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Original Article

Beyond trastuzumab: New treatment options for HER2-positive breast cancer

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SUMMARY

HER2-positive tumors comprise 15% to 20% of all breast cancers (BC) and are associated with worse clinical outcomes. Trastuzumab is a humanized monoclonal antibody designed to target the extracellular domain of the HER2 receptor, and is the foundation of care of women with early and advanced HER2-positive BC. However, a significant proportion of patients with this type of BC display either primary or secondary resistance to trastuzumab. Therefore, in an effort to overcome such resistance and further improve the outcome of patients with HER2-positive disease, several new anti-HER2 agents are currently being developed. These include small molecules that inhibit the HER2 tyrosine kinase activity (lapatinib, neratinib), monoclonal antibodies directed at other epitopes of the HER2 extracellular domain (pertuzumab), antibody-drug conjugates (trastuzumab-DM1), and heat shock protein 90 inhibitors (tanespimycin).
A great deal of interest has been generated by recent data from the randomized neo-adjuvant studies NeoALTTO and NeoSphere, which have shown that dual blockade of the HER2 receptor with anti-HER2 agents is significantly superior to using one agent alone. If these results are validated in larger ongoing and planned phase III studies in early BC, they could lead to a paradigm shift in treatment strategy. Therefore, to avoid unnecessary toxicities and costs, it is critical to intensify the research for biomarkers that can identify those patients most likely to benefit from specific targeted therapies.

Sitaron se aage jahan aur bhi hain …
[Beyond the stars lie other worlds – unexplored, unconquered …]
Iqbal, Urdu poet, 1908

HER2 receptor biology and downstream signaling

HER2 belongs to the epidermal growth factor receptor (EGFR; also known as ErbB) family of receptor tyrosine kinases (RTK). In humans, this family consists of four structurally related receptors HER1 (EGFR, ErbB1), HER2, HER3 (ErbB3) and HER4 (ErbB4). The HER receptors play an important physiological role in regulating cell growth, survival, and differentiation.

The HER2 proto-oncogene is located on chromosome 17 and encodes a 185 kDa membrane-spanning protein composed of a ligand-binding extracellular domain (ECD), an α-helical transmembrane segment, and an intracellular tyrosine kinase domain.[1,2] HER2 is found in normal cells, but is present in abnormally high quantities in a subset of breast cancers (BC) characterized by an aggressive natural history.[3,4] Although HER2-positive BCs usually display several genomic aberrations, they remain critically dependent on HER2 signaling for survival and growth. Blocking the HER2 receptor (or its key downstream effectors) can lead to cell apoptosis, often translating into striking clinical benefit. HER2 thus represents a robust target that clearly drives oncogenesis, and anti-HER2 therapies exploit this phenomenon of "oncogene addiction".

Approximately 15% to 20% of all BCs are HER2-positive and display HER2 protein overexpression (detected by immunohistochemistry),[4] which is almost always associated with HER2 gene amplification (detected by fluorescent in-situ hybridization). Of note, up to 40% of inflammatory breast cancers may be HER2-positive.[5] Cleavage of the HER2 ECD results in a truncated version of the HER2 receptor (so-called p95) that may be associated with resistance to trastuzumab.

In the quiescent state, HER receptors are inactive monomers, but signaling activity requires that they undergo dimerization. The pairing may occur among molecules of the same HER receptor subtype (homodimerization) or different HER receptor subtypes (heterodimerization). HER2 has no known ligand; moreover, it exists only in a constitutively active conformation, making it the

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preferred heterodimerization partner for other HER receptors.[6] In contrast, HER3 lacks tyrosine kinase activity and can only form heterodimers. Growth factor binding changes the conformation of the HER receptors and exposes the dimerization domain. Dimer formation results in the functional activation of the intracellular catalytic domain by tyrosine kinase phosphorylation. The HER2-HER3 dimer has higher signaling potency than other HER homo-and heterodimers and is thought to be the important oncogenic unit that signals constitutively to PI3K and Akt (see below).[7] HER1/EGFR does not seem to drive the oncogenesis of HER2-positive BC, as suggested by the low clinical efficacy of gefitinib in HER2-positive metastatic BC (MBC).[8] HER dimerization sets in motion a complex cascade of downstream signaling that consists of two main canonical pathways, the phosphatidyl inositol 3 kinase (PI3K) and the mitogen-activated protein kinase (MAPK) (Fig. 1).

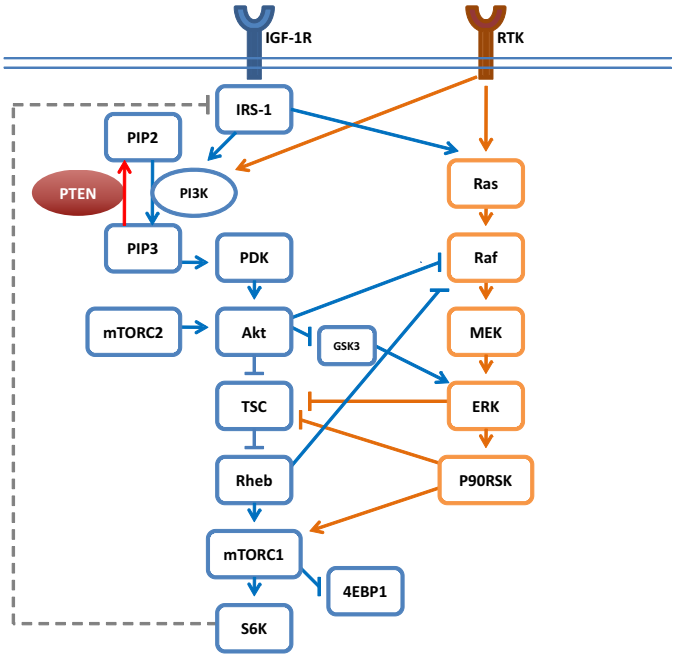

Fig. 1. A schematic representation of the phosphatidyl inositol 3 kinase (PI3K) pathway in blue, the mitogen-activated protein kinase (MAPK) pathway in orange, and cross-talk between them. PTEN, the negative regulator of the PI3K pathway, is displayed in red. The dashed line represents the negative feedback loop from S6K to IRS-1.

The PI3K pathway plays a key role in controlling several physiological cellular functions. A number of extracellular signals, including HER2 activation, can activate this pathway. PI3K phosphorylates phosphatidylinositol 4,5-diphosphate (PIP2) to form phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 then sequentially activates phosphoinositide-dependent kinase 1 (PDK1), Akt, tuberous sclerosis complex (TSC), Rheb, and mammalian target of rapamycin complex 1 (mTORC1). The tumor suppressor gene (TSG) phosphatase and tensin homolog (PTEN) located on chromosome 10 dephosphorylates PIP3 to PIP2 and is thus the principal negative regulator of the PI3K pathway. Mutations of the PIK3CA gene that codes for PI3K may be seen in about 25% of BCs and are particularly associated with the estrogen receptor (ER) positive and HER2-positive subtypes.[9,10]

MAPK pathway signaling is triggered by ligand binding to HER2, which sequentially activates Ras, Raf kinases (ARaf, BRaf, CRaf), mitogen-activated and extracellular-signal regulated kinase kinase (MEK), and extracellular signal regulated kinase (ERK).[11] Although mutations of Ras and Raf are uncommon in BC, deregulation of this pathway at the gene expression level has been noted, especially in the so-called triple-negative BCs or "basal-like" tumors.[12]

HER2 signaling occurs mainly via the PI3K/Akt/mTOR pathway, though the Raf/MEK/ERK pathway is also involved. Significant cross-talk exists between the PI3K/Akt and the MAPK pathways, and also with other molecular pathways.[13] Therefore, blocking one molecular target may still allow an escape via alternate signaling cascades. This phenomenon has led to the hypothesis that a dual blockade of HER2 and another molecular substrate may improve the clinical efficacy of anti-HER2 therapies. Dual targeting may be "vertical" (if the second target lies downstream of HER2, for example, in the PI3K or MAPK pathways), or "horizontal" (if the second target is a part of another cascade, for example, the ER signaling pathway). Many clinical trials are already underway to test this hypothesis. For example, cross-talk between the ER and HER2 pathways may be a primary contributor to the development of resistance to hormonal therapy.[14] A strategy of "horizontal dual blockade" that targets ER and HER2 has already shown promising results in clinical trials.[15,16]

Trastuzumab

Trastuzumab was the first agent developed to target the HER2 pathway. It is a humanized monoclonal antibody that binds to domain IV on the juxtamembrane region of the ECD of HER2[17] and inhibits tumor cell growth *in vitro* and *in vivo* via several mechanisms. These include inhibition of ECD cleavage, activation of antibody-dependent cellular cytotoxicity (ADCC), inhibition of intracellular signaling, reduction of angiogenesis, and decreased DNA repair.[18–20] The predominant mechanism by which trastuzumab disrupts HER2 signaling is by uncoupling the HER2/HER3/PI3K complex in the absence of ligands. An increasing body of data suggests that trastuzumab efficacy could be, at least in part, related to the induction of immune response against the HER2 protein.[21] Trastuzumab has the ability to bind to fragment C receptor (FcγR) present on macrophages and natural killer cells.[22] Single-agent trastuzumab has also been shown to cause cellular apoptosis in patients with locally advanced BC.[23]

The introduction of trastuzumab to the treatment of HER2-positive BC represents an important milestone in the history of oncology. The pivotal study by Slamon et al. showed that the addition of trastuzumab to chemotherapy in MBC patients significantly improved response rate (50% vs. 32%; $p < 0.001$), duration of response (9.1 months vs. 6 months; $p < 0.001$), and time to disease progression (7.4 months vs. 5.6 months; $p < 0.001$), with a significant 20% reduction in the risk of death (HR 0.80; $p = 0.046$).[24] Recent trials have shown further synergy and efficacy of combining trastuzumab with aromatase inhibitors in patients with metastatic, endocrine-responsive, HER2-positive BC.[16,25]

More impressively, the addition of trastuzumab to adjuvant chemotherapy has resulted in a striking reduction in the risk of relapse and death by 50% and 30% respectively.[26–29] These results led to the approval of trastuzumab for use in the early and advanced BC settings, establishing it as the most vital agent available today to manage HER2-positive BC. Trastuzumab use in the neoadjuvant setting has also shown promising results, and is discussed later in this manuscript.

Cardiac toxicity remains the hallmark side effect associated with trastuzumab. This has been attributed to the essential role of HER2 in embryonic cardiac development and its importance in the growth, survival and inhibition of myocyte apoptosis.[30–33] This was clearly demonstrated in the H0648g pivotal trial in which the co-administration of trastuzumab and doxorubicin resulted in an extremely high incidence of congestive heart failure (CHF) reaching 16%.[24] In the adjuvant setting, the sequential use of trastuzumab and anthracyclines is also associated with a risk of congestive heart failure (CHF) ranging from 1% to 4%; however, this may constitute an acceptable level of risk when weighted against its impressive efficacy results.[34,35]

While the introduction of trastuzumab has revolutionized the management of HER2-positive BC, several questions remain unanswered. These include the optimal duration of adjuvant trastuzumab (2 years, 1 year, 6 months, or 9 weeks) and its role in very early disease (T < 1cm and N0). There are several ongoing trials addressing these important questions, but until their results are available, adjuvant trastuzumab should be administered for a total duration of one year. The decision to start trastuzumab with chemotherapy or following its completion, or to use it to treat very early disease, should rely on other prognostic factors, co-morbidities, and patient preference.[36,37]

Given the high cost of trastuzumab, the risk of cardiotoxicity and the possibility of developing resistance, a major challenge is to prospectively identify patients likely to derive benefit from this drug. Clinical experience has already shown that a large fraction of patients develop either primary or secondary resistance to the antibody.[38] Several mechanisms of resistance have been suggested, including impaired receptor-antibody binding, loss of PTEN, truncated HER2 protein, co-expression of insulin growth factor receptor, gene mutations, or signaling via alternate pathways.[39–43] Such resistance mechanisms to trastuzumab have major clinical implications; hence novel anti-HER2 therapies are needed.

Lapatinib

Lapatinib is an oral small-molecule reversible dual inhibitor of the HER1 and HER2 tyrosine kinase (TK), which directly inhibits the kinase activity of both receptors. Although it blocks HER1 and HER2 TK with the same potency, its therapeutic benefit is restricted to HER2-positive BC.[44,45] Although inhibition of HER2 autophosphorylation and signaling via MAPK and PI3K/Akt pathways may be necessary for clinical response to lapatinib, it is not sufficient to induce apoptosis of tumor cells.[45,46] The antitumor effect of lapatinib on HER2-overexpressing BC cells appears to be linked to the down-regulation of survivin.[47] Moreover, recent preclinical data suggest that lapatinib can also inhibit HER2-HER3 mediated cell growth.[48,49]

Lapatinib gained approval from the USA Food and Drug Administration (FDA) and the European Medicines Agency in 2007 after Geyer et al. reported positive results in heavily pretreated HER2-positive BC patients.[50] In the EGF100151 trial, 321 patients with HER2-positive tumors who had previously failed trastuzumab therapy were randomized to receive capecitabine alone or capecitabine plus lapatinib. In the combination arm, time to progression was almost doubled when compared to capecitabine alone ($p < 0.001$). Later the EGFR30008 trial showed that progression-free survival (PFS) in patients with endocrine responsive, HER2-positive MBC more than doubled when lapatinib was combined with letrozole compared to letrozole alone (8.2 months vs. 3 months; $p = 0.019$).[15]

Unlike trastuzumab, lapatinib is a small molecule that may easily cross the blood-brain barrier and thus could be effective in dealing with brain metastasis. Preclinical data from a mouse xenograft model have shown that lapatinib reduces the migration and growth of BC cells in the brain.[51] Reporting the results of a phase II trial involving 242 patients who had progressive brain metastasis following trastuzumab-based therapy and brain irradiation,[52] Lin et al. demonstrated that lapatinib caused at least 20% central nervous system volumetric tumor reduction in 21% of patients.

The cardiotoxicity profile of lapatinib appears to differ from that of trastuzumab.[33] In a pooled analysis of over 3500 patients treated with lapatinib, only 0.2% of patients developed symptomatic drop in left ventricular ejection fraction (LVEF).[53] The analysis included a selected patient population (long time since prior anthracycline exposure, pretreatment with trastuzumab), which possibly contributed to the lower incidence of cardiac toxicity than that found with trastuzumab. Therefore, although lapatinib seems to be associated with a lower incidence of cardiac events, this should be carefully analyzed and prospectively confirmed in ongoing randomized trials.[33] Some authors have alluded to the different effects trastuzumab and lapatinib exert on the cardiac bioenergetics.[54] Increased ATP production by the cardiac mitochondria has been identified in association with lapatinib, for example, but not with trastuzumab.[55]

While the cardiac risk of lapatinib appears to be lower than that of trastzumab, lapatinib has been associated with a high incidence of diarrhea, hepatic and skin toxicity, particularly skin rash.[56,57] Diarrhea is the most commonly encountered adverse event, with around 60% of patients developing diarrhea when lapatinib is given in combination with chemotherapy.[50,58] The same incidence was reported when combining lapatinib with letrozole in the EGF30008 trial (16% of diarrhea was of grade 3 or 4).[15] In a recent study by Dang et al., combining lapatinib with trastuzumab and paclitaxel following dose-dense anthracycline-based regimen was deemed not feasible due to excessive diarrhea.[59]

The lack of complete cross-resistance between lapatinib and trastuzumab suggests that their mechanisms of resistance differ. This is particularly true for alterations in PTEN and p95HER, in which lack of PTEN expression and/or expression of p95HER2 confer resistance to trastuzumab, but not to lapatinib.[60,61] Hence, the concurrent administration of both drugs is very appealing. The premise of this strategy is that the two active agents will abrogate the same signaling pathway and thus overcome the potential resistance associated with either of them.

The first published trial investigating this approach was reported by Blackwell and colleagues. In their study, 295 patients with trastuzumab-refractory BC were randomized to receive either lapatinib or the combination of lapatinib and trastuzumab.[62] The combination of two anti-HER2 drugs showed a significant reduction in the risk of disease progression (HR: 0.73 [95% CI, 0.57–0.93]; $p = 0.008$) and a trend towards an improved overall survival (HR: 0.75 [95% CI, 0.53–1.07]; $p = 0.106$) when compared to lapatinib alone. These results reinforced the merit of a combined HER2 blockade and its potential, which has been subsequently tested and confirmed in two randomized neoadjuvant trials, NeoALTTO (Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization, BIG 1–06, NCT00553358) and NeoSphere (Neoadjuvant treatment with Herceptin® and pertuzumab, NCT00545688). Initial results from these studies were presented at the San Antonio Breast Cancer Symposium in December 2010, and are discussed later in this manuscript (Table 1).

Many of the fundamental questions associated with a dual HER2 blockade and the best use of adjuvant HER2 therapies are being further investigated in the context of the ALTTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimization, BIG 2–06, NCT00490139), an ongoing phase III clinical trial testing adjuvant chemotherapy alone with one year of anti-HER2 therapy (lapatinib alone, trastuzumab alone, their sequence or their combination).[69]

Pertuzumab

Pertuzumab is a first-in-class recombinant, humanized monoclonal antibody that binds to domain II of the HER2 receptor, thus inhibiting HER2 heterodimerization with HER1, HER3, and HER4.[70] Blocking HER2-HER3 dimerization is clinically the most relevant action of pertuzumab, and this can effectively block HER2-mediated cell signaling. The efficacy of single-agent pertuzumab in HER2-negative BC was very limited,[71] but the mechanism of action of pertuzumab is complementary to that of trastuzumab, as shown in preclinical studies.[72] Clinical evidence of activity of pertuzumab in combination with trastuzumab was provided by a phase II

Table 1
First and second generation of neoadjuvant targeted therapies in HER2-positive breast cancer patients

Studies and sample size	Study design	pCR* rates, p values
M.D. Anderson[63], N = 64	(1) T → FEC	26.3
	(2) T + H → FEC + H	60
		P = NR
NOAH[64], N = 235	(1) AT → T → CMF	19
	(2) AT + H → T + H → CMF + H	38
		P = 0.001
GeparQuattro[65], N = 1495*	(1) 4 EC → 4 D100 4 EC → 4 D75 + 4 X 4 EC → 4 D75 → 4 X	15.7
	(2) 4 ECH → 4 D100H → H 4 ECH → 4 D75H + 4 XH → H 4 ECH → 4 D75H → 4 XH → H	31.7 P = NR
NeoALTTO[66],N = 455	(1) H qw ×6 → T qw ×12 + H qw ×12 → surgery → FEC q3w ×3 → Hq3w until w 52	29.5
	(2) L qd → T qw ×12 → surgery → FEC q3w ×3 → L qd until w 52	24.7 P = 0.34 (2 vs 1)
	(3) H qw ×6 + L qd → T qw ×12 + H qw ×12 + Lqd → surgery → FEC q3w ×3 → H q3w + L qd until w 52	51.3 (3 vs 1) P = 0.0001
NeoSphere[67], N = 417	(1) H q3w + D q3w ×4 → surgery → FEC → H until w 52	29
	(2) H q3w + PZ q3w + D q3w ×4 → surgery → FEC → H until w 52	45.8 p = 0.0141 (2 vs 1)
	(3) H q3w + PZ q3w ×4 → surgery → H q3w + D q3w ×4 → FEC → H until w 52	16.8 P = 0.0198 (3 vs 1)
	(4) PZ q3w + D q3w ×4 → surgery → FEC → H until w 52	24 P = 0.03 (4 vs 2)
GeparQuinto[68], N = 620	(1) EC q3w ×4 + H → D q3w ×4 + H → surgery	31.3
	(2) EC q3w ×4 + L → D q3w ×4 + L → surgery	21.7 P < 0.05

Abbreviations: A = doxorubicin; C = cyclophosphamide; D = docetaxel; E = epirubicin; F = fluorouracil; H = trastuzumab; L = lapatinib; NR = not reported; PZ = pertuzumab; pCR = pathologic complete response; T = taxane; X = capecitabine.
Comments: MD Anderson, NOAH and GeparQuattro studies have chemotherapy-only arms compared with chemotherapy plus trastuzumab (first generation studies). NeoALTTO, NeoSphere and GeparQuinto represent the second generation of neoadjuvant studies with anti-HER2 regimens in all study arms. pCR definitions: MD Anderson – No evidence of invasive cancer in breast or axilla; NOAH – Total pCR in breast and axillary nodes; GeparQuattro – no invasive or in situ residual tumor in the breast; NeoALTTO – non invasive cancer in the breast or only non invasive in situ cancer; NeoSphere – pathologic complete response in the breast; GeparQuinto – no microscopic evidence of residual viable cells in any specimen.
*1050 HER2-negative patients were used as a reference group in the GeparQuattro study.

study involving 11 patients with HER2-positive MBC, where partial response of 18% and stable disease of 27% was observed.[73]

Another phase II trial of pertuzumab in combination with trastuzumab in 66 patients with HER2-positive MBC whose disease had progressed during prior trastuzumab therapy showed a PFS of 5.5 months, an objective response rate of 24.2% and a clinical benefit rate (objective response plus stable disease) of 50%.[74] The combination of both antibodies was well tolerated, with diarrhea, fatigue, nausea, and rash being the most frequent adverse events (AEs; mostly grade 1 and 2). Three patients had an LVEF drop of ≥10 percentage points and to a less than 50% absolute value, but no patient withdrew from the study as a result of cardiac-related AEs. In an ongoing phase III study CLEOPATRA (NCT00567190) in first line MBC, 808 patients will be randomized to receive docetaxel plus trastuzumab with or without pertuzumab.

Similar to the dual combination of lapatinib and trastuzumab discussed earlier, the dual blockade of trastuzumab and pertuzumab has already shown impressive results in the neoadjuvant setting, and it will soon be tested in early BC. A large, randomized phase III, double-blind, placebo-controlled study is being planned to compare the efficacy and safety of chemotherapy plus trastuzumab and placebo with that of chemotherapy plus trastuzumab and pertuzumab as adjuvant therapy in patients with operable, HER2-positive, primary breast cancer (BIG 04–11/BO25126/TOC 4939G). Recruitment is expected to begin in the third quarter of 2011.

Trastuzumab-DM1 (T-DM1)

A new approach to targeting the HER2 receptor is to couple an anti-HER2 monoclonal antibody with a cytotoxic agent, thereby specifically directing the cytotoxicity to the target. An antibody-cytotoxic conjugate consists of the monoclonal antibody, the cytotoxic molecule, and a linker to join the two. In T-DM1, a highly stable thioether linker, N-maleimidomethyl cyclohexane-1-carboxylate (MCC) is joins trastuzumab with an antimicrotubule drug maytansinoid, and this conjugate has shown promising clinical efficacy and acceptable toxicity. Activation of cytotoxicity of this conjugate requires internalization into the cell after binding to HER2. The non-internalized conjugate remains inactive, thus limiting the systemic toxicity. This is the reason why this approach has been also dubbed "targeted chemotherapy". In T-DM1, trastuzumab not only delivers the drug to HER2-overexpressing cells, but it also retains its native properties, namely the inhibition of HER2 signalling and induction of ADCC.[75]

A phase I study (n = 24) identified the maximum tolerated dose (MTD) of T-DM1 as 3.6 mg/kg every 3 weeks, with grade 4 thrombocytopenia being the dose-limiting toxicity.[76] Other AEs

included increased liver enzymes, fatigue, nausea, and anemia. A single-arm, phase II trial (n = 112 MBC patients whose disease progressed on trastuzumab) showed at a follow-up of ≥12 months a median PFS of 4.6 months (95% CI, 3.9 to 8.6) and an overall response rate of 26%.[77] The HER2 expression levels of the patients with HER2-positive disease were tested by reverse transcriptase polymerase chain reaction and, interestingly, the response was higher in patients who had ≥median HER2 levels than in those who did not. The response was also higher (34%) in patients with centrally confirmed HER2-positive tumors. Hypokalemia, thrombocytopenia, and fatigue were the most common observed adverse events. No dose-limiting cardiotoxicity was reported.

T-DM1 is undergoing further testing in the context of several other studies. An open-label, phase III randomized trial (EMILIA, NCT00829166) is comparing single-agent T-DM1 with the combination of capecitabine and lapatinib in patients whose HER2-positive disease has progressed on trastuzumab. In another phase III trial MARIANNE (NCT01120184), T-DM1 monotherapy is being compared to trastuzumab plus a taxane (docetaxel or paclitaxel). Finally, a pilot phase II study is assessing the feasibility of administering T-DM1 sequentially with anthracycline-based chemotherapy as adjuvant or neoadjuvant therapy for patients with early stage HER2-positive BC (NCT01196052).

Neratinib

Neratinib/HKI-272 is an oral, irreversible, small molecule inhibitor of EGFR/HER1, HER2, and HER4. A phase I study determined the MTD of once daily, oral neratinib to be 320 mg, with diarrhea being its most common – but also unacceptably high – toxicity.[78] In light of these findings, 240 mg daily was the dose selected for phase II development. In an open-label, phase II study, patients with advanced HER2-positive BC with and without prior trastuzumab treatment received neratinib.[79] The 16-week PFS was 59% for patients with prior trastuzumab (n = 63) and 78% for those without (n = 64), with the median PFS 22.3 and 39.6 weeks, respectively. The most frequent AEs were diarrhea, nausea, vomiting, and fatigue. Grade 3 or 4 diarrhea occurred in 30% of patients with prior trastuzumab therapy, leading to neratinib dose reduction in 29% of this cohort.

A phase III randomized study (NCT00915018) of paclitaxel with either neratinib or trastuzumab in MBC is ongoing, as is a randomized phase II study (NCT00777101) of neratinib alone versus the combination of capecitabine and lapatinib. Neratinib is also being tested in patients with early BC who have received prior trastuzumab therapy in a phase III, placebo-controlled trial (NCT00878709). In the neoadjuvant setting, an ongoing phase II randomized trial (NCT01008150) evaluating neoadjuvant neratinib or trastuzumab followed by postoperative trastuzumab is enrolling women with locally advanced HER2-positive BC.

Afatinib

Afatinib/BIBW 2992 is an oral irreversible small molecule inhibitor of EGFR/HER1 and HER2. In a phase I study including 53 patients the most common adverse events were diarrhea, nausea, vomiting, rash, and fatigue.[80] Dose-limiting toxicities were grade 3 rash (n = 2) and reversible dyspnea secondary to pneumonitis (n = 1). The recommended dose for subsequent studies was 50 mg/day. An open-label phase II study recruited advanced HER2-positive BC patients to receive afatinib after failure of treatment to trastuzumab.[81] A total of 41 patients were included and received afatinib 50 mg/day as monotherapy. Of the 34 evaluable patients, PR was observed in 12%, SD in 41% and clinical benefit (CR + PR + SD) in 53%. Subsequent phase II studies demonstrated modest activity of afatinib monotherapy among ER-positive and HER2-negative BC patients.[82,83]

Afatinib is under evaluation in a phase III randomized study in HER2 Overexpressing Metastatic Breast Cancer Patients (NCT01125566). In this study, 780 HER2-positive MBC patients who experience progression on prior trastuzumab treatment will be randomized to receive afatinib plus vinorelbine or trastuzumab plus vinorelbine.

Heat shock protein 90 (Hsp90) inhibitors

Hsp90 is a molecular chaperone that facilitates the folding, maturation, conformation, and stability of the HER2 protein and downstream substrates like Raf and Akt.[84] Geldanamycin and its derivative tanespimycin/17-AAG have antitumor properties and can downregulate client proteins of Hsp90, including HER2. Hsp90 is highly expressed in most cells, yet Hsp90 inhibitors display selective killing of cancer cells with relative sparing of normal cells. It has been shown that Hsp90 derived from cancer cells has a 100-fold higher binding affinity for tanespimycin/17-AAG than does Hsp90 from normal cells.[85] Tanespimycin/17-AAG reduces HER2 levels and inhibits proliferation of the trastuzumab resistant breast tumor cell line JIMT-1.[86]

A phase I study (n = 25) has examined different doses of tanespimycin/17-AAG in combination with trastuzumab in heavily pre-treated patients with solid tumors.[87] Drug-related grade 3 AEs included emesis, increased ALT, hypersensitivity reactions, and drug-induced thrombocytopenia. Pharmacodynamic testing confirmed Hsp70 induction (a marker of Hsp90 inhibition) in lymphocytes.

Another Hsp90 inhibitor, IPI-504, is under investigation in combination with trastuzumab in a phase II study (NCT00817362) evaluating the efficacy and safety of this regimen for treatment of patients with advanced HER2-positive BC. The results of this trial are still awaited.

Accelerating the discovery of new anti-HER2 therapies: advantages of the neoadjuvant approach

Well-designed neoadjuvant studies incorporating serial biopsies and biomarkers have the potential to allow translational researchers to accurately measure target inhibition and downstream pathways blockade, as well as to correlate these findings with tumor response. They can also provide an early read-out of drug efficacy, and thus represent an optimal platform for new drug development. Importantly, pathologic complete response (pCR) identifies the subgroup of patients most likely to survive longer, and is frequently used as the primary endpoint in neoadjuvant studies.[88]

In the "first generation" of neoadjuvant anti-HER2 trials, patients were randomized to receive chemotherapy with or without trastuzumab.[63–65] These seminal studies confirmed the superiority of neoadjuvant trastuzumab in combination with chemotherapy, showing pCR rates at least two times higher in the trastuzumab containing arms (summarized in Table 1).[63–65] In the M.D. Anderson trial, a higher three-year disease-free survival (DFS) rate was observed in patients treated with chemotherapy plus trastuzumab when compared to chemotherapy alone (n = 45 pts; 100% vs. 85.3%, respectively; p = 0.041).[63] In the Neoadjuvant Herceptin (NOAH) study, with three years of median follow-up, higher event-free survival was observed in patients treated with chemotherapy plus trastuzumab compared to chemotherapy alone (71% vs. 56%, respectively; HR = 0.59; p = 0.013).[64] The German Breast Group/Gynecologic Oncology Study Group trial (GeparQuattro) showed a pCR rate of 31.7% in the subgroup of HER2-positive BC patients and 15.7% in the HER2-negative subgroup.[65]

In the "second generation" of neoadjuvant anti-HER2 trials, patients were randomized to receive trastuzumab with or without another anti-HER2 agent in an effort to further improve the clinical benefit achieved with trastuzumab alone (Table 1).[66–68] A great deal of interest has been generated by the recently reported results of the randomized NeoALTTO[66] and NeoSphere[67] trials, both of which confirmed that using a dual blockade with anti-HER2 agents in early BC is markedly superior to treatment with one agent alone.

In NeoALTTO a significantly higher pCR rate was observed in patients treated with lapatinib in combination with trastuzumab plus chemotherapy than in those receiving trastuzumab plus chemotherapy (pCR 51.3% vs. 29.5%, respectively; $p = 0.0001$).[66] Unique about this trial is its "biological window" of anti-HER2 therapies: trastuzumab, or lapatinib, or trastuzumab plus lapatinib were given for six weeks without chemotherapy. This window provides an opportunity to better evaluate the tumor changes and clinical response to anti-HER2 therapies alone. Specifically, biopsies in all patients were taken at week 2 of the biological window, which will enable us to compare gene expression and biomarkers at baseline, week 2 and surgery (for correlation with pCR).

The NeoSphere study also reported a significantly higher pCR rate for patients who were treated with pertuzumab, trastuzumab and chemotherapy rather than just trastuzumab plus chemotherapy alone (pCR 45.8% vs. 29%, respectively; $p = 0.0141$).[67] Interestingly, 16.8% of patients treated with trastuzumab plus pertuzumab for 12 weeks (without chemotherapy) achieved pCR. A subgroup analysis according to hormone receptor (HR) status suggests that the benefit of dual HER2 blockade with trastuzumab and pertuzumab is mainly confined to the HR-negative population. 29.1% of patients with HR-negative BC achieved pCR, as opposed to only 6% in the HR-positive patients. These results suggest that a subset of patients could be spared the toxicity of chemotherapy and be treated with anti-HER2 agents alone.

The GeparQuinto study failed to show a superior pCR rate of lapatinib in combination with chemotherapy when compared to treatment with trastuzumab plus chemotherapy (pCR 21.7% vs. 31.3%, respectively; $p < 0.05$).[68] Although the reasons for this failure are unclear, it is possible that it was caused by the high incidence of lapatinib interruption during neoadjuvant therapy due to AEs (~34%), the important synergism between anthracycline chemotherapy and trastuzumab, or the contribution of an immune response to the activity of trastuzumab.

To summarize the initial results of NeoALTTO, NeoSphere and GeparQuinto, superior pCR rates have been demonstrated in the subsets of patients with HER2-positive, ER-negative BC, as detailed in Fig. 2.[66–68] These trials indicate that the dual HER2 blockade combined with chemotherapy may be superior to a single HER2 blockade. The ongoing National Surgical Adjuvant Breast and Bowel Project B41 study (NCT00486668) will compare the combination of trastuzumab plus lapatinib to trastuzumab and to lapatinib administered with weekly paclitaxel following anthracyclines and cyclophosphamide.

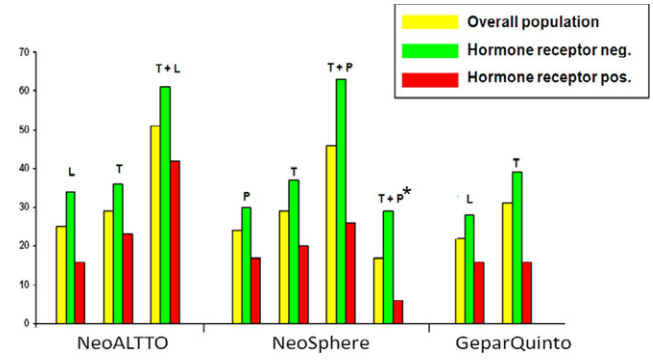

Fig. 2. Pathologic complete response rates to anti-HER2 therapy were higher in the hormone receptor negative patient population in the NeoALTTO, NeoSphere, and GeparQuinto studies. neg = negative; pos = positive; T = trastuzumab, L = lapatinib, P = pertuzumab. *Chemotherapy not given.

The huge translational research efforts incorporated into these neoadjuvant studies are eagerly awaited. For example, NeoALTTO incorporates a comprehensive translational research component for all patients as well as modern imaging techniques (PET-scan) and circulating tumour cells in a subset of patients. The results from these analyses will likely to shed light on complex issues such as anti-HER2 resistance and sensitivity, as well as the identification of the BC subgroups most likely to benefit from the different approaches to anti-HER2 therapy.

While these second generation neoadjuvant studies hold considerable promise, it must also be acknowledged that the pCR definitions used across these trials were not homogeneous (pCR definitions are described in Table 1), which will limit cross-trial analysis considerably. This is regrettable and underscores the need for better standardisation of clinical trials endpoints in our next generation of trials.

Conclusions

HER2-positive BC encompasses a heterogeneous group of diseases with differing biological characteristics and clinical outcomes.[89] The current clinical strategy, by which all HER2-positive patients are treated using a broadly similar approach, remains suboptimal. There is urgency for us to define the characteristics of the "various diseases" that comprise the HER2 group and, most importantly, to tailor their treatments accordingly. Novel anti-HER2 agents are currently under clinical investigation; however, there is a lack of reliable biomarkers to prospectively identify subgroups of HER2-positive BC most likely to benefit from specific anti-HER2 agents. The translational research embedded in the recently reported neoadjuvant trials could enable us to select those patients who should be treated with anti-HER2 single-agent therapy in combination with chemotherapy regimens or, more ambitiously, as a dual blockade of anti-HER2 drugs, whereby chemotherapy can be spared. Achieving these objectives will move us closer to an era of truly tailored therapy.[90] Importantly, the hypotheses generated by these neoadjuvant trials will certainly be validated in the ongoing or planned large phase III adjuvant trials.

The introduction of trastuzumab has lead to a paradigm shift in treatment of HER2-positive BC. Now, we must aim still higher.[69] With several new anti-HER2 agents beckoning on the horizon, we can be hopeful that the full benefit of HER2 receptor blockade will soon be realized. In a 2005 editorial of the The New England Journal of Medicine, Dr Gabriel Hortobagyi commented that the results of the pivotal adjuvant trastuzumab trials in HER2-positive breast cancer were "not evolutionary but revolutionary".[91] It is now time for the next revolution.

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Conflict of interest statement

M. Piccart: Honoraria from Roche. K.S. Saini, H.A. Azim Jr, O. Metzger-Filho, S. Loi, C. Sotiriou and E. de Azambuja have no conflict of interest to declare.

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