Mini review

Herceptin: mechanisms of action and resistance

Rita Nahta\textsuperscript{a}, Francisco J. Esteva\textsuperscript{a,b,*}

\textsuperscript{a}Department of Breast Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030-4009, USA
\textsuperscript{b}Department of Molecular and Cellular Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030-4009, USA

Received 25 January 2005; accepted 30 January 2005

Abstract

HER-2 is overexpressed in 20–25\% of invasive breast cancers and is associated with an aggressive tumor phenotype and reduced survival rate. The HER-2 status of a tumor is the critical determinant of response to the HER-2-targeted antibody Herceptin. Thus, accurate assessment of HER-2 expression levels is essential for identifying breast cancer patients who will benefit from HER-2-targeted therapy. Herceptin combined with chemotherapy increases response rates, time to disease progression, and survival. However, the majority of cancers that initially respond to Herceptin begin to progress again within 1 year. This review describes mechanisms by which Herceptin inhibits cell growth in breast cancers that overexpress HER-2 and highlights possible mechanisms contributing to Herceptin resistance.

\textcopyright 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: Breast; Monoclonal antibodies; Trastuzumab; Tyrosine kinase receptor; p27\textsuperscript{kip1}; IGF-IR

1. Introduction

The \textit{her-2 (erbB2/neu)} gene encodes an epidermal growth factor receptor- (EGFR)-related tyrosine kinase that is overexpressed in 20–25\% of invasive breast cancers\cite{1,2}. The oncogenic potential of HER-2 was demonstrated in part by its ability to transform normal fibroblasts\cite{3} and to produce breast cancer in transgenic mice when overexpressed under the control of the MMTV promoter\cite{4–6}. Overexpression of HER-2 occurs primarily through amplification of the wild-type \textit{her-2} gene and is associated with poor disease-free survival\cite{1,7–11} and resistance to certain chemotherapeutic agents\cite{12–14}.

HER-2 has become an important therapeutic target in breast cancer for several reasons. (1) HER-2 levels correlate strongly with the pathogenesis and prognosis of breast cancer. (2) The level of HER-2 in human cancer cells with gene amplification is much higher than in normal adult tissues, potentially reducing the toxicity of HER-2-targeting drugs. (3) HER-2 is present in a very high proportion of tumor cells\cite{15}, and tumors with high expression (i.e. an IHC score of 3+) often show uniform, intense immunohistochemical staining\cite{16}, suggesting that anti-HER-2 therapy would target most cancer cells in a given patient. (4) HER-2 overexpression is found both in the primary...
tumor and in metastatic sites [17], indicating that anti-HER-2 therapy may be effective in all disease sites.

2. Mechanisms of action of Herceptin

Herceptin™ (trastuzumab; Genentech; South San Francisco, CA), a recombinant humanized monoclonal antibody (MAb) directed against the extracellular domain (ECD) of the HER-2 protein (Fig. 1), was engineered by inserting the complementary determining regions of a murine antibody (clone 4D5) into the framework of a consensus human IgG1 [18]. Currently, Herceptin is the only HER-2-targeted therapy approved by the United States Food and Drug Administration for the treatment of metastatic breast cancer (MBC). Although the mechanisms by which Herceptin induces regression of HER-2-overexpressing tumors are incompletely defined, several molecular and cellular effects have been observed in experimental in vitro and in vivo models (Table 1).

2.1. Diminished receptor signaling

HER-2 activates multiple cellular signaling pathways, including the PI3 kinase (PI3K) and MAP kinase (MAPK) cascades. Herceptin reduces signaling from these pathways, and thus promotes cell cycle arrest and apoptosis. Diminished receptor signaling may result from Herceptin-mediated internalization and degradation of the HER-2 receptor [19,20]. However, it is unclear whether Herceptin actually downregulates HER-2, as some groups have demonstrated that receptor levels are unchanged in response to Herceptin treatment [21–23]. An alternative mechanism by which Herceptin may block PI3K signaling was recently described. Nagata et al. [24] demonstrated that the interaction between HER-2
and the Src tyrosine kinase is disrupted in response to Herceptin treatment, leading to inactivation of Src with subsequent activation of the PI3K inhibitor PTEN. Thus, Herceptin activates the PTEN phosphatase, which results in rapid Akt dephosphorylation and inhibits cell proliferation.

2.2. G1 arrest: modulation of p27 kip1

Cells treated with Herceptin undergo arrest during the G1 phase of the cell cycle, with a concomitant reduction in proliferation. Cell cycle arrest is accompanied by reduced expression of proteins involved in sequestration of the cyclin-dependent kinase (cdk) inhibitor p27 kip1, including cyclin D1. This results in the release of p27 kip1, allowing it to bind and inhibit cyclin E/cdk2 complexes [20,25,26]. Herceptin treatment also results in an accumulation of p27 kip1, although this seems to occur secondary to formation of p27 kip1-cdk2 complexes, perhaps as a result of decreased targeting of p27 kip1 to the ubiquitin–proteasome [26].

2.3. Induction of apoptosis

Single-agent Herceptin can dramatically reduce tumor size in patients with HER-2-overexpressing metastatic breast cancer. Whether this cytotoxic effect is a direct effect on the cancer cells or it is mediated by indirect mechanisms (i.e. anti-angiogenic activity or the immune system) is not well defined. In vivo studies indicate that Herceptin may induce apoptosis in breast cancers. Chang et al. [27] treated patients with locally advanced breast cancer with preoperative Herceptin and obtained tissue before and after treatment. Preliminary data from that clinical trial indicate that Herceptin therapy results in a significant increase in apoptotic cell death, whereas no change was noted in Ki67 expression. Gennari et al. [28] conducted a similar study using preoperative Herceptin in patients with HER-2-overexpressing operable breast cancer. This study showed no change in HER-2 expression before and after treatment, and no decrease in proliferation in breast cancer tissue. Therefore, more research is needed to characterize the direct cytotoxic effects of Herceptin in cancer cells.

2.4. Inhibition of angiogenesis

Overexpression of HER-2 in human tumor cells is closely associated with increased angiogenesis and expression of vascular endothelial growth factor (VEGF) [29,30]. Treatment of HER-2-overexpressing breast cancers with Herceptin reduced tumor volume and decreased microvessel density in vivo [31,32] and reduced endothelial cell migration in vitro [32]. Furthermore, expression of multiple pro-angiogenic factors was reduced, while expression of anti-angiogenic factors was increased in Herceptin-treated tumors relative to control-treated tumors in vivo [31-33]. The combination of Herceptin with paclitaxel inhibited angiogenesis-associated events to an even greater degree than did Herceptin alone [32], an observation that may reflect more efficient drug delivery due to normalized tumor vasculature after Herceptin treatment [31].

2.5. Immune mechanisms

In vivo breast cancer models and clinical trials have demonstrated that Herceptin, in contrast to many targeted agents, possesses cytotoxic properties. This ability to not only block proliferation but also to actually promote cell death may be related in part to

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed mechanisms of Herceptin action</td>
</tr>
<tr>
<td>Internalization and degradation of HER-2. Disrupts receptor dimerization; disrupts downstream signaling pathways</td>
</tr>
<tr>
<td>G1 arrest and reduced proliferation. Induces p27 kip1-cdk2 complex formation; induces p27 kip1 levels</td>
</tr>
<tr>
<td>Apoptosis. Inhibits Akt activity</td>
</tr>
<tr>
<td>Suppresses angiogenesis. Reduces tumor vasculature in vivo; reduces expression of pro-angiogenic VEGF, TGF-α, Ang-1, PAI-1; induces anti-angiogenic TSP-1</td>
</tr>
<tr>
<td>Immune-mediated responses. ADCC; stimulates natural killer cells</td>
</tr>
<tr>
<td>Inhibits HER-2 ECD proteolysis</td>
</tr>
<tr>
<td>Inhibits DNA repair</td>
</tr>
</tbody>
</table>
induction of an immune response. Herceptin activated an antibody-dependent cellular cytotoxicity (ADCC) response in multiple breast cancer cell lines [18,34,35]. Natural killer (NK) cells, a principal immune cell type involved in ADCC, express the Fc gamma receptor, to which the Fc domain of the Herceptin IgG1 binds, activating NK-mediated cell lysis. Mice bearing BT474 HER-2-overexpressing xenografts demonstrated a tumor regression rate of 96% when treated with Herceptin. In contrast, mice lacking the Fc receptor (FcR\(\kappa\)\(\kappa\)) lost much of the protective effect of Herceptin, with only 29% tumor growth inhibition observed [36]. Thus, NK cells and ADCC are important contributors to the cytotoxic activity of Herceptin but are not solely responsible, as partial tumor regression was still obtained in FcR\(\neg\neg\) mice. This immune function of Herceptin was the focus of two recent clinical trials. In Gennari’s study [28], a strong lymphoid infiltration was noted in all patients treated with preoperative Herceptin, and ADCC correlated with response to therapy. Repka et al. [37] used low-dose interleukin-2 (IL-2) in combination with Herceptin in patients with HER-2-overexpressing breast cancer patients who had progressed on multiple prior systemic therapies. IL-2 increases NK cell numbers and enhances ADCC. In this small cohort of patients, no correlation was observed between clinical response and NK cell expansion or degree of ADCC activity. However, it should be noted that patients with advanced metastatic breast cancer are immunosuppressed and may not be the optimal population to study. Additional studies are needed to better understand the importance of ADCC in mediating the response to Herceptin.

2.6. Inhibition of HER-2 ECD cleavage

The full-length HER-2 receptor undergoes a slow proteolytic cleavage in HER-2-overexpressing cells, yielding a 110-kDa ECD, which can be detected in cell culture medium [38–40], and a 95-kDa amino-terminally truncated membrane-associated fragment with increased kinase activity [41]. The HER-2 ECD can also be detected in the serum of breast cancer patients [42–44], and HER-2 p95 has been found in some breast tumors [41], indicating that HER-2 ECD shedding occurs in vivo. This process may be of clinical importance, because high serum levels of HER-2 ECD correlate with poor prognosis, increased metastasis [45], and decreased responsiveness to endocrine therapy and chemotherapy in patients with advanced breast cancer [42–44]. Herceptin has been shown to block HER-2 ECD proteolytic cleavage and shedding in vitro [46]. Furthermore, the response to Herceptin may depend in part on ECD levels prior to treatment initiation [47,48].

2.7. Inhibition of DNA repair

In vitro studies show that Herceptin is synergistic with a variety of chemotherapies [49,50]. Although not all of the mechanisms of synergy are known, synergy with DNA-damaging drugs is thought to be due to Herceptin-mediated inhibition of DNA repair. Pietras et al. [51] refer to this synergy as receptor-enhanced chemosensitivity, demonstrating that Herceptin partially inhibits repair of DNA adducts in vitro after treatment with cisplatin [51,52] and blocks unscheduled DNA synthesis (a measure of DNA repair) after radiation [53]. The molecular mechanism by which Herceptin blocks DNA repair may involve modulation of p21\(^\text{WAF1}\), such that Herceptin blocks cisplatin-associated induction of p21 [52] and inhibits tyrosine phosphorylation of p21 after radiation [53]. In addition, Herceptin was noted to promote an increase in DNA strand breaks specifically in HER-2-overexpressing BT474 and SKBR3 cells [54] and to increase transcription of genes whose products are involved in DNA repair [55]. These studies suggest that Herceptin promotes DNA damage either independently or in association with therapeutic agents and subsequently inhibits DNA repair, resulting in apoptosis.

3. Assessment of HER-2 status

The American Society of Clinical Oncology recommends evaluation of HER-2 status in all primary breast tumors, either at the time of diagnosis or upon recurrence [56]. The HER-2 status of a tumor provides prognostic information and is the critical determinant of response to Herceptin. Thus, accurate assessment of HER-2 expression levels is essential for identifying breast cancer patients who will most benefit from Herceptin. Several methods for assessing
the HER-2 status of tumors are listed in Table 2. Currently, the two most common methods of measuring HER-2 levels in the clinical setting are immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) [8,9,11,57–60].

### 3.1. IHC

IHC, the most widely used method, entails staining paraffin-embedded tissue with a HER-2-specific antibody. When commercially available kits such as HercepTest (Dako, Carpinteria, CA) and Pathway HER2 (Ventana, Tucson, AZ) are used, staining is graded semiquantitatively on a scale from 0 (no detectable HER-2) to 3+ (high HER-2 expression) on the basis of comparison with cell lines of known HER-2 receptor density. Tumors with a staining score of 3+ are the most responsive to Herceptin [9,48,61–63]. The disadvantages of IHC include the subjective interpretation and the semiquantitative nature of the results. Currently available IHC kits provide control slides against which samples are compared. Such standardization is essential to ensuring accurate assessment of HER-2 status [9].

### 3.2. FISH

FISH detects her-2 gene amplification and is more specific and sensitive than IHC [11,64]. Importantly, FISH offers quantitative results, possibly eliminating subjectivity and variability among different laboratories. Furthermore, FISH more accurately predicts prognosis and response to Herceptin than does IHC, as the subset of patients whose tumors overexpress HER-2 in the absence of gene amplification are less likely to respond to Herceptin-based therapy [9,48,65]. In general, IHC and FISH demonstrate a concordance rate of approximately 80% [61,66,67]. The FDA has approved the use of IHC and FISH for selecting patients for Herceptin-based therapy. Although IHC is the more widely used method, FISH should be performed on tumors scoring 2+ by IHC (HercepTest scoring system) because FISH status improves the prediction of likelihood of response to Herceptin [58].

### 3.3. HER-2 ECD

Another method under investigation for predicting response to Herceptin is the quantification of serum levels of the HER-2 ECD. The HER-2 ECD is shed into the blood and is readily measured using ELISA as a circulating tumor antigen in the serum of 70% of patients with HER-2-overexpressing MBC [48,66]. The advantage of this method is that blood is relatively easy to collect, allowing real-time monitoring of changes in HER-2 status in response to HER-2-targeted therapies. Our group previously showed that the rate of response to docetaxel and Herceptin therapy was higher for patients whose levels of HER-2 ECD were high at baseline than for patients who had low HER-2 ECD levels before initiation of treatment [48]. Monitoring HER-2 ECD may also have clinical utility because a significant reduction in serum levels predicts improved response rates and
time to progression [68,69]. An ongoing prospective multicenter study is evaluating the role of the HER-2 ECD assay for patients with MBC who are receiving Herceptin-based therapy.

4. Clinical trials with Herceptin

Initial phase I trials of Herceptin showed that the antibody was safe and that its pharmacokinetics were reliable [70]. Response rates to Herceptin given as a single agent ranged from 12 to 34%, in part depending on the method used to determine HER-2 status and the prior treatment received by the patients [63,71,72]. In a pivotal randomized clinical trial, Slamon et al. [62] showed that combining Herceptin with either doxorubicin plus cyclophosphamide (AC) or with single-agent paclitaxel produced longer time to progression, higher response rates, and improved survival rates than did chemotherapy alone. However, the administration of AC plus Herceptin caused severe cardiac dysfunction [62,73,74]. Although HER-2 is not overexpressed in cardiomyocytes, HER-2, together with its co-receptor HER-4 and the ligand heregulin, is essential for normal development of the heart ventricle. Conditional knockout mice lacking HER-2 gene expression in ventricular cardiomyocytes developed severe dilated cardiomyopathy [75]. Clinical trials are under way to evaluate the safety of epirubicin and liposomal anthracyclines in combination with Herceptin [76]. Non-anthracycline-containing Herceptin-based regimens that have shown promising results include cisplatin [77], paclitaxel administered weekly [61], docetaxel [48], vinorelbine [78], and gemcitabine [79]. Combinations of taxanes, platinum salts, and Herceptin (TCH) are highly synergistic in vitro [50,80,81]. Preliminary data from phase II studies of TCH have shown a high response rate and an extended time to progression [82]. A phase III, randomized trial showed an improvement in time to progression (13 months) for patients treated with Herceptin, paclitaxel, and carboplatin compared with results for patients receiving Herceptin and paclitaxel (7 months) [83]. Slamon and colleagues [84] recently reported a time to progression of 17 months for patients with HER-2-amplified MBC treated with docetaxel, carboplatin, and Herceptin. A randomized trial of docetaxel and Herceptin with and without carboplatin is ongoing.

Perhaps the most promising application of Herceptin therapy will be in the adjuvant setting. Cooperative groups are conducting large randomized trials. The National Surgical Adjuvant Breast and Bowel Project (NSABP)-B31 protocol is randomizing node-positive, HER-2-positive breast cancer patients to four cycles of AC followed by paclitaxel with or without Herceptin. The Intergroup protocol N9831 is testing whether Herceptin should be administered either concomitantly with paclitaxel or after completion of AC and paclitaxel therapy. Both studies initially allowed HER-2 testing at local hospitals. However, a significant number of false-positive results were noted, and a more centralized testing approach was implemented to ensure proper patient selection [85,86]. The Breast Cancer International Research Group (BCIRG protocol 006) is evaluating the role of docetaxel with and without Herceptin following AC chemotherapy. A third experimental arm incorporates the TCH regimen. This protocol includes node-positive and high-risk node-negative patients; HER-2 status is determined using FISH at a central laboratory. The HERceptin Adjuvant (HERA) trial is a large-scale international clinical trial led by the Breast International Group (BIG), in which patients are randomly assigned to Herceptin versus no further treatment after completion of adjuvant/completion of surgery, radiation therapy and adjuvant chemotherapy. Patients receiving Herceptin are randomly assigned to 1 or 2 years of Herceptin therapy.

Several studies have evaluated the role of Herceptin as preoperative therapy in patients with early-stage breast cancer. Burstein et al. [87] reported a phase II study of preoperative Herceptin in combination with paclitaxel (four cycles total), and reported a pathologic complete response (pCR) rate of 18%. Buzdar et al. [88] conducted a randomized clinical trial of paclitaxel (four cycles) followed by fluorouracil, epirubicin, and cyclophosphamide (FEC, four cycles), versus the same chemotherapy regimen in combination with Herceptin. The pCR rate was 25% for patients receiving eight cycles of chemotherapy and 65% for the chemotherapy/Herceptin group. The study was stopped early because of these dramatic results. If the pCR rates translate into long disease-free and overall survival, this approach may be preferable to the adjuvant, postoperative approach. However, long-term safety and efficacy data are
needed before Herceptin is applied widely to patients with early-stage breast cancer.

5. Mechanisms of resistance

Clinical studies established that Herceptin is active against HER-2-overexpressing MBC [1,2], leading to its approval in 1998 by the US Food and Drug Administration. However, the objective response rates to Herceptin monotherapy were low, ranging from 12 to 34% for a median duration of 9 months [2]. Currently Herceptin is administered in combination with chemotherapies such as paclitaxel [3,4] or docetaxel [5], which increase response rates, time to disease progression, and overall survival compared with Herceptin monotherapy. However, the majority of patients who achieve an initial response to Herceptin-based regimens generally acquire resistance within 1 year [48,62]. Elucidating the molecular mechanisms by which tumors escape Herceptin-mediated cytotoxicity is critical to improving the survival of MBC patients whose tumors overexpress HER-2. Multiple mechanisms contributing to Herceptin resistance have been proposed (Table 3).

5.1. Altered receptor–antibody interaction

Somatic mutations in the region of the egrf gene encoding the tyrosine kinase domain correlated with response to the EGFR kinase inhibitor gefitinib (Iressa; AstraZeneca, Wilmington, DE) in lung cancer patients [89]. Similarly, mutations in the region encoding the ECD domain may be present in the her-2 gene, preventing Herceptin from binding HER-2. Mutation of the her-2 gene was reported in a small proportion of human lung cancers [90]. However, no data are available regarding mutation status and response to Herceptin breast cancer patients. Interaction between receptor and antibody may also be inhibited if HER-2 levels decline over time. However, immunohistochemical studies demonstrate that HER-2 overexpression is generally maintained in breast cancer cells obtained from patients who fail to achieve a complete pathologic response to Herceptin-based therapy [28,91].

5.2. Increased cell signaling

The EGFR type I growth factor receptor tyrosine kinase family consists of EGFR, HER-2, HER-3, and HER-4. All except HER-3 contain a cytoplasmic tyrosine kinase region, and all except HER-2 bind specific ligands at their extracellular domain. Upon ligand binding, receptors dimerize using HER-2 as the preferred binding partner [92]. Heterodimerization induces tyrosine kinase activity and the downstream MAPK and PI3K signaling pathways. While Herceptin reduces HER-2-mediated signaling through these pathways, it does not reduce signaling mediated from other HER receptors. Thus, cells with EGFR/HER-3 heterodimers or EGFR homodimers may demonstrate mitogenic PI3K and MAPK signaling even in the presence of Herceptin. For this reason, agents that target multiple HER receptors may be effective in Herceptin-resistant cancer cells. The dual kinase inhibitor GW572016 (GlaxoSmithKline; Research Triangle Park, NC) inhibits EGFR and HER-2. Current clinical studies are examining the efficacy of this agent in Herceptin-refractory breast cancer. A previous study demonstrated that cancers with high AKT and phosphorylated MAPK responded to GW572016 but not to Herceptin [93]. Additionally, recently developed HER-2-targeted antibodies that inhibit dimerization, such as pertuzumab (Genentech), may prove to be beneficial in breast cancers that have progressed while patients received Herceptin. In fact, we demonstrated that Herceptin and pertuzumab synergistically inhibit survival of HER-2-overexpressing breast cancer cells, suggesting that they may act via different mechanisms to induce cytotoxicity [23].

<table>
<thead>
<tr>
<th>Table 3: Proposed mechanisms of Herceptin resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Altered receptor–antibody interaction</strong></td>
</tr>
<tr>
<td><strong>Increased cell signaling</strong></td>
</tr>
<tr>
<td><strong>Modulation of p27kip1</strong></td>
</tr>
<tr>
<td><strong>Increased IGF-IR signaling</strong></td>
</tr>
</tbody>
</table>
Constitutive Akt cell signaling was previously shown to inhibit cell cycle arrest and apoptosis mediated by Herceptin [94]. Another recent publication also suggests that increased PI3K signaling contributes to Herceptin resistance. Nagata et al. [24] demonstrated that downregulation of PTEN blocks Herceptin-mediated inhibition of proliferation in HER-2-overexpressing breast cancer cells with an increase in PI3K signaling. Importantly, these authors showed that patients with PTEN-deficient HER-2-overexpressing breast tumors have a poor response to Herceptin-based therapy. They also suggested that PI3K inhibitors should be explored preclinically as potential therapies in Herceptin-resistant tumors possessing low PTEN levels.

5.3. Modulation of p27kip1

The growth inhibitory properties of Herceptin depend in part upon effects on the cdk-inhibiting protein p27kip1. Herceptin increases the half-life of p27kip1 by decreasing cyclin E/cdk2-mediated phosphorylation of p27kip1 and blocking subsequent ubiquitin-dependent degradation [21]. Herceptin also increases association between p27kip1 and cdk2 complexes, resulting in G1 arrest [26]. Importantly, antisense oligonucleotides [94] and small interfering RNA [21] that reduced p27kip1 expression levels also blocked Herceptin-mediated growth arrest in SKBR3 HER-2-overexpressing breast cancer cells. We recently showed that Herceptin-resistant pools derived from the SKBR3 line after continuous drug exposure express reduced p27kip1 levels with elevated cdk2 activity. Transfection of p27kip1 or pharmacologic induction of p27kip1 restored Herceptin sensitivity to these cells, confirming that p27kip1 is a critical mediator of Herceptin response [22]. Because resistant pools were sensitive to the proteasome inhibitor MG132, which restored p27kip1 levels [22], downregulation of p27kip1 is likely due to increased protein degradation. Cellular localization of p27kip1 may also be important for Herceptin response, because Herceptin-resistant BT474 HER-2-overexpressing cells demonstrated loss of nuclear p27kip1 expression [95]. Thus, p27kip1 may serve as a marker of Herceptin response and as a therapeutic target in a subset of breast cancers that have progressed while patients received Herceptin.

5.4. Increased insulin-like growth factor-I receptor (IGF-IR) signaling

The IGF mitogenic signaling pathway is an attractive therapeutic target in breast cancer, as its ligands and receptors are frequently overexpressed and implicated in cellular proliferation, transformation, and metastasis [96]. High levels of IGFs prevent apoptosis in response to chemotherapeutics and radiation. Interestingly, overactive IGF-IR signaling was associated with resistance to Herceptin in HER2-overexpressing breast cancer cells. Lu et al. [97] demonstrated that Herceptin-mediated growth arrest was lost in SKBR3 cells engineered to overexpress IGF-IR, but this arrest was regained when the IGF-IR-inhibiting IGF binding protein 3 was added to the cell culture medium. Growth factor receptors of the type I class (HER family) and the IGF-IR signal through common pathways, including MAPK and PI3K. Cross-talk between various growth factor receptors occurs in cancer cells, and it is possible that IGF-IR cross-signals to HER-2. Such cross-talk would activate mitogenic signaling cascades despite blockade of HER-2 by Herceptin, resulting in tumor progression in the presence of Herceptin.

6. Future directions with Herceptin

In most patients who initially respond to Herceptin, disease progression is noted within 1 year. Combining Herceptin with novel agents and novel strategies for targeting HER-2 may increase the magnitude and duration of response. Many new agents are currently in preclinical or early clinical stages of development [98].

6.1. Novel combinations

Herceptin plus the anti-EGFR tyrosine kinase inhibitor (TKI) gefitinib produced complete remission of BT474 breast tumor xenografts [99]. A combination of Herceptin, gefitinib, and pertuzumab resulted in complete remission of breast cancer xenografts [100]. Because HER-2 and EGFR co-expression occurs in 10 to 36% of mammary carcinomas and defines one of the most aggressive tumor phenotypes, blockade of both receptors is an
important therapeutic strategy. Blockade of EGFR may prevent transactivation of HER-2, improving response rates to Herceptin. Such a combination may also be considered for Herceptin-resistant tumors, in which compensatory signaling by EGFR may inhibit the response to Herceptin.

In preclinical studies, the FTI R115777 (tipifarnib, Zarnestra; Janssen Pharmaceutica, Titusville, NJ) has demonstrated activity in breast cancer cells [101] and is being studied in combination with Herceptin. Although breast cancers rarely demonstrate Ras mutations, aberrant Ras signaling via activated growth factor receptors such as HER-2 and EGFR may be a target for FTIs and may be inhibited to a greater degree when FTIs are combined with Herceptin. Another novel combination being tested in patients with MBC is Herceptin plus the cdk inhibitor flavopiridol, which together have been shown to synergistically inhibit the survival of HER-2-overexpressing breast cancer cells [102–104]. Inhibitors of the Akt cell survival pathway are also being explored as therapies in HER-2-overexpressing breast cancer. Constitutive Akt signaling is often observed in growth factor receptor-positive tumors and may contribute to Herceptin resistance. However, there are no specific Akt inhibitors in clinical trials due to their excessive toxicity in preclinical models. This pathway may be inhibited by small molecules that inactivate the kinase m-TOR, downstream from Akt [105]. Two of the mTOR inhibitors in clinical trials for patients with breast cancer and other solid tumors are CCI-779 (Wyeth-Ayerst; Madison, NJ) and RAD001 (Novartis, New York, NY) [106].

6.2. Novel HER-2-targeted agents

Novel HER-2-targeting agents, including MAbs, TKIs, and vaccines, are being developed and tested in patients with MBC [107] (Table 4). The recombinant humanized HER-2 MAb pertuzumab sterically blocks dimerization of HER-2 with other HER receptors [108]. Thus, pertuzumab should block signaling from HER-2/HER-3 and HER-2/EGFR heterodimers. Cho et al. [109] recently described the crystal structure of HER-2 complexed with Herceptin. The HER-2 conformation confirms its ability to interact with other HER receptors in the absence of ligand. Altering HER-2 heterodimers has the potential to block compensatory signaling in HER-2-overexpressing tumor cells treated with Herceptin, as well as to inhibit signaling in cells that express normal levels of HER-2. Phase 1 clinical trials of pertuzumab are currently being conducted in breast cancer and other solid tumors and include patients whose tumors express normal HER-2 levels.

To increase the potency of antibody-directed therapy, the specificity of the antigen-binding site has been combined with a wide variety of effector agents, including toxins [110]. Using this approach, HER-2-targeted antibodies have been linked with the toxin DM-1 in ongoing preclinical studies.

Table 4

<table>
<thead>
<tr>
<th>Agent</th>
<th>Type of agent</th>
<th>Phase of development</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab-DM1</td>
<td>MAb-toxin conjugate</td>
<td>Preclinical</td>
<td>Genentech</td>
</tr>
<tr>
<td>Pertuzumab (2C4)</td>
<td>Mab</td>
<td>I</td>
<td>Genentech</td>
</tr>
<tr>
<td>CI-1033</td>
<td>TKI</td>
<td>II</td>
<td>Pfizer</td>
</tr>
<tr>
<td>GW572016</td>
<td>TKI</td>
<td>II</td>
<td>Glaxo smithkline</td>
</tr>
<tr>
<td>E1A</td>
<td>Transcriptional inhibitor</td>
<td>I</td>
<td>Targeted genetics</td>
</tr>
<tr>
<td>2B1</td>
<td>Bispecific antibody against HER-2 and Fc RI</td>
<td>II</td>
<td>Chiron</td>
</tr>
<tr>
<td>MDX-H210</td>
<td>Bispecific antibody against HER-2 and Fc RI</td>
<td>II</td>
<td>Medarex</td>
</tr>
<tr>
<td>Autovac</td>
<td>DNA vaccine</td>
<td>II</td>
<td>Pharmexa</td>
</tr>
<tr>
<td>HER-2/neu recombinant therapeutic vaccine</td>
<td>Recombinant vaccine</td>
<td>I</td>
<td>Corixa and Glaxo Smithkline</td>
</tr>
</tbody>
</table>

MAb, monoclonal antibody; Fc RI, type I Fc receptors for immunoglobulin G (IgG) (Fc gamma RI); Fc RIII, type III Fc receptors for immunoglobulin G (IgG) (Fc gamma RIII); TKI, tyrosine kinase inhibitor.
Additionally, recombinant molecules in which the antibody-combining site is fused directly to the toxin have been developed and show strong selectivity for HER-2 binding [110–113]. Recombinant toxins show promise in that they can be safely delivered to experimental animals at effective doses and may penetrate tumors more effectively than does Herceptin alone [114,115]. However, one limitation facing the development of toxin targeting is the potential for immune response to the protein.

In addition to antibodies targeting the HER-2 ECD, TKIs that directly inhibit the cytoplasmic tyrosine kinase domain of growth factor receptors are being developed. Several of these agents inhibit more than one HER/erbB receptor. CI-1033 (PD183805; Pfizer, New York, NY) is an orally available pan-HER TKI that irreversibly inhibits all HER receptors. Homologous kinase domains shared by the HER receptors can be targeted by small molecule pan-HER inhibitors to simultaneously block signaling from all active receptors [116]. Phase I trials of single-agent CI-1033 in which pre- and post-treatment tumor biopsy specimens were studied for biomarkers revealed a 40–50% reduction in EGFR and HER-2 phosphorylation, which correlated with decreased proliferation. Although partial remissions and stable disease occurred primarily in patients with squamous cell skin cancer and advanced-stage non-small cell lung cancer (NSCLC), respectively, one heavily pretreated patient with breast cancer has remained in a CI-1033 phase I trial for more than 6 months without disease progression [117]. Current clinical trials include testing of CI-1033 in patients with MBC whose disease did not respond to Herceptin therapy.

GW572016 inhibits both the EGFR and HER-2 tyrosine kinases and is currently undergoing clinical testing in breast cancer patients. This agent has shown remarkable in vitro and in vivo activity, leading to growth arrest and/or apoptosis in EGFR- and HER-2-dependent tumor cell lines. GW572016 markedly reduced tyrosine phosphorylation of EGFR and HER-2 and inhibited activation of Erk1/2 and AKT, which are downstream effectors of proliferation and cell survival, respectively [118]. Ongoing studies are evaluating the safety and efficacy of GW572016 as a single agent and in combination with other biologic agents. A multicenter phase II study is evaluating the efficacy of GW572016 as monotherapy for patients who develop progressive disease while receiving Herceptin-based therapy [119]. Because Herceptin resistance is a considerable clinical problem that may be due to compensatory signaling by other HER receptors, pan-HER inhibitors such as CI-1033 and GW572016 may offer a new therapeutic strategy in this patient population.

In addition to the previously discussed strategies that target the HER-2 protein, strategies that prevent the synthesis of HER-2 mRNA are also being developed. One such strategy derives from the finding that the HER-2 gene can be repressed by the introduction of the adenovirus E1A gene [120]. Delivery of E1A expression constructs into human tumor cell lines using liposomes has resulted in inhibition of HER-2 expression and loss of tumorigenicity [121]. A phase I clinical trial of E1A therapy showed that intracavitary injection of the EIA gene complexed with DC-Chol cationic liposome (DCC-EIA; Targeted Genetics) is feasible in patients with breast cancer [122].

Two approaches to immunotherapy that rely on targeting by anti-HER-2 antibodies have been developed; both are designed to deliver immune effector cells to the tumor. The first approach is to use a single chimeric protein molecule that features two antibody-binding specificities: one that binds HER-2 and one that binds an immune cell via CD16, Fc receptor III [123], or CD3 [124]. The toxicity of this therapy has been assessed in phase I clinical studies, and there is evidence that a biologically relevant concentration of the experimental therapeutic can be achieved [125,126].

DNA and peptide-based vaccine strategies designed to specifically boost HER-2 immunity are being tested in patients with MBC. Initial results demonstrated that significant levels of HER-2 immunity can be generated with active immunization and that the T-cells generated against HER-2 do not produce an autoimmune response against cells with normal HER-2 levels [127]. However, initial strategies employing single HLA binding epitopes to induce cytotoxic CD8+ T-cells produced transient responses [127,128]. More recent approaches generating active immunization against HER-2 with CD4+ T-helper epitopes resulted in the development of T-cell immunity in 92% of patients with MBC, ovarian cancer, and NSCLC, with responses persisting in 38% of patients at 1-year.
follow-up [129]. The clinical role of cancer vaccines remains to be defined. HER-2 vaccines may be useful as adjuvant therapies to prevent relapse by establishing an effective memory response or as treatments for patients whose disease has progressed during treatment with Herceptin [126,128].

7. Conclusion

Currently, the optimal duration of Herceptin treatment is unknown. In most patients who initially respond to Herceptin, disease progression begins again within a year. A clearer understanding of the mechanisms that contribute to Herceptin resistance is needed to increase the magnitude and duration of response. Elucidating the molecular changes that occur as tumors progress during Herceptin therapy will allow for the design of targeted therapies to be used in combination with or after Herceptin.

Acknowledgements

The authors wish to acknowledge funding from the AACR-Amgen, Inc. Fellowship in Clinical/Translational Cancer Research (R. Nahta), the M.D. Anderson Cancer Center Odyssey Special Fellowship, and the Theodore N. Law Award for Scientific Achievement (R. Nahta). We thank the Nellie B. Connally Breast Cancer Research Fund for supporting the Breast Cancer Translational Research Laboratory at M.D. Anderson Cancer Center, and the NCI for the Cancer Center Core Grant (CA16672). The authors thank Michael Worley for editorial assistance.

References


[42] R. Colomer, S. Montero, A. Luch, B. Ojeda, A. Barnadas, A. Casado, et al., Circulating HER2 extracellular domain


[66] L.N. Harris, V. Liotecheva, G. Broadwater, M.J. Ramirez, P. Maimonis, S. Anderson, et al., Comparison of methods of


S. S. Bacus, Y. Smith, Y. Yarden, N. Specter, Differences in response of breast cancer molecular profiles of patients likely to respond to either tyrosine kinase inhibitors or to erbB targeted therapies, J. Clin. Oncol. 22 (2004) 3097.


