Advances in Targeting Human Epidermal Growth Factor Receptor-2 Signaling for Cancer Therapy

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Abstract

Human epidermal growth factor receptor (HER)-2 is a member of the HER tyrosine kinase family, which regulates cell growth and proliferation. HER-2 is overexpressed in 20% to 30% of breast cancers and has been associated with an aggressive phenotype and a poorer prognosis, making it an appealing therapeutic target. Since 1998, the anti-HER-2 antibody trastuzumab has been used for the treatment of women with HER-2-positive metastatic breast cancer. Results from large trials have established a role for trastuzumab in the adjuvant setting for the treatment of high-risk primary breast cancer as well. Tyrosine kinase inhibitors that target HER-2 are also very promising therapies and are likely to be incorporated into clinical practice in the near future. HER-2-targeted therapies represent a major step forward in achieving our goal of delivering individualized targeted therapy for breast cancer. However, there are many unanswered questions about the optimal use of these agents. Ongoing research will better elucidate the best combination therapies to overcome resistance to HER-2-targeted agents and will help identify patients at high enough risk to warrant their toxicity.

Background

Human epidermal growth factor receptor (HER)-2 (neu, c-erb-B2) is a ligand orphan receptor tyrosine kinase that amplifies the signal provided by other members of the HER family (HER-1, HER-3, and HER-4) by forming heterodimers with them. HER-2 activates several downstream signaling cascades, including the mitogen-activated protein kinase and phosphatidylinositol-3-OH kinase (PI3K) pathways (Fig. 1), and new downstream HER-2-signaling proteins continue to be identified (1). Variations in the activating ligand and the composition of the HER-2 dimer lead to the diversity of downstream signaling. HER-2 activation causes alterations in gene expression mediated through alterations in transcription, translation, and protein stability. These alterations in turn affect cell growth, proliferation, migration, adhesion, and survival.

The neu gene was first recognized as a potent oncogenic mutant in neuroglioblastomas that developed in carcinogen-exposed rats (2) HER-2 overexpression increases cell proliferation, anchorage-independent cell growth, cell migration, and invasiveness, up-regulates the activities of the matrix metalloproteinases MMP-2 and MMP-9, enhances the expression of chemokine receptor CXCR4, and increases cyclooxygenase-2 levels and aromatase activity (3–7). Introduction of the HER-2 gene into transgenic mice induces mammary tumors (8, 9). Overexpression of HER-2 increases tumorigenicity, vascular endothelial growth factor and Src production, angiogenesis, and metastatic potential (7, 10–13). It also confers a survival advantage on cancer cells by making them resistant to apoptosis induced by certain proapoptotic stimuli (14, 15).

HER-2 is amplified and overexpressed in 20% to 30% of breast cancers, and several studies have found that breast cancers that overexpress HER-2 have a more aggressive course and higher relapse and mortality rates. HER-2 is also overexpressed in other cancers, including ovarian, lung, gastric, and oral cancers (16–20). Therefore, strategies are being investigated to target HER-2 for cancer therapy.

Monoclonal Anti-HER-2 Antibodies

Monoclonal antibodies were the first anti-HER-2 strategy to be brought to the clinic. One of the most potent murine monoclonal antibodies against HER-2 is 4D5, which interacts with the extracellular domain (21). 4D5 was humanized by fusing its antigen-binding region to the framework of human immunoglobulin G (22) and was named trastuzumab (Herceptin, Genentech, Inc., San Francisco, CA).

The role of trastuzumab in cancer therapy. The antitumor activity of trastuzumab is attributable to several mechanisms. In some models, trastuzumab down-regulates HER-2 expression on the cell surface (23). It also can partially block heregulin-induced activation of HER-2/HER-3 complexes and induce the cyclin-dependent kinase 2 inhibitor p27 and Rb-related protein p130 (24). Trastuzumab sensitizes tumor cells to the effects of tumor necrosis factor (25) and restores E-cadherin and integrins to normal levels (26). Trastuzumab inhibits tumor angiogenesis by decreasing the production of vascular endothelial growth factor and activating antiangiogenic...
thrombospondin-1 (27, 28). Immune responses, particularly through natural killer cells, may also play a role in the effects of trastuzumab (29). In addition, trastuzumab sensitizes cells to the cytotoxic effects of chemotherapeutic agents in some models (30).

Trastuzumab is active as a single agent in women with HER-2-positive metastatic breast cancer (31–34). In randomized trials, treatment of HER-2-positive metastatic breast cancer with chemotherapy plus trastuzumab was associated with significantly higher response rates, a longer duration of response, a longer time to treatment failure, and improved survival compared with chemotherapy alone (35, 36). These results led to the approval of trastuzumab by the Food and Drug Administration in 1998.

That approval was followed by large multicenter trials to test the role of trastuzumab in adjuvant therapy. In these studies, the use of trastuzumab dramatically (by about one half) reduced the risk of recurrence (37, 38). These data led to the incorporation of trastuzumab into adjuvant therapy for patients with node-positive or high-risk, node-negative HER-2-positive breast cancer. Trastuzumab plus chemotherapy has also been studied in the neoadjuvant setting, where it was associated with a significant increase in complete pathologic response rates compared with chemotherapy alone (39). Furthermore, trastuzumab is considered in high-risk node-negative patients with HER-2-positive breast cancer.

These studies have clearly established a role for trastuzumab in cancer therapy, but many questions remain. Although the addition of trastuzumab seems to be more effective than chemotherapy alone in HER-2-positive breast cancer cases, the incremental benefit decreases with advancing age, higher cardiac toxicity, and lower risk of recurrence (40). Preclinical studies suggest that synchronous delivery of trastuzumab and chemotherapy is more effective than sequential therapy (41). However, the toxicity of synchronous delivery, especially for therapy involving anthracyclines, remains a concern. Thus, the best treatment sequence remains to be determined. The duration of trastuzumab therapy required for maximal efficacy is also still unknown; short-term (9 weeks) administration with chemotherapy has been tested (42), and the ongoing HERA trial is comparing 1 versus 2 years of treatment. It is likely that trastuzumab will be most effective in combination therapy;

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**Fig. 1.** Selected HER-2 signaling pathways. HER-2 can interact with different members of the HER family and activate mitogenic and antiapoptotic pathways.
however, which agents are best to deliver in combination with trastuzumab is still being studied (42, 43). Ongoing clinical trials include combinations of trastuzumab with capecitabine, irinotecan, ixabepilone, epothilone D, ABI-007, oxaliplatin, vinflunine, PTK787, gefitinib, erlotinib, bevacizumab, imatinib, PS-341, fulvestrant, faslodex, RAD001, lapatinib, and pertuzumab (44).

Mechanisms of resistance to trastuzumab. Trastuzumab is most effective in patients who are HER-2 positive by fluorescence in situ hybridization analysis (45). However, even in patients with HER-2-amplified tumors, the objective response rate to single-agent trastuzumab as first-line therapy for metastatic breast cancer is only ~34% (34). Furthermore, many patients who have an initial response acquire resistance within a year. Thus, many ongoing studies are focused on the mechanisms of intrinsic and acquired trastuzumab resistance.

Trastuzumab resistance is likely multifactorial. In vitro effect of trastuzumab is at least in part mediated through G1 arrest, with induction of the cyclin-dependent kinase inhibitor p27. Cell lines with acquired trastuzumab resistance have lower p27 levels (46), but whether these low p27 levels can be used as a marker of intrinsic resistance to trastuzumab remains unknown. Many signaling pathways, including HER-1 and insulin-like growth factor-I receptor (IGF-IR), converge on p27, and continued PI3K and mitogen-activated protein kinase signaling through the other HER family members in the presence of trastuzumab represents a potential mechanism of resistance. Thus, agents targeting multiple HER family members, alone or in combination with trastuzumab, may overcome this resistance.

In preclinical models, increased IGF-IR signaling is associated with trastuzumab resistance (47). In vitro, IGF-IR/HER-2 heterodimerization contributes to trastuzumab resistance (48). Thus, strategies that target IGF-IR signaling, such as anti-IGF-IR antibodies (e.g., CP-751871; Pfizer, New York, NY) or IGF-IR kinase inhibitors (e.g., NVP-AEW541; Novartis, Basel, Switzerland), may prevent or delay the development of trastuzumab resistance.

Another potential mechanism of trastuzumab resistance is activated PI3K signaling through alterations in PTEN/PI3K/Akt. Nagata et al. (49) showed that PTEN activation contributes to tumor inhibition by trastuzumab, and reducing PTEN conferred trastuzumab resistance in vitro and in vivo. Furthermore, patients with PTEN-deficient tumors had significantly poorer responses to trastuzumab. Therefore, PTEN status needs to be further studied as a potential marker of trastuzumab resistance. The activation status of PI3K signaling could also be assessed as a potential pharmacodynamic marker of response to therapy. Other activators of PI3K signaling, such as PI3K mutations and Akt amplification, have not yet been explored as markers of trastuzumab resistance. Combination therapy with trastuzumab and PI3K/Akt inhibitors can also be pursued when PI3K and Akt inhibitors enter clinical trials. Another potential point of therapeutic intervention in PI3K signaling is mammalian target of rapamycin. Mammalian target of rapamycin is not only a critical effector of Akt but, complexed with rictor, it also phosphorylates Akt (50, 51). The combination of trastuzumab and mammalian target of rapamycin inhibitor RAD001 (Novartis) is already in clinical trials (44).

Other molecular aberrations in HER-2-positive tumors may also affect trastuzumab sensitivity. In the NSABP B-31 trial, trastuzumab was more effective in terms of recurrence- and disease-free survival in patients with c-Myc coamplifications (52). Additional potential predictors of response are also being pursued.

Somatic mutations in the HER-2 kinase domain have been described in 4% of lung, 5% of gastric, 3% of colorectal, and 4% of breast cancers (53, 54). In preclinical models, cells harboring HER-2 kinase mutations exhibit a gain-of-function phenotype; however, whether they are as sensitive to HER-2-targeted agents, such as trastuzumab, is controversial (55, 56). Trastuzumab response has been reported in a patient with non–small cell lung cancer with mutations in HER-1 and exon 21 of HER-2 (57), whereas exon 21 mutations were reported in three breast cancer patients with acquired trastuzumab resistance (58). The effect of mutations in the HER-2 kinase domain and the extracellular domain on intrinsic and acquired trastuzumab resistance needs to be investigated further.

Recently, COOH-terminal fragments of HER-2 generated by alternative translation initiation have been described (59). In preclinical models, tumors dependent on COOH-terminal fragments are sensitive to inhibitors of HER-2 kinase activity but do not respond to trastuzumab. The role of HER-2 COOH-terminal fragments in clinical trastuzumab resistance has not yet been determined.

Pertuzumab as a potential alternative to trastuzumab. Another HER-2-targeted monoclonal antibody is pertuzumab (Omnitarg, rhu mAb-2C4, Genentech). Pertuzumab binds to the dimerization domain of HER-2 and blocks HER-2 homodimerization and heterodimerization with other HER-1 family members, thus blocking ligand-activated HER-2 signaling (60). In preclinical studies, pertuzumab, unlike trastuzumab, showed activity in non–HER-2-overexpressing tumors (61). Phase II trials evaluating the efficacy of pertuzumab in a variety of cancers are under way. Pertuzumab has shown synergy with trastuzumab in vitro (46), and a phase II trial is investigating the effect of pertuzumab plus trastuzumab in HER-2-overexpressing breast cancer (62).

Tyrosine Kinase Inhibitors

Another therapeutic approach is the use of tyrosine kinase inhibitors that inhibit specific HER receptors. For example, erlotinib (OSI-774) and gefitinib (ZD1839) inhibit HER-1; lapatinib (GW572016), PKI-166, and PD168393 inhibit HER-1 and HER-2; and PD12878 and CI-1033 (PD183805) inhibit all members of the HER family. Of the HER-2 inhibitors, the most clinically advanced is lapatinib, a reversible small-molecule inhibitor. In vitro, lapatinib inhibits breast cancer cell proliferation in a concentration-dependent fashion, and response was correlated with HER-2 expression and inhibition of HER-2, Raf, Akt, and Erk phosphorylation (63, 64). The combination of lapatinib and trastuzumab is synergistic (63), with lapatinib showing activity as a first-line therapy when administered in combination with trastuzumab (65, 66). Lapatinib has also shown activity in patients with HER-2-positive cancer whose disease progressed after treatment with trastuzumab (67). Patients with HER-2-overexpressing metastatic breast cancer who are treated with trastuzumab are still at risk for central nervous system progression (68). These patients may especially benefit from tyrosine kinase
inhibitors, such as lapatinib, a strategy being pursued in clinical trials (69).

Recently, in a phase III trial comparing capcitabine alone with capcitabine in combination with lapatinib in advanced, HER-2-positive breast cancer that had progressed after trastuzumab therapy, a significant improvement in time to disease-free progression was reported in the combination arm, leading to early closure of the study (70).

In a randomized phase III trial of lapatinib versus hormone therapy in advanced renal cell carcinoma, lapatinib resulted in longer overall survival in patients with cancers that overexpressed HER-1 (strong positive immunohistochemical results; ref. 71). In contrast, in inflammatory breast cancer, HER-2 overexpression alone, but not HER-1 expression, was predictive of sensitivity to lapatinib (72). Based on these data, lapatinib is likely to be incorporated into clinical practice. However, further work is needed to identify and validate the best predictors of response to lapatinib.

**Other HER-2-Targeted Therapies**

Several other strategies are being investigated to target HER-2. 17-Allylamino-17-demethoxygeldanamycin, the first Hsp90 inhibitor to be tested in clinical trials, leads to degradation of HER-2 as well as other Hsp90 targets. Gene therapy with transcriptional factors, PEA3 (73), and adenosine type 5 E1A protein (74) is another approach to regulating HER-2 expression. Other preclinical approaches have targeted HER-2 mRNA through ribozymes, antisense, and small interfering RNA strategies. Vaccines provoking a response to HER-2 antigen are already in clinical trials. Methods of in vivo imaging of HER-2 expression are also being pursued (75). The clinical value of these strategies has yet to be defined.

HER-2 overexpression represents an excellent example for development of targeted therapy. Many encouraging outcomes, such as development of trastuzumab and lapatinib, have resulted in clinical benefits. Molecular mechanisms for resistance to trastuzumab are also gradually unraveled. However, there is still much that we need to learn to further develop effective therapeutic strategies. More studies on signaling pathways downstream of HER-2 will assist in the development of combination therapies of trastuzumab, tyrosine kinase inhibitors, and other therapeutic candidates and will likely enhance the success of cancer therapy targeting HER-2-overexpressing tumors.

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