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Resistance to HER2-directed antibodies and tyrosine kinase inhibitors

Mechanisms and clinical implications

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The antibody trastuzumab and the tyrosine kinase inhibitor lapatinib are approved by the FDA for the treatment of HER2-overexpressing breast cancer. These anti-HER2 drugs are changing the natural history of HER2-overexpressing breast cancer. However, therapeutic resistance to trastuzumab or lapatinib, as either single-agents or in combination with chemotherapy in the metastatic setting, typically occurs within months of starting therapy. Several mechanisms of trastuzumab-resistance have been reported that include signaling from other HER receptors, signaling from receptor tyrosine kinases (RTKs) outside of the HER (ErbB) family, increased phosphatidylinositol-3-kinase signaling, and the presence of truncated forms of HER2. Mechanisms of resistance to lapatinib also point to increased phosphatidylinositol 3-kinase signaling as well as derepression/activation of compensatory survival pathways. In this review, we discuss how these models and mechanisms enhance our understanding of the clinical resistance to HER2-directed therapies.

Introduction

HER2 (ErbB2) is a member of the ErbB family of transmembrane receptor tyrosine kinases, which also includes the epidermal growth factor receptor (EGFR, ErbB1), HER3 (ErbB3) and HER4 (ErbB4). Binding of ligands to the extracellular domain of EGFR, HER3 and HER4 induces the formation of kinase active homo- and heterodimers to which activated HER2 is recruited as a preferred partner.[1] HER2 does not bind any of the ErbB ligands directly; however, its catalytic activity can potently amplify signaling by ErbB-containing heterodimers via increasing ligand binding affinity and/or receptor recycling and stability.[2-5] HER2/HER3 heterodimers are the most transforming of this receptor network.[6,7] HER3, which lacks intrinsic kinase activity, is able to potently activate the phosphatidylinositol-3 kinase (PI3K)/Akt signaling pathway[8,9] via its six docking sites for the p85 adaptor subunit of PI3K, whereas HER2 is unable to directly bind to and thus activate PI3K-Akt. Loss of HER3 inhibits viability of HER2-overexpressing breast cancer cells[10,11] and HER2-overexpressing cells are particularly sensitive to apoptosis induced by PI3K inhibitors,[12] thus suggesting the HER3-PI3K axis is essential for survival of HER2-dependent cells.

Amplification of the *HER2* gene occurs in approximately 25% of invasive breast cancers and is associated with poor patient outcome.[13] HER2 is an appealing therapeutic target in breast cancers because of the correlation between overexpression and poor prognosis and because normal cells have relatively low HER2 expression. Trastuzumab (Herceptin), a humanized monoclonal IgG_1 that binds to the juxtamembrane region of HER2, induces clinical responses in HER2-overexpressing breast cancers and prolongs patient survival (see below). The clinical efficacy of trastuzumab appears limited to breast cancers that overexpress HER2 as measured by intense membrane staining in the majority of tumor cells with HER2 antibodies (3+ by immunohistochemistry [IHC]) or excess copies of the *HER2* gene determined by fluorescent in situ hybridization (FISH). Therefore, HER2 overexpression by IHC and/or FISH is the biomarker predictive of good odds of response to treatment with the antibody.

Resistance to Trastuzumab

Trastuzumab binds to an epitope in the juxtamembrane region of the HER2 receptor tyrosine kinase. This binding induces uncoupling of ligand-independent HER2-HER3 heterodimers and inhibition of downstream signaling[14] as well as antibody-dependent, cell-mediated cytotoxicity (ADCC).[15] Several large randomized adjuvant trials (NCCTG N9831, NSABP B-31, BCIRG 006 and HERA) have shown that the addition of trastuzumab to standard chemotherapy reduces disease recurrence and the risk of death compared to chemotherapy alone in patients with surgically-resected tumors.[16-19] In the N-9831 trial, a recent interim analysis showed that the benefit of concurrent trastuzumab and chemotherapy was more pronounced than that of chemotherapy followed by trastuzumab.[20] Based on these data, the addition of trastuzumab to adjuvant chemotherapy has become standard of care in women with $HER2^+$ early breast cancer. Although it is anticipated that many patients treated with adjuvant trastuzumab will be cured of their disease, it is also expected that many will recur. Trastuzumab in combination with chemotherapy is also indicated for the treatment of $HER2^+$

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metastatic breast cancer.[21] Nevertheless, response rates to single-agent trastuzumab are short lived.[16] Thus, a large proportion of patients with HER2$^+$ tumors either does not respond to trastuzumab or develops acquired tolerance to the antibody, suggesting both de novo and acquired mechanisms of drug resistance.

Most preclinical models have reported that *HER2* gene amplification and RNA/protein overexpression are maintained in trastuzumab-resistant HER2$^+$ clones,[22,23] thus implying that HER2-overexpressing tumor cells that bypass trastuzumab action continue to depend on the HER2 oncogene. Several studies have reported potential mechanisms of resistance to trastuzumab, including signaling from RTKs outside of the HER (ErbB) family, increased PI3K signaling, amplification of signaling by other ErbB receptors and the presence of altered forms of HER2 that are not recognized or bound by trastuzumab.

Cross-talk with heterologous RTKs and amplification of ErbB signaling. A potential mechanism of trastuzumab resistance involves RTKs outside of the HER family modulating levels of the Cdk inhibitor $p27^{KIP1}$, such as the IGF-I receptor. For example, overexpression of IGF-IR or increased levels of IGF-IR/HER2 heterodimers,[24,25] which potently activate PI3K and its downstream effector AKT, abrogate trastuzumab action when transfected into antibody-sensitive breast cancer cells. In a neoadjuvant trial of chemotherapy plus trastuzumab, high levels of IGF-IR as measured by IHC correlated with a poor clinical response.[26] MET (HGF receptor) has also been implicated in trastuzumab resistance. HER2 overexpressing cells upregulate MET following exposure to trastuzumab. Further, activation of MET protects cells against trastuzumab by abrogating the induction of p27.[27] In a cohort of patients with HER2$^+$ breast cancers, overexpression of the EphA2 RTK was associated with reduced disease-free and overall survival. Treatment of resistant cells with trastuzumab induced phosphorylation of Src and EphA2 resulting in the activation of PI3K/AKT and MAPK. Administration of a neutralizing EphA2 antibody restored sensitivity to trastuzumab in vivo.[28] Finally, the receptor for erythropoietin (EpoR) is co-expressed in a proportion of cell lines and primary tumors that also harbor *HER2* gene amplification. In those cells, treatment with recombinant human erythropoietin (rHuEPO) activates Jak and Src leading to inactivation of PTEN and attenuation of the response to trastuzumab. Interestingly, the concurrent administration of rHuEPO and trastuzumab correlated with a shorter progression-free and overall survival in patients with HER2+ metastatic breast cancer.[29]

Other members of the ErbB receptor network are thought to play a role in trastuzumab resistance. Exogenous ligands of the EGFR and HER3/4 co-receptors have been shown to rescue from the anti-proliferative effect of the antibody.[30,31] This is consistent with structural data using ErbB receptor ectodomains, which show that trastuzumab is unable to block ligand-induced EGFR/HER2 and HER2/HER3 heterodimers.[32,33] Our laboratory reported trastuzumab-resistant HER2-overexpressing BT-474 human breast cancer cells generated in vivo. The resistant cells retained *HER2* gene amplification and trastuzumab binding. They exhibited higher levels of phosphorylated EGFR and HER3 and EGFR/HER2 heterodimers as well as overexpression of EGFR, TGFα, HB-EGF and heregulin RNAs compared to the parental trastuzumab-sensitive cells,[23] thus suggesting enhanced EGFR- and HER3-mediated activation of HER2. The HER2 tyrosine kinase inhibitor (TKI) lapatinib and the HER2 antibody, pertuzumab, which blocks HER2 heterodimerization with ErbB co-receptors,[34,35] inhibited growth of the antibody-resistant cells suggesting that, although resistant to trastuzumab, the cells were still dependent on HER2-dependent interactions with the ErbB receptor network.[23] In line with this report, the activation of TGFβ receptors, a pathway amplified in metastatic mammary tumors, has been shown to induce phosphorylation of the sheddase TACE/ADAM17 resulting in increased secretion of TGFα, amphiregulin and heregulin. These changes are followed by enhanced coupling of p85 and HER3, activation of PI3K/AKT and resistance to trastuzumab. Further, a gene signature induced by expression of a constitutively active, mutant type I TGFβ receptor correlated with resistance to trastuzumab in a panel of HER2$^+$ breast cancer cells lines and with poor clinical outcome in patients with invasive breast cancer.[36]

Amplification of the PI3K/AKT pathway. Resistance to trastuzumab may occur as a result of aberrant activation of signaling pathways downstream of the receptor, such as PI3K/Akt. Molecular alterations involving this pathway are considered the most frequent in breast cancer, together encompassing over 30% of invasive tumors. Alterations in breast cancer resulting in hyperactivity of the PI3K pathway include gain-of-function mutations in *PIK3CA* (the gene encoding the PI3K catalytic subunit p110α),[37,38] mutations in *AKT1,*[39] amplifications of *AKT2,*[40] loss of the PTEN lipid phosphatase,[41,42] and loss of the tumor suppressor *INPP4B* (inositol polyphosphate 4-phosphatase type II).[43] *PIK3CA* mutations in primary breast tumors have been associated with lymph node metastases, the presence of ER and PgR and HER2 overexpression.[44,45] It is generally accepted that anti-HER2 therapies should inhibit PI3K/Akt signaling downstream the HER2 receptor in order to inhibit tumor growth.[14,46]

Using a large-scale RNA interference screen, Berns et al. identified PTEN as the only gene whose knockdown resulted in trastuzumab resistance,[47] consistent with the previous observation that in antibody-sensitive cells, trastuzumab increases the phosphatase activity of PTEN via inhibition of Src and Src-mediated (inhibitory) phosphorylation of PTEN.[48] This same report also showed that oncogenic mutants of *PIK3CA*, the gene encoding for the catalytic subunit of PI3K p110α, conferred resistance to trastuzumab to cells in culture. In patients with breast cancer, the presence of oncogenic *PIK3CA* mutations and low PTEN expression measured by IHC identified those patients with the worst outcome following chemotherapy plus trastuzumab.[47] Supporting aberrant PI3K signaling and causality to drug resistance, in more recent preclinical studies, the addition of PI3K inhibitors to trastuzumab has inhibited growth of HER2$^+$/PIK3CA mutant tumors resistant to anti-HER2 therapy.[49-51] Interestingly, inhibitors of mTOR, a serine-threonine kinase downstream PI3K, have shown activity after progression on trastuzumab. Dalenc et al. recently reported a multicenter phase II study of 55 women with HER2$^+$ MBC whose tumors were resistant to trastuzumab and taxanes. Patients were treated with the TOR inhibitor everolimus, paclitaxel and

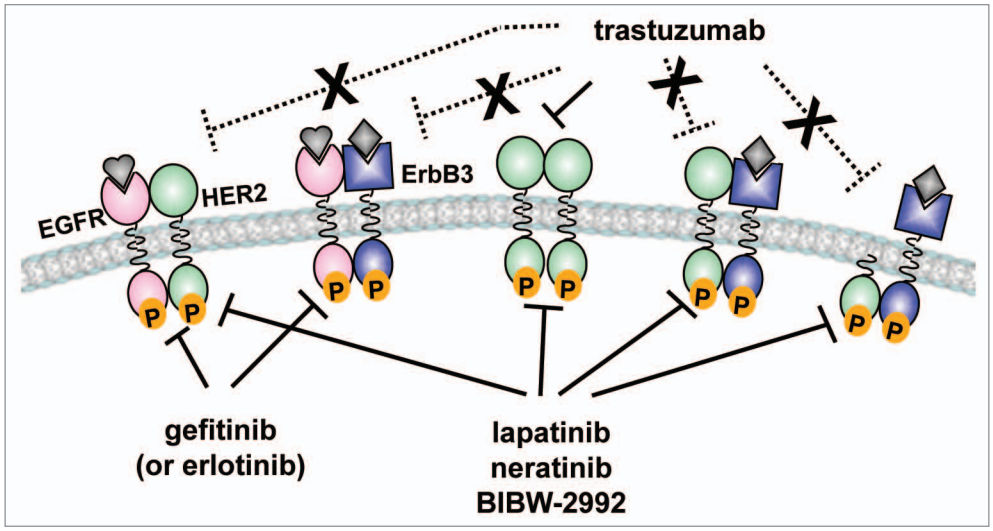

Figure 1. Diagram of mechanisms of resistance to trastuzumab. Trastuzumab can block HER2 homodimers. However, it is unable to interfere with ligand-activated EGFR-HER2 and HER2-HER3 heterodimers (dotted lines). The HER2/EGFR dual inhibitor lapatinib should be able to block the signaling output of HER2-containing heterodimers. Trastuzumab cannot bind kinase-active cytosolic fragments of HER2 ($p95^{HER2}$), whereas lapatinib and other irreversible TKIs, such as neratinib and BIBW2992, can inhibit the catalytic activity of $p95^{HER2}$.

trastuzumab, exhibiting an impressive partial response rate of 19% and an overall clinical benefit rate of 81%.[52]

Alterations in binding of trastuzumab to HER2. Another potential mechanism of resistance is the presence of truncated forms of HER2 that trastuzumab does not recognize. Anido et al. reported the presence of HER2 C-terminal fragments, which result from alternative translation initiation from methionines near the transmembrane domain of the full-length receptor molecule.[53] These fragments are kinase-active but lack the trastuzumab binding epitope and therefore, can potentially allow the cancer cell to escape antibody action.[54] Analysis of a cohort of patients with $HER2^+$ metastatic breast cancer treated with trastuzumab and chemotherapy showed a very low response rate in tumors with cytosolic $p95^{HER2}$ compared to those without.[54] Lapatinib has been shown to inhibit the catalytic activity of $p95^{HER2}$. Therefore, patients with $p95^{HER2}$-positive breast cancers treated with lapatinib alone or in combination with capecitabine exhibited a similar progression-free survival and overall response rate compared to $p95^{HER2}$-negative tumors,[55] suggesting a clinical setting where a HER2 TKI might be advantageous over trastuzumab (**Fig. 1**).

In addition to truncated forms of HER2, overexpression of the membrane-associated glycoprotein mucin-4 (MUC4),[56,57] has been shown to mask trastuzumab binding epitopes in the HER2 receptor, resulting in acquired resistance. Finally, an oncogenic splice isoform with an in-frame deletion of exon 16 (HER2Δ16) is found in some HER2-overexpressing breast cancer cell lines and primary breast cancers.[58,59] Loss of exon 16 results in a constitutively dimerized and active HER2 receptor, enhanced Src activity and accelerated transformation. Cells expressing HER2Δ16 are resistant to trastuzumab; this resistance is abrogated by co-treatment with Src inhibitors.[60] It has not been shown yet whether HER2Δ16 is a mechanism of resistance to trastuzumab in patients with $HER2^+$ tumors.

Resistance to Tyrosine Kinase Inhibitors

Another approach to block HER2 is the use of ATP-competitive, small molecule TKIs. The dual EGFR/HER2 TKI lapatinib is active as first line monotherapy in patients with $HER2^+$ metastatic breast cancer and in combination with chemotherapy improves progression free survival compared to chemotherapy alone.[61,62] In the latter registration trial, fewer brain metastases occurred in women in the combination than in the monotherapy arm, suggesting a potential difference between lapatinib and trastuzumab as it applies to recurrences in the CNS.[62] In the registration study and in a second randomized trial of paclitaxel ± lapatinib in patients with metastatic breast cancer, the clinical benefit of lapatinib was limited to patients with HER2 overexpression as scored by IHC and/or FISH.[63] Like lapatinib, the HER2/EGFR dual TKI neratinib[64] has shown clinical activity in patients with $HER2^+$ metastatic breast cancer who have progressed on trastuzumab. As with trastuzumab, it is generally accepted that in order to exert an antitumor effect in $HER2^+$ cancers, treatment with lapatinib should inhibit the PI3K/Akt pathway.[23,65]

Proposed mechanisms of resistance to lapatinib involve recovery through derepression and/or activation of compensatory survival pathways. For example, in HER2-overexpressing BT474 cells selected for acquired resistance to lapatinib, the resistant cells continued to show inhibition of HER2, HER3, MAPK and AKT phosphorylation upon treatment with lapatinib. In these cells, inhibition of AKT with lapatinib resulted in derepression of FoxO3a thus leading to increased ERα transcription and ER signaling.[66,67] Co-treatment with lapatinib and the ER downregulator fulvestrant prevented the outgrowth of drug resistant cells. Further, lapatinib was shown to induce ER signaling in tumor biopsies from patients with $HER2^+/ER^+$ but not $HER2^+/ER^-$ negative breast cancers. The same group also found calcium-dependent increased levels of phosphorylated RelA, the

pro-survival subunit of NFκB, upon lapatinib treatment of HER2+ breast cancer cell lines.[68] Using either small interfering RNA constructs targeting RelA or an intracellular calcium chelator enhanced the apoptotic effects of lapatinib, suggesting a possible role for RelA in adaptation to the HER2 TKI.

Using HER2+ cells selected in culture, another study identified overexpression of AXL as a mechanism of resistance to lapatinib.[69] AXL is an RTK with a kinase domain closely resembling MET and an extracellular domain resembling neural cell adhesion molecules.[70] BT474 cells rendered drug-resistant by chronic exposure to lapatinib exhibited increased expression and activation of AXL. GSK1363089 (foretinib), a multikinase inhibitor of AXL, MET and VEGFR, restored lapatinib and trastuzumab sensitivity in the AXL-overexpressing, drug-resistant cells.[69]

Other studies have shown upregulation of HER3 transcription and protein levels and recovery of HER3 phosphorylation after short-term inhibition of HER2 with the TKIs gefitinib and lapatinib.[71,72] As with ERα, this recovery of HER3 is also explained by derepression of FoxO upon inhibition of PI3K/AKT downstream of HER2 and upregulation of FoxO-dependent HER3 transcription (Garret et al. submitted). In one study, HER3-PI3K-AKT activity was completely inhibited by higher, pulsatile doses of lapatinib both in vitro and in vivo.[72] While the complete inactivation of the HER2 kinase may require doses of lapatinib that are not tolerated in vivo, it is still possible that high intermittent doses might be more effective than the currently approved (lower dose) daily regimens. These alternative treatment schedules are currently being explored in clinical trials.

As with trastuzumab, activating PIK3CA mutations, loss of the tumor suppressor PTEN and alternative signaling pathways that activate PI3K-AKT are reported mechanisms for escape from lapatinib. Using a large-scale loss-of-function short hairpin RNA screen to identify novel modulators of resistance to lapatinib, Eichhorn et al. identified the tumor suppressor PTEN as a gene whose loss reduced the sensitivity to the TKI both in vitro and in vivo.[50] In addition, two dominant activating mutations in PIK3CA (E545K and H1047R), which are prevalent in breast cancer, also conferred resistance to lapatinib. These authors also showed that the resistance to lapatinib induced by the PI3K mutants can be abrogated through the use of BEZ235, a dual inhibitor of PI3K/mTOR.[50]

HER2 Gene Mutations: Possible Role in Acquired Resistance

A common mechanism of clinical resistance to TKIs that target RTKs other than HER2 is the development of mutations in the targeted receptor. For example, some lung cancers that acquire resistance to EGFR inhibitors harbor T790M alleles that comprise <5% of the total *EGFR* alleles;[73] these mutations are generally detected in cancers that progress after an initial response to an EGFR TKI.[74-76] Additional examples include mutations in BCR/ABL and c-kit in chronic myeloid leukemia and gastrointestinal stromal tumors, respectively, which result in resistance to imatinib, a specific BCR/ABL and c-kit kinase inhibitor.[77,78] It is possible that as a result of selective pressure from anti-HER2 therapies, breast cancers will acquire or will be 'enriched' for mutations in *HER2*, which may be present in only a fraction of the HER2 alleles. Intragenic somatic mutations in the *HER2* gene were reported in about 4% of non-small-cell lung cancers (NSCLC). These involve in-frame duplications/insertions in a small stretch within exon 20 of *HER2*.[79,80] Two studies did not find HER2 kinase domain mutations[79,81] though it is possible that direct gene sequencing methods used in these studies may have missed mutations present in a few HER2 alleles in tumors with *HER2* gene amplification. Only one report has identified a low frequency of HER2 mutations in breast cancer.[82] Interestingly, one of these mutations, a YVMA insertion at G776 in exon 20, was found to confer de novo resistance to trastuzumab and lapatinib. Cells expressing this mutant still responded to CI-1033, an irreversible covalent inhibitor of the HER2 kinase.[83] This inhibitor is very similar to neratinib, the irreversible HER2 kinase inhibitor in late clinical development.[64] These acquired alterations have yet to be detected in metastatic lesions recurring after an initial response to primary anti-HER2 therapy or following adjuvant therapy.

Dual HER2 Blockade and Abrogation of Drug Resistance

As indicated above, HER2 TKIs have shown clinical activity in patients with HER2+ breast cancer who progress on trastuzumab. These data suggest that trastuzumab-resistant tumors continue to be dependent on the HER2 tyrosine kinase after escaping trastuzumab action. However, the clinical responses to single agent TKIs such as lapatinib or neratinib tend to be short-lived.[61,62] Further, these patients may still need trastuzumab beyond progression as suggested by a recent study where the combination of lapatinib and trastuzumab was superior to lapatinib alone at improving progression-free survival, clinical response and overall survival in patients with HER2+ metastatic breast cancer who had progressed on trastuzumab.[84] The activity of trastuzumab beyond progression is not limited to combinations with TKIs as it has also been shown in a study where the combination of trastuzumab plus capecitabine was clearly superior to capecitabine alone.[85]

A second piece of evidence supporting continued dependence on HER2 after progression on anti-HER2 therapy is provided by clinical data with the antibody-toxin fusion trastuzumab-DM1 T-DM1, T-DM1 is an antibody-drug conjugate in which one molecule of trastuzumab is covalently coupled via a non-cleavable linker to three molecules of the microtubule polymerization inhibitor Derivative of Maytansine 1 (DM1).[86] T-DM1 binds to HER2 with similar affinity as trastuzumab. It is proposed that after binding to the receptor, the T-DM1/HER2 complex is internalized followed by degradation in the lysosome, release of DM1 and subsequent cell lysis. Although used at lower doses and frequency than trastuzumab, T-DM1 retains the ability to inhibit signaling and engaging immune effectors that mediate ADCC and is active against lapatinib-resistant xenografts.[87] Phase I–II studies of T-DM1 demonstrated mild, reversible toxicity and a remarkable clinical response rate in excess of 25% in patients with heavily pretreated HER2-overexpressing metastatic breast cancer who had progressed after trastuzumab and lapatinib.[88,89] T-DM1

Table 1. Molecular and clinical characteristics and mechanisms of antitumor action of HER2-targeted drugs

	Trastuzumab	Pertuzumab	Lapatinib	Neratinib
Type of molecule	Humanized IgG_1, binds to juxtamembrane domain IV	Humanized IgG_1, binds to heterodimerization domain II	Reversible, ATP- competitive, small molecule TKI	Irreversible, covalent, small molecule TKI
Administration, half-life	Intravenous, weeks	Intravenous, weeks	Oral, 24 h	Oral, ~16 h
Cell surface HER2 levels	Reduces	No change	Increases	May increase
Receptor dimers	Inhibits HER2 homodimers and ligand-independent HER2-HER3 dimers	Inhibits ligand-induced HER2-containing heterodimers	May increase cell surface dimers	May increase cell surface dimers
Downstream signaling	Weak inhibitor	Weak inhibitor	Potent inhibitor	Potent inhibitor
HER2 ectodomain cleavage	Inhibits	Does not inhibit	No effect	No effect
Inhibits $p95^{HER2}$	No	No	Yes	Yes
ADCC*	Yes	Yes	No	No
Exon 20 insertion and gatekeeper mutation	Inactive	Probably inactive	Inactive	Active
Cardiotoxicity	Rare	No	Rare	Rare
Diarrhea	Rare	Dose-limiting toxicity	Dose-limiting toxicity	Dose-limiting toxicity

*antibody-dependent cell mediated cytotoxicity.

is being further evaluated in two large phase III randomized studies in the first- and second-line metastatic disease settings.

Taken together, these data imply that even in advanced stages, $HER2^+$ breast cancers remain dependent on HER2 and that single-agent trastuzumab and lapatinib are not adequate to inhibit the HER2 signaling network completely. They also imply that the use of combinations of HER2-targeted agents delivered early against $HER2^+$ breast cancer should be widely considered. Several preclinical and early clinical data have already suggested combinations of HER2 inhibitors, which because of their distinct properties and (thus) mechanisms by which they interact with HER2 cells, provide an opportunity for synergy. These characteristics and mechanisms are summarized for four anti-HER2 agents in **Table 1**.

Along these lines, Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization (ALTTO) and Neo-ALTTO are two ongoing large international adjuvant and neoadjuvant studies, respectively, comparing trastuzumab vs. lapatinib vs. dual HER2 blockade using both drugs. Results on the neoadjuvant study were reported recently. Neo-ALTTO is a 450 patient study in which $HER2^+$ tumors measuring >2 cm were randomized to trastuzumab, lapatinib or the combination for 6 weeks, at which time paclitaxel is added to each of the arms for an additional 12 weeks. After surgery, patients in all three arms receive adjuvant chemotherapy with FEC followed by the respective HER2 inhibitor either alone or in combination for 34 weeks. About half of the patients enrolled had ER^+ tumors. There was increased but manageable toxicity in the lapatinib arms, mostly diarrhea and transaminitis. Pathological complete response (path CR, defined as no invasive cancer in the breast or only DCIS in the breast specimen) was significantly higher in the combination arm (51.3%) vs. 29.5 and 24.7% in the trastuzumab and lapatinib arms, respectively. In all three arms, the pathological CR rate was lower in the ER^+ vs. the ER^- tumors.[90] This study is the best demonstration to date of superiority of dual HER2 blockade over single agent trastuzumab or lapatinib. Whether the combination is superior at abrogating resistance and, hence, prolonging disease-free and overall survival compared to single agent anti-HER2 therapy, as suggested by the higher pathological CR rate in Neo-ALTTO, would require longer follow-up.

A second approach that improves inactivation of the HER2 network is the combination of trastuzumab and pertuzumab. For example, in trastuzumab-resistant xenografts and in patients with $HER2^+$ breast cancer that have progressed on trastuzumab, only the combination of both but not each antibody alone exhibited clinical activity.[91,92] These data suggest that both HER2 antibodies, each binding to a different epitope in HER2 (**Table 1**), might be required to completely inhibit HER2-HER3 dimerization in situ, potentially explaining their clinical activity in combination. To test this hypothesis, the phase III Cleopatra study is currently randomizing patients with $HER2^+$ metastatic breast cancer to trastuzumab and docetaxel ± pertuzumab as first line therapy in the metastatic setting using progression-free survival as a primary endpoint. Notably, in the recently reported NeoSphere trial in patients with $HER2^+$ primary breast cancer, the pathological CR rate was 45.8 vs. 29% ($p = 0.01$) in patients treated with neoadjuvant docetaxel/trastuzumab/pertuzumab vs. docetaxel/trastuzumab, respectively.[93] Currently, the HER3 monoclonal antibodies AMG-888,[94] and MM-121,[95] are completing phase I testing. We anticipate that like pertuzumab, they may also exert a synergistic effect in combination with trastuzumab or lapatinib in patients with $HER2^+$ breast cancer.

Conclusions

At this time, only trastuzumab and lapatinib are approved by the FDA for the treatment of patients with $HER2^+$ overexpressing breast cancer. Although not approved in combination, data summarized above clearly suggest dual HER2 blockade is feasible and superior to each HER2 inhibitor alone. In addition, there are a

Table 2. Drugs in development that target HER2 or pathways proposed to contribute or to mediate resistance to trastuzumab and/or lapatinib

Drug	Mechanism
Pertuzumab	Humanized HER2 IgG_1, binds to heterodimerization domain II
Trastuzumab-DM1	Inhibition of microtubule polymerization (apoptosis) after internalization; ADCC
Irreversible HER2/EGFR TKIs (neratinib, BIBW-2992)	Covalent binding to ATP pocket in HER2 and EGFR
HER3 monoclonal antibodies (MM-121, AMG-888)	Block heregulin binding and partially downregulate HER3
PI3K inhibitors (BKM120, GDC-0941, XL147)	ATP competitive inhibitors of p110
Dual PI3K/TOR inhibitors (BEZ235, XL765)	
Rapalogs: everolimus (RAD001), temsirolimus (CI-779), ridaforolimus (MK-8669)	Non-catalytic inhibitors of TORC1
Catalytic mTOR inhibitors (OSI-027, AZD8055)	Inhibit TORC1 and TORC2
AKT inhibitors (MK-2206, AZD5363)	Allosteric or catalytic inhibitors of AKT1/2
IGF-IR TKIs (OSI-906, AEW541)	Inhibition of InsR and IGF-IR tyrosine kinases
IGF-IR monoclonal antibodies (MK-0646, R1507)	IGF-IR downregulation; inhibition of IGF-I binding to IGF-IR
HSP90 inhibitors	Induce degradation of HER2 and signal transducers
MET, Src, TGFβ inhibitors	Block mechanisms of resistance
MMP inhibitors	Prevent ectodomain shedding (HER2 cleavage)

plethora of agents that either target HER2 by different mechanisms or inhibit those mechanisms of resistance summarized above. All these drugs (**Table 2**) are currently in different phases of clinical development. It is anticipated that an increasing number of these agents will eventually be combined with the approved anti-HER2 therapies. We propose that the increasing use of dual HER2 blockade with trastuzumab and lapatinib as well as the development of novel anti-HER2 combinations will markedly limit or eventually abrogate acquired resistance to primary anti-HER2 therapy. On the other hand, molecular profiling of $HER2^+$ metastatic recurrences following anti-HER2 therapy should provide important leads as to which of the molecular mechanisms of resistance summarized above are the more relevant and targetable in the clinic.

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