HER-2-Targeted Therapy: Lessons Learned and Future Directions

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Abstract

HER-2 is overexpressed in 20–25% of invasive breast cancers and is associated with an aggressive tumor phenotype and reduced survival rates. The HER-2 status of a tumor is the critical determinant of response to the HER-2-targeted antibody trastuzumab. Thus, accurate assessment of HER-2 expression levels is essential for identifying breast cancer patients who will benefit from HER-2-targeted therapy. Trastuzumab combined with chemotherapy increases response rates, time to progression, and survival. However, the majority of cancers that initially respond to trastuzumab begin to progress again within 1 year. This minireview describes HER-2 targeting strategies currently in use or in stages of development for the treatment of breast cancer.

Introduction

The primary goal of novel anticancer drug design is to directly target specific molecular lesions found in tumor cells in the hopes of improving cancer cure rates and reducing cytotoxicity in normal cells. Advances in molecular biology have facilitated the identification of tumor markers that not only predict prognosis and therapeutic response but may also function as potential therapeutic targets (1, 2).

HER-2 (erbB2/neu) is an EGFR2-related tyrosine kinase receptor that is overexpressed in 20–25% of invasive breast cancers (3, 4). The oncogenic potential of HER-2 was demonstrated in part by its ability to transform normal fibroblasts (5) and to produce breast cancer in transgenic mice when overexpressed under the control of the mouse mammary tumor virus promoter (6–8). Overexpression of HER-2 occurs primarily through amplification of the wild-type her-2 gene and is associated with poor disease-free survival (3, 9–13) and may be associated with resistance to certain types of chemotherapy (14–16).

HER-2 has become an important therapeutic target in breast cancer for several reasons. (a) HER-2 levels correlate strongly with the pathogenesis and prognosis of breast cancer. (b) The level of HER-2 in human cancer cells with gene amplification is much higher than that in normal adult tissues, potentially reducing the toxicity of HER-2-targeting drugs. (c) HER-2 is present in a very high proportion of tumor cells (17), and tumors with high expression (i.e., an IHC score of 3+) often show uniform, intense immunohistochemical staining (18), suggesting that anti-HER-2 therapy would target most cancer cells in a given patient. (d) HER-2 overexpression is found in both the primary tumor and metastatic sites (19), indicating that anti-HER-2 therapy may be effective in all disease sites.

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 (20). The HER-2 status of a tumor provides prognostic information and is the critical determinant of response to the HER-2-targeted Ab trastuzumab. Thus, accurate assessment of HER-2 expression levels is essential for identifying breast cancer patients who will benefit from trastuzumab.

Several methods for assessing the HER-2 status of tumors are listed in Table 1. Currently, the two most common methods of measuring HER-2 levels in the clinical setting are IHC and FISH (11–13, 21–24). IHC is the most widely used method and entails staining paraffin-embedded tissue with a HER-2-specific Ab. When using commercially available kits such as HercepTest (Dako, Carpinteria, CA) and Pathway HER2 (Ventana, Tucson, AZ), 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 trastuzumab (12, 25–27). The disadvantages of IHC include the subjective interpretation and semiquantitative nature of 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 (12).

FISH detects her-2 gene amplification and is more specific and sensitive than IHC (11, 28). Importantly, FISH offers quantitative results, possibly eliminating subjectivity and variability among different laboratories. Furthermore, FISH more accurately predicts prognosis and response to trastuzumab than does IHC, because the subset of patients whose tumors overexpress HER-2 in the absence of gene amplification are less likely to respond to trastuzumab-based therapy (12, 27, 29). In general, IHC and FISH demonstrate a concordance rate of approximately 80% (30–32). The FDA has approved the use of IHC and FISH for selecting patients for trastuzumab-based therapy. Although IHC is the more widely used method, FISH should be performed...
Another method under investigation for predicting response to trastuzumab is the quantification of serum levels of the HER-2 ECD. The HER-2 ECD is shed into blood and is readily measured using ELISA as a circulating tumor antigen in molecular and cellular effects have been observed in vitro (23). This method also provides a measure of treatment response to trastuzumab (23).

Mechanisms of Action of Trastuzumab

Trastuzumab (Herceptin; Genentech, South San Francisco, CA), a recombinant humanized MAb directed against the ECD of the HER-2 protein, is the only HER-2-targeted therapy approved by the FDA for the treatment of MBC. Although the mechanisms by which trastuzumab induces regression of HER-2-overexpressing tumors are incompletely defined, several molecular and cellular effects have been observed in vitro (Table 2). Trastuzumab and the murine MAb 4D5, from which trastuzumab is derived, induce HER-2 receptor internalization and degradation in a dose-dependent manner in the BT474 and SKBR3 HER-2-overexpressing breast cancer cell lines (34, 35). Down-regulation of HER-2 disrupts receptor dimerization and signaling through the downstream phosphatidylinositol 3'-kinase cascade (36). Cells treated with trastuzumab undergo arrest during the G1 phase of the cell cycle, with a concomitant reduction in proliferation (35). Cell cycle arrest is accompanied by induction of the cdk inhibitor p27kip1 and increased formation of p27kip1-cdk2 complexes (35, 37, 38). Additional mechanisms of trastuzumab that have been demonstrated in vivo include suppression of angiogenesis via induction of antiangiogenic factors and repression of proangiogenic factors (39), activation of Ab-dependent cellular cytotoxicity (40–42), and inhibition of proteolytic cleavage of the HER-2 ECD (34, 43). In vitro studies showed that trastuzumab is synergistic with a variety of chemotherapies (44), and Pietras et al. (45) showed that treatment with trastuzumab prevented DNA repair following the impact of DNA-damaging drugs. However, the mechanism of synergies observed with other chemotherapy agents in vitro is unknown.

Clinical Trials with Trastuzumab

Initial Phase I trials of trastuzumab showed that the Ab was safe and that its pharmacokinetics were reliable (46). Response rates to trastuzumab given as a single agent ranged from 12% to 34%, depending in part on the method used to determine HER-2 status and the prior treatment received by the patients (26, 47, 48). In a pivotal randomized clinical trial, Slamon et al. (25) showed that combining trastuzumab with either AC or single-

### Table 1: Methods of assessing HER-2 status

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Clinical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western blot</td>
<td>Widely available; relatively inexpensive</td>
<td>Semiquantitative; Ab variability; tumor extract is required</td>
<td>Not in clinical use</td>
</tr>
<tr>
<td>PCR</td>
<td>Rapid; specific; sensitive; small amount of starting material</td>
<td>Semiquantitative</td>
<td>Not in clinical use</td>
</tr>
<tr>
<td>IHC</td>
<td>Widely available; relatively inexpensive</td>
<td>Semiquantitative; Ab variability; subjective interpretation</td>
<td>FDA-approved; most frequently used clinically</td>
</tr>
<tr>
<td>FISH</td>
<td>Specific; quantitative; strong correlation with response to trastuzumab</td>
<td>Expensive; requires specialized equipment not widely available</td>
<td>FDA-approved; valuable for confirmation of HER-2 status if IHC score is 2+</td>
</tr>
<tr>
<td>ECD ELISA</td>
<td>Serum easily obtained</td>
<td>ECD levels do not always correlate with tumor load</td>
<td>FDA-approved to monitor response to chemotherapy; multicenter prospective study ongoing in patients on trastuzumab</td>
</tr>
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</table>

### Table 2: Proposed mechanisms of action of trastuzumab

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Ref. no.</th>
</tr>
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<tbody>
<tr>
<td>Internalization and degradation of HER-2: disrupts receptor dimerization; disrupts downstream signaling</td>
<td>89</td>
</tr>
<tr>
<td>G1 arrest and reduced proliferation: induces p27kip1-cdk2 complex formation; induces p27kip1 levels</td>
<td>37 and 38</td>
</tr>
<tr>
<td>Apoptosis: inhibits Akt activity</td>
<td>36 and 90</td>
</tr>
<tr>
<td>Suppresses angiogenesis: reduces tumor vasculature in vivo; reduces expression of proangiogenic VEGF, TGF-α, Ang-1, PAI-1; induces antiangiogenic TSP-1</td>
<td>39 and 91</td>
</tr>
<tr>
<td>Immune-mediated responses: ADCC; stimulates natural killer cells</td>
<td>92</td>
</tr>
<tr>
<td>Inhibits HER-2 ECD proteolysis</td>
<td>43</td>
</tr>
</tbody>
</table>

* VEGF, vascular endothelial growth factor; TGF, transforming growth factor; Ang-1, angiopoietin 1; PAI-1, plasminogen-activator inhibitor 1; TSP-1, thrombospondin 1; ADCC, antibody-dependent cellular cytotoxicity.
agent paclitaxel produced longer time to progression, higher response rates, and improved survival rates compared with chemotherapy alone. However, the administration of AC plus trastuzumab caused severe cardiac dysfunction (25, 49, 50). Although HER-2 is not overexpressed in cardiomyocytes, HER-2, together with its coreceptor, 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 (51). Clinical trials are under way to evaluate the safety of epirubicin and liposomal anthracyclines in combination with trastuzumab (52). Non-anthracycline-containing trastuzumab-based regimens that have shown promising results include cisplatin (53), paclitaxel administered weekly (32), docetaxel (27), vinorelbine (54), and gemcitabine (55). Combinations of TCH are highly synergistic in vitro (56, 57). Preliminary data from Phase II studies of TCH have shown a high response rate and an extended time to progression (58). A Phase III, randomized trial showed an improvement in median time to progression for patients treated with trastuzumab, paclitaxel, and carboplatin (13 months) compared with patients receiving trastuzumab and paclitaxel [7 months (59)]. Slamon et al. (60) recently reported a time to progression of 17 months for patients with HER-2-amplified MBC treated with docetaxel, carboplatin, and trastuzumab. A randomized trial of docetaxel and trastuzumab with and without carboplatin is ongoing.

Perhaps the most promising application of trastuzumab therapy will be in the adjuvant setting. Cooperative groups are conducting large randomized trials. The National Surgical Adjuvant Breast and Bowel Project-B31 protocol is randomizing node-positive, HER-2-positive breast cancer patients to four cycles of AC followed by four cycles of paclitaxel with or without trastuzumab. The Intergroup Protocol N9831 is testing a similar approach using weekly paclitaxel. In addition, trastuzumab is being administered either concomitantly with paclitaxel or after completion of AC and paclitaxel therapy. Both studies allowed HER-2 testing at local hospitals initially. However, a significant number of false-positive results were noted, and a more centralized testing approach was implemented to assure proper patient selection (61, 62). The Breast Cancer International Research Group (BCIRG Protocol 006) is evaluating the role of docetaxel with and without trastuzumab after 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 Trial is a large-scale international clinical trial led by the Breast International Group in which patients are randomized to trastuzumab versus no further treatment after completion of adjuvant/neoadjuvant chemotherapy. Patients receiving trastuzumab will be randomly assigned to 1 year or 2 years of trastuzumab therapy.

Future Directions

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

Trastuzumab plus the anti-EGFR TKI ZD1839 (Iressa; AstraZeneca, Wilmington, DE) produced complete remission of BT474 breast tumor xenografts (63). Because HER-2 and EGFR coexpression occurs in 10–36% of mammary carcinomas and defines one of the most aggressive tumor phenotypes, blockade of both receptors is an important therapeutic strategy. The Eastern Cooperative Oncology Group is conducting a Phase II trial in which patients with HER-2-overexpressing, trastuzumab-naive MBC will be treated with combined ZD1839 and trastuzumab (64). Blockade of EGFR may prevent transactivation of HER-2, improving response rates to trastuzumab. Such a combination may also be considered for trastuzumab-resistant tumors, in which compensatory signaling by EGFR may inhibit the response to trastuzumab.

In preclinical studies, the FTI R115777 (tipifarnib, Zanestra; Janssen Pharmaceutica, Titusville, NJ) has demonstrated activity in breast cancer cells (65) and is being studied in combination with trastuzumab. 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 trastuzumab. Another novel combination being tested in patients with MBC is trastuzumab plus the cdk inhibitor flavopiridol, which together have been shown to synergistically inhibit the survival of HER-2-overexpressing breast cancer cells (66, 67). 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 trastuzumab resistance. One of the AKT inhibitors undergoing clinical testing is CCI-779 (Wyeth-Ayerst, Madison, NJ), a water-soluble ester analogue of rapamycin that inhibits the kinase mTOR downstream from Akt (68). Clinical trials of CCI-779 documented objective responses in patients with refractory breast cancer (69). Ongoing biomarker studies are evaluating the molecular mechanisms of CCI-779 in patients with early-stage breast cancer.

Novel HER-2-targeting agents, including MAbs, TKIs, and vaccines, are being developed and tested in patients with MBC (Table 3). The recombinant humanized HER-2 MAb 2C4 (Genentech) sterically blocks dimerization of HER-2 with other HER receptors (70). Thus, 2C4 should block signaling from HER-2/HER-3 and HER-2/EGFR heterodimers. Cho et al. (71) recently described the crystal structure of HER-2 complexed with trastuzumab. 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 trastuzumab and inhibit signaling in cells that express normal levels of HER-2. Phase I clinical trials of 2C4 in breast cancer are currently being conducted and include patients whose tumors express normal HER-2 levels.

To increase the potency of Ab-directed therapy, the specificity of the antigen-binding site has been combined with a wide variety of effector agents, including toxins (72). Using this approach, trastuzumab has been linked with the toxin DM-1 in ongoing preclinical studies. Additionally, recombinant mole-
cules in which the Ab-combining site is fused directly to the toxin have been developed and show strong selectivity for HER-2 binding (72, 73). Recombinant toxins show promise in that they can be safely delivered to experimental animals at effective doses and may penetrate tumors more effectively than trastuzumab alone (74, 75). However, one limitation facing the development of toxin targeting is the potential for immune response to the protein.

In addition to Abs 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 (76). Phase I trials of single-agent CI-1033 in which pre- and posttreatment 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, 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 (77). Current clinical trials include testing of CI-1033 in patients with MBC whose disease did not respond to trastuzumab therapy. GW572016 (GlaxoSmithKline, Research Triangle Park, NC) is another novel inhibitor of the EGFR and HER-2 tyrosine kinases 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 erbB2 and inhibited activation of extracellular signal-regulated kinase 1/2 and AKT, downstream effectors of proliferation and cell survival, respectively (78). Ongoing studies are evaluating the safety and efficacy of GW572016 as a single agent and in combination with other biological agents. A multicenter, Phase II study is evaluating the efficacy of GW572016 as monotherapy for patients who develop progressive disease while on trastuzumab-based therapy. Because trastuzumab 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 is derived from the finding that the HER-2 gene can be repressed by the introduction of the adenovirus E1A gene (79). Delivery of E1A expression constructs into human tumor cell lines using liposomes has resulted in inhibition of HER-2 expression and loss of tumorigenicity (80). A Phase I clinical trial of E1A therapy showed that intracavitary injection of the E1A gene complexed with DC-Chol cationic liposome (DCC-E1A; Targeted Genetics) is feasible in patients with breast cancer (81). Two approaches to immunotherapy that rely on targeting by anti-HER-2 Abs 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 Ab-binding specificities: (a) one that binds HER-2; and (b) one that binds an immune cell via CD16, Fc receptor III (82), or CD3 (83). 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 (84, 85).

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 (86). However, initial strategies using single HLA binding epitopes to induce cytotoxic CD8+ T cells produced transient responses (86, 87). 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 non-small cell lung cancer, with responses persisting in 38% of these patients at a follow-up time of 1 year (88). 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 HER-2 MAb (85, 87).

### Conclusions

Currently, the optimal duration of HER-2-targeted treatment is unknown. In most patients who initially respond to trastuzumab, disease progression begins again within 1 year. A clearer understanding of the mechanisms that contribute to trastuzumab resistance is needed to increase the magnitude and duration of response. Elucidating the molecular changes that

### Table 3  Novel HER-2-targeting agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Class of compound</th>
<th>Phase of development in MBC</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>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>ElA</td>
<td>Transcriptional inhibitor</td>
<td>I</td>
<td>Targeted Genetics</td>
</tr>
<tr>
<td>2B1</td>
<td>Bispecific Ab against HER-2 and Fc</td>
<td>II</td>
<td>Chiron</td>
</tr>
<tr>
<td>AutoVac</td>
<td>DNA vaccine</td>
<td>II</td>
<td>Pharmexa</td>
</tr>
</tbody>
</table>
occurs as tumors progress on trastuzumab therapy will allow the design of targeted therapies to be used in combination with or after trastuzumab. Additionally, new HER-2 targeting strategies are in preclinical and clinical development stages.

Acknowledgments

We thank Kate O’Suilleabhain for editorial assistance.

References


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