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Current status of anti-her2 therapies: predicting and overcoming herceptin resistance

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Introduction

Human epidermal growth factor receptor 2-overexpressing (Her2+) breast cancer represents 20–25% of breast cancer and has been shown to be associated with high relapse rates and poor prognosis. [1–3] The introduction of Trastuzamab (Herceptin) for the adjuvant treatment of Her2+ breast cancer has significantly reduced relapse rates. [4–7] However, some patients with Her2+ tumors have de novo resistance and do not respond to Trastuzamab. There is currently no reliable means to predict which patients will be resistant to Trastuzamab. Additionally, among patients with Her2+ metastatic breast cancer the majority of those who initially respond to Trastuzamab acquire resistance within a year.[4, 5, 8, 9] Most resistance is believed to occur via persistent signaling through the HER 2 pathway.

The mechanism of Her2 signaling has been the focus of extensive research in an attempt to identify additional targeted therapies for patients with Trastuzamab-resistant Her2+ breast cancer. All of these therapies target various downstream components of the pathway associated with Her2 signaling. The effects of many of these drugs are short-lived, and acquired resistance will continue to be a challenge. In this review we discuss Her2+ breast cancer, possible mechanisms of Trastuzamab resistance, and various drugs that have been introduced to overcome Trastuzamab resistance. We propose to explore an alternative cause of increased risk or drug resistance that has not been widely investigated: the basal phenotype.

Trastuzamab

The Her2 gene

The Her2 gene is part of the ErbB family of receptor tyrosine kinases that contain an extracellular ligand-binding domain, a single transmembrane span, and intracellular tyrosine kinase and regulatory domains. Upon ligand binding, these receptors dimerize with themselves or other ErbB family members and undergo phosphorylation of several tyrosine residues within the regulatory domain leading to recruitment of signaling molecules involved in intracellular signal transduction cascades. These, in turn, modulate the activity of regulatory proteins that control cell proliferation, survival, and differentiation, such as the phosphatidylinositol triphosphate kinase (PI3K)/Protein Kinase B (Akt) pathway and the mitogen-activated protein kinase (MAPK/ERK) cascade.[10, 11] The Her2 receptor can undergo ligand-independent dimerization and is the preferred hetero-dimerization partner for the other ErbB family members.[12] Overexpression of Her2 secondary to gene

amplification leads to spontaneous homo-dimerization and dysregulation of downstream signaling networks which promotes tumor cell growth and survival.[13]

Proposed mechanisms of action

Trastuzamab is a monoclonal antibody that targets the Her2 extracellular domain, induces uncoupling of heterodimers, and inhibits downstream signaling.[14, 15] The exact mechanism of anti-tumor activity in Her2+ breast cancer is unknown. Possible mechanisms include activation of antibody-dependent cellular cytotoxicity (ADCC), increased intracellular degradation of HER 2 via binding of Herceptin, inhibition of proteolytic cleavage of the Her2 extracellular domain, inhibition of intracellular signal transduction, or inhibition of tumor-induced angiogenesis.

Evidence supporting involvement of immune effects in Trastuzamab's molecular mechanism of action includes data from pilot clinical and preclinical studies. Strong lymphoid infiltration was demonstrated in patients treated with neoadjuvant Trastuzamab, and ADCC activity correlated with response to therapy. [16]In preclinical studies, Trastuzamab has been shown to contain an IgG1 Fc receptor and binding of this receptor to the Fc gamma receptor of natural killer cells has been shown to lead to recruitment of immune effector cells to attack target cells, leading to activation of natural killer cell-mediated lysis.[17–19] Data from xenograft models demonstrated near complete tumor regression when treated with Trastuzamab in mice, whereas those lacking the natural killer Fc receptor had significantly less inhibition of tumor growth. Trastuzamab has also been shown to activate ADCC in multiple breast cancer cell lines.[15, 19]

Through the adaptive immune system, Trastuzamab forms complexes with Her2 that are internalized rapidly allowing Her2 to undergo intracellular degradation. This results in formation of Her2 epitopes that can be recognized by HLA class I molecules that when bound to the Her2 fragment can cause tumor cell lysis by circulating T lymphocytes.[20, 21] Additionally, Perez and colleagues found that circulating CD4+ and CD25+ regulatory T cells (Treg) occur at higher frequency in Her2+ patients compared to both Her2-negative patients and healthy donors. Trastuzumab therapy resulted in a progressive decrease of circulating Treg and this correlated with either objective clinical response or stable disease, whereas increased frequency of Treg during Trastuzumab therapy coincided with disease progression.[22] Furthermore, Horlock and colleagues demonstrated that Trastuzamab alters the balance between circulating Treg and Th17 cells, defined as an immune subset of CD4+ lymphocytes, in patients with Her2+ breast cancer being treated with Trastuzamab.[23]

Another proposed mechanism of action includes inhibition of Her2 cellular domain proteolysis. In vitro studies demonstrated that Trastuzamab inhibits basal and activated Her2 extracellular domain cleavage which, when it occurs, produces a membrane-bound fragment that is constitutively activated.[24] In a Phase II clinical trial of patients with metastatic Her2+ breast cancer, variations in serum extracellular domain levels correlated with response rate to treatment with Trastuzamab and Docetaxel.[25]

Inhibition of intracellular signal transduction has been shown to occur as a result of Trastuzamab treatment. Overexpression of Her2 results in activation of the PI3K-Akt and MAPK signaling pathways which regulate cell-cycle progression and apoptosis. Treatment of various Her2+ breast cancer cell lines with Trastuzamab has been shown to result in inhibition of PI3K and phosphorylated Akt levels.[26] In vitro studies of tissue specimens from patients with Her2+ breast cancer, Trastuzamab inhibited PI3K-Akt and MAPK-Erk signaling and decreased survivin levels and apoptosis resistance.[27] In addition, specific genes contributing to cell cycle progression and cell growth are regulated by Trastuzamab via PI3K-Akt pathways.[28] Trastuzamab has been shown to activate PTEN, an anti-

survival mechanism which negatively regulates Akt activity in breast cancer cells, leads to cell death, and is the basis of the therapeutic benefit of Trastuzumab.[29] Finally, expression of $p27^{Kip1}$, a cyclin-dependent kinase inhibitor, is downregulated by Her2, leading to growth inhibition.[30] Preclinical studies suggest that Trastuzamab increases cell-cycle arrest via increasing $p27^{Kip1}$ levels.[31, 32]

Her2 overexpression in breast cancer is closely linked to intratumoral vascular endothelial growth factor (VEGF) expression, which induces cancer cells to undergo angiogenesis to support tumor growth. Treatment of Her2+ breast cancer xenograft models with Trastuzamab led to reduction in VEGF levels and regression of tumor vasculature.[33] As a result, tumor growth was significantly inhibited and the animal survival rate was significantly improved. The association of VEGF and Her2 has been explored in a clinical cohort of over 600 patients with primary invasive breast cancer. Investigators found a strong correlation between VEGF and Her2 overexpression in tumor lysates and that VEGF expression was a significant prognostic indicator for survival.[34]

Inhibition of Her2-signaling disrupts the DNA repair process. After treatment with Cisplatin chemotherapy, treatment with Trastuzamab resulted in a significant reduction in repair of Cisplatin reaction products and unscheduled DNA synthesis in human xenograft models.[35, 36]

Trastuzamab in clinical trials

Trastuzamab has demonstrated its efficacy in clinical trials in the metastatic, adjuvant and neoadjuvant settings. Addition of Trastuzamab to chemotherapy in patients with metastatic Her2+ breast cancer, as defined by 3+ HER 2 staining by immunohistochemistry (IHC) or gene amplification by fluorescence in situ hybridization (FISH), was associated with a longer progression-free survival (PFS) (median, 7.4 vs. 4.6 months; $P<0.001$), a higher rate of objective response (50 percent vs. 32 percent, $P<0.001$), a longer duration of response (median, 9.1 vs. 6.1 months; $P<0.001$), a lower rate of death at 1 year (22 percent vs. 33 percent, $P=0.008$), longer overall survival (OS) (median OS, 25.1 vs. 20.3 months; $P=0.01$), and a 20 percent reduction in the risk of death.[4] Trastuzamab has also been shown to prolong survival and reduce relapse rates as first-line monotherapy in women with Her2+ metastatic breast cancer and as single agent therapy in women with metastatic disease which progressed after chemotherapy.[5, 9] There have been four major clinical trials (NSABP-B31, HERA, NCCTG N9831, and BCIRG) in the adjuvant setting that demonstrated significant improvement in disease-free survival (DFS), ranging from 36–52%, and OS of up to 35% when 1 year of Trastuzamab was added to adjuvant chemotherapy.[6, 7, 37] Use of Trastuzamab in neoadjuvant trials has resulted in higher rates of complete pathologic response (cPR) of 30–40% and combination with Lapatinib (Tykerb) has increased the rate of cPR to over 50%.[38–40]

Trastuzamab Resistance

Although Trastuzamab has dramatically reduced recurrence rates in Her2+ breast cancer, de novo or acquired resistance is still observed in 66–88% of Her2+ metastatic breast cancer.[5, 8, 9] Several mechanisms of Trastuzamab resistance have been identified in preclinical studies (see Table 1) with very little data validated in the clinical setting.

Epitope Masking

There are several means by which alteration of the receptor-antibody interaction binding site may occur, acting as a possible mechanism of resistance. Increased expression of proteins such as mucin-4 (MUC4), which is a membrane-associated glycoprotein that may mask Her2 and disrupt the binding of Herceptin to the Her2 protein.[41–43] By interacting with

Her2 by means of an epidermal growth factor-like domain, MUC4 prevents it from binding to Herceptin and may increase phosphorylation of Her2 potentially altering signals sent from the Her2 receptor.CD44, a transmembrane receptor for hyaluronan, is another protein that has been implicated in the masking of Her2.[44] When endogenous hyaluronan binds to CD44, the PI3K/Akt pathway is activated. Inhibition of CD44 by anti-CD44 antibodies resulted in inhibition of the PI3K/Akt-mediated tumor cell growth in mouse breast cancer models. Interference with receptor-antibody interaction may also be caused by expression of a truncated form of the Her2 protein, known as p95Her2, which has been demonstrated in a number of breast cancer cell lines that are resistant to Trastuzamab.[45] These breast cancer cells activated growth and survival signals through p95Her2-HER3 heterodimers when treated with Trastuzamab. In addition, p95Her2 expression in patients with metastatic breast cancer was associated with worse clinical outcome with Trastuzamab treatment compared to those who had the full-length receptor.[45, 46] Interaction between Her2 and Trastuzamab may also be interrupted by mutations in the Her2 gene that could prevent antibody recognition or physical binding of the drug to the receptor protein. Mutations of the tyrosine kinase domain of the Her2 gene have been identified in lung cancer with some data suggesting that the presence of these mutations correlates with response to treatment, but there is limited data on such mutations in breast cancer. [47, 48]

Upregulation of Her2 downstream signaling pathways

Upregulation of Her2 downstream signaling pathways is another proposed mechanism of Trastuzamab resistance demonstrated in preclinical models. Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene that normally inhibits PI3K activation. Mutations resulting in the loss of PTEN can lead to activation of the PI3K signaling pathway and stimulate tumorigenesis. Loss of PTEN has been demonstrated in several solid tumors, including ovarian, endometrial, prostate, glioblastomas and breast cancer.[49] Nagata and colleagues demonstrated that anti-tumor activity of Herceptin may occur by activation of PTEN in breast cancer cell lines.[29] Trastuzamab treatment of cell lines resulted in increased PTEN membrane localization and phosphatase activity, by inhibiting Src tyrosine kinase from associating with ErbB2. The eventual response was a reduction of PTEN that conferred resistance to Trastuzamab in vitro and in vivo. In a study of patients with locally advanced Her2+ breast cancer, PTEN deficiency was associated with a diminished response to neoadjuvant Trastuzamab, providing clinical support that activation of the PI3K/Akt pathway via PTEN mutations is associated with Trastuzamab resistance.[50]

Other pathways that upregulate the PI3K/Akt pathway have been implicated in Trastuzamab resistance. PI3K mutations can lead to activation of the PI3K/Akt pathway and may confer Trastuzamab resistance. PIK3CA is the gene that encodes the catalytic subunit of p100-alpha of PI3K. PIK3CA mutations have been shown to result in Trastuzamab resistance when overexpressed in breast cancer cell lines.[51] Trastuzamab blocks Her1/Her3/PI3K complex formation which inhibits Akt. Mutation of PI3K has been shown to disrupt the Her1/Her3/PI3K complex, preventing Akt inhibition.[52] Phosphoinotiside-1(PDK1) is linked to Akt signaling. PDK1 inhibitors when given in combination with Trastuzamab enabled Akt inhibition and an antiproliferative effect in Trastuzamab-resistant breast cancer cell lines.[53] This data demonstrated a synergism between PDK1 inhibitors and Trastuzamab, suggesting that PDK1 may be involved in Trastuzamab resistance.

Because the Her2 signaling pathway is a complex biological network where redundancy and crosstalk between pathways regulates cell growth and survival in tumors, multiple escape mechanisms circumventing inhibition of the Her system have been proposed as possible mechanisms of resistance. For example, inhibition of the PI3K and MAPK signaling may occur in the presence of other Her family receptors, such as Her1 and Her3. Trastuzamab was ineffective in blocking Her2/Her3 heterodimerization in cells expressing EGFR and

ErbB2 treated with Trastuzamab.[54] Her2/Her3 dimerization promotes activation of PI3K and Src, favoring cell survival and may account for Trastuzamab resistance.[55] Overexpression of EGFR can result in EGFR-Her2 dimerization which has been shown to increase MAPK activation and downstream signaling. Ligands of the Her family may be involved in formation of Her2 heterodimers. These include transforming growth factor-alpha, epidermal growth factor, and heregulin. Increased expression of these Her ligands may lead to stimulation of Her3 and activation of the PI3K pathway.[56–58] Her2 interacts with other membrane receptors, possibly triggering survival signaling pathways that result in Trastuzamab resistance. Increased signaling from IGF1R activated by IGF1 activates the PI3K signaling pathway.[59]

Alteration of ADCC

There is both in vitro and in vivo evidence suggesting that the anti-tumor activity of Trastuzamab mediated by ADCC may be altered inducing Trastuzamab resistance. Two types of cells, Trastuzamab-resistant (acquired from Trastuzamab-resistant cell lines) and Trastuzamab-sensitive tumor cells, injected in athymic mice and treated with Trastuzamab caused tumor growth inhibition, suggesting that resistance in vitro is not predictive of resistance in vivo.[60] ADCC was assessed in both the Trastuzamab-resistant and Trastuzamab-sensitive cells treated with and without Trastuzamab. Immune-mediated killing of tumor cells was equal between both cell lines in the presence of Trastuzamab and both cell lines showed similar susceptibility to ADCC despite having different growth responses to Trastuzamab in vitro. Yoshida et al found that long-term exposure of Her2+ breast cancer cells to Trastuzamab induced drug resistance that was associated with down-regulation of Her2 expression and impairment of ADCC activity.[61] They then re-sensitized the Trastuzamab-resistant cells to Trastuzamab by inducing exogenous Her2-ECD expression which enhanced ADCC activity in low Her2-expressing or Trastuzamab-resistant human cancer cells. Barok et al demonstrated that tumor growth was inhibited by Trastuzamab in xenografts in severe combined immunodeficient (SCID) mice derived from human Her2+ JIMT-1 cancer cells, which are intrinsically resistant to Trastuzamab via ADCC. Moreover, in vitro ADCC reaction of human leukocytes was equally strong against breast cancer cells intrinsically sensitive (SKBR-3) or resistant (JIMT-1) to Trastuzamab or even against a subline of JIMT-1 that was established from xenograft tumors growing despite Trastuzamab treatment.[62, 63] The investigators studied the effect of Trastuzamab on the number of circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) in this xenograft model at a time when the primary tumor was already resistant to Trastuzamab and found that the number of CTCs and DTCs was reduced. They concluded that Her2+ CTCs and DTCs may be sensitive to Trastuzamab-mediated ADCC even if the primary tumor is resistant.

Alternative Drugs for Trastuzamab-resistant Her2+ breast cancer (Table 2)

Lapatinib

Lapatinib is a small molecule tyrosine kinase inhibitor of EGFR/HER1 and HER2 that binds to the intracellular domains of HER1 and HER2 to reversibly inhibit receptor phosphorylation and subsequent activation of downstream signaling pathways.[64, 65] Its use has been approved in combination with endocrine therapy and Capecitabine for the treatment of hormone receptor-positive Her2+ metastatic breast cancer in patients who have progressed on chemotherapy and Trastuzamab.[66, 67] Preclinical studies have demonstrated that combining Lapatinib with Trastuzamab results in synergistic anti-tumor activity compared with either agent alone by targeting intracellular and extracellular Her2 domains.[64] A Phase III randomized clinical trial has recently demonstrated that the combination of Lapatinib and Trastuzamab resulted in longer PFS compared to monotherapy and offered a significant 4.5 month median OS advantage in patients with heavily pretreated

Her2+ metastatic breast cancer.[68] A neoadjuvant study of Lapatinib in early stage Her2+ breast cancer recently reported that adding Lapatinib to Trastuzamab and chemotherapy in the neoadjuvant setting resulted in a higher cPR rate (51.3%) compared to either agent alone (29.5% for Trastuzamab; 24.7% for Lapatinib).[39] Additional clinical trials evaluating Lapatinib and Trastuzamab are currently ongoing. Long-term follow-up data on this drug combination is not yet available and the longevity of these beneficial effects remains to be seen.

Pertuzamab

Pertuzamab is a monoclonal antibody that targets an epitope of the extracellular domain of Her2 that is distinct from that which binds to Trastuzamab. It inhibits homo- and heterodimerization within the Her family and IGF-1R.[69–71] A single-arm Phase II study evaluated Pertuzamab in 66 patients with Her2+ metastatic breast cancer who had received up to 3 prior Trastuzamab-containing regimens and found a 24% response rate with 50% of patients demonstrating stable disease, suggesting that Pertuzamab may have a role in treating Trastuzamab-resistant Her2+ breast cancer. [72] Pertuzamab has recently been approved for use in metastatic Her2+ breast cancer in combination with Docetaxel for patients who have not received prior anti-Her2 targeted therapy or chemotherapy for metastatic disease. This was based on a Phase III multi-center randomized clinical trial, the CLEOPATRA (Clinical Evaluation of Pertuzamab and Trastuzamab) study, of 808 patients comparing the combination of Pertuzamab with Trastuzamab and Docetaxel with placebo plus Trastuzamab and Docetaxel as first-line therapy in Her2+ metastatic breast cancer.[73] This trial demonstrated a significant prolongation of PFS in the Pertuzamab arm (18.5 months vs. 12.4 months, $p < 0.001$) with an objective response rate of 69.3% in the control arm compared to 80.2% in the Pertuzamab arm. A phase II neoadjuvant trial of Pertuzamab and Trastuzamab with chemotherapy in Her2+ breast cancer, the NeoSphere study, demonstrated a significantly higher rate of cPR in the Pertuzamab arm (45.8%) compared to the control arm (29.0%).[74] While Pertuzamab appears to have anti-tumor activity, the combination of Pertuzamab with Trastuzamab was demonstrated to be more efficacious than Pertuzamab monotherapy in a clinical trial of patients with advanced Her2+ breast cancer who had previously progressed on Trastuzamab.[75]

Several of these investigators studied biomarkers as predictive and prognostic factors in Her2+ patients from the CLEOPATRA and NeoSphere trials, the results of which were reported recently at the 35th Annual San Antonio Breast Cancer Symposium. Baselga and colleagues performed biomarker analysis on tissue and serum samples from patients in the CLEOPATRA trial to determine qualitative associations between biomarkers (HER2, IGF-1R, PTEN, EGFR, and TGF-α) and benefit from pertuzamab treatment. They found that all patients benefited from the addition of pertuzamab to the treatment regimen, regardless of the level of expression of any of the biomarker candidates. In a prognostic analysis of the treated patients, expression of HER2, HER3 and HER3 messenger RNA were associated with better prognoses, and PI3KCA mutations were associated with worse prognoses ($p<0.05$).[76] Gianni and colleagues performed gene expression profiling of the tumors in the NeoSphere study to assess the association of pre-selected adaptive immune functions and key immune regulatory genes with cPR to neoadjuvant therapy.[77] They found that high expression of PD-L1, programmed death ligand 1, which plays a role in inhibiting proliferation of CD8+ T cells possibly allowing cancer cells to evade the host immune system, was consistently associated with low pCR in all chemotherapy containing arms. These findings support the notion that the immune gene signatures may be predictive of response to therapy.

T-DM1

Trastuzamab-emtansine (T-DM1) is an antibody-drug conjugate linking Trastuzamab to Emtansine, a cytotoxic agent that has anti-microtubule activity. This drug uses Trastuzamab's monoclonal antibody to optimize delivery of the chemotherapeutic drug, Emtansine to Her2+ breast cancer cells. Preclinical trials have shown a synergistic effect of combining Trastuzamab with anti-microtubulin agents. [78] Studies performed in vitro and in vivo demonstrated that T-DM1 showed greater activity compared with non-conjugated Trastuzamab while maintaining selectivity for Her2+ tumor cells. In these trials, Trastuzumab linked to DM1 through a nonreducible linker offered improved efficacy and pharmacokinetics and reduced toxicity over other reducible disulfide linkers evaluated, Trastuzumab-MCC-DM1 was selected for clinical development.[79] To investigate whether T-DM1 is active in patients with treatment-refractory metastatic Her2+ breast cancer, Krop and colleagues tested its efficacy in a Phase II trial of 110 patients whose disease had progressed on all standard Her2-directed therapies as well as a taxane, an anthracycline, and Capecitabine. In this single-arm, phase II study, treatment with T-DM1 was well tolerated and resulted in an overall response rate of 34.5% and a median PFS duration of 6.9 months. Interestingly, patients with confirmed Her2+ primary tumors had an overall response rate of 41.3%, compared with 20% in patients with non-Her2+ tumors, suggesting that Her2 status of a tumor may change over time and that even clinically undetectable levels of Her2 may be sufficient to render a tumor sensitive to T-DM1. Given that this cohort of patients had received a median of 7 prior treatments for metastatic breast cancer, these findings indicate that T-DM1 may represent a therapeutic option for refractory Her2+ disease.[80] Results of the phase III EMILIA trial were recently reported at the 2012 ASCO Plenary Session, demonstrating a 3.2 month improvement in PFS for patients with locally advanced or metastatic Her2+ breast cancer (who previously received taxane with Trastuzamab) receiving T-DM1 compared to those who received Capecitabine and Lapatinib (9.6 versus 6.4 months, $p<0.0001$). OS favored the group that received T-DM1 with HR=0.621. This drug was well tolerated compared to the other drugs and is proving to be a promising alternative therapy for Her2+ breast cancer.[81, 82] The results of this trial led to the approval by the FDA for the use of T-DM1 for the treatment of metastatic Her2+ breast cancer whose disease has progressed with Trastuzamab and taxane therapy in February of 2013.

Biomarker analysis of the EMILIA trial revealed that the only predictor of response to TDM-1 appeared to be the extent of HER 2 over-expression, while the presence or absence of mutations in the PIK3CA pathway did predict response to lapatinib/capecitabine. [83] These findings suggest that the efficacy and mechanism of action of TDM-1 is primarily dependent on the targeted delivery of emtansine to HER-2 expressing cells, with a possible "bystander" effect on nearby cells as well, and is not affected by resistance through upregulation of downstream signaling. In addition, as all patients enrolled were required to have displayed progression on trastuzumab, the observation of responses to TDM-1 following progression on trastuzumab suggested that ADCC may not play a primary role in the activity of TDM-1.

Neratinib

Neratinib is a low-molecular weight, orally administered irreversible Erb-B receptor tyrosine kinase inhibitor. This drug has activity against Her1, Her2 and Her4. Neratinib was shown in preclinical studies to inhibit proliferation of Her2+ breast cancer cell lines and Her1-overexpressing epidermal carcinoma cell lines.[84] In an open-label phase II trial of patients with advanced Her2+ breast cancer (with and without prior Trastuzamab treatment) treated with Neratinib as monotherapy, the 16-week PFS rates were 59% for patients with prior Trastuzumab treatment and 78% for patients with no prior Trastuzumab treatment. Median

PFS was 22.3 and 39.6 weeks respectively.[85] The drug was very well tolerated with diarrhea being reported as the most frequent grade 3/4 adverse event. Several phase I/II trials of Neratinib in combination with other drugs, such as Vinorelbine and Capecitabine, have been performed in the pretreated Her2+ metastatic setting demonstrating acceptable tolerability of the drug.[86–88] Several Phase III trials investigating Neratinib in the adjuvant and metastatic setting, including the ExteNET (placebo vs. Neratinib), NEFERTT (Paclitaxel + Herceptin vs. Paclitaxel + Neratinib) are currently underway.

M-TOR inhibitors

There has been a strong interest in investigating therapeutic targets against the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway in Her2+ breast cancer. M-Tor plays a role in mediating cell growth and proliferation by means of downstream activation of the PI3K/AKT signaling pathway. Because inhibition of one aspect of this signaling pathway may lead to compensatory activation of another aspect of this pathway, combination therapy has been proposed to overcome drug resistance that may occur with monotherapy.[89] The combination of m-TOR inhibitor, RAD001, with Trastuzamab resulted in greater reduction in cell growth compared to single agent anti-Her2 therapies, including Trastuzamab and Lapatinib, in Her2+ breast cancer cell lines. These results were confirmed in a mouse model of Her2+ breast cancer, where the combination of RAD001 with Trastuzamab induced more tumor cell death compared to either agent alone.[90, 91] Everolimus and Temsirolimus are m-TOR inhibitors that have been under investigation in Phase I/II clinical trials. Temsirolimus was shown to have an objective response rate of 9.2% in heavily pretreated patients with locally advanced or metastatic breast cancer with median time to progression of 12 weeks and minimal grade 3/4 toxicity.[92] Several phase I/II studies of Everolimus in combination with Trastuzamab with or without chemotherapy have demonstrated clinical benefit with limited toxicity in pre-treated patients with recurrent or metastatic Her2+ breast cancer.[93–96] The objective response rates in these studies ranged from 15–44% with PFS ranging from 4.1 months – 8.5 months. Patients are currently being enrolled in phase III randomized, double-blind, placebo-controlled trials of Everolimus, including the BOLERO-1 trial (NCT00876395), which is investigating the efficacy of Everolimus with Trastuzamab and Paclitaxel as first-line therapy in the metastatic setting, and the BOLERO-3 trial (NCT01007942), which is assessing the use of Vinorelbine and Trastuzamab with or without Everolimus in pretreated patients with locally advanced or metastatic Her2+ breast cancer. Everolimus has recently been approved by the FDA for the treatment of pretreated post-menopausal women with metastatic hormone-sensitive, Her2-negative breast cancer when used in combination with Exemestane based on the results of the BOLERO-2 (NCT00863655) trial.[97]

Inhibition of PI3K in Her2+ breast cancer cell lines has been shown to inhibit AKT, but this leads to compensatory up-regulation of ERK signaling which activates Her family receptors.[98] Combined anti-Her2 or anti-MEK therapy with PI3K inhibitors has been suggested to overcome this compensatory up-regulation of ERK signaling. Hyper-activation of the PI3K/mTOR pathway caused by PI3K oncogenic mutations implicated in Trastuzamab resistance was reversed by dual PI3K/m-TOR inhibitor NVP-BEZ235 in vitro.[99] This drug also inhibited proliferation of Trastuzamab-resistant Her2-amplified cells expressing these mutations and inhibited PI3K signaling in Trastuzamab-resistant xenografts resulting in potent anti-tumor activity. PI3K/mTOR inhibition was also shown to increase Lapatinib sensitivity in Trastuzamab-resistant Her2+ breast cancer cell lines.[100]

Anti-angiogenesis agents

Her2 overexpression has been shown to cause increased expression of vascular endothelial growth factor (VEGF) at both the RNA and protein levels and exposure of Her2+ breast cancer cells to Trastuzamab significantly decreases VEGF expression.[101, 102] Bevacizumab is a humanized monoclonal antibody that targets VEGF, forming a complex which is incapable of further binding to VEGF receptor sites. This reduces available VEGF, inhibits angiogenesis and delays tumor growth. In preclinical studies, VEGF has been shown to be upregulated in tumors resistant to Trastuzamab both in vitro and in vivo. In these studies, tumor growth observed in Trastuzamab-resistant SCID mouse tumor models of Her2+ breast cancer was inhibited by Bevacizumab.[103] In a phase II trial of patients with metastatic Her2+ breast cancer treated with Bevacizumab and Lapatinib, a clinical benefit rate of 30.8% was observed with an overall response rate of 13.3% and median PFS of 24.7 weeks with minimal toxicity.[104] Bevacizumab was used in combination with Trastuzamab and chemotherapy in the neoadjuvant setting in a Phase II trial of patients with non-metastatic Her2+ inflammatory breast cancer. CPR was observed in 63.5% of cases. [105] While there have been promising results with Bevacizumab, there has been controversy as to whether this drug should be approved for use in breast cancer. Its use was approved by the Food and Drug Administration (FDA) in 2008 for the treatment of Her2-negative metastatic breast cancer and this decision was reversed in 2010 due to safety concerns, with reported increases in hypertension, proteinuria, thrombosis and hemorrhage. [106] This decision remains controversial in the oncology community, and investigators continue to enroll patients in phase III trials of Bevacizumab in breast cancer. The NSABP-B44 trial (BETH; NCT00625898) evaluating Bevacizumab added in the adjuvant setting to chemotherapy and Trastuzamab in node-positive or high-risk node-negative Her2+ operable breast cancer has completed accrual and we await a report of the results.

Other agents

Other agents that are being developed for Trastuzamab-resistant Her2+ breast cancer include the irreversible tyrosine kinase inhibitor BIBW2992, Metformin, which promotes inhibition of m-TOR, and MM-III, a novel monoclonal antibody against Her2 and Her3 that prevents their dimerization. The data on the efficacy of these drugs for Trastuzamab-resistant breast cancer is currently pending.

Potential mechanism of increased risk and Trastuzamab resistance outside of Her2 signaling

As described in this review, the mechanism of Her2 signaling has been the focus of extensive research in an attempt to identify additional targeted therapies for patients with Trastuzamab-resistant Her2+ breast cancer, and all of these therapies target various downstream components of the pathway associated with Her2 signaling. However, due to redundancy and crosstalk in the Her2 signaling pathway, the effects of many of these drugs may be short-lived, and acquired resistance may continue to be a challenge as the data on these newer drugs become fully mature. This raises the question whether mechanisms outside of the Her2 signaling pathway should be investigated to target Her2+ breast cancer. In addition, there is evidence that the degree of Her2 overexpression is inconsistent within Her2+ breast tumors and that over time some Her2+ tumors cease to overexpress Her2, becoming Her2-negative (Her2-) tumors. Pectasides and colleagues[107] demonstrated that 37% of patients with Her2+ primary breast cancer no longer exhibit Her2 overexpression in their recurrent metastatic lesions. Investigators who studied the effect of preoperative chemotherapy and Trastuzamab in patients with locally advanced Her2+ breast cancer reported conversion of 32–43% of patients from Her2+ to Her2- upon evaluation of surgically resected specimens.[108, 109] Relapse-free survival was superior in the patients

who retained Her2 overexpression. These findings suggest that there is heterogeneity in Her2 overexpression within Her2+ tumors and that there may be specific biologic features in Her2 primary breast cancer that predict which tumors exhibit more aggressive behavior. In fact, intratumoral genetic heterogeneity within Her2+ breast tumors has been well documented in up to 30% of cases and should be an important factor in selecting patients for Trastuzamab therapy.[110–112] It is possible that those identified to be at higher risk for recurrence are also more likely to develop resistance to therapies that target the Her2 signaling pathway. There may be distinct biologic properties of Her2+ breast cancer that are predictors of increased risk of recurrence and resistance to Trastuzamab. Recent clinical evidence supports the basal-Her2 phenotype as a potential factor in increased risk and Trastuzamab resistance in certain subtypes of Her2+ breast cancer.

Basal-Her2 phenotype

Molecular profiling of breast tumors has led to a new classification of breast cancer based on expression of certain biologic markers within the primary tumor. There are 5 major subtypes, including luminal A, luminal B, basal, Her2+, and normal-like, with those carrying the basal phenotype predicted to have the worst outcomes.[113–115] The luminal phenotypes are characterized by estrogen-receptor (ER)and/or progesterone-receptor (PR) positivity, and the basal phenotype, which is characterized by expression of basal cytokeratins CK5/6, 14, and EGFR, is typically associated with triple negative breast cancer (does not express ER, PR or Her2). However, there can be overlap of ER, PR and Her2 expression among the various subtypes; for example, some ER+ tumors may express basal cytokeratins, and some luminal tumors do not express ER.

A basal-Her2 phenotype, defined by expression of basal associated genes, such as CK5/6, EGFR, CK 14, and overexpression of Her2 as defined by IHC or FISH has recently been described and preliminary work in our laboratory has demonstrated that those tumors expressing the basal-Her2 phenotype appear to be associated with a worse prognosis than those with luminal-Her2 or Her2+ tumors. [116] In a cohort of 131 patients with operable Her2+ breast cancer, we identified 9% whose primary tumor expressed one or more of the basal CK5/6 and CK14. This subgroup had a 65% 5-year OS compared to 94% and 96% 5-year OS demonstrated in the luminal and Her2+ subtypes, ($p=0.0035$ and $p=0.0031$, respectively). These findings are consistent with results reported by Blows et al.[117] This group pooled molecular profiling data from over 10,000 cases from 12 studies and demonstrated the basal-Her2 phenotype in 8.1% of cases. This subgroup had a worse prognosis compared to the luminal Her2 subgroup and the basal-Her2 group had similar survival to those with the triple-negative phenotype. Liu et al [118] demonstrated that the basal-Her2 subtype had the worst 5-year survival among 713 ER-negative breast cancers. These data indicate that the basal-Her2 phenotype is associated with a higher risk of recurrence. Data regarding Trastuzamab therapy was inconsistent or not reported in these studies; therefore, little is known about how those with the basal-Her2 subtype respond to Trastuzamab.

There has been accumulating clinical and preclinical evidence suggesting that the basal molecular subtype may play a role in Trastuzamab resistance of certain Her2+ tumors. Harris and colleagues [119] investigated the relationship between molecular subtypes and response to neoadjuvant Trastuzamab and Vinorelbine in patients with operable Stage II/III Her2+ breast cancer and found that those with the basal phenotype were more likely to be resistant to the drug regimen. Lesniak et al. [120] identified B1-intergrin, an adhesion molecule involved in cell migration, invasion and progression in basal tumors[121], to be an independent prognostic factor for shorter time to progression in patients with Her2+ metastatic breast cancer who were treated with Trastuzamab-based chemotherapy. Expression of beta integrins has also been shown to combine with ErbB2 to amplify its

signaling ability and promote tumor progression in mouse models of Her2+ breast cancer. [122] Oliveras-Ferraros et al. [123] performed a low-scale proteomic analysis to explore expression of various biomarkers associated with Trastuzamab resistance in a model of basal Her2+ breast cancer, the JIMT-1 cell line. They applied antibody-based array technology to the extracellular milieu of the Trastuzamab-resistant JIMT-1 and the Herceptin-sensitive cell line, SKBR3, and identified increased secretion of a number of growth factors, including amphiregulin, EGF, IGFBP-6, VEGF, and TGFB. HCC1569 is a basal-Her2 cell line that demonstrated minimal growth inhibition when treated with the combination of Trastuzamab and Lapatinib, suggesting de novo resistance to both of these drugs in vitro.[124]

The current data on the basal-Her2 phenotype comprise a compelling argument for further investigation as potential biologic predictors of worse prognosis and possible resistance to therapy among patients with Her2+ breast cancer.

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Table 1

Proposed mechanisms of Herceptin Resistance.

Methods of Resistance	Protein	Mechanism
Epitope Masking	MUC4[1]	Disrupts binding of Herceptin to Her2
	CD44	Binding to hyaluronan activates PI3K/Akt[2]
	p95Her2 (truncated form of Her2)	Dimerizes with Her3 to interfere w receptor-antibody binding
Upregulation of Her2 downstream signals	PTEN[3] mutation	Loss pf PTEN allows PI3K activation
	PI3K mutation	Disrupts binding of Herceptin with Her1/Her3/PI3K allowing Akt activation
	PDK1[4]	Akt signaling
Overexpression of Her family members	Her1, Her3, EGFR [5], ErbB2	Increased MAPK[6], PI3K signaling
Overexpression of Her ligands	TGF-α[7], EGF[8], heregulin	Activation of PI3K
Alteration of ADCC[9]	Impaired immune-mediated mechanism	Interferes with Herceptin-mediated ADCC

[1] mucin-4 protein;

[2] Phosphatidylinositol 3-kinase/protein kinase B;

[3] Phosphatase and tensin homolog;

[4] Phosphoinotiside-1;

[5] Epidermal growth factor receptor;

[6] Mitogen-activated protein kinase;

[7] Transforming growth factor-α;

[8] Epidermal growth factor;

[9] Antibody-Dependent Cell-Mediated Cytotoxicity

Table 2

Drugs developed to target Herceptin-resistant Her2+ breast cancer.

Anti-Her2 drug	Mechanism of Action	Status
Lapatinib	small molecule tyrosine kinase inhibitor of EGFR[1]/ Her1/Her2 that binds to intracellular domain of Her1 & Her2	Approved in combination with endocrine therapy and capecitabine for the treatment of hormone receptor-positive Her2+ metastatic breast cancer in patients who have progressed on chemotherapy and Herceptin
Pertuzamab	monoclonal antibody that targets an epitope of the extracellular domain of Her2	Approved for use in metastatic Her2+ breast cancer in combination with docetaxel as first-line therapy
TDM-1[2]	antibody-drug conjugate linking Herceptin to emtansine	Results of Phase III studies in patients with pre-treated locally advanced and metastatic Her2+ breast cancer recently reported
Neratinib	low-molecular weight, irreversible tyrosine kinase inhibitor with activity against Her1, Her2 & Her4	Several Phase III trials of Neratinib combination therapy in adjuvant and metastatic setting underway (ExteNET[3], NEFERRT[4])
mTOR inhibitors	targets against the PI3K/AKT/mTOR[5] pathway in Her2+ breast cancer	Phase III trials of Everolimus with Herceptin and Paclitaxel (BOLERO-1[6]) or vinorelbine (BOLERO-3[7]) in pretreated locally advanced or metastatic Her2+ breast cancer underway
Bevucizamab	monoclonal antibody that targets VEGF[8], inhibits angiogenesis and delays tumor growth	BETH trial (Bevucizamab + Herceptin + chemo) in adjuvant setting in node-positive or high-risk node-negative Her2+ breast cancer has completed accrual; report of results pending

[1] Epidermal growth factor;

[2] Trastuzamab emtansine;

[3] Study Evaluating The Effects Of Neratinib After Adjuvant Trastuzumab In Women With Early Stage Breast Cancer;

[4] Study Evaluating Neratinib Plus Paclitaxel VS Trastuzumab Plus Paclitaxel In ErbB-2 Positive Advanced Breast Cancer;

[5] Phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin;

[6] A randomized, phase III, double-blind, placebo-controlled multicenter trial of everolimus in combination with trastuzumab and paclitaxel as first-line therapy in women with HER2-positive, locally advanced or metastatic breast cancer;

[7] Daily Everolimus in Combination With Trastuzumab and Vinorelbine in HER2/Neu Positive Women With Locally Advanced or Metastatic Breast Cancer;

[8] Vascular endothelial growth factor