF, Finn RS, Cameron D, Di Leo A, Geyer CE, Villalobos IE, Santiago A, Guzman R, Gasparyan A, Ma Y, Danenberg K, Martin AM, Williams L, Oliva C, Stein S, Gagnon R, Arbushites M, Koehler MT. HER-2 gene amplification, HER-2 and epidermal growth factor receptor mRNA and protein expression, and lapatinib efficacy in women with metastatic breast cancer. *Clin Cancer Res*. 2008 Dec 01; 14(23):7861–7870. [PubMed: 19047115]
15. Park S, Jiang Z, Mortenson ED, Deng L, Radkevich-Brown O, Yang X, Sattar H, Wang Y, Brown NK, Greene M, Liu Y, Tang J, Wang S, Fu Y-X. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. *Cancer Cell*. 2010 Aug 09; 18(2):160–170. [PubMed: 20708157]
16. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat Med*. 2000 Apr; 6(4):443–446. [PubMed: 10742152]
17. Le X-F, Lammayot A, Gold D, Lu Y, Mao W, Chang T, Patel A, Mills GB, Bast RC. Genes affecting the cell cycle, growth, maintenance, and drug sensitivity are preferentially regulated by anti-HER2 antibody through phosphatidylinositol 3-kinase-AKT signaling. *J Biol Chem*. 2005 Jan 21; 280(3):2092–2104. [PubMed: 15504738]
18. Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL. Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. *Cancer Res*. 2002 Jul 15; 62(14):4132–4141. [PubMed: 12124352]
19. Mittendorf EA, Liu Y, Tucker SL, McKenzie T, Qiao N, Akli S, Biernacka A, Liu Y, Meijer L, Keyomarsi K, Hunt KK. A novel interaction between HER2/neu and cyclin E in breast cancer. *Oncogene*. 2010 Jul 8; 29(27):3896–3907. [PubMed: 20453888]
20. Lane HA, Beuvink I, Motoyama AB, Daly JM, Neve RM, Hynes NE. ErbB2 potentiates breast tumor proliferation through modulation of p27(Kip1)-Cdk2 complex formation: receptor overexpression does not determine growth dependency. *Mol Cell Biol*. 2000 May; 20(9):3210–3223. [PubMed: 10757805]
21. 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 Jun 05; 15(5):429–440. [PubMed: 19411071]

22. Mohsin SK, Weiss HL, Gutierrez MC, Chamness GC, Schiff R, Digiovanna MP, Wang C-X, Hilsenbeck SG, Osborne CK, Allred DC, Elledge R, Chang JC. Neoadjuvant trastuzumab induces apoptosis in primary breast cancers. *J Clin Oncol*. 2005 Apr 10; 23(11):2460–2468. [PubMed: 15710948]
23. Konecny GE, Pegram MD, Venkatesan N, Finn R, Yang G, Rahmeh M, Untch M, Rusnak DW, Spehar G, Mullin RJ, Keith BR, Gilmer TM, Berger M, Podratz KC, Slamon DJ. Activity of the dual kinase inhibitor lapatinib (GW572016) against HER-2-overexpressing and trastuzumab-treated breast cancer cells. *Cancer Res*. 2006 Feb 1; 66(3):1630–1639. [PubMed: 16452222]
24. Xia W, Mullin RJ, Keith BR, Liu L-H, Ma H, Rusnak DW, Owens G, Alligood KJ, Spector NL. Anti-tumor activity of GW572016: a dual tyrosine kinase inhibitor blocks EGF activation of EGFR/erbB2 and downstream Erk1/2 and AKT pathways. *Oncogene*. 2002 Sep 12; 21(41):6255–6263. [PubMed: 12214266]
25. Spector NL, Xia W, Burris H, Hurwitz H, Dees EC, Dowlati A, O'Neil B, Overmoyer B, Marcom PK, Blackwell KL, Smith DA, Koch KM, Stead A, Mangum S, Ellis MJ, Liu L, Man AK, Bremer TM, Harris J, Bacus S. Study of the biologic effects of lapatinib, a reversible inhibitor of ErbB1 and ErbB2 tyrosine kinases, on tumor growth and survival pathways in patients with advanced malignancies. *J Clin Oncol*. 2005 Apr 10; 23(11):2502–2512. [PubMed: 15684311]
26. Dave B, Migliaccio I, Gutierrez MC, Wu M-F, Chamness GC, Wong H, Narasanna A, Chakrabarty A, Hilsenbeck SG, Huang J, Rimawi M, Schiff R, Arteaga C, Osborne CK, Chang JC. Loss of phosphatase and tensin homolog or phosphoinositol-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2-overexpressing locally advanced breast cancers. *J Clin Oncol*. 2011 Feb 10; 29(2):166–173. [PubMed: 21135276]
27. Engelman JA, Mukohara T, Zejnullahu K, Lifshits E, Borrás AM, Gale C-M, Naumov GN, Yeap BY, Jarrell E, Sun J, Tracy S, Zhao X, Heymach JV, Johnson BE, Cantley LC, Jänne PA. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. *J Clin Invest*. 2006 Oct 1; 116(10):2695–2706. [PubMed: 16906227]
28. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*. 2001 Aug 3; 293(5531):876–880. [PubMed: 11423618]
29. Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, Halmos B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2005 Feb 24; 352(8):786–792. [PubMed: 15728811]
30. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med*. 2005 Mar 1.2(3):e73. [PubMed: 15737014]
31. Kosaka T, Yatabe Y, Endoh H, Yoshida K, Hida T, Tsuboi M, Tada H, Kuwano H, Mitsudomi T. Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. *Clin Cancer Res*. 2006 Oct 1; 12(19):5764–5769. [PubMed: 17020982]
32. Tamborini E, Bonadiman L, Greco A, Albertini V, Negri T, Gronchi A, Bertulli R, Colecchia M, Casali PG, Pierotti MA, Pilotti S. A new mutation in the KIT ATP pocket causes acquired resistance to imatinib in a gastrointestinal stromal tumor patient. *Gastroenterology*. 2004 Jul; 127(1):294–299. [PubMed: 15236194]
33. Anido J, Scaltriti M, Bech Serra JJ, Santiago Josef B, Todo FR, Baselga J, Arribas J. Biosynthesis of tumorigenic HER2 C-terminal fragments by alternative initiation of translation. *EMBO J*. 2006 Jul 12; 25(13):3234–3244. [PubMed: 16794579]
34. Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, Di Cosimo S, Matias-Guiu X, Ramon y Cajal S, Arribas J, Baselga J. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst*. 2007 May 18; 99(8):628–638. [PubMed: 17440164]
35. Scaltriti M, Chandarlapaty S, Prudkin L, Aura C, Jimenez J, Angelini PD, Sánchez G, Guzman M, Parra JL, Ellis C, Gagnon R, Koehler M, Gomez H, Geyer C, Cameron D, Arribas J, Rosen N, Baselga J. Clinical benefit of lapatinib-based therapy in patients with human epidermal growth factor receptor 2-positive breast tumors coexpressing the truncated p95HER2 receptor. *Clin Cancer Res*. 2010 Jun 01; 16(9):2688–2695. [PubMed: 20406840]

36. Xia W, Liu Z, Zong R, Liu L, Zhao S, Bacus SS, Mao Y, He J, Wulfkühle JD, Petricoin EF 3rd, Osada T, Yang XY, Hartman ZC, Clay TM, Blackwell KL, Lysterly HK, Spector NL. Truncated ErbB2 expressed in tumor cell nuclei contributes to acquired therapeutic resistance to ErbB2 kinase inhibitors. *Mol Cancer Ther.* 2011 Aug; 10(8):1367–1374. [PubMed: 21673090]
37. Kwong KY, Hung MC. A novel splice variant of HER2 with increased transformation activity. *Mol Carcinog.* 1998 Oct; 23(2):62–68. [PubMed: 9808159]
38. Castiglioni F, Tagliabue E, Campiglio M, Pupa SM, Balsari A, Ménard S. Role of exon-16-deleted HER2 in breast carcinomas. *Endocr-Relat Cancer.* 2006 Apr; 13(1):221–232. [PubMed: 16601290]
39. Mitra D, Brumlik MJ, Okamgba SU, Zhu Y, Duplessis TT, Parvani JG, Lesko SM, Brogi E, Jones FE. An oncogenic isoform of HER2 associated with locally disseminated breast cancer and trastuzumab resistance. *Mol Cancer Ther.* 2009 Aug; 8(8):2152–2162. [PubMed: 19671734]
40. Buttitta F, Barassi F, Fresu G, Felicioni L, Chella A, Paolizzi D, Lattanzio G, Salvatore S, Camplese PP, Rosini S, Iarussi T, Mucilli F, Sacco R, Mezzetti A, Marchetti A. Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. *Int J Cancer.* 2006 Dec 1; 119(11):2586–2591. [PubMed: 16988931]
41. Lee JW, Soung YH, Seo SH, Kim SY, Park CH, Wang YP, Park K, Nam SW, Park WS, Kim SH, Lee JY, Yoo NJ, Lee SH. Somatic mutations of ERBB2 kinase domain in gastric, colorectal, and breast carcinomas. *Clin Cancer Res.* 2006 Jan 1; 12(1):57–61. [PubMed: 16397024]
42. Mounawar M, Mukeria A, Le Calvez F, Hung RJ, Renard H, Cortot A, Bollart C, Zaridze D, Brennan P, Boffetta P, Brambilla E, Hainaut P. Patterns of EGFR, HER2, TP53, and KRAS mutations of p14arf expression in non-small cell lung cancers in relation to smoking history. *Cancer Res.* 2007 Jun 15; 67(12):5667–5672. [PubMed: 17575133]
43. Shigematsu H, Takahashi T, Nomura M, Majmudar K, Suzuki M, Lee H, Wistuba II, Fong KM, Toyooka S, Shimizu N, Fujisawa T, Minna JD, Gazdar AF. Somatic mutations of the HER2 kinase domain in lung adenocarcinomas. *Cancer Res.* 2005 Mar 1; 65(5):1642–1646. [PubMed: 15753357]
44. Stephens P, Hunter C, Bignell G, Edkins S, Davies H, Teague J, Stevens C, O'Meara S, Smith R, Parker A, Barthorpe A, Blow M, Brackenbury L, Butler A, Clarke O, Cole J, Dicks E, Dike A, Drozd A, Edwards K, Forbes S, Foster R, Gray K, Greenman C, Halliday K, Hills K, Kosmidou V, Lugg R, Menzies A, Perry J, Petty R, Raine K, Ratford L, Shepherd R, Small A, Stephens Y, Tofts C, Varian J, West S, Widaa S, Yates A, Brasseur F, Cooper CS, Flanagan AM, Knowles M, Leung SY, Louis DN, Looijenga LHJ, Malkowicz B, Pierotti MA, Teh B, Chenevix-Trench G, Weber BL, Yuen ST, Harris G, Goldstraw P, Nicholson AG, Futreal PA, Wooster R, Stratton MR. Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature.* 2004 Sep 30; 431(7008):525–526. [PubMed: 15457249]
45. Willmore-Payne C, Holden JA, Layfield LJ. Detection of epidermal growth factor receptor and human epidermal growth factor receptor 2 activating mutations in lung adenocarcinoma by high-resolution melting amplicon analysis: correlation with gene copy number, protein expression, and hormone receptor expression. *Hum Pathol.* 2006 Jun 1; 37(6):755–763. [PubMed: 16733218]
46. Willmore-Payne C, Holden JA, Layfield LJ. Detection of EGFR- and HER2-activating mutations in squamous cell carcinoma involving the head and neck. *Mod Pathol.* 2006 May 1; 19(5):634–640. [PubMed: 16547470]
47. Wang SE, Narasanna A, Perez-Torres M, Xiang B, Wu FY, Yang S, Carpenter G, Gazdar AF, Muthuswamy SK, Arteaga CL. HER2 kinase domain mutation results in constitutive phosphorylation and activation of HER2 and EGFR and resistance to EGFR tyrosine kinase inhibitors. *Cancer Cell.* 2006 Jul 1; 10(1):25–38. [PubMed: 16843263]
48. Zito CI, Riches D, Kolmakova J, Simons J, Egholm M, Stern DF. Direct resequencing of the complete ERBB2 coding sequence reveals an absence of activating mutations in ERBB2 amplified breast cancer. *Genes Chromosomes Cancer.* 2008 Jul; 47(7):633–638. [PubMed: 18418848]
49. Nagy P, Friedländer 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 Feb 15; 65(2):473–482. [PubMed: 15695389]

50. Price-Schiavi SA, Jepson S, Li P, Arango M, Rudland PS, Yee L, Carraway KL. Rat Muc4 (sialomucin complex) reduces binding of anti-ErbB2 antibodies to tumor cell surfaces, a potential mechanism for herceptin resistance. *Int J Cancer*. 2002 Jul 20; 99(6):783–791. [PubMed: 12115478]
51. Fessler SP, Wotkowicz MT, Mahanta SK, Bamdad C. MUC1* is a determinant of trastuzumab (Herceptin) resistance in breast cancer cells. *Breast Cancer Res Treat*. 2009 Nov; 118(1):113–124. [PubMed: 19415485]
52. Li Y, Yu WH, Ren J, Chen W, Huang L, Kharbanda S, Loda M, Kufe D. Heregulin targets gamma-catenin to the nucleolus by a mechanism dependent on the DF3/MUC1 oncoprotein. *Mol Cancer Res*. 2003 Aug; 1(10):765–775. [PubMed: 12939402]
53. Hikita ST, Kosik KS, Clegg DO, Bamdad C. MUC1* mediates the growth of human pluripotent stem cells. *PloS one*. 2008; 3(10):e3312. [PubMed: 18833326]
54. Mahanta S, Fessler SP, Park J, Bamdad C. A minimal fragment of MUC1 mediates growth of cancer cells. *PloS ONE*. 2008; 3(4):e2054. [PubMed: 18446242]
55. Motoyama AB, Hynes NE, Lane HA. The efficacy of ErbB receptor-targeted anticancer therapeutics is influenced by the availability of epidermal growth factor-related peptides. *Cancer Res*. 2002 Jul 01; 62(11):3151–3158. [PubMed: 12036928]
56. Moulder SL, Yakes FM, Muthuswamy SK, Bianco R, Simpson JF, Arteaga CL. Epidermal growth factor receptor (HER1) tyrosine kinase inhibitor ZD1839 (Iressa) inhibits HER2/neu (erbB2)-overexpressing breast cancer cells in vitro and in vivo. *Cancer Res*. 2001 Dec 15; 61(24):8887–8895. [PubMed: 11751413]
57. Agus DB, Akita RW, Fox WD, Lewis GD, Higgins B, Pisacane PI, Lofgren JA, Tindell C, Evans DP, Maiese K, Scher HI, Sliwkowski MX. Targeting ligand-activated ErbB2 signaling inhibits breast and prostate tumor growth. *Cancer Cell*. 2002 Aug; 2(2):127–137. [PubMed: 12204533]
58. Wang SE, Xiang B, Guix M, Olivares MG, Parker J, Chung CH, Pandiella A, Arteaga CL. Transforming growth factor beta engages TACE and ErbB3 to activate phosphatidylinositol-3 kinase/Akt in ErbB2-overexpressing breast cancer and desensitizes cells to trastuzumab. *Mol Cell Biol*. 2008 Sep; 28(18):5605–5620. [PubMed: 18625725]
59. Adams CW, Allison DE, Flagella K, Presta L, Clarke J, Dybdal N, McKeever K, Sliwkowski MX. Humanization of a recombinant monoclonal antibody to produce a therapeutic HER dimerization inhibitor, pertuzumab. *Cancer Immunol Immunother*. 2006 Jul; 55(6):717–727. [PubMed: 16151804]
60. Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell*. 2004 May; 5(4):317–328. [PubMed: 15093539]
61. 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 Dec 19; 93(24):1852–1857. [PubMed: 11752009]
62. Nahta R, Yuan LXH, 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 Dec 01; 65(23):11118–11128. [PubMed: 16322262]
63. Huang X, Gao L, Wang S, McManaman JL, Thor AD, Yang X, Esteva FJ, Liu B. Heterotrimerization of the growth factor receptors erbB2, erbB3, and insulin-like growth factor-I receptor in breast cancer cells resistant to herceptin. *Cancer Res*. 2010 Feb 1; 70(3):1204–1214. [PubMed: 20103628]
64. Browne BC, Crown J, Venkatesan N, Duffy MJ, Clynes M, Slamon D, O'Donovan N. Inhibition of IGF1R activity enhances response to trastuzumab in HER-2-positive breast cancer cells. *Ann Oncol*. 2011 Jan; 22(1):68–73. [PubMed: 20647220]
65. Nahta R, Yuan LX, Du Y, Esteva FJ. Lapatinib induces apoptosis in trastuzumab-resistant breast cancer cells: effects on insulin-like growth factor I signaling. *Mol Cancer Ther*. 2007 Feb; 6(2):667–674. [PubMed: 17308062]

66. Liu B, Fan Z, Edgerton SM, Yang X, Lind SE, Thor AD. Potent anti-proliferative effects of metformin on trastuzumab-resistant breast cancer cells via inhibition of erbB2/IGF-1 receptor interactions. *Cell Cycle*. 2011 Sep 01; 10(17):2959–2966. [PubMed: 21862872]
67. Harris LN, You F, Schnitt SJ, Witkiewicz A, Lu X, Sgroi D, Ryan PD, Come SE, Burstein HJ, Lesnikowski B-A, Kamma M, Friedman PN, Gelman R, Iglehart JD, Winer EP. Predictors of resistance to preoperative trastuzumab and vinorelbine for HER2-positive early breast cancer. *Clin Cancer Res*. 2007 Mar 15; 13(4):1198–1207. [PubMed: 17317830]
68. Kostler WJ, Hudelist G, Rabitsch W, Czerwenka K, Muller R, Singer CF, Zielinski CC. Insulin-like growth factor-1 receptor (IGF-1R) expression does not predict for resistance to trastuzumab-based treatment in patients with Her-2/neu overexpressing metastatic breast cancer. *J Cancer Res Clin Oncol*. 2006 Jan; 132(1):9–18. [PubMed: 16184380]
69. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, Lindeman N, Gale C-M, Zhao X, Christensen J, Kosaka T, Holmes AJ, Rogers AM, Cappuzzo F, Mok T, Lee C, Johnson BE, Cantley LC, Janne PA. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science*. 2007 Jun 18; 316(5827):1039–1043. [PubMed: 17463250]
70. Shattuck DL, Miller JK, Carraway KL, Sweeney C. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. *Cancer Res*. 2008 Apr 01; 68(5):1471–1477. [PubMed: 18316611]
71. 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 Feb 01; 70(1):299–308. [PubMed: 20028874]
72. Liang K, Esteva FJ, Albarracín C, Stemke-Hale K, Lu Y, Bianchini G, Yang C-Y, Li Y, Li X, Chen C-T, Mills GB, Hortobagyi GN, Mendelsohn J, Hung M-C, Fan Z. Recombinant human erythropoietin antagonizes trastuzumab treatment of breast cancer cells via Jak2-mediated Src activation and PTEN inactivation. *Cancer Cell*. 2010 Nov 16; 18(5):423–435. [PubMed: 21075308]
73. Amin DN, Sergina N, Ahuja D, McMahon M, Blair JA, Wang D, Hann B, Koch KM, Shokat KM, Moasser MM. Resiliency and vulnerability in the HER2-HER3 tumorigenic driver. *Sci Transl Med*. 2010 Feb 27; 2(16):16ra7.
74. Garrett JT, Olivares MG, Rinehart C, Granja-Ingram ND, Sanchez V, Chakrabarty A, Dave B, Cook RS, Pao W, McKinley E, Manning HC, Chang J, Arteaga CL. Transcriptional and posttranslational up-regulation of HER3 (ErbB3) compensates for inhibition of the HER2 tyrosine kinase. *Proc Natl Acad Sci U S A*. 2011 Mar 22; 108(12):5021–5026. [PubMed: 21385943]
75. 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 Feb 25; 445(7126):437–441. [PubMed: 17206155]
76. 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 Sep 01; 69(17):6871–6878. [PubMed: 19671800]
77. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, Konishi H, Karakas B, Blair BG, Lin C, Peters BA, Velculescu VE, Park BH. The PIK3CA gene is mutated with high frequency in human breast cancers. *Cancer Biol Ther*. 2004 Aug 1; 3(8):772–775. [PubMed: 15254419]
78. Bellacosa A, de Feo D, Godwin AK, Bell DW, Cheng JQ, Altomare DA, Wan M, Dubeau L, Scambia G, Masciullo V, Ferrandina G, Benedetti Panici P, Mancuso S, Neri G, Testa JR. Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. *Int J Cancer*. 1995 Aug 22; 64(4):280–285. [PubMed: 7657393]
79. Campbell IG, Russell SE, Choong DYH, Montgomery KG, Ciavarella ML, Hooi CSF, Cristiano BE, Pearson RB, Phillips WA. Mutation of the PIK3CA gene in ovarian and breast cancer. *Cancer Res*. 2004 Nov 1; 64(21):7678–7681. [PubMed: 15520168]
80. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian Y-W, Zeckner DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai M-HT, Blanchard KL, Thomas JE. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature*. 2007 Jul 26; 448(7152):439–444. [PubMed: 17611497]

81. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science*. 1997 Mar 28; 275(5308):1943–1947. [PubMed: 9072974]
82. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, Yu JS, Malmström P-O, Mansukhani M, Enoksson J, Hibshoosh H, Borg A, Parsons R. PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. *Cancer Res*. 2005 Apr 1; 65(7):2554–2559. [PubMed: 15805248]
83. Gewinner C, Wang ZC, Richardson A, Teruya-Feldstein J, Etemadmoghadam D, Bowtell D, Barretina J, Lin WM, Rameh L, Salmena L, Pandolfi PP, Cantley LC. Evidence that inositol polyphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. *Cancer Cell*. 2009 Aug 04; 16(2):115–125. [PubMed: 19647222]
84. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, Linn SC, Gonzalez-Angulo AM, Stemke-Hale K, Hauptmann M, Beijersbergen RL, Mills GB, van de Vijver MJ, Bernards R. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell*. 2007 Oct; 12(4):395–402. [PubMed: 17936563]
85. Nagata Y, Lan K-H, Zhou X, Tan M, Esteva FJ, Sahin AA, Klos KS, Li P, Monia BP, Nguyen NT, Hortobagyi GN, Hung M-C, Yu D. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell*. 2004 Aug; 6(2):117–127. [PubMed: 15324695]
86. 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 Oct; 177(4):1647–1656. [PubMed: 20813970]
87. Ginestier C, Adelaide J, Goncalves A, Repellini L, Sircoulomb F, Letessier A, Finetti P, Geneix J, Charafe-Jauffret E, Bertucci F, Jacquemier J, Viens P, Birnbaum D. ERBB2 phosphorylation and trastuzumab sensitivity of breast cancer cell lines. *Oncogene*. 2007 Nov 1; 26(50):7163–7169. [PubMed: 17525746]
88. 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 Jun; 9(6):1489–1502. [PubMed: 20501798]
89. Eichhorn PJA, Gili M, Scaltriti M, Serra V, Guzman M, Nijkamp W, Beijersbergen RL, Valero V, Seoane J, Bernards R, Baselga J. Phosphatidylinositol 3-kinase hyperactivation results in lapatinib resistance that is reversed by the mTOR/phosphatidylinositol 3-kinase inhibitor NVP-BEZ235. *Cancer Res*. 2008 Nov 15; 68(22):9221–9230. [PubMed: 19010894]
90. 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 Feb 1; 67(3):1170–1175. [PubMed: 17283152]
91. Chakrabarty A, Rexer BN, Wang SE, Cook RS, Engelman JA, Arteaga CL. H1047R phosphatidylinositol 3-kinase mutant enhances HER2-mediated transformation by heregulin production and activation of HER3. *Oncogene*. 2010 Sep 16; 29(37):5193–5203. [PubMed: 20581867]
92. Serra V, Markman B, Scaltriti M, Eichhorn PJA, Valero V, Guzman M, Botero ML, Llonch E, Atzori F, Di Cosimo S, Maira M, Garcia-Echeverria C, Parra JL, Arribas J, Baselga J. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res*. 2008 Oct 01; 68(19):8022–8030. [PubMed: 18829560]
93. 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 Oct 1; 13(19):5883–5888. [PubMed: 17908983]

94. Dalenc F, Campone M, Hupperets P, O'Regan R, Manlius C, Vittori L, Mukhopadhyay P, Massacesi C, Sahmoud T, Andre F. Everolimus in combination with weekly paclitaxel and trastuzumab in patients (pts) with HER2-overexpressing metastatic breast cancer (MBC) with prior resistance to trastuzumab and taxanes: A multicenter phase II clinical trial. *J Clin Oncol*. 2010; 28 suppl(15s) abstr 1013.
95. Rexer BN, Ham AJ, Rinehart C, Hill S, de Matos Granja-Ingram N, Gonzalez-Angulo AM, Mills GB, Dave B, Chang JC, Liebler DC, Arteaga CL. Phosphoproteomic mass spectrometry profiling links Src family kinases to escape from HER2 tyrosine kinase inhibition. *Oncogene*. 2011 Oct 6; 30(40):4163–4174. [PubMed: 21499296]
96. Zhang S, Huang W-C, Li P, Guo H, Poh S-B, Brady SW, Xiong Y, Tseng L-M, Li S-H, Ding Z, Sahin AA, Esteva FJ, Hortobagyi GN, Yu D. Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nat Med*. 2011 May; 17(4):461–469. [PubMed: 21399647]
97. Faber A, Corcoran R, Ebi H, Sequist L. BIM expression in treatment-naïve cancers predicts responsiveness to kinase inhibitors. *Cancer Discovery*. 2011 Sep; 1(4):352–365. [PubMed: 22145099]
98. Tanizaki J, Okamoto I, Fumita S, Okamoto W, Nishio K, Nakagawa K. Roles of BIM induction and survivin downregulation in lapatinib-induced apoptosis in breast cancer cells with HER2 amplification. *Oncogene*. 2011 Sep 29; 30(39):4097–4106. [PubMed: 21499301]
99. Xia W, Bacus S, Hegde P, Husain I, Strum J, Liu L, Paulazzo G, Lyass L, Trusk P, Hill J, Harris J, Spector NL. 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 Jun 16; 103(20):7795–7800. [PubMed: 16682622]
100. Valabrega G, Capellero S, Cavalloni G, Zaccarello G, Petrelli A, Migliardi G, Milani A, Peraldo-Neia C, Gammaitoni L, Sapino A, Pecchioni C, Moggio A, Giordano S, Aglietta M, Montemurro F. HER2-positive breast cancer cells resistant to trastuzumab and lapatinib lose reliance upon HER2 and are sensitive to the multitargeted kinase inhibitor sorafenib. *Breast Cancer Res Treat*. 2011 Nov; 130(1):29–40. [PubMed: 21153051]
101. Oliveras-Ferraro C, Vazquez-Martin A, Cufí S, Torres-Garcia VZ, Sauri-Nadal T, Barco SD, Lopez-Bonet E, Brunet J, Martin-Castillo B, Menendez JA. Inhibitor of apoptosis (IAP) survivin is indispensable for survival of HER2 gene-amplified breast cancer cells with primary resistance to HER1/2-targeted therapies. *Biochem Biophys Res Commun*. 2011 May 08; 407(2):412–419. [PubMed: 21402055]
102. Faber AC, Li D, Song Y, Liang M-C, Yeap BY, Bronson RT, Lifshits E, Chen Z, Maira S-M, Garcia-Echeverria C, Wong K-K, Engelman JA. Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition. *Proc Natl Acad Sci U S A*. 2009 Nov 17; 106(46):19503–19508. [PubMed: 19850869]
103. Scaltriti M, Eichhorn PJ, Cortes J, Prudkin L, Aura C, Jimenez J, Chandarlapaty S, Serra V, Prat A, Ibrahim YH, Guzman M, Gili M, Rodríguez O, Rodríguez S, Pérez J, Green SR, Mai S, Rosen N, Hudis C, Baselga J. Cyclin E amplification/over-expression is a mechanism of trastuzumab resistance in HER2+ breast cancer patients. *Proc Natl Acad Sci U S A*. 2011 Apr 01; 108(9):3761–3766. [PubMed: 21321214]
104. Nahta R, Takahashi T, Ueno NT, Hung M-C, Esteva FJ. P27(kip1) down-regulation is associated with trastuzumab resistance in breast cancer cells. *Cancer Res*. 2004 Jul 01; 64(11):3981–3986. [PubMed: 15173011]
105. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, Missale G, Laccabue D, Zerbini A, Camisa R, Bisagni G, Neri TM, Ardizzoni A. 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 May 10; 26(11):1789–1796. [PubMed: 18347005]
106. Gennari R, Menard S, Fagnoni F, Ponchio L, Scelsi M, Tagliabue E, Castiglioni F, Villani L, Magalotti C, Gibelli N, Oliviero B, Ballardini B, Da Prada G, Zambelli A, Costa A. Pilot study of the mechanism of action of preoperative trastuzumab in patients with primary operable breast tumors overexpressing HER2. *Clin Cancer Res*. 2004 Sep 01; 10(17):5650–5655. [PubMed: 15355889]

107. Varchetta S, Gibelli N, Oliviero B, Nardini E, Gennari R, Gatti G, Silva LS, Villani L, Tagliabue E, Menard S, Costa A, Fagnoni FF. Elements related to heterogeneity of antibody-dependent cell cytotoxicity in patients under trastuzumab therapy for primary operable breast cancer overexpressing Her2. *Cancer Res.* 2007 Dec 15; 67(24):11991–11999. [PubMed: 18089830]
108. von Minckwitz G, du Bois A, Schmidt M, Maass N, Cufer T, de Jongh FE, Maartense E, Zielinski C, Kaufmann M, Bauer W, Baumann KH, Clemens MR, Duerr R, Uleer C, Andersson M, Stein RC, Nekljudova V, Loibl S. 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 Apr 20; 27(12):1999–2006. [PubMed: 19289619]
109. von Minckwitz G, Schwedler K, Schmidt M, Barinoff J, Mundhenke C, Cufer T, Maartense E, de Jongh FE, Baumann KH, Bischoff J, Harbeck N, Lück H-J, Maass N, Zielinski C, Andersson M, Stein RC, Nekljudova V, Loibl S. investigators ObotGB-sgap. Trastuzumab beyond progression: overall survival analysis of the GBG 26/BIG 3-05 phase III study in HER2-positive breast cancer. *Eur J Cancer.* 2011 Oct; 47(15):2273–2281. *Eur J Cancer.* 2011. [PubMed: 21741829]
110. Burstein HJ, Sun Y, Dirix LY, Jiang Z, Paridaens R, Tan AR, Awada A, Ranade A, Jiao S, Schwartz G, Abbas R, Powell C, Turnbull K, Vermette J, Zacharchuk C, Badwe R. Neratinib, an irreversible ErbB receptor tyrosine kinase inhibitor, in patients with advanced ErbB2-positive breast cancer. *J Clin Oncol.* 2010 Apr 10; 28(8):1301–1307. [PubMed: 20142587]
111. Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J, Baselga J, O'Shaughnessy J. Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. *J Clin Oncol.* 2010 Apr 01; 28(7):1124–1130. [PubMed: 20124187]
112. Baselga J, Bradbury I, Eidtmann H, Di Cosimo S, Aura C, De Azambuja E, Gomez H, Dinh P, Fauria K, Van Dooren V, Paoletti P, Goldhirsch A, Chang T-W, Lang I, Untch M, Gelber R, Piccart-Gebhart M. Abstract S3-3: first results of the NeoALTTO trial (BIG 01-06/EGF 106903): a phase III, randomized, open label, neoadjuvant study of lapatinib, trastuzumab, and their combination plus paclitaxel in women with HER2-positive primary breast cancer. *Cancer Res.* 2011 Apr 26; 70(24 Supplement):S3–S3. 2011.
113. Burris HA, Rugo HS, Vukelja SJ, Vogel CL, Borson RA, Limentani S, Tan-Chiu E, Krop IE, Michaelson RA, Girish S, Amler L, Zheng M, Chu Y-W, Klencke B, O'Shaughnessy JA. 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 Mar 01; 29(4):398–405. [PubMed: 21172893]
114. Krop IE, Beeram M, Modi S, Jones SF, Holden SN, Yu W, Girish S, Tibbitts J, Yi J-H, Sliwkowski MX, Jacobson F, Lutzker SG, Burris HA. 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 Jul 01; 28(16):2698–2704. [PubMed: 20421541]
115. Junttila TT, Li G, Parsons K, Phillips GL, Sliwkowski MX. Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. *Breast Cancer Res Treat.* 2011 Jul; 128(2):347–356. [PubMed: 20730488]
116. Baselga J, Gelmon KA, Verma S, Wardley A, Conte P, Miles D, Bianchi G, Cortes J, McNally VA, Ross GA, Fumoleau P, Gianni L. 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 Apr 01; 28(7):1138–1144. [PubMed: 20124182]
117. Scheuer W, Friess T, Bartscher H, Bossenmaier B, Endl J, Hasmann M. Strongly enhanced antitumor activity of trastuzumab and pertuzumab combination treatment on HER2-positive human xenograft tumor models. *Cancer Res.* 2009 Dec 15; 69(24):9330–9336. [PubMed: 19934333]
118. Gianni L, Pienkowski T, Im Y-H, Roman L, Tseng L-M, Liu M-C, Lluch-Hernandez A, Semiglazov V, Szabo T, Ross G. Abstract S3-2: neoadjuvant pertuzumab (P) and trastuzumab (H): antitumor and safety analysis of a randomized phase II study ('NeoSphere'). *Cancer Res.* 2011 Apr 26. 70(24 Supplement) 2011; S3–2.

Figure 1.

Mechanisms of resistance to HER2 inhibitors. A. Resistance mechanisms involving alterations in HER2 that abrogate trastuzumab binding include expression of alternative isoforms of HER2 (p95, $\Delta 16$) or expression of mucins that block the binding of trastuzumab. B. Increase in ligand production enables signaling through other ErbB family members to overcome inhibition of HER2. C. Mutations that activate intracellular signaling pathways downstream of HER2 and uncouple signaling from HER2 inhibition include mutations in *PIK3CA* and/or loss of function of PTEN. D. Signaling through bypass pathways (e.g., emanating from MET, IGF-1R, EphA2, or EpoR) engage the downstream

effectors of HER2 such as PI3K-Akt either directly or through intracellular kinases such as Src.

TABLE 1**Two Drug Combinations of Anti-HER2 Therapies and Mechanisms of Action**

Combination	Mechanism(s) of action
trastuzumab + lapatinib (or neratinib, afatinib)	ADCC, partial disruption of HER2-HER3 dimers, inhibition of HER2/EGFR kinases
trastuzumab + pertuzumab	Disruption of ligand-dependent and -independent HER2-HER3 dimers, ADCC
T-DM1 + pertuzumab	ADCC, disruption of ligand-dependent and -independent HER2-HER3 dimers, inhibition of microtubules with targeted chemotherapy (DM1)
trastuzumab + PI3K/AKT pathway inhibitor	ADCC, partial disruption of HER2-HER3 dimers, inhibition of PI3K/AKT
T-DM1 + PI3K/AKT pathway inhibitor	ADCC, partial disruption of HER2-HER3 dimers, inhibition of PI3K/AKT, inhibition of microtubules with targeted chemotherapy (DM1)
Lapatinib (or neratinib, afatinib) + PI3K/AKT pathway inhibitor	Simultaneous inhibition of HER2/EGFR kinases and PI3K/AKT
trastuzumab + Src inhibitor	ADCC, partial disruption of HER2-HER3 dimers, inhibition of Src tyrosine kinase
trastuzumab + TORC1 inhibitor (i.e., everolimus)	ADCC, partial disruption of HER2-HER3 dimers, inhibition of TORC1 kinase
trastuzumab or lapatinib + HER3 neutralizing antibody	Adds blockade of HER3 ligand binding and/or HER3 receptor down-regulation to trastuzumab or lapatinib