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Targeting HER2 for the Treatment of Breast Cancer

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Abstract

HER2 (ErbB2), a member of the HER family of tyrosine kinase receptors (HER1–4), is a major driver of tumor growth in 20% of breast cancers. Treatment with the anti-HER2 monoclonal antibody trastuzumab has revolutionized the outcome of patients with this aggressive breast cancer subtype, but intrinsic and acquired resistance is common. Growing understanding of the biology and complexity of the HER2 signaling network and of potential resistance mechanisms has guided the development of new HER2-targeted agents. Combinations of these drugs to more completely inhibit the HER receptor layer, or combining HER2-targeted agents with agents that target downstream signaling, alternative pathways, or components of the host immune system, are being vigorously investigated in the preclinical and clinical settings. As a result, the list of more effective and well tolerated FDA-approved new regimens for patients with HER2+ tumors is constantly growing.

INTRODUCTION

Carcinoma of the breast is a heterogeneous group of cancers. Therapeutically, it is subclassified into three groups: estrogen receptor (ER) positive, HER2 positive (amplified for the ERBB2 gene), and ER negative or triple negative. The term triple-negative breast cancer (TNBC) refers to the absence of ER, progesterone receptor (PR), and HER2. HER2-positive (HER2+) tumors account for 20% of breast cancers, and they overexpress the HER2 protein, which drives their growth. These tumors are aggressive with rapid growth, early metastasis with frequent spread to the central nervous system, and a relatively poor prognosis for the patient. They are typically treated with therapies that inhibit HER2 signaling together with chemotherapy. About half of HER2+ tumors express ER, and their treatment also includes endocrine therapy. Clarification of the factors that activate HER2 and the signaling pathways regulated by it has led to new targeted therapies that have dramatically changed the outcome of patients with this subtype of the disease.

THE HER2 NETWORK

HER2 is a member of a family of four membrane tyrosine kinase (TK) receptors (HER1–4) (**Figure 1**) (1). HER receptors have an extracellular ligand-binding domain, a transmembrane domain, and an intracellular TK domain. These receptors work together to activate multiple signaling pathways that regulate proliferation, apoptosis, invasion and metastasis, angiogenesis, and cell differentiation.

The HER network is complex with many redundant control circuits (1). HER1 (EGF receptor), HER3, and HER4 are activated at the cell membrane by the binding of different ligands. Ligand binding induces a change in receptor conformation that facilitates homo- or heterodimerization with another HER family member to activate theTKs; these then initiate an intracellular phosphorylation cascade downstream via several pathways including the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K) pathways. Eventually a variety of transcription factors in the nucleus alter expression of genes that contribute to the malignant phenotype. Although HER2 does not have its own ligand, it already exists in the proper open conformation for dimerization, and because it is abundant in HER2-amplified tumors, it is the preferred dimer partner with other family members or with itself. HER3 has a ligand but its TK is inactive. Thus, to activate the pathway it must heterodimerize with another family member. HER3 has several docking sites for PI3K, and therefore HER3 heterodimers are potent activators of this pathway.

To further complicate this signaling network, there are ligand-independent mechanisms that can activate the HER pathway. Very high levels of HER2 can result in homodimer formation and downstream signaling (1). Other membrane receptors, such as insulin-like growth factor 1 receptor (IGF-1R) (2) and MET (3), have been shown to dimerize with HER receptors and activate the phosphorylation cascade. ER can also activate HER signaling when bound by estrogen (4). Finally, alterations in signaling molecules downstream of the receptors (5) can activate the pathway, including reduced levels of the tumor suppressor genes *INPP4-B* (for inositol polyphosphate-4-phosphatase, type II) and *PTEN* (for phosphatase and tensin homolog), which normally inactivate PI3K, or activating mutations in *PIK3CA* (for phosphatidylinositor-4,5-bisphosphate 3-kinase), the gene that encodes the catalytic subunit of PI3K (6). These alternative mechanisms to activate the HER network may account for resistance to therapies that target only the receptor layer. With four receptors, multiple ligands, several feedback loops in the MAPK and PI3K pathways, and multiple levels of control, the HER network, like a robust electronic circuit, is complex, redundant, capable of fine tuning, adaptable, and difficult to block completely (1).

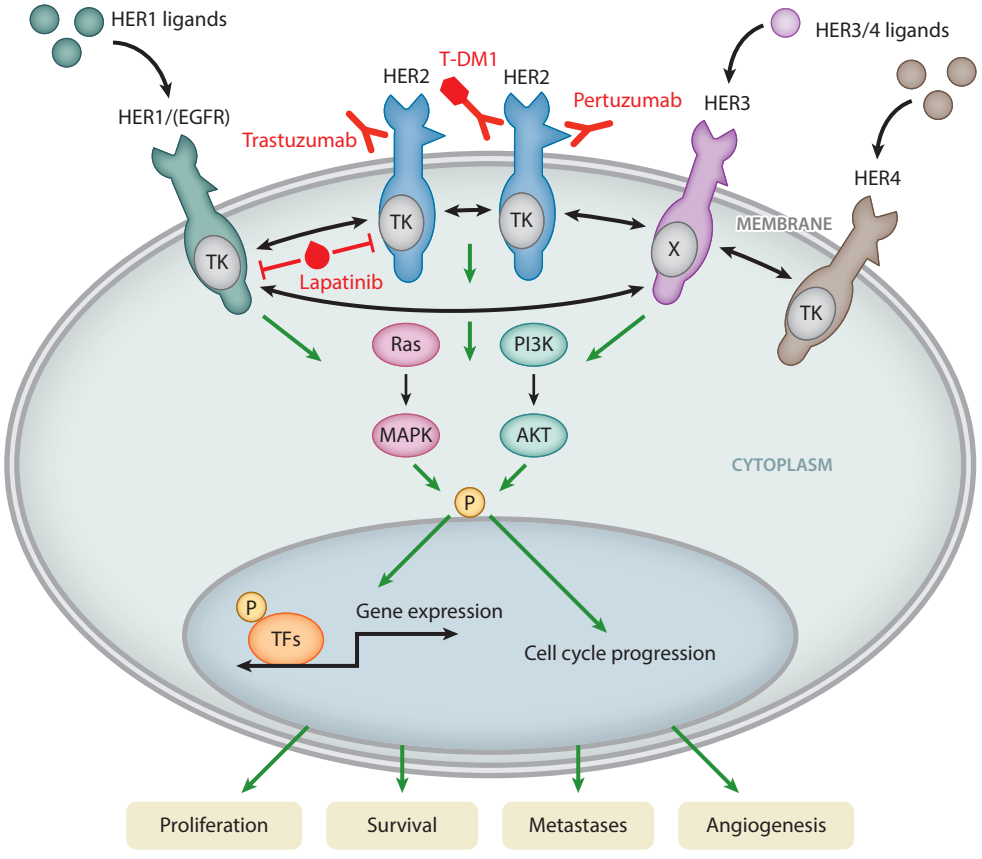

Figure 1

The HER2 network and HER2-targeted therapy for HER2+ breast cancer. The robust and redundant HER2 network includes four membrane tyrosine kinase (TK) receptors (HER1–HER4) and >10 ligands. Ligand binding to HER1, HER3, and HER4 induces a conformational change that facilitates homo- or heterodimerization with another receptor of the family. HER2, which is gene-amplified and/or overexpressed in 20% of breast cancers, does not have a ligand but resides in the membrane in an open conformation. HER2 is therefore activated by heterodimerizing with other ligand-bound HER members or by homodimerizing when it is overexpressed. Dimerization of the HER receptors results in the activation of their TKs and the initiation of a phosphorylation cascade, which activates downstream signaling including Ras/p42/44 MAPK and PI3K/AKT pathways. Activated downstream kinases, in turn, modify and phosphorylate (P) transcription factors (TF) and other components of the transcriptional and cell-cycle machineries. This enables them to alter gene expression, and to promote tumor cell proliferation and survival as well as metastatic and angiogenic capacities. HER3's TK is inactive (X) and therefore activation of downstream signaling by HER3 requires heterodimerization with other HER members. The HER2-targeted drugs approved by the US Food and Drug Administration for HER2+ breast cancer are the monoclonal antibodies trastuzumab and pertuzumab, the trastuzumab drug conjugate T-DM1, and the dual HER1/HER2 TK inhibitor lapatinib.

INHIBITING HER2 THERAPEUTICALLY

Oncogene Addiction Hypothesis

HER2-amplification in breast cancer is an excellent illustration of the oncogene addiction hypothesis, which argues that some cancers are driven by a single oncogene that harbors an activating

mutation or is overexpressed through gene amplification (7). As a consequence of this single dominant driver, the activity of other survival pathways in the cell is downregulated because they are not necessary for the cell to thrive, and potent inhibition of the dominant driver should then kill the cell. If other survival pathways remain or become active, then resistance to the therapy evolves and the cell survives. Moreover, inhibition of the HER2 antiapoptosis activity by targeted therapy provides additive or synergistic effects when combined with certain chemotherapy drugs (8, 9).

Targeting HER2 with Trastuzumab

The first approved targeted therapy for HER2+ breast cancer was the humanized monoclonal antibody trastuzumab (Herceptin). Trastuzumab binds to the extracellular domain of HER2 and was originally shown to inhibit proliferation of cultured HER2+ breast cancer cells (10). The molecular mechanism by which it inhibits cell signaling is not totally clear, although data suggest that it is more potent in inhibiting HER2 homodimers than heterodimers (11, 12). Trastuzumab may also activate antibody-dependent cellular cytotoxicity (13). Preclinical data demonstrate synergism with certain chemotherapy drugs for the reason discussed above (8, 9).

Trastuzumab was first studied as monotherapy in unselected patients with heavily pretreated metastatic breast cancer. Response rates were not impressive, with 10–15% of patients having an objective partial response and a rare patient a complete response. Later, a single-agent trial in previously untreated HER2-overexpressing metastatic disease showed slightly more partial and complete responses (26%) in patients with tumors that were HER2+ as assessed by gene amplification or by intense membrane staining (3+) (14). These data demonstrated that only tumors with oncogene addiction as defined by gene amplification were responsive to HER2 inhibition. These early studies also revealed that although trastuzumab was well tolerated, cardiomyopathy was a potential side effect, especially in elderly patients, those previously treated with doxorubicin, or those with poorly controlled hypertension (15, 16).

Chemotherapy Combined with Trastuzumab

The next-generation studies of trastuzumab in metastatic disease were based on preclinical data showing a synergistic or additive effect with several classes of cytotoxic chemotherapies (8, 9) including alkylating agents, platinum drugs, topoisomerase inhibitors, anthracyclines, and taxanes. The most important early trial of trastuzumab in HER2+ patients with metastatic disease compared chemotherapy with doxorubicin and cyclophosphamide or paclitaxel with the same chemotherapy plus trastuzumab (16). A significant increase in response rate (50% versus 32%), time to progression (TTP) (7.4 versus 4.6 months), and overall survival (OS) (25 versus 20 months) led to the approval of trastuzumab by the US Food and Drug Administration (FDA). Importantly, the trial also demonstrated an unacceptable rate of cardiac dysfunction when trastuzumab is given with doxorubicin.

Many other trials of trastuzumab in patients with HER2+ metastatic disease were completed in the past decade, demonstrating increased activity when combined with several other agents such as vinorelbine, docetaxel, and gemcitabine (17). The cumulative data led to the routine practice of continuing trastuzumab even after disease progression together with a series of chemotherapy agents (17, 18). This strategy is used less frequently since the introduction of other HER2-targeting agents.

Other Drugs Targeting HER Receptors

Because HER2 requires other HER family members to activate downstream cell survival and proliferation pathways, other drugs were developed to more completely block the receptor family. Dual inhibitors such as lapatinib, afatinib, and neratinib inhibit HER1 or epidermal growth factor receptor (EGFR) and HER2 TKs. Lapatinib is the most studied of these agents and is approved for treatment of metastatic disease. It has activity as a single agent in patients whose tumors are progressing on trastuzumab. Its toxicity profile is different, with diarrhea and skin rash, expected with an EGFR inhibitor, being the most frequent toxicities (19). Cardiac toxicity is not typically seen with dual HER inhibitors.

Single-agent activity of lapatinib in refractory metastatic HER2+ breast cancer is modest, but based on clues from preclinical studies, it was combined with chemotherapy drugs such as capecitabine. In a phase III randomized trial, the combination of lapatinib with capecitabine was superior to capecitabine alone (TTP 8.4 versus 4.4 months), leading to approval of this regimen by the FDA (20, 21). Lapatinib has also been combined with docetaxel and paclitaxel. The addition of lapatinib to paclitaxel significantly improved the response rate, progression-free survival (PFS), and OS compared to paclitaxel plus placebo (22).

Again based on preclinical observations suggesting that more complete blockade of the HER receptor layer using combinations of HER inhibitors is superior to single agents, studies of trastuzumab combined with lapatinib were initiated first in metastatic disease and then in the neoadjuvant and adjuvant settings. A phase III trial compared lapatinib added to trastuzumab with lapatinib alone in HER2+ patients progressing on trastuzumab (23). This study showed superior PFS and OS with the combination.

The other dual kinase inhibitors are just now being studied in HER2+ breast cancer. Afatinib and neratinib have the potential advantage of being more potent inhibitors of EGFR than lapatinib (24). Furthermore, they are irreversible inhibitors of the HER2 TK and have been shown to be effective in tumors harboring HER2 mutations that render cells resistant to lapatinib (25). Their role in the treatment of HER2+ breast cancer remains to be determined.

The monoclonal antibody pertuzumab binds to the heterodimerization domain of HER2 and prevents dimerization with EGFR and particularly HER3. HER2:HER3 dimers are potent activators of the PI3K pathway. Although pertuzumab has single-agent activity, most clinical trials have focused on combining it with trastuzumab, again to obtain a more complete blockade of HER receptor signaling. In patients whose tumors were progressing on trastuzumab, the combination was found to be superior to pertuzumab monotherapy and well tolerated (26). This led to a phase III trial of pertuzumab as first-line therapy in patients with metastatic disease treated with docetaxel who were randomized to trastuzumab with placebo or trastuzumab with pertuzumab (27, 28). The combination showed significantly superior PFS and OS, leading to its approval by the FDA and to trials in early-stage disease.

HER2-Targeted Therapy Plus Endocrine Therapy in HER2+/ER+ Breast Cancer

There is considerable cross-talk between ER and HER2 signaling in breast cancer cells expressing both receptors (4, 29). ER+/HER2+ tumors are less endocrine sensitive than ER+/HER2− tumors, but ER can provide an escape pathway when HER2 is blocked. These data suggested that targeting both ER and HER2 in such patients would be superior to either therapy alone. Two large randomized trials compared aromatase inhibitors as single agents versus in combination

with HER2-targeted therapy in metastatic breast cancer patients. One of these trials compared anastrozole alone to anastrozole with trastuzumab (30); the other compared letrozole with placebo to letrozole with lapatinib (31). Both showed the superiority of the combination approach and suggested that endocrine therapy should be added to HER2-targeted therapy in tumors positive for both receptors.

HER2 Antibody Drug Conjugates

The hypothesis that a HER2 antibody–cytotoxic drug conjugate would deliver the cytotoxic agent directly to tumor cells, thereby reducing side effects on normal cells while preserving the anti-HER2 activity of the antibody, has been confirmed. Trastuzumab-DM1 (T-DM1) is an antibody conjugate with emtansine, a potent microtubule inhibitor that is released intracellularly after the conjugate binds to HER2 on the membrane. T-DM1 is active in HER2-overexpressing tumors, even those resistant to trastuzumab itself (32–34). Remarkably, the drug has only modest toxicity, most frequently consisting of thrombocytopenia, fatigue, and nausea, with no significant cardiac toxicity. In a phase III direct comparison with lapatinib plus capecitabine, T-DM1 showed superior PFS and OS (35), and it has been approved by the FDA. In the first-line setting in patients with HER2+ metastatic disease, T-DM1 was also superior to docetaxel plus trastuzumab and significantly less toxic (36). Additional trials of T-DM1 in combination with pertuzumab and in other stages of disease are under way.

TRASTUZUMAB THERAPY IN THE ADJUVANT SETTING

Because of the promising activity of trastuzumab with chemotherapy in metastatic HER2+ breast cancer, several randomized trials were designed to investigate whether trastuzumab in the adjuvant setting would further decrease the risk of recurrence (**Table 1**). Three of these trials first reported in 2005: NSABP B31, Intergroup N9831, and HERA.

The B31 and N9831 trials were analyzed jointly, which was reasonable because both trials were conducted in North America on patients with HER2+ tumors and their designs and treatment

Table 1 Key randomized adjuvant trastuzumab trials in HER2+ breast cancer

Study (Reference)	N	Treatment arms	DFS HR (p-value)	OS HR (p-value)
B-31/N9831 joint analysis (38)	3,551	AC × 4 → Pac × 4 +/− T[a]	0.60 ($p < 0.0001$)	0.63 ($p < 0.0001$)
HERA (39)	5,102	Chemotherapy alone, or followed by T for 1 or 2 years	0.76 ($p < 0.0001$)	0.76 ($p < 0.0005$)
BCIRG 006 (45)	3,222	AC × 4 → Doc × 4 AC × 4 → Doc × 4 + T Doc + Carbo × 6 + T	0.64 (versus control arm, $p < 0.0001$) 0.75 (versus control arm, $p = 0.04$)	0.63 (versus control arm, $p < 0.001$) 0.77 (versus control arm, $p = 0.04$)
FinHer (44)	232	V × 3 or Doc × 3 +/− T for 9 weeks followed by FEC × 3	0.65 ($p = 0.12$)	0.55 ($p = 0.09$)
PACS-04 (40)	528	FEC × 6 or ED × 6 alone or followed by T	0.86 ($p = 0.41$)	1.27 ($p =$ NS)

Abbreviations: A, doxorubicin; BCIRG, Breast Cancer International Research Group; C, cyclophosphamide; Carbo, carboplatin; DFS, disease-free survival; Doc, docetaxel; E, epirubicin; F, 5-fluorouracil; HR, hazard ratio; NS, not statistically significant; OS, overall survival; P, paclitaxel; T, trastuzumab; V, vinorelbine. Table adapted from Reference 100.

[a]Trastuzumab was administered for one year, unless otherwise specified.

regimens were similar. The chemotherapy backbone contained an anthracycline followed by a taxane. Trastuzumab was given in both trials for one year. This analysis reported 3,551 patients at a median follow-up of two years and showed a 52% reduction in the disease-free survival (DFS) events and 33% reduction in the risk of death, both of which were highly statistically significant (37). This led to FDA approval of trastuzumab in the adjuvant setting. The final results, at a median follow-up of more than eight years, confirmed continued benefit from trastuzumab. The absolute improvement in OS (82% versus 74.2%) meant that trastuzumab saved one life out of every 14 patients who received it (38).

HERA was a global trial that also studied the addition of trastuzumab to adjuvant chemotherapy. The trial did not specify the chemotherapy regimen received by study participants. In the experimental arms, trastuzumab was also administered after completion of chemotherapy for one or two years. The results reported in 2005 showed a significant 46% improvement in DFS in the one-year arm of trastuzumab versus the control arm (39). A smaller trial with a similar design did not show a comparable benefit, presumably due to its small size (40). At a median follow-up of eight years, the HERA trial showed a significant 24% improvement in both DFS and OS (41). The trastuzumab two-year versus one-year comparison was more recently reported and showed no benefit in DFS or OS for longer treatment (42). Another trial showed that a shorter treatment duration of six months for trastuzumab is slightly inferior to one year of treatment (43). This was disappointing because the FinHer trial gave trastuzumab for only nine weeks and yet reported a significant reduction in the risk of recurrence (44). Taken together, data from these trials support a treatment duration of one year of trastuzumab as the standard of care while awaiting results from other trials exploring optimal treatment durations.

The Breast Cancer International Research Group (BCIRG) 006 study is another global trial that evaluated trastuzumab in the adjuvant setting (45). This trial used two different chemotherapy backbones with trastuzumab, a standard anthracycline-containing regimen and a nonanthracycline regimen. With over 3,200 patients enrolled, this trial showed that both trastuzumab-containing arms improved DFS and OS compared to the control arm. Although the trial did not establish equivalence of the nonanthracycline regimen to the anthracycline-containing regimen, it provided an effective nonanthracycline regimen as a therapeutic option.

Important findings from a single-arm phase II trial were recently reported (46). This trial explored a less intense chemotherapy regimen consisting of paclitaxel with trastuzumab in patients with smaller and lower-risk tumors. With 400 participants and a median follow-up of three years, there was a very low recurrence rate (2.5%), suggesting that this regimen might be a less toxic option for selected patients meeting the trial's eligibility criteria.

TRASTUZUMAB THERAPY IN THE NEOADJUVANT SETTING

Trastuzumab was also evaluated in the neoadjuvant setting, in which systemic therapy is given before surgery to allow assessment of response at surgery. Randomized trials were designed to assess the effect of adding trastuzumab to chemotherapy on the rate of pathologic complete response (pCR), defined in some trials as the absence of invasive cancer in the breast and in others as the absence of tumor in the breast and axillary nodes. These trials showed clinically meaningful increases in pCR in trastuzumab-containing arms over chemotherapy alone (47–49). These findings support the role of neoadjuvant trials in drug development to identify effective treatments years before large, expensive adjuvant trials are completed.

The introduction of trastuzumab in the adjuvant and neoadjuvant settings has dramatically improved outcomes for women with HER2+ breast cancer. Despite that, a substantial number

of women suffer a recurrence of their disease. Two important questions today are how tumors develop resistance to anti-HER2 therapy and how to abrogate or prevent that resistance.

MECHANISMS OF RESISTANCE TO HER2-TARGETED THERAPIES

Preclinical and clinical studies in the last decade provide insights into potential mechanisms of resistance to trastuzumab. More recent research examines resistance to more potent HER2 inhibition using additional drugs or their combinations. Overall, these studies suggest that to survive and evade HER2 inhibition, HER2+ breast cancer cells either (*a*) reactivate the HER pathway or its downstream signaling via compensatory, redundant, or mutated elements of the pathway, or (*b*) they switch to alternative survival pathways that can bypass HER network inhibition. Most of these mechanisms stem from the tumor cells themselves, but studies also highlight a role for the host and tumor microenvironment in resistance. Multiple signaling molecules and pathways implicated in resistance have been thoroughly reviewed recently (25, 50–55); here we briefly discuss these key mechanisms. Although described in individual categories below, these mechanisms share overlapping components and may coexist in a single tumor.

Alterations and Compensatory Mechanisms within the HER Network Receptor Layer

Changes in HER2 interactions with trastuzumab or other HER receptor family members often result in (re)activation of the HER receptor layer and consequent potential intrinsic or acquired resistance to trastuzumab. These changes include genetic and epigenetic alterations in the HER2 receptor itself, which have the following results:

1. Truncated forms of HER2 that lack the trastuzumab-binding epitope [e.g., p95-HER2 (56)].
2. More constitutively activated receptors, e.g., the HER2 extracellular-domain splice variant Δ16, which increases stabilization of HER2 homodimers (57); or activating mutations in the kinase and the extracellular domains of HER2, recently described in HER2– tumors and in some primary and metastatic HER2+ breast cancers (5, 25, 58, 59).
3. Loss or increased expression of HER2 (53).

Similarly, increased expression of HER1 (60, 61) and HER3 (61), or excess ligands for these receptors (52), have also been described in the context of trastuzumab resistance.

Resistance to trastuzumab and to other single anti-HER2 agents may commonly arise from incomplete inhibition of the HER receptor layer, allowing compensatory activation of the pathway by other HER members (1). Indeed, a combination of two or three agents with different mechanisms of action that together block all HER1–3 dimers results in a more complete inhibition of the HER pathway, and in eradication of HER2+ xenografts in mice (62–64).

Activation of Compensatory and Alternative Signaling and Survival Pathways

Receptor tyrosine kinase. Multiple preclinical studies suggest that interactions of HER2 with receptor tyrosine kinases (RTKs) outside of the HER family, such as IGF-R1 (2), MET, and EphA2 (3, 52), may also lead to resistance to trastuzumab or other anti-HER therapies. These RTKs can heterodimerize with and activate HER2 or other HER members to sustain HER2 signaling in the presence of its inhibitory drugs. Alternatively, independent of HER2, these RTKs can directly activate common downstream signaling components, such as PI3K/AKT (50, 53), providing alternative survival and proliferative stimuli that support tumor growth when HER2 is

effectively inhibited. Combining agents targeting these other RTKs with trastuzumab or other anti-HER2 therapies could potentially circumvent drug resistance (50, 61).

Mucins. Preclinical studies suggest that the membrane-associated mucin glycoproteins MUC4 and MUC1 are implicated in resistance to trastuzumab and potentially to additional anti-HER2 therapies (65). MUC4 can potentiate signaling through the HER2 pathway (66) via direct interaction with HER2/3 complexes and can impede the binding of trastuzumab to its epitope on HER2, rendering trastuzumab less able to antagonize the pathway (67). Cleaved forms of MUC1 (termed MUC1* or MUC1-C), which are increased upon acquired resistance to trastuzumab, also associate with HER2 and contribute to its constitutive activation (68, 69). Treating trastuzumab-resistant cancer cells with MUC1* antagonists reverses this drug resistance (68, 69). Additional research is needed to assess the role of these mucins in resistance to HER2-targeted therapies in the clinical setting.

ER and additional transcription factors. Cross-talk between HER2 and ER occurs at multiple levels as each pathway can activate or repress the other (4). Recent results from our and other preclinical studies and neoadjuvant trials suggest that ER acts as an escape mechanism to bypass HER2 inhibition (29, 64, 70). In breast cancer cell cultures, potent HER2-targeted therapy, such as lapatinib-containing regimens, restores redundant survival pathways including ER (64), presumably by means of prolonged inhibition of the PI3K/AKT pathway and consequent upregulation of the FOXO family of transcription factors required for ER synthesis (71). In these resistant lines, where HER signaling remains inhibited, activated ER signaling and its downstream antiapoptotic BCL2 protein become the dominant escape pathway, sensitizing the cells to anti-ER therapies. In vivo, potent anti-HER2 drug combinations can eradicate ER+/HER2+ xenografts only when combined with endocrine therapy (62–64). Parallel activation and increased levels of ER and BCL2 were also shown in patients using specimens from a lapatinib neoadjuvant clinical trial (72).

Similar to derepression of ER signaling, a cytoprotective stress response triggered by lapatinib in HER2+ breast cancer cell lines can also activate the prosurvival NF-κB RelA subunit, which in turn causes resistance by rescuing cells from drug-induced proapoptotic effects (73).

Hyperactivation of Downstream Kinase Pathways

PTEN/PI3K/AKT. Activation of signaling downstream of HER receptors can counteract HER2 inhibition. Compelling preclinical evidence suggests that constitutive activation of the PI3K/AKT pathway, achieved by reducing levels of its tumor suppressor PTEN or by activating mutations in PIK3CA, results in resistance to trastuzumab and other anti-HER2 therapies (6, 74). Activation of PIK3CA signaling may also contribute to acquired resistance to lapatinib in experimental models (75). Other studies, however, suggest that PTEN status may not affect sensitivity of HER2+ breast cancer cells to lapatinib (76). Similarly, in prior neoadjuvant trials that included chemotherapy, we found that PIK3CA mutations and PTEN loss were associated with resistance to trastuzumab but not lapatinib (77). In fact, clinical trials report conflicting results concerning the role of PTEN in response to HER2-targeted therapies in different settings, potentially due to different assays and cutoff values for measuring PTEN levels (78). Our recent preliminary findings in a neoadjuvant trial of lapatinib plus trastuzumab without chemotherapy suggest that low levels of PTEN, but not necessarily complete loss, can activate the PI3K pathway and reduce treatment efficacy (79). Recent data from several large neoadjuvant trials confirmed that PIK3CA mutations compromise response to lapatinib, trastuzumab, and their combination (80, 81). Interestingly,

our lapatinib plus trastuzumab neoadjuvant study found that tumors with activating PIK3CA mutations failed to achieve pCR (79), a surrogate marker for long-term outcome. Preclinical studies suggest that the addition of PI3K/mTOR/AKT inhibitors to anti-HER2 treatment can overcome resistance in tumors with PIK3CA mutations (74, 75), a strategy currently being investigated in the clinic.

SRC family kinases. Recent studies have pointed to SRC as a common signaling mediator in trastuzumab resistance caused by different mechanisms, including hyperactive growth factor receptors or PTEN loss (52, 82). The role of additional members of the SRC family, specifically Yes, was recently reported in lapatinib-resistant cell lines (52). Preclinical evidence for the ability of SRC inhibitors to restore sensitivity to anti-HER2 therapies supports the translation of this strategy to the clinic (52).

Deregulation of Apoptotic and Cell-Cycle Control Regulators

Owing to the central oncogenic role of HER2 in HER2+ breast cancer, inhibition of this pathway induces both cell-cycle growth arrest and apoptosis. As such, deregulation of cell-cycle regulators can promote resistance. Indeed, upregulation of cell-cycle-positive regulators [e.g., amplification of cyclin E (83)] and downregulation of negative regulators of the cell cycle [e.g., the CDK inhibitor $p27^{Kip1}$ (84)] have been shown to negate the antiproliferative effect of trastuzumab, leading to resistance.

Consistent with the cytotoxic effects of anti-HER2 therapies, alterations in the normal apoptotic machinery have also been implicated in resistance (52, 71, 72). Decreased levels of proapoptotic molecules and upregulation of prosurvival factors were shown to be associated with resistance to anti-HER2 therapies.

Host and Tumor Microenvironment-Associated Mechanisms of Resistance

In addition to its direct effects on signaling in cancer cells, trastuzumab triggers antibody-dependent cellular cytotoxicity by interacting with the Fc receptors on immune effector cells. The host immune components involved in this process can contribute to trastuzumab efficacy as well as resistance (13, 85–87). Preclinical and clinical data provide evidence for the role of the host leukocyte Fc gamma receptors in modulating the clinical efficacy of trastuzumab (13, 87), although the suggested correlation between specific Fc gamma receptor polymorphisms and response to trastuzumab is still controversial (88). Recent data also suggest that higher levels of tumor-infiltrating lymphocytes are significantly associated with superior clinical benefit from trastuzumab in combination with chemotherapy and that trastuzumab may exert its antitumor activity partly by relieving tumor-mediated immunosuppression (80, 89). These intriguing findings support testing whether the addition of immunomodulators such as T cell checkpoint inhibitors can enhance trastuzumab efficacy (89, 90).

Additional components of the tumor microenvironment, including structural elements of the extracellular matrix (ECM) and soluble factors (growth factors and cytokines), may also influence response or resistance to anti-HER2 therapies. Several reports have shown that the β1 and β4 integrins, two key transmembrane receptors at the tumor–ECM microenvironment interface, can interact with EGFR/HER2 to negate their inhibition or can provide alternative survival signals to protect cells from apoptosis induced by anti-HER2 treatment (91–94). As such, disruption of integrins and FAK/SRC downstream signaling by pharmacological or genetic short hair-pin

RNA (shRNA) strategies has been shown to sensitize breast cancer cells to anti-HER2 drugs and overcome resistance (91–93).

COMBINATION NEOADJUVANT TRIALS

The neoadjuvant (or preoperative) approach has been successfully utilized to test combination anti-HER2 therapies. Several trials tested the hypothesis that more potent inhibition of the receptor layer using drug combinations would increase treatment efficacy, as suggested by preclinical evidence discussed above (62–64). Serial tumor biopsies obtained from patients on these trials also provide a valuable resource for biomarker discovery. The most important clinical trials that tested this concept are summarized in **Table 2**. These trials investigated the addition of a second anti-HER2 agent, either lapatinib or pertuzumab, to trastuzumab.

As a dual TK inhibitor of HER1 and HER2, lapatinib should theoretically inhibit the HER receptor layer potently by itself. However, the GeparQuinto trial showed that in the neoadjuvant setting, the combination of chemotherapy with lapatinib was not only inferior in efficacy but also more toxic (95).

The NeoALTTO trial randomized 455 patients with HER2+ cancers to a six-week run-in of lapatinib, trastuzumab, or the combination followed by the same anti-HER2 therapy combined with paclitaxel (96). At the time of surgery, the rate of pCR in the breast was significantly higher in the arm that received the combination of lapatinib and trastuzumab with paclitaxel (51%) over either agent alone (25% and 30%, respectively) (96).

The NSABP B41 trial also compared trastuzumab, lapatinib, or their combination together with paclitaxel. However, in this study all patients also received an initial course of anthracycline-based chemotherapy. The rate of pCR in the breast was higher in patients who received the combination (62%) than in patients randomized to trastuzumab (52%) or lapatinib (53%), although the difference was not statistically significant (97).

Table 2 Key randomized neoadjuvant clinical trials investigating anti-HER2 drug combinations

Study	Patients (N)	Neoadjuvant treatment	pCR[a] RateChemotherapy + single anti-HER2	pCR RateChemotherapy + dual anti-HER2	pCR by ER expression	
					ER+	ER–
NeoALTTO	455	Pac+T, Pac+L, Pac+LT	30%[b] (T), 25% (L)	51%[c]	42%	61%
NSABP B-41	519	AC → Pac+T, Pac+L, or Pac+LT	53%[b] (T), 53% (L)	62% (NS)	56%	73%
NeoSphere	417	Doc+T, Doc+P, Doc+PT, PT[d]	29%[b] (T), 24% (P)	46%[c]	26%	63%
Tryphaena	225	FEC → Doc+PT	N/A	57%	49%	65%
		FEC+PT → Doc+PT		62%	46%	79%
		Doc-Carbo+PT		66%	50%	84%

Abbreviations: A, doxorubicin; C, cyclophosphamide; Carbo, carboplatin; Doc, docetaxel; E, epirubicin; ER, estrogen receptor; F, 5-fluorouracil; L, lapatinib; N/A, not applicable; P, pertuzumab; Pac, paclitaxel; pCR, pathologic complete response; T, trastuzumab.

[a]pCR rate defined as no residual carcinoma in the breast, unless otherwise specified.

[b]Control arm.

[c]Statistically significant over control arm.

[d]Results in **Table 3.**

Table 3 Neoadjuvant clinical trials targeting HER2+ tumors with potent anti-HER2-targeted regimens without chemotherapy

Trial	N	Targeted therapy	Concurrent endocrine therapy	Overall pCR	pCR in ER positive	pCR in ER negative
NeoSphere (targeted therapy)	107	P+T	None	17%	6%	29%
TBCRC 006	64	L+T	Estrogen deprivation for ER positive tumors[a]	27%	21%	36%

Abbreviations: P, pertuzumab; T, trastuzumab; L, lapatinib; pCR, pathologic complete response; ER, estrogen receptor.
[a]Estrogen deprivation: aromatase inhibitor alone (in postmenopausal women) or with ovarian suppression (in premenopausal women).

The NeoSphere study randomized 417 women with HER2+ cancer to docetaxel with trastuzumab, pertuzumab, or their combination. In a targeted-therapy-only arm, patients received pertuzumab and trastuzumab without chemotherapy. The rate of pCR in the breast was 46% in the arm that received chemotherapy with the two antibodies, significantly higher than in the arms that received chemotherapy with either antibody alone (98).

The Tryphaena study tested administration of the two antibodies, trastuzumab and pertuzumab, with various chemotherapy backbones. This trial also showed a high pCR rate for all arms containing chemotherapy and the two antibodies (99). These studies led to FDA approval of pertuzumab in the neoadjuvant setting in combination with trastuzumab and chemotherapy. Taken together, these trials demonstrate that combination anti-HER2 therapy for potent inhibition of the receptor layer results in meaningfully increased efficacy as determined by pCR rates. Confirmatory results from large adjuvant trials (ALTTO and APHINITY) testing the same regimens are eagerly awaited.

Another interesting finding from these trials has been the difference in pCR rate by ER subgroup. Across all of these neoadjuvant trials, ER+ tumors consistently showed a lower pCR rate than ER– tumors. ER may act as an escape pathway for these tumors, and concomitant inhibition of ER along with potent anti-HER2 therapy and chemotherapy may abrogate that difference in pCR between ER+ and ER– tumors. This concept is currently being tested in the NSABP B52 trial.

Another approach that is biologically sound and clinically less toxic is to target HER2+ tumors with potent anti-HER2-targeted agents without chemotherapy. **Table 3** summarizes results from the two trials that explored this concept, NeoSphere (which included a targeted-therapy-alone arm) and TBCRC 006 (70, 98). The latter was a multicenter single-arm trial of lapatinib and trastuzumab without chemotherapy. The 64 patients treated with that regimen had a large median tumor size of 6 cm. Endocrine therapy was also administered in patients whose tumors were ER+. Serial tumor biopsies were obtained. The targeted-therapy arm of NeoSphere administered the two antibodies trastuzumab and pertuzumab without chemotherapy. Endocrine therapy was not administered. Lapatinib/trastuzumab combined with endocrine therapy produced significantly higher pCR rates, especially in the ER+ subset. Although it is hazardous to compare across trials, it has been speculated that the more comprehensive inhibition of the HER receptor layer by lapatinib (which inhibits HER1), the cotargeting of ER, or both may explain the higher rate of pCR noted in TBCRC 006 (70).

Both of these trials confirm that, in a subgroup of patients, pCR can be achieved in a significant proportion of patients without chemotherapy. Tissue acquired through serial tumor biopsies from patients on these trials is helping identify molecular markers to determine which patients would benefit most from a targeted-therapy-alone approach. In the future, we predict that chemotherapy will continue to play an important role in the management of many, but not all, patients.

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