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EXPERT REVIEW

Resistance to HER2-Targeted Therapy in HER2+ Breast Cancer

Elizabeth R. Kessler[1] and Anthony D. Elias[2]

[1]Fellow in Medical Oncology, University of Colorado School of Medicine, [2]The Martha Cannon Dear Professor of Medicine, Division of Medical Oncology, University of Colorado School of Medicine, MS F-724, 1635 Aurora Court, Aurora, CO 80045. Corresponding author email: anthony.elias@ucdenver.edu

Abstract: Breast cancer is the most commonly diagnosed cancer in women and one of the earliest tumor types for which we have used molecular characterization of the tumor to guide treatment. Approximately one quarter of breast tumors show overexpression of HER2, a transmembrane receptor tyrosine kinase. This review focuses on the HER2 pathway and consequences of overexpression, or activation, of this signal. Trastuzumab, the first line monoclonal antibody directed at HER2 will also be described in terms of mechanism of action and influence on patient care. Additional focus will be paid to understanding mechanisms of primary and secondary resistance to the agent. We then attempt to describe the current milieu of therapeutic options for patients resistant or refractory to trastuzumab. There are certainly many new targeted agents as well as exciting preclinical data which may offer some direction for treatment of patients in whom trastuzumab is not an effective targeted agent.

Keywords: breast cancer, Her2+, treatment, resistance

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Introduction

Breast cancer is the most commonly diagnosed cancer in women with almost half a million new cases diagnosed each year. Through molecular analysis we can now subcategorize this diagnosis into phenotypes with varying prognoses and responses to therapy. Breast cancer is one of the earliest tumor types for which targeted therapy has existed.

In this review, we will first describe the HER2 pathway and the molecular consequences of its activation, the therapeutic results when incorporating trastuzumab into the treatment algorithm, and finally delineate the mechanisms of resistance, the agents and clinical trials that provide us guidance to treat tumors once resistant to trastuzumab-containing therapy. In this discussion, we will outline future directions and exciting experimental designs.

HER2, an epidermal growth factor receptor, has shown to be overexpressed in 15%–25% of breast cancer tumors and has been associated with more aggressive tumor types.[1] The HER/EGF receptor family is made up of four cell surface receptors labeled either as HER or ErbB. HER family members activate the RAS/RAF/mitogen activated protein kinase (MEK)/extracellular signal related kinases (ERK)[2] These pathways function to promote cellular survival, growth, differentiation, tumor invasion, metastasis and angiogenesis.[3,4]

Trastuzumab is a humanized monoclonal antibody directed at the HER2 receptor and functions to block the cell survival and proliferative effects of receptor activation. It is an effective form of treatment, however, primary and secondary resistance has been encountered.[5,6] There are numerous studies underway to investigate the mechanism of resistance and options for further therapy of HER2 positive breast cancer.

The phosphatidylinositol 3-kinase PI3K/Akt pathway.[2] This pathway functions to promote cellular survival, growth, differentiation, tumor invasion, metastasis and angiogenesis.[3,4]

HER Pathway

HER2 is a transmembrane tyrosine kinase receptor without an identified natural ligand.[7,8] It does not bind canonical growth factors, but functions through dimerization with other members of the HER family.[9,10] It activates multiple pathways including PI3K and MAPK important in cell survival and proliferation. By heterodimer formation with other HER receptors, HER2 can amplify signaling, increase growth factor affinity for the heterodimer, and broaden the ligand binding specificity.[11] Upon dimerization, the tyrosine residue within the cytoplasmic domain serves as a docking site for adaptor proteins, which then activate nuclear effectors. Through amplification, not receptor mutation, HER2 is partially responsible for the progression from normal epithelia into invasive cancer in both breast and gastric cancer. HER ligands have been shown to transform mouse fibroblasts and seem to play an important role in human oncogenesis.[12] Given these characteristics, this receptor is a target of many small molecule inhibitors both approved and in development.

Mechanism of Trastuzumab

Trastuzumab is a recombinant humanized monoclonal antibody that binds to the extracellular domain of HER2 thus blocking the cell proliferative effects of HER2 signaling.[13] The exact mechanism of action is yet to be understood, yet some key features have been described.

Trastuzumab binds to the extracellular domain of HER2 resulting in antibody-dependent cell-mediated cytotoxicity.[14] Decreased receptor expression occurs as trastuzumab aids in the internalization and degradation of HER2 resulting in G1 cell cycle arrest and angiogenesis inhibition.[11,15] By binding to HER2, trastuzumab stimulates signals that result in the ubi-quitination and eventual proteosomic degradation of HER2.[15] Trastuzumab also causes decreased signaling through the PI3K/Akt/mammalian target of rapamycin (mTOR) and MAPK pathways. This decreased signaling increases the interaction of p27 with cyclinD-kinase 2 (CDK2), which then promotes cell cycle arrest and apoptosis. Trastuzumab also decreases activity in the Akt pathway by dissociating Src from HER2, thus preventing phosphorylation of phosphatase tensin homolog (PTEN) and allowing for further tumor growth suppression.[16,17] These various mechanisms of action decrease cell survival and proliferation and serve as targets of possible resistance.

Trastuzumab in the Clinical Realm

Metastatic setting

Trastuzumab has proven efficacious as a single agent and in combination with chemotherapies.[6,18–20] As a single agent, 23% of patients with HER2 overexpressing metastatic cancer responded to trastuzumab used as

first line therapy for their disease, with a clinical benefit rate of 38% and a median time to progression of 3.5 months.[18] In heavily pretreated patients with metastatic disease, the overall response rate (ORR) was approximately 15% in the intention to treat grouping.[21] Compared with chemotherapy alone, trastuzumab in combination with chemotherapy, resulted in a reduction in the risk of death of 20%; time to progression (TTP) was also lengthened from 4.6 to 7.4 months ($P < 0.001$) and overall survival improved from 20.3 months to 25.1 months ($P = 0.046$). Similar results were seen comparing trastuzumab plus docetaxel in the metastatic setting versus docetaxel alone with an ORR of 61% ($P = 0.002$) and a survival benefit of 31.2 months versus 22.7 months ($P = 0.0325$).[19]

Adjuvant setting

The role of trastuzumab in combination with chemotherapy in the adjuvant setting has been assessed in more than four large trials including over 6000 patients.[22,23] The National Surgical Breast and Bowel Project (NSABP) B-31 and North Central Cancer Treatment Group (NCCTG) N9831 trials were designed as parallel trials evaluating chemotherapy versus chemotherapy plus trastuzumab. The combined results in a recent report of 3.9 years of median follow-up continue to show statistically significant reduction in disease free survival (DFS) of 48% favoring trastuzumab ($P < 0.001$) and a 39% reduction in death rate ($P < 0.001$).[24] These results were also substantiated by the Herceptin Adjuvant (HERA) trial in which patients with HER2 positive tumors were randomized to adjuvant trastuzumab of varying lengths.[22] The two year follow up data showed an unadjusted hazard ration (HR) for the risk of death with trastuzumab compared with observation alone when following chemotherapy of 0.66 (95% CI 0.47–0.91; $P = 0.0115$), and HR for DFS with trastuzumab was 0.64 (0.54–0.76; $P < 0.0001$).[25] In general, adjuvant trastuzumab has shown to decrease the risk of early recurrence by approximately 50% and reduce the risk of death by 33%–37%. The FinHer study was unique in that it suggests that a similar degree of risk reduction in the Her2 + BC subset could be obtained with only 9 weeks of trastuzumab given concurrently with either docetaxel or vinorelbine followed by 3 cycles of FEC chemotherapy.[122] Of 232 patients with Her2 amplification, 112 patients received docetaxel. In this subset, the 5y distant DFS was improved with trastuzumab from 74.1% to 92.5.3% (HR 0.32, $P = 0.029$). The Her2 + BC population was similarly a subset in the PACS04 trial.[123] Following 6 cycles of FEC, 528 patients with node-positive Her2 + BC were randomly allocated to sequential every 3-weekly trastuzumab or observation for a year. Only a 14% reduction in risk of relapse was observed at a median follow-up of 47 months, which was not statistically significant, although few events were recorded. 10% of the patients assigned to trastuzumab never received it.

Neoadjuvant setting

Given its success in the adjuvant setting, trastuzumab is also recommended for neoadjuvant therapy, especially as HER2 positive cancer generally has earlier relapse after treatment with surgery. The two largest trials have demonstrated that the addition of trastuzumab significantly improves pathologic complete response (pCR) to almost twice that of the standard chemotherapy group.[26–29] No overall survival benefit has yet been demonstrated. The "Neoadjuvant Herceptin" (NOAH) trial looked at the addition of trastuzumab to chemotherapy and found the 3-year event free survival was improved to 71% vs. 56% (HR 0.59, $P = 0.013$).[27] Additionally, Buzdar et al looked at the neoadjuvant treatment of HER2 positive tumors and found an improved pCR rate of 25% to 66.7% ($P = 0.02$).[26] The addition of trastuzumab to preoperative docetaxel has been shown to result in a pCR of 54% in patients with confirmed HER-2-over-expressing (3+) and/or fluorescence in situ hybridization (FISH) -positive tumors.[30,31] Neoadjuvant therapy has shown to be effective in improving pathologic responses and event free survival confirmed by the GEPARquattro trial.[121] Of 445 patients with T3-4 Her2 + BC, including 114 patients with inflammatory BC, pCR was achieved in 31.7%. An addition 14.2% had only residual in situ cancer.

Pathway Resistance

Much as with other targets of small molecules such as endothelial growth factor receptor (EGFR) or breakpoint cluster region/Abelson (BCR/Abl), resistance is encountered in HER2 targeted therapy. Of patients with HER2 over-expression, only 30% of patients

respond to trastuzumab and many of those patients will become resistant within a year of initiating therapy.[18,32] Among the proposed mechanisms, alterations have been identified in the HER2 receptor, thus influencing the binding of trastuzumab, but interestingly few point mutations have been identified. Additional resistance occurs from alteration within the pathway or network of pathways.

HER2 receptor alteration

P95 truncation

Truncated isoforms of HER receptors exist, arising from alternatively spliced transcripts of *HER2*, or from cleavage of full length *HER2*.[33–35] In certain breast cancer cell lines, overexpression of HER2 leads to cleavage of the receptor, release of the extracellular domain, and production of a membrane bound or soluble fragment. This results in constitutive activity of the remaining truncated receptor. The fragments were first labeled p95HER2, based on molecular weight, but are also known as carboxy terminal fragments (CTFs). Activity is greater when membrane bound where they can then dimerize with full length HER2 or HER3.[36] The quantitative levels of these fragments correlate with response to trastuzumab. Complete HER2 and truncated HER2 have been transfected into MCF-7 breast cancer cells and exposed to both lapatinib and trastuzumab, and have shown resistance only to trastuzumab.[37] Expression of p95HER2 in surgically excised breast cancer tumors has been retrospectively correlated with poor responsiveness to trastuzumab, nodal metastasis, and worse outcome.[33,37,38] These findings were not confirmed in the CHER-LOB study.[39] No clear therapeutic target exists to nullify these fragments, but they may function as biomarkers suggestive of poor prognosis or early trastuzumab resistance perhaps indicating dual therapy with lapatinib.

Δ16 splice variation

Three HER2 splice variants have been described, one of those being the Δ16HER2 that encodes a receptor with transforming activity. This variant is reported in 4%–9% of HER2 overexpressing breast cancer tumors.[40,41] ΔHER2 represents a constitutively active form of HER2 that may confer a more aggressive phenotype and resistance to trastuzumab.[42]

Heterodimerization

Members of the HER receptor family may interact to modify intracellular signaling. HER3 has no tyrosine kinase activity on its own yet does have at least six docking sites for the regulatory unit of PI3K. The combined signal of HER2/HER3 may be more functional than that of the individual receptors. Breast cancer cell lines expressing higher levels of these receptors have a higher degree of Akt phosphorylation and thus greater cell survival.[43] Cell lines overexpressing EGFR and HER2 are less sensitive to trastuzumab and pertuzumab.[11] The heterodimerization with other Her receptors is a druggable target that is currently under exploration in trastuzumab resistance.

MUC4

While not validated in a clinical setting, masking of the HER2 receptor by the membrane-associated glycoprotein mucin-4 (MUC4) prevents effective binding by trastuzumab.[44] MUC4 is a member of the mucin family of proteins that form protective barriers on epithelial cells such as mammary epithelia. In oncologic literature, MUC4 has been described as inhibiting immune recognition of cancer cells, promoting progression of metastasis, and suppressing apoptosis and activating HER2.[45] MUC4 is thought to directly interact with HER2 and increase the phosphorylation of a tyrosine residue which may contribute to transformation to an oncoprotein.[46] In vitro studies have demonstrated MUC4 mediated disruption of trastuzumab in resistant cell lines.[44] The MUC family certainly may influence the sensitivity of certain cells to trastuzumab.

Alteration of Growth Factor Pathways

Activation of other growth factor pathways can bypass the HER2 blockade. This upregulation of IGF1R, cMET or even the nuclear steroid receptor family such as ER can be associated with resistance to HER2 blockade. Additionally, abnormal activation of downstream key enzymes in the growth factor signaling pathway such as PTEN/PI3K, Akt, mTOR and p27 contribute to resistance.

Upregulation of other pathways

IGF-IR

The insulin-like growth factor-I receptor (IGF1R) is a transmembrane tyrosine kinase receptor expressed

in human breast cancer cells and involved in the promotion of proliferation and metastasis through the PI3K/Akt and MAPK pathways. Trastuzumab does not have any direct effect on IGF1R as it is not a member of the HER family, however, overexpression of IGF1R has shown to be important in resistance to trastuzumab. IGF1R overexpression upregulates Skp2, a ubiquitin ligase that decreases p27.[47] Liu et al has demonstrated heterotrimers of HER2, Her3 and IGF1R.[48] The role of these trimers and whether they occur in actual human tumors (outside of cell lines) is not known. As mentioned p27 is important in trastuzumab mediated cell cycle arrest. Additionally, cross talk between the IGF1R and HER2 receptors in trastuzumab resistant cell lines has been shown to induce phosphorylation of HER2, and more rapid signaling through IGF1 in comparison to the sensitive parental lines.[49] Lines with low levels of IGF1R also lose their sensitivity to trastuzumab upon overexpression, and IGF1R overexpression has been shown in resistant clones. A clinical trial of neoadjuvant therapy looked at biomarkers and found IGF1R overexpression correlated with reduced response rates from 97% to 50%.[50] Lu et al reported that IGF1R inhibitors and trastuzumab were effective in growth inhibition of MCF-7/HER2 cells lines over-expressing both HER2 and IGF1R.[51] Expression of IGF1R may serve not only as a predictive marker but a therapeutic target to enhance the efficacy of trastuzumab. CP-751871 and NVP-AEW541 are agents being tested.[52] There are multiple Phase I and II trials looking at the efficacy of IGF1R inhibitors as monotherapy or in combination in the treatment of breast cancer.[53]

cMET

cMet, a receptor tyrosine kinase, has also been implicated in trastuzumab resistance.[54] HER2 overexpressing breast cancer cells upregulate the cMET receptor upon trastuzumab exposure, limiting the in vitro activity of the drug. Shattuck et al examined HER2 overexpressing breast cancer tumors and cell lines and found higher levels of MET present in samples resistant to the combination of vinorelbine and trastuzumab.[54] Additionally, the cell line analysis showed that exogenous hepatocyte growth factor (HGF), the ligand of cMET, inhibited trastuzumab effects and upregulated p27. By treating with trastuzumab and cMET inhibitors, or by knocking down cMET, a synergistic growth inhibitory effect resulted. This upregulation of cMET may occur just upon exposure herceptin, and up-front pharmacologic inhibition may prevent early resistance.

ER

There is in vitro evidence supporting cross talk between HER2 and ER.[55] The proportion of patients with ER/HER2 positive breast tumors is approximately 9%. HER2 signaling eventually leads to activation of MEKK1 that can activate ER and stimulate the agonist activity of tamoxifen.[56] Retrospective clinical analysis has noted that patients with HER2 positive tumors respond poorly to antiestrogens with roughly similar effects seen with aromatase inhibitors or tamoxifen.[57,58,120] For example, pathologic complete response (CR) to neoadjuvant chemotherapy without trastuzumab in HER2+/ER– tumors is seen in 27%–45% of cases, but HER2+/ER+ tumors have an 8% rate of CR.[18] Time to progression of patients with HER2 positive breast cancer, treated with first line hormonal therapy is generally less than six months.[59]

PI3K and PTEN

The PI3K pathway mediates cell survival, and has shown to be important in many cancers.[60] Mutations of *PIK3CA (*PI3K) may result in hyperactivation independent of HER2 overexpression, leading to translocation of Akt to the membrane and subsequent phosphorylation of downstream targets,[61] including mTOR.

The PTEN phosphatase is a tumor suppressor that represses PI3K. Trastuzumab causes the dissociation of HER2 and Src, which then activates PTEN. Loss of PTEN, or low levels, is associated with resistance to trastuzumab through heightened PI3K activation, Akt phosphorylation and activation of mTOR.

Trastuzumab functions to reduce signaling through the HER2/PI3K pathways and to help promote apoptosis.[62] Activation of PI3K independent of the HER2/HER3 signaling pathway is associated with trastuzumab resistance.[63,64] Thus far various mutations have been identified in *PIK3CA* that are associated with transformation of epithelial cells.[60,65] The alpha subunit activating mutation occurs in 8%–40% of breast cancers but rarely in HER2 overexpressing tumors.[65–70] Analysis of patient samples showed *PIK3CA* mutations correlated with reduced time to progression after trastuzumab therapy.[64] Given the close association of

the PI3K pathway and mTOR, the efficacy of mTOR inhibitors has been explored in preclinical models in correlation with *PIK3CA* mutations.[71] Analysis of 31 breast cancer cell lines showed that *PIK3CA* mutational status predicted response to mTOR inhibitors. HER2 receptors also signal through mTOR in cancer cells, yet may act independently in cells with low PTEN.[71,72] Loss of PTEN occurs in one third of breast cancer, and 31.6% of HER2+.[67,73–76] Nagata et al describe the importance of decreased PTEN expression through 47 HER2 overexpressing xenograft tumors treated with trastuzumab and taxane chemotherapy.[77] PTEN level was significantly higher (35.7% vs. 66.7%) in the responders. Combined treatment with trastuzumab and PI3K inhibitor overcame the resistance. Preclinical studies have shown that inhibition of mTOR inhibits slows tumor growth in mice with PTEN and HER2 overexpressing tumors, and can sensitize them to trastuzumab therapy.[72] PTEN may serve as a marker of response rather than a druggable target.

PI3K and mTOR inhibitors are also being explored in clinical trials. A combination mTOR/PI3K inhibitor, BEZ235, is currently in phase I/II trials without efficacy data available.[78] This agent has been looked at in preclinical studies showing excellent inhibition of downstream effectors and antitumor activity in xenograft models.[79–81] Presentation of the Phase I data have shown tolerability with the most numerous adverse events being nausea, vomiting, fatigue and some hematologic effects. 14 of the 59 patients have breast cancer, with 4 of those 14 (29%) exhibiting stable disease $>$ 4 months and one partial response. Numerous mTOR inhibitors are already FDA approved for other tumor types yet are interesting targets in breast cancer given their association with the PI3K pathway. Preclinical data has evaluated rapalogues and shown efficacy especially in lines with PIK3CA mutations. Temsirolimus and everolimus are being explored as breast cancer therapies, regardless of HER2 status.[82–84] Additionally a phase I study showed everolimus combined with paclitaxel resulted in stable disease in 11 of 16 patients with breast cancer resistant to trastuzumab.[85] Two phase I/II trials focused on HER2 positive tumors evaluated everolimus in combination with trastuzumab and either paclitaxel or vinorelbine. 5 of 138 patients had a CR, 10 patients with PR and 16 with SD for an ORR of 19% and a disease control rate of 83%.[86] A recent phase I/II trial evaluated trastuzumab in combination with everolimus in patients with metastatic breast cancer,

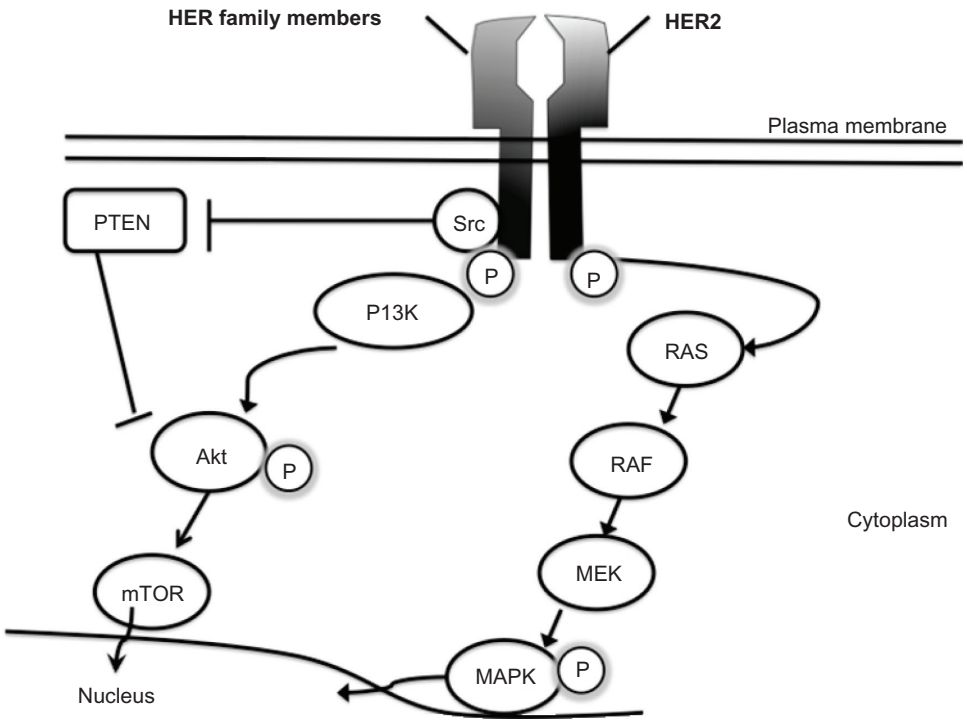

Figure 1. HER2 signaling pathway with common downstream effectors. (Adapted from Biocarta).

Table 1. Selected Her2 pathway inhibitors in clinical trials.

Agent*	Target	Phase
Lapatinib	TKI, HER2/EGFR	Approved
Pertuzumab	mAb, HER2	II/III
Neratinib	TKI, HER2/EGFR/HER3	III
CI1033	HER2/EGFR/Her3	Discontinued
Trastuzumab-DM1	mAb-Toxin	II/III
BEZ235	mTOR/PI3K	I/II
Temsirolimus	mTOR	II/III for breast
Everolimus	mTOR	II/III for breast

Note: *selected from the literature and clinicaltrials.gov.

HER2+, who had progressed on trastuzumab. The 47 enrolled patients continued on trastuzumab with the addition of everolimus with a 15% PR, SD in 19% for a clinical benefit rate of 34%.[87] Much earlier in development is AP23573, another mTOR inhibitor recently evaluated in a phase II clinical trial in combination with trastuzumab for patients with trastuzumab refractory metastatic breast cancer.[88] Thus far the drug is well tolerated and some early PRs have been seen. Akt signaling is certainly intertwined in this pathway. Since HSP90 inhibitors can reduce Akt signaling, tanespimycin (KOS953) has demonstrated anti-tumor activity when combined with trastuzumab in trastuzumab-refractory HER2 + BC.[89]

P27

HER2 overexpression is also associated with decreased expression of p27 through ubiquitination and enhanced degradation. Trastuzumab increases the half life of p27 and allows it to influence cdk2 and stimulate G1 cell cycle arrest.[90,91] However, cell lines resistant to trastuzumab generally have a decrease in p27 making the cell cycle arrest less effective.[92] No clear pharmaceutical target is identified but low levels of p27 may be predictors of resistance to trastuzumab.

Overcoming Resistance

As in many tumor types, we have found that one targeted agent may not be sufficient to overcome all forms of secondary resistance. There does not seem to be a single driving force or mutation to the transformation and growth of breast tumors. Additional HER2 therapies and molecules targeted at involved pathways may serve as treatment options for the proportion of patients that have tumors resistant to trastuzumab.

Lapatinib (Tykerb) is a reversible small molecule tyrosine kinase inhibitor of EGFR and HER2. Lapatinib binds to the intracellular domain of EGFR and HER2 and inhibits MAPK and Akt.[93] This effect has been seen in cells, xenografts and biopsy samples. It is functional in tumors that normally express HER2 and has shown some synergy with trastuzumab in the preclinical setting.[94] Single agent lapatinib has also been used in patients thought to be resistant to trastuzumab.[95,96] Lapatinib has been studied in combination with capecitabine and shown to be more efficacious than capecitabine alone. This patient population included those previously unresponsive to trastuzumab and showed a TTP of 19.7 weeks versus 36.9 with lapatinib, HR 0.51 (p 0.00016).[97] The EGF30008 trial reported the combination of letrozole plus lapatinib in patients with ER+/PR+ advanced or metastatic disease.[98] Patients with metastatic disease were randomized to first-line therapy with letrozole versus letrozole plus lapatinib. At early analysis, the PFS for the study is improved with the addition of lapatinib from 13 to 35.4 weeks, HR 0.71 (p 0.019) and the HER2 subset showed a response rate of 27.9% versus 14.8%.

Other agents also block HER2 such as HKI 272 (neratinib), an irreversible inhibitor of HER2 and EGFR that has shown activity in a phase II trial.[99] Phase I trials have shown a 40%–50% reduction in EGFR and HER2 phosphorylation and decreased proliferation, however phase II trials showed no clinical benefit.[100,101] Thus, lapatinib and other tyrosine kinase inhibitors may be an effective treatment option for patients resistant to trastuzumab.

Dual targeting of the HER2 pathway

In patients whose tumors progressed on trastuzumab-containing therapy, a trial compared switching to lapatinib vs. continuing trastuzumab with the addition of lapatinib.[102] At 6-month analysis 28% of patients in the combination arm had not progressed versus 13% in the lapatinib monotherapy arm with a HR of 0.73 (p 0.008) for the combination arm, the updated survival analysis in the 2009 San Antonio Breast Conference showed a median overall survival benefit of 14 months versus 9.5 months ($P = 0.026$).[102]

Preoperative combined HER2 blockade appears promising. In the absence of chemotherapy, neoadjuvant trastuzumab and lapatinib plus endocrine treatment as indicated achieved a pCR (no residual invasive tumor in breast) in 28% (21% for HER2+/

ER+ and 40% for HER2 + ER–).[103] Two phase II trials randomized patients to single vs. dual HER2 inhibition combined with chemotherapy. With FEC chemotherapy, dual inhibition increased the pCR rate from 54% and 45% to 74%.[104] In the CHER-LOB study, pCR (no invasive disease in breast and nodes) rates increased from 26% and 28% to 43% for trastuzumab, lapatinib, or the combination, respectively.[39] In each of these trials, dosing of lapatinib was limited by severe diarrhea.[105] Phase III studies, CALGB 40601, EORTC 10054, and NSABP B-41, are nearing completion.

Pertuzumab is a HER dimerization inhibitor that binds HER2 and sterically hinders its association with other HER receptors.[106] Trastuzumab and Pertuzumab bind to different epitopes in the extracellular domain of HER2.[107] Pertuzumab has shown pre-clinical activity in non-HER2 overexpressing tumors as well as activity in trastuzumab resistant cell lines, or synergistic effect in vitro with trastuzumab, and a phase I clinical trial assessed tolerability.[108] A study by Baselga et al evaluated pertuzumab plus trastuzumab and found an overall response rate of 18% with SD in 21% and clinical benefit in a total of 39%.[109] NEOSPHERE, a randomized phase II neoadjuvant trial, compared 12 weeks of trastuzumab or pertuzumab alone or in combination. The combination was best, achieving a 17% pCR rate in both breast and nodes.[110] Another phase II trial is underway looking at first line therapy with trastuzumab, docetaxel with or without pertuzumab. A phase III trial is currently investigating trastuzumab plus capecitabine, plus or minus pertuzumab in patients who have progressed on single agent trastuzumab in the metastatic setting.[111] Various phase III trials are currently underway evaluating pertuzumab in combination with additional agents.[112]

Trastuzumab Emtansine (T-DM1) is composed of trastuzumab and DM1, a potent antitubulin, linked by a stable thioether[113] Importantly, systemic exposure to free DM1 is minimal, thus peripheral neuropathy is typically minor. As a single agent, the MTD is 3.6 mg/kg given every 3 weeks or 2.4 mg/kg given weekly.[114,115] The safety profile includes thrombocytopenia and mild/moderate transaminitis. Each of the phase I studies demonstrated over 50% clinical benefit rates in HER2 + BCs previously treated with both trastuzumab and lapatinib. Two phase II trials in previously treated BC demonstrated major responses in 34%–41% of HER2 + BCs and much lower benefit rates in HER2 normal breast cancers.[113,116–118] Preliminary results of a randomized phase II comparing T-DM1 to trastuzumab plus docetaxel in first line patients demonstrated response rates of 47.8% vs. 41.4%,[119] although this was not deemed sufficient to obtain accelerated approval by the FDA. Two phase III studies are evaluating T-DM1. EMILIA randomizes 1:1 T-DM1 vs. lapatinib plus capecitabine in patients with metastatic HER2 + BC previously treated with trastuzumab and taxanes. MARIANNE compares T-DM1 plus placebo vs, T-DM1 plus pertuzumab vs. trastuzumab plus a taxane in first line HER2 + metastatic breast cancers.[112] Phase I trials are combining T-DM1 and pertuzumab with taxanes.

Conclusion

The use of targeted therapies has added to the treatment possibilities in oncology. The targeted therapy of trastuzumab has numerous mechanisms by which it blocks the cell proliferative signals of HER2. However, as with many agents, sensitivity is not 100% in HER2 overexpressors, and eventually, tumors learn to alter other sibling pathways in order to continue to grow. Through understanding the HER2 signaling pathway, and the mechanisms by which trastuzumab works, one may then look to additional therapies when it fails. There are new agents in development with promising results both singularly and in combination with continuing trastuzumab HER2 blockade. Combined blockade of the HER2 pathway appears to be more effective than single agents, but how these will be applied clinically will depend on the balance of therapeutic benefit vs. increased toxicity.

Author Contributions

ERK and ADE were responsible for literature search and review. ERK and ADE prepared the manuscript. All authors read and approved the final manuscript.

Disclosures

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to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest. Provenance: the authors were invited to submit this paper.

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