Review

Advances in systemic therapy for HER2-positive metastatic breast cancer

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Abstract

Human epidermal growth factor receptor (HER)2 over-expression is associated with a shortened disease-free interval and poor survival. Although the addition of trastuzumab to chemotherapy in the first-line setting has improved response rates, progression-free survival, and overall survival, response rates declined when trastuzumab was used beyond the first-line setting because of multiple mechanisms of resistance. Studies have demonstrated the clinical utility of continuing trastuzumab beyond progression, and further trials to explore this concept are ongoing. New tyrosine kinase inhibitors, monoclonal antibodies, PTEN (phosphatase and tensin homolog) pathway regulators, HER2 antibody-drug conjugates, and inhibitors of heat shock protein-90 are being evaluated to determine whether they may have a role to play in treating trastuzumab-resistant metastatic breast cancer.

Introduction

As knowledge about the treatment of breast cancer has grown, attention has increasingly focused on developing a targeted approach to this diverse disease. In particular, treatment of human epidermal growth factor receptor (HER)2/neu-positive breast cancer has undergone significant advances since the cloning of the HER2 oncogene in 1984 [1].

The HER2 oncogene encodes one of four transmembrane receptors within the erbB family. Its over-expression, which occurs in approximately 25% of all breast cancer tumors, is associated with a shortened disease-free interval and poor survival [2]. Following ligand binding, the glycoprotein receptor is activated through homodimerization or heterodimerization, leading to a cascade of events that involves activation of the tyrosine kinase domain, Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, and phosphatidylinositol-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR). This sequence promotes the rapid cell growth, differentiation, survival, and migration that are associated with HER2-positive breast cancers (Figure 1). Thus, women with HER2-positive breast cancers exhibit significantly decreased disease-free survival and overall survival (OS) [2-5].

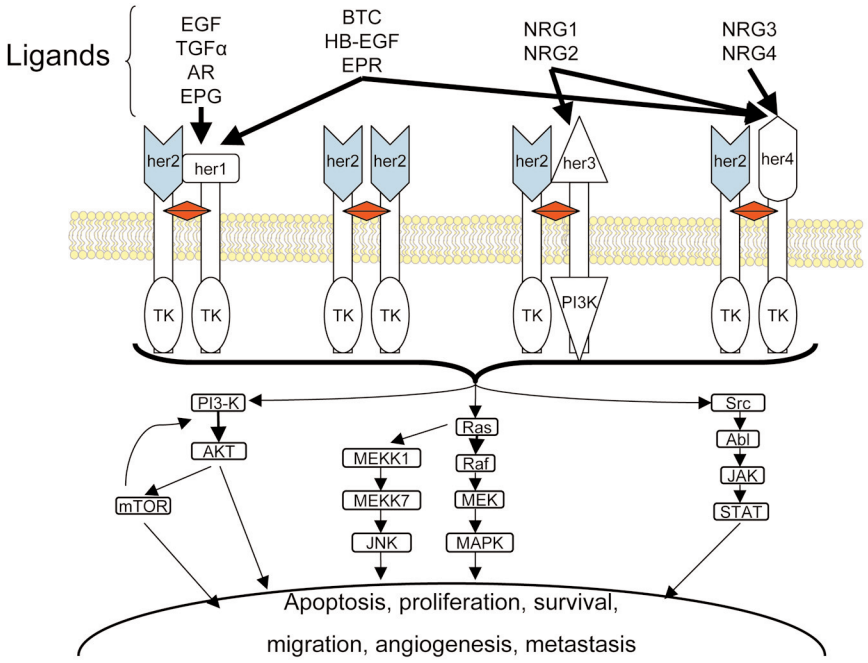
This review discusses progress in the treatment of HER2-positive metastatic breast cancer since the discovery of the HER2 oncogene, with particular focus upon the mechanisms of resistance to trastuzumab, treatment with trastuzumab beyond progression, use of lapatinib, and new biologic agents that may provide further therapeutic options in patients with metastatic HER2-positive breast cancer.

Use of trastuzumab in the treatment of metastatic breast cancer

Trastuzumab is a humanized recombinant monoclonal antibody, of the IgG_1 type, which binds with high affinity to the extracellular domain of the HER2 receptor. The mechanism underlying trastuzumab's efficacy in the treatment of HER2-positive breast cancer is multifaceted and incompletely understood. *In vivo* breast cancer models have demonstrated that trastuzumab induces antibody-dependent cellular cytotoxicity through activation of Fc receptor expressing cells (for example, macrophages and natural killer cells), leading to lysis of tumor cells [6,7]. Trastuzumab has also been shown to downregulate $p185^{ErbB2}$ [8]. In addition, trastuzumab blocks the release of the extracellular domain of HER2 by inhibiting cleavage of the HER2 protein by ADAM (a disintegrin and metalloproteinase domain) metalloproteinases [9]. Significant declines in serum HER2 levels are a predictor of outcome after trastuzumab-based therapy [10-12]. Furthermore, trastuzumab inhibits downstream PI3K-Akt signaling, leading to apoptosis [13]. It has also been shown that trastuzumab downregulates proteins that are involved in $p27^{kip1}$ seques-

17-AAG = 17-(allylamino)-17-demethoxygeldanamycin; CR = complete response; EGFR = epidermal growth factor receptor; HER = human epidermal growth factor receptor; HR = hazard ratio; IGF-IR = insulin-like growth factor receptor-I; MAPK = mitogen-activated protein kinase; mTOR = mammalian target of rapamycin; OS = overall survival; PI3K = phosphatidylinositol-3-kinase; PR = partial response; PTEN = phosphatase and tensin homolog; TTP = time to progression; VEGF = vascular endothelial growth factor.

Figure 1



The HER2 family and interrelated signaling and events. The binding of ligands, including epidermal growth factor and transforming growth factor-α, leads to the activation of signaling cascades involving Ras/Raf/MAPK, PI3K/Akt/mTOR, and JAK/STAT. This sequence of events promotes the apoptosis, proliferation, survival, migration, angiogenesis, and metastasis of HER2-over-expressing breast cancers. BTC, betacellulin; EGF, epidermal growth factor; EPG, epigen; EPR, epiregulin; HB-EGF, heparin-binding EGF-like growth factor; HER, human epidermal growth factor receptor; JAK, Janus kinase; JNK, c-Jun N-terminal kinase 1; mTOR, mammalian target of rapamycin; MAPK, mitogen-activated protein kinase; MEK, mitogen-induced extracellular kinase; MEKK, mitogen-activated protein/ERK kinase kinase; NRG, neuregulin; PI3K, phosphatidylinositol 3-kinase; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TK, tyrosine kinase.

tration, causing release of $p27^{kip1}$ and enabling inhibition of cyclin E/Cdk2 complexes and subsequent G_1 arrest [14]. Moreover, trastuzumab has been shown to exert antiangiogenic effects through normalization of microvessel density [15].

Although the mechanism that accounts for trastuzumab's antitumor activity remains incompletely understood and requires further elucidation, the results of the inclusion of trastuzumab in the treatment of HER2-positive breast cancer are clear. Slamon and colleagues [16] found that addition of trastuzumab to chemotherapy, in the first-line setting, resulted in a significantly improved objective response, time to disease progression, and OS. Combinations of trastuzumab with taxanes, platinum salts, vinorelbine, and capecitabine have yielded benefits in the treatment of HER2-positive metastatic breast cancer [17-23]. However, other trials demonstrated that response rates declined markedly when trastuzumab was used beyond the first-line setting, indicating the development of resistance to this agent.

Mechanisms of resistance to trastuzumab

PTEN/PI3K/mTOR/Akt pathways

PTEN (phosphatase and tensin homolog) is a tumor suppressor gene that causes dephosphorylation of phosphotidylinositol-3,4,5 triphosphate, which is the site that recruits the pleckstrin-homology domain of Akt to the cell membrane [24,25]. PTEN inhibits the ability of PI3K to catalyze the production of phosphotidylinositol-3,4,5 triphosphate and thus antagonizes the Akt cascade [26]. Loss of PTEN function occurs in approximately 50% of all breast cancers [27]. Restoration of PTEN expression impedes Akt activation and increases apoptosis [28]. Nagata and coworkers [24] demonstrated that inhibition of PTEN expression by antisense oligonucleotides resulted in trastuzumab resistance *in vitro* and *in vivo*. Specifically, tumors in which PTEN expression was abrogated by antisense oligonucleotides exhibited tumor growth patterns that were unaffected by trastuzumab administration. Patients with PTEN-deficient tumors demonstrated significantly lower complete response (CR) and partial response (PR) rates to trastuzumab plus taxane therapy than those who had PTEN-expressing tumors.

Independent of PTEN mutations, constitutive Akt phosphorylation may also occur in HER2-over-expressing tumors [29].

MUC4 overexpression

MUC4 is a membrane-associated, glycosylated mucin that has been shown to be over-expressed in breast cancer cells

[30]. In rat models, MUC4 forms a complex with the HER2 protein and potentiates its phosphorylation, leading to cell proliferation [30,31]. In addition to its ligand activity, MUC4 inhibits binding of trastuzumab to the HER2 receptor. Utilizing JIMT-1, a HER2-over-expressing cell line with primary resistance to trastuzumab, Nagy and colleagues [32] demonstrated that MUC4 expression was higher in this trastuzumab-resistant cell line than in trastuzumab-sensitive cell lines, and that the level of MUC4 expression inversely correlated with the trastuzumab-binding capacity of single tumor cells. Because the researchers found no mutations in the coding sequence of HER2 in the JIMT-1 cell line, they hypothesized that inhibition of trastuzumab binding was mediated by MUC4-induced masking of the trastuzumab-binding epitope [32].

Truncation of the HER2 receptor

Truncation of the HER2 receptor, as a result of proteolytic shedding of the HER2 extracellular domain or via alternative initiation from methionines located near to the transmembrane domain of the full-length molecule, can yield a 95-kDa fragment [33-35]. The p95HER2 fragment maintains constitutive kinase activity and correlates with poor outcome in patients with HER2-positive breast cancer [35]. This truncated form of HER2 does not possess the extracellular trastuzumab-binding epitope, and its expression has been associated with trastuzumab resistance [36].

Activation of the insulin-like growth factor receptor-1 pathway

The insulin-like growth factor receptor-I (IGF-IR) is a transmembrane tyrosine kinase receptor whose activation is associated with mitogenesis and cell survival [37]. Lu and colleagues [38] showed that trastuzumab was rendered ineffective by genetic alteration of the SKBR3 human breast cancer cell lines to enable IGF-IR over-expression. Subsequently, addition of an IGF-binding protein that reduced IGF-IR signaling restored trastuzumab's ability to inhibit cell growth. Utilizing the SKBR3 cell lines, Nahta and coworkers [39] demonstrated that IGF-I stimulation led to increased phosphorylation of HER2 in trastuzumab-resistant cells but not in parental trastuzumab-sensitive cells. In addition, inhibition of IGF-IR tyrosine activity reduced HER2 phosphorylation only in trastuzumab-resistant cells. Thus, there exists substantial cross-talk between IGF-IR and HER2 in trastuzumab-resistant cells.

Increased expression of vascular endothelial growth factor

Du Manoir and colleagues [40] compared the levels of vascular endothelial growth factor (VEGF) protein expression in cell lines that had never been exposed to trastuzumab with those cell lines that had acquired resistance to trastuzumab. They found that VEGF protein expression was increased in three out of four of the resistant cell lines; however, no significant difference in VEGF mRNA expression was present, implying that the increase in VEGF protein expression was mediated by post-transcriptional changes. In addition, when bevacizumab was added to a trastuzumab-based regimen for mice bearing tumors of the 231-H2N cell line, a HER2-over-expressing variant of the MDA-MB-231 cell line, tumor growth was delayed significantly. Their cumulative findings indicate that upregulation of VEGF may contribute to the mechanism of trastuzumab resistance, and dual inhibition of these pathways is currently being studied in phase I trials.

Treatment with trastuzumab beyond progression

Disease progression after treatment with a trastuzumab-based regimen in the first-line metastatic setting is met with two options: continuation of trastuzumab alongside another chemotherapeutic agent that is not cross-resistant, or changing to a completely different (non-trastuzumab-based) regimen. Evidence supporting the former approach first appeared in an extension study, in which patients who had progressed during the pivotal trial of trastuzumab plus chemotherapy were offered further trastuzumab-based treatment [41]. Concurrent chemotherapy was chosen at the discretion of the treating physician. Researchers found that the group of patients who had previously received chemotherapy alone during the pivotal trial had a 14% objective response rate and 32% clinical benefit rate (CR plus PR plus stable disease for ≥6 months) during the extension trial. Those who had already received a trastuzumab-based regimen during the pivotal trial had a 11% objective response rate and 22% clinical benefit rate. Median duration of response was greater than 6 months in both groups. These findings suggest that trastuzumab continues to demonstrate clinical utility in the treatment of HER2-positive metastatic breast cancer beyond progression. Four retrospective studies have corroborated these results.

Stemmler and coworkers [42] found that patients who received two or more trastuzumab-based regimens had a significantly longer OS than did those who received only one regimen (62.4 months versus 38.5 months; $P = 0.01$), indicating that use of trastuzumab beyond progression is a valid option in this group of patients. In addition, the Hermine retrospective cohort study [43] demonstrated that, when compared with patients who discontinued trastuzumab upon progression of disease, those who continued to receive a trastuzumab-based regimen beyond progression exhibited an improvement both in time to progression (TTP) and in median OS. Another study, conducted by Gelmon and colleagues [44], revealed that overall response rate to trastuzumab alone or with a taxane as the first regimen was 39%; a further 30% of patients had stable disease as the best response. These rates declined only slightly (36% and 38% for overall response rate and stable disease, respectively) after a second regimen of trastuzumab (alone or in combination with paclitaxel or vinorelbine) was administered. Patients who received further trastuzumab-based regimens beyond disease

progression demonstrated a significant improvement in OS when compared with those who received only one trastuzumab-based regimen (38.5 months versus 62.4 months; $P = 0.01$).

A fourth study - a retrospective analysis of 101 patients who continued to receive a trastuzumab-containing regimen beyond progression - was reported this year [45]. It demonstrated a trend toward improvement in response rates (35% versus 16%) and median OS rates (70 months versus 56 months) in the patients who continued on trastuzumab, but the findings were not statistically significant.

Although the results of these retrospective studies are encouraging, these analyses suffer from the inherent limitations of retrospective chart review, lack of uniformity in chemotherapy choice, and evaluation of adverse events in only the retrospective setting [46].

Recently, interim results from three prospective trials were reported that support the use of trastuzumab beyond disease progression on a trastuzumab-based regimen [47]. Bachlot and colleagues [48] presented the results of a phase II trial that evaluated the response rate to second-line treatment with the combination of trastuzumab and vinorelbine, after progression of disease on a first-line regimen of trastuzumab plus taxane. They found that the overall response rate in this second-line setting was 29%, with two patients showing a CR. Furthermore, von Minckwitz and coworkers [49] presented the results of the TBP (Treatment Beyond Progression) trial, in which women who had progressed while on a trastuzumab-based regimen were randomly assigned to treatment with capecitabine monotherapy or capecitabine plus trastuzumab. The women who received both capecitabine and trastuzumab demonstrated an improvement in TTP (33 weeks versus 24 weeks), although this finding was not statistically significant ($P = 0.178$). O'Shaughnessy and colleagues [50] conducted a randomized phase III trial of lapatinib versus lapatinib plus trastuzumab in heavily pretreated patients who had progressed after trastuzumab-based therapy. In this study, the TTP was improved in patients treated with trastuzumab plus lapatinib ($P = 0.029$).

Given the importance of this issue, two other randomized phase III trials are poised to address the use of trastuzumab beyond progression: THOR (Trastuzumab Halted or Retained) and Pandora. The THOR and Pandora trials are phase III trials that will randomly assign patients, who progressed on a first-line regimen of trastuzumab plus chemotherapy, to continue or discontinue trastuzumab while receiving a second-line chemotherapy of the investigator's choice.

New agents in the treatment of HER2-positive breast cancer

Since the identification of the HER2 oncogene, development of new agents targeting the HER2 pathway has led to significant improvement in the treatment of HER2-positive metastatic breast cancer (Table 1).

Tyrosine kinase inhibitors

Lapatinib

Lapatinib is an orally bioavailable, small-molecule dual inhibitor of the tyrosine kinase activity of epidermal growth factor receptor (EGFR) and HER2. It reversibly binds to the cytoplasmic ATP-binding site of the intracellular tyrosine kinase, preventing receptor phosphorylation and downstream signaling of several pathways, including Akt and MAPK [51]. Its targeting of the kinase domain of HER2 enables this agent to retain activity in trastuzumab-resistant tumor cells that overexpress the truncated HER2 receptor (p95HER2). Treatment of p95HER2-expressing cells with lapatinib has been shown to reduce p95HER2 phosphorylation, impede downstream activation of Akt and MAPK, and inhibit cell proliferation [36]. In addition, an analysis of the tumor samples of 46 patients with metastatic breast cancer who were treated with trastuzumab demonstrated that only one out of nine patients who expressed p95HER2 responded to trastuzumab, whereas 19 of the 37 patients with tumors expressing full-length HER2 achieved either a CR or a PR ($P = 0.029$) [36]. These findings indicate that there may be a role for lapatinib, or another tyrosine kinase inhibitor, in the treatment of p95HER2-related trastuzumab resistance.

The preclinical studies of lapatinib eventually led to a pivotal phase III trial that involved patients with HER2-positive advanced or metastatic breast cancer who had progressed after treatment with a regimen that included an anthracycline, a taxane, and trastuzumab [52]. The study randomly assigned these patients to receive treatment with capecitabine monotherapy versus lapatinib plus capecitabine, and an interim analysis demonstrated that the combination of lapatinib plus capecitabine yielded a significant improvement in TTP (hazard ratio [HR] = 0.49; one-sided $P = 0.00016$). However, it should be noted that OS was equivalent in the treatment arms. Researchers noted a trend toward improvement in the occurrence of cerebral nervous system metastases in the combination group. Updated results of this trial confirmed the improved TTP due to the combination of lapatinib plus capecitabine (27 weeks versus 19 weeks), with a HR of 0.57 ($P = 0.00013$) [53]. Furthermore, the updated findings demonstrated a trend toward an improvement in OS, although this result was not statistically significant (HR = 0.78, $P = 0.177$). The results of this trial led to the US Food and Drug Administration's approval of lapatinib, in combination with capecitabine, in the treatment of patients with advanced or metastatic HER2-positive breast cancer that progressed after trastuzumab, anthracyclines, and taxanes.

A recent phase III trial randomly assigned patients with metastatic breast cancer, which was negative or untested for HER2, to receive first-line treatment with paclitaxel monotherapy versus paclitaxel and lapatinib [54]. Overall, the study

Table 1

Therapeutic agents targeting the HER2 pathway in breast cancer

Agent	Molecule	Target/mechanism	Status
Trastuzumab	Monoclonal antibody	Blocks extracellular domain of HER2	US FDA approved in metastatic and adjuvant settings [16]
Pertuzumab (2C4)	Monoclonal antibody	Blocks HER2 and HER3 dimerization	Phase III (with trastuzumab)
Trastuzumab-DM1	Antibody-drug conjugate	Binds HER2, conjugate is internalized, and DM1 toxin causes cancer cell death	Phase III
Ertumaxomab	Monoclonal antibody	Trifunctional anti-HER2 x anti-CD3 antibody	Phase II
Lapatinib	Small molecule TKI	Reversible inhibitor of EGFR and HER2 TK	FDA approved in metastatic setting [52]
Gefitinib	Small molecule TKI	Reversible inhibitor of EGFR	Phase II (discontinued because of poor results)
Erlotinib	Small molecule TKI	Reversible inhibitor of EGFR	Phase II
HKI-272	Small molecule TKI	Irreversible inhibitor of the HER-2 and EGFR TK	Phase I/II
BIBW2992	Small molecule TKI	Irreversible inhibitor of the HER-2 and EGFR TK	Phase II
Temsirolimus (CCI779)	Rapamycin analogue	mTOR inhibitor and Akt deactivation	Phase III (with letrozole)
Everolimus (RAD001)	Rapamycin analogue	mTOR inhibitor and Akt deactivation	Phase III (with paclitaxel)
Tanespimycin (17AAG)	Derivate of geldanamycin	Hsp-90 inhibitor (anti-downstream pathway element)	Phase II
Tipifarnib	Analogue of imidazole	farnesyltranstransferase inhibitor (downstream pathway element)	Phase II (with chemo and/or homonaltherapy)
GDC-0941	Small molecule	PI3K inhibitor	Phase I

EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; HER, human epidermal growth factor receptor; Hsp, heat shock protein; mTOR, mammalian target of rapamycin; TK, tyrosine kinase; TKI, tyrosine kinase inhibitor.

found no significant difference in TTP, event-free survival, or OS with the addition of lapatinib to paclitaxel therapy. However, in a planned subgroup analysis, the authors found that - among patients who were HER2 positive - the addition of lapatinib to paclitaxel resulted in significant improvements in TTP (HR = 0.53; $P = 0.005$) and event-free survival (HR = 0.52; $P = 0.004$). In these HER2-positive patients, OS was also increased in the paclitaxel-lapatinib arm, but this difference was not statistically significant ($P = 0.365$). These findings support further studies exploring the use of lapatinib-based regimens in the first-line treatment of HER2-positive metastatic breast cancer.

Based upon preclinical data indicating that the combination of trastuzumab and lapatinib may produce synergistic inhibition of cell proliferation, a phase I study involving this combination was conducted in patients with advanced or metastatic HER2-positive breast cancer [55,56]. The most frequent drug-related grade 3 events were diarrhea (17%), fatigue (11%), and rash (6%). The combination regimen resulted in one CR and seven PRs. All eight responders had received prior trastuzumab therapy in combination with cytotoxic chemotherapy. Furthermore, six patients had stable disease for longer than 6 months. Because of these encouraging results, a double-blind, placebo-controlled, phase III study was initiated to determine whether the addition of lapatinib to a combination of trastuzumab and paclitaxel will render an improvement in TTP [57]. This trial will also evaluate the incidence of central nervous system metastases among these groups, given the encouraging findings in the previous pivotal trial, in order to investigate further the hypothesis that the small molecule tyrosine kinase inhibitors, unlike the monoclonal antibodies, may be able to cross the blood-brain barrier effectively.

HKI-272

HKI-272 is a potent and irreversible inhibitor of the tyrosine kinase domains of EGFR and HER2, resulting in arrest at the G_1-S (Gap 1/DNA synthesis) phase [58]. Preliminary results of an open-label, phase II study involving HKI-272 in the treatment of women with advanced or metastatic HER2-positive breast cancer [59] revealed six confirmed PRs, as well as additional patients with unconfirmed PRs. Diarrhea was the only grade 3 to 4 treatment-related adverse event that occurred in more than one patient. This trial continues to accrue patients.

Gefitinib and erlotinib

Gefitinib and erlotinib are small-molecule EGFR tyrosine kinase inhibitors. Previous preclinical studies have been contradictory. For example, in one study [60] the combination of gefitinib and trastuzumab resulted in a synergistic inhibition of the SK-Br-3 and BT-474 breast carcinoma cell lines, both of which express EGFR and ErbB-2. However, these findings were not confirmed in breast cancer xenograft models, which demonstrated that gefitinib did not further reduce tumor cell viability in trastuzumab-treated tumors [61]. In phase II clinical trials of single-agent gefitinib and single-agent erlotinib, the response rates were less than 5% [62]. A phase I/II trial was conducted in which patients with HER2-positive metastatic breast cancer who had previously received between 0 to 2 chemotherapy regimens were treated with a combination of trastuzumab (2 mg/kg per week) and gefitinib (250 to 500 mg/day). Within the chemotherapy-naïve group, one CR, one PR, and seven instances of stable disease were reported. However, no responses were seen among patients who had previously been treated. Thus, at the time of the interim analysis, the TTP did not meet predetermined statistical end-points required for study continuation, and the study was halted [63]. Moreover, the abbreviated TTP (2.5 to 2.9 months) observed in this trial suggests a possible antagonistic interaction between gefitinib and trastuzumab.

Pazopanib

Pazopanib is a tyrosine kinase inhibitor of VEGF receptor-1, -2 and -3, platelet-derived growth factor-α and -β, and c-kit. A phase I study evaluating the use of pazopanib in various solid tumors demonstrated that the agent was well tolerated [64]. Preliminary efficacy results revealed a minimal response in four patients; six other patients exhibited stable disease for longer than 6 months. Based upon encouraging preclinical data supporting the combination of lapatinib and pazopanib, a phase I open-label trial involving 33 patients with solid tumors was initiated [65]. It demonstrated that 10 patients experienced stable disease for longer than 16 weeks, and three patients had PRs. Thus, a phase II, open-label randomized trial began in 2006 and is ongoing. This study randomly assigned patients to receive treatment with lapatinib plus pazopanib, versus lapatinib alone, in the first-line treatment of advanced or metastatic HER2-positive breast cancer [66,67]. Preliminary efficacy results revealed that the progressive disease rate was 69% in the combination group versus 27% in the lapatinib monotherapy group.

Monoclonal antibodies

Pertuzumab

Pertuzumab is an IgG_1 monoclonal antibody that binds to HER2 at the dimerization domain, sterically preventing heterodimerization [68,69]. The pertuzumab-binding site does not overlap with the trastuzumab epitope on HER2 [70,71]. Preclinical studies demonstrated that trastuzumab and pertuzumab synergistically inhibited the survival of BT474 cells, reduced levels of total and phosphorylated HER2 protein, and blocked receptor signaling through Akt [72]. A phase I dose escalation study of pertuzumab in 21 patients with advanced cancers showed that pertuzumab was well tolerated and led to a partial response in two patients and stable disease in six patients [73]. Thus, a single-arm, two-stage phase II trial, involving patients with HER2-positive metastatic breast cancer who had received up to three lines of prior therapy and had developed disease progression during trastuzumab-based therapy, is in progress. Interim results revealed that, among the 33 evaluable patients, one CR and five PRs had occurred [74,75]. In addition, seven patients exhibited stable disease for longer than 6 months, and 10 patients had stable disease for less than 6 months. Recruitment into the second stage of this study is ongoing.

Bevacizumab

Bevacizumab is a monoclonal antibody to VEGF, which was approved this year by the US Food and Drug Administration to be used in combination with paclitaxel for the treatment of metastatic HER2-negative breast cancer. Encouraging preclinical studies demonstrated the benefit of adding bevacizumab to trastuzumab, as compared with treatment with trastuzumab alone, in order to inhibit tumor growth further in a breast cancer xenograft model [40]. A phase I, open-label, dose-escalation trial assessed the optimal dose schedule and safety of bevacizumab in combination with trastuzumab [76]. The study showed that co-administration of these agents did not alter the pharmacokinetics of either drug, and no grade 3 or 4 adverse events occurred. Preliminary efficacy results showed one CR, four PRs, and two patients with stable disease among nine evaluable patients.

Based upon these encouraging results, a phase II extension study was initiated to evaluate the efficacy of this combination in the first-line treatment of patients with HER2-positive metastatic breast cancer. Of the 37 patients who were evaluated in this trial, preliminary findings demonstrated one CR and 19 PRs; the combination of bevacizumab and trastuzumab without chemotherapy produced an overall response rate of 54%. Furthermore, a phase III study has been initiated that will randomly assign patients to receive either bevacizumab and trastuzumab and docetaxel, versus trastuzumab plus docetaxel [77]. The primary end-point of this study will be progression-free survival; secondary outcome measures will include OS, best overall response, duration of response, time to treatment failure, quality of life, and cardiac safety.

Regulators of the PTEN/P13K/mTOR/Akt pathways

Everolimus is a macrolide that selectively inhibits mTOR. Because PTEN deficiency, which occurs in approximately 50% of all breast cancers, leads to constitutive activation of Akt and subsequent activation of mTOR, inhibitors of mTOR add potential additive benefit to biologic or chemotherapy. Preclinical testing demonstrated that, after the breast cancer

cell line BT474M1 was rendered PTEN deficient and trastuzumab resistant through transfection with PTEN antisense oligonucleotides, the combination of RAD001 and the Akt inhibitor triciribine restored trastuzumab sensitivity and markedly increased growth inhibition by trastuzumab [78]. A phase I/II trial, involving the use of RAD001 in combination with trastuzumab for the treatment of patients with HER2-positive metastatic breast cancer that has progressed on trastuzumab-based therapy, is ongoing at our institution.

HER2 antibody-drug conjugates

Trastuzumab-DM1 is a novel HER2 antibody-drug conjugate in which trastuzumab is chemically linked to a highly potent maytansine-derived antimicrotubule drug (DM1). Xenograft models have established the efficacy of this compound in both trastuzumab-sensitive and trastuzumab-resistant breast tumors [79]. Recently, the results of a phase II trial involving trastuzumab-DM1 in the treatment of metastatic breast cancer that had progressed on HER2-directed therapy (trastuzumab, lapatinib, or both) were presented [80]. Twenty-five percent of patients demonstrated a confirmed objective response, and 35% of patient demonstrated clinical benefit, which consisted of either a confirmed objective response or stable disease lasting for at least 6 months.

Inhibitors of heat shock protein-90

The heat shock protein-90 is a chaperone protein that enables the proper folding of newly synthesized client proteins, such as HER2, into a stable tertiary conformation. Geldanamycin, an ansamycin antibiotic, was first isolated from *Streptomyces hygroscopicus* and noted to have inhibitory activity against heat shock protein-90 [81,82]. However, because of geldamycin's narrow therapeutic widow, 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) was derived from the initial compound [82]. Preclinical studies involving 17-AAG revealed that it reduced cellular proliferation of the trastuzumab-resistant cell line JIMT-1 [83]. The antiproliferative effect of 17-AAG was positively correlated with phosphorylation and downregulation of HER2, and was dominated by apoptosis. Thus, a phase I dose-escalation study was initiated, in which patients with advanced solid tumors who had progressed during standard therapy were treated with weekly trastuzumab followed by intravenous tanespimycin (17-AAG). Among the 25 patients enrolled, one PR, four minor responses, and four patients with stable disease were noted [84]. The authors observed that tumor regressions were seen only in patients with HER2-positive metastatic breast cancer.

Economic implications of targeted therapy

Unfortunately, most countries experience economic pressures on health care, resulting in the need to evaluate the cost-effectiveness of utilizing targeted therapy for the treatment of HER2-positive metastatic breast cancer. Specifically, trastuzumab is being closely studied in this setting. Norum and colleagues [85] conducted a cost-effectiveness study in which they reviewed cost per life year in clinical trials involving chemotherapy with or without trastuzumab. They found that the cost per life year saved with the use of trastuzumab ranged between €63,137 and €162,417, depending on survival gain and discount rate employed. However, this study has been criticized for its failure to account for the ability of trastuzumab to reduce the incidence, and thus the cost of treatment, of relapses [86]. A French open-control study evaluated the cost-effectiveness ratio of trastuzumab in combination with paclitaxel when compared with conventional chemotherapy [87]. This study found that the additional cost per saved year of life of trastuzumab, expressed as the incremental cost-effectiveness ratio, was €15,370. Although this figure may be acceptable in a developed country that has adequate resources, the Breast Health Global Initiative Therapy Focus Group has noted that the price of monoclonal antibodies may exceed the resources of low-resource countries [88].

Conclusions

In the years that have intervened since the discovery of the HER2 oncogene, the use of trastuzumab has revolutionized the treatment of HER2-positive breast cancer. However, as the success of trastuzumab has grown, its limitations have become apparent in a parallel manner. Although mechanisms of trastuzumab resistance have been described, a complete understanding of these pathways requires elucidation of the interactions within and between their members. Multifaceted novel strategies that target these alternative pathways are necessary to overcome the adaptive mechanisms of this genetically diverse population and thus increase the likelihood of establishing lasting antitumor efficacy.

Competing interests

FJE has served as a consultant for Genentech, GSK and Novartis. FJE has been the principal investigator on a clinical trial funded by GSK, and another clinical trial funded by Novartis. Research funding for the trials was administered by The University of Texas M. D. Anderson Cancer Center. PKHM and FZ have no competing interests.

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