Overview: HER2 is a member of the human epidermal growth factor family of receptor tyrosine kinases that plays a key role in the pathogenesis of breast cancer. When overexpressed as a result of erbB2 gene amplification in as many as 20% of human breast cancers, HER2 correlates with a particularly aggressive clinical phenotype. As such, HER2 represents an archetyped therapeutic target. In this review, we explore the clinical evolution of HER2-targeted therapeutics, with emphasis on more recent controversies involving clinical development of anti-HER2 antibodies in the adjuvant setting, and efforts targeting resistance pathways involved in resistance to HER2-targeted agents.

At first glance, the assertion that response to HER2-targeted therapies is dependent on aberrant HER2 expression (frequently in association with amplification of the erbB2 gene) may seem trivial, because it is based on countless experimental observations, both preclinical and clinical (level 1) evidence. Figure 1 demonstrates the effect of trastuzumab on HER2-amplified/overexpressing BT-474 breast carcinoma cells as compared to HER2-negative MCF7 control cells. In this experiment, trastuzumab has no measurable effect on cell proliferation in HER2-negative cells. Moreover, the reported synergism between trastuzumab and cytotoxic DNA damaging agents is also HER2-dependent. Similarly, in xenograft models, only HER2-overexpressing human breast carcinoma xenografts showed in vivo response to trastuzumab. Indeed, even immunologic antibody-dependent cell-mediated cytotoxicity (ADCC) shows a dose-dependent relationship between HER2 protein abundance on the cell surface and cytotoxic response to human immune effector cells. In the clinic, retrospective analysis of the original trastuzumab pivotal trial (in HER2-positive first-line metastatic breast cancers [MBC]) demonstrated that only those subjects with erbB2 gene amplification demonstrable by fluorescence in situ hybridization (FISH) had a survival benefit in association with trastuzumab plus chemotherapy treatment, whereas FISH-negative subjects had no such survival benefit. Subsequently, a prospective randomized phase III study was conducted by the Cancer and Leukemia Group B (CALGB) comparing weekly to every three week paclitaxel, which included a HER2-negative MBC patient population (that are actually HER2-negative), one might expect that by excluding true HER2-negative patients from the data set, efficacy results from a HER2-targeted adjuvant therapeutic agent might look even better, absent the confounding effects of false-positive HER2 subjects. Such an experiment was done by the National Surgical Adjuvant Breast and Bowel Project (NSABP) for trial B-31, an adjuvant trastuzumab study of doxorubicin/cyclophosphamide followed by paclitaxel with or without trastuzumab (during the paclitaxel treatment sequence and for a total of 1 year). Surprisingly, in this study, however, the hazard ratio for the HER2 FISH-negative subset was not significantly different from that in the FISH-positive subset (Fig. 2). But perhaps this FISH-negative subset is composed predominantly of single-copy HER2 protein overexpressers. Resolving this subset in more detail based on repeat immunohistochemical testing, Paik and colleagues then demonstrated that patients classified as central HER2-negative by any assay still had a hazard ratio for benefit from trastuzumab that did not differ significantly from that of the intent-to-treat analysis. These alarming results next begged the question: Could there be laboratory error in the central retesting lab? After all, a local laboratory somewhere had originally classified these tumors as HER2-positive, as it was an eligibility criteria for participation in the study protocol. Moreover, there is now precedent in the literature demonstrating that even commercial central laboratory testing in multicenter trials may yield results with significant discordance to academic reference laboratories. To test
this possibility, Paik and colleagues reanalyzed the B-31 samples using real-time quantitative polymerase chain reaction in order to measure HER2 status in these samples at the transcript level. This confirmed that tumors classified by the NSABP central laboratory as HER2-negative indeed had significantly lower transcript levels compared to HER2-positive samples. Paik and colleagues went one step further to repeat FISH studies on these samples using an alternative FISH probe near, but adjacent to, the erbB2 gene cod-

**KEY POINTS**

- HER2 is a member of the human epidermal growth factor family of receptor tyrosine kinases that plays a key role in the pathogenesis of breast cancer, which when overexpressed as a result of erbB2 gene amplification in as many as 20% of human breast cancers, correlates with a particularly aggressive clinical phenotype.

- Aberrant HER2 expression is required for response to HER2-targeted therapeutics.

- A significant challenge for clinical/translational investigators will be to define who is clinically resistant versus who is not, seek which mechanism or mechanisms apply to a given individual with clinically resistant disease, and exploit known mechanisms of resistance with targeted agents aimed at particular resistance factors or pathways.

- A substantial amount of research has been dedicated to the elucidation of molecular mechanisms of response and resistance to trastuzumab, as well as other HER2-targeted therapeutic approaches.

- Preclinical and some clinical studies have been performed to identify potential methods for overcoming resistance to otherwise effective HER2-targeted therapies, including targeting alternate p185HER2 epitopes, and the combined inhibition of multiple signaling components and/or pathways.

**Fig 1.** Trastuzumab blocks G1/S progression only in cells that overexpress HER2. There is no evidence of apoptosis. (Courtesy Gail Lewis Phillips, Genentech, Inc., South San Francisco.)

**Fig 2.** Relapse risk for various IHC and FISH subsets in NSABP B-31. Statistical test for interaction p = 0.60 for FISH; interaction p = 0.26 for IHC. Abbreviations: RR, relapse risk; ACT, doxorubicin/cyclophosphamide + paclitaxel; ACTH, doxorubicin/cyclophosphamide + paclitaxel + trastuzumab; DFS, disease-free survival. (Courtesy Dr. Soon Paik, NSABP.11)
statistically significant (HR, 0.51; p = 0.14). For these reasons, should the U.S. cooperative groups elect to proceed with an adjuvant trastuzumab trial in HER2-normal subjects (NSABP B-47), it should be done with caution. Based on lessons learned in the laboratory and in the metastatic disease setting with multiple HER2-targeted agents, many authorities in the field predict the results from such an effort will be negative. Admittedly, however, if this “high-risk, high-gain” trial is successful, it would indeed be revolutionary in the history of targeted therapy for human malignancies. Greater enthusiasm for participation in NSABP B-47 could be garnered by more efforts to establish proof of concept in experimental systems. There are certainly immunocompetent transgenic mouse models of spontaneously arising neu-negative breast cancers that could be manipulated with murine anti-neu antibodies. There are metastatic models of HER2-negative orthotopic human breast carcinoma xenografts that could be experimentally treated with or without murine anti-HER2 antibodies to model the primary to metastatic transition in vivo. And there are opportunities in human metastatic disease to study potential impact of trastuzumab on circulating tumor cells in HER2-negative disease. Lacking these (or any other) proof-of-concept studies, enthusiasm for participation in NSABP B-47 may be diminished.

Mechanism(s) of Resistance to HER2-targeted Therapy

As noted above, one trivial example of drug resistance to HER2-targeted therapy is the lack of aberrant overexpression of the drug target p185HER2. In addition to this simplistic mechanism, as shown in Table 1, numerous other mechanisms of resistance have been elucidated, including (but not limited to) 1) proteolytic cleavage of the HER2 extracellular domain or alternative translation of HER2—yielding truncated HER2 species lacking therapeutic antibody binding epitopes; 2) steric hindrance of therapeutic antibody binding by mucins 1 or 4; 3) activation of parallel signaling receptors such as HER3, insulin-like growth factor 1 receptor (IGF1R), Met, or Axl; 4) activation of downstream signaling events caused, for example, by phosphatase and tensin homolog loss or somatic mutation of the PI3 kinase; 5) expression of low-affinity Fcγ receptor polymorphisms on immune effector cells; 6) activation of steroid receptor (ER) signaling; 7) decreased expression of the cyclin-dependent kinase inhibitor p27; and 8) anatomic resistance (e.g., the blood–brain barrier) resulting in sanctuary sites of resistant disease caused by limited penetration of macromolecular biologic HER2-targeting agents. What is notable about most of these observations is that they are based largely on cell-line data, and in most cases observations are restricted to just one type of HER2 inhibitor (i.e., antibody compared with kinase inhibitor); thus, for many of these resistance mechanisms, we do not yet know whether there is cross-resistance between one class of HER2-targeted agent and another. Even more sobering, based on the data in Table 1, is the fact that very few of these putative mechanisms have been subject to rigorous clinical investigation. And for those that have, clinical validation data sets are virtually nonex-
CHALLENGES IN HER2-POSITIVE BREAST CANCER

The author would like to thank Michelle Gallas, PhD, for her expert technical assistance.

**Table 1. Mechanisms of Trastuzumab and Lapatinib Resistance***

<table>
<thead>
<tr>
<th>Mechanism of Resistance</th>
<th>Publications with Association to Trastuzumab Resistance</th>
<th>Publications with Association to Lapatinib Resistance</th>
<th>Clinical Evidence/Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of truncated HER2</td>
<td>Zabrecky et al(^{31}); Christiannson et al(^{32});</td>
<td>N/A</td>
<td>All et al(^{38}); Pegram et al(^{39}); Lennon et al(^{40}); Witzel et al(^{41}); Scaltriti et al(^{42}); Prognosis only: Pedersen et al(^{43})</td>
</tr>
<tr>
<td>Regulation of the stability of HER2 by HSP90 [decreases receptor turnover thereby potentiating HER2 signaling]</td>
<td>Chanderlapaty et al(^{44})</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Steric hindrance/target occlusion: interaction between HER2 and trastuzumab becomes disrupted by overexpression of molecules such as MUC 4 and MUC1</td>
<td>Nagy et al(^{45}); Paliy-Kreek(^{46}); Price-Schiavi et al(^{47}); Fessler et al(^{48}); Workman et al(^{49})</td>
<td>N/A</td>
<td>ND</td>
</tr>
<tr>
<td>Altered downstream signaling affecting PTEN, P27 (kip1), and Akt activity</td>
<td>PTEN: Eichhorn et al(^{50}); P27 (kip1): Le et al(^{51}); Nahta et al(^{52}); Akt: Yakes et al(^{53})</td>
<td>Chan et al(^{54}); Chen et al(^{55})</td>
<td>Nagata et al(^{56})</td>
</tr>
<tr>
<td>Increased signaling from the ER in ER-positive HER2-overexpressing breast cancers</td>
<td>Xia et al(^{57})</td>
<td>Xia et al(^{57})</td>
<td>ND</td>
</tr>
<tr>
<td>Compensatory signaling: increased signaling from other receptor [Axl, IGFR, EGFR, MET, p-HER3], causing inhibition of HER family heterodimerization</td>
<td>IGFR: Lu et al(^{58}); Nahta et al(^{59}); Huang et al(^{60}); EGFR: Dua et al(^{61}); Yotsumoto et al(^{62}); MET: Shattuck et al(^{63})</td>
<td>Axl: Liu et al(^{64}); p-HER3: Sergina et al(^{65}); Amin et al(^{66})</td>
<td>ND</td>
</tr>
<tr>
<td>Targeting of immune cells to HER2-positive tumor cells/Fc gamma polymorphisms and ADCC response</td>
<td>Junttila et al(^{67}); Koninki et al(^{68})</td>
<td>N/A</td>
<td>Musolin et al(^{69}); Tamura et al(^{70})</td>
</tr>
<tr>
<td>Inability to cross blood-brain barrier [intracerebral metastases]</td>
<td>Grossi et al(^{71}); Kinoshita et al(^{72})</td>
<td>N/A</td>
<td>Lin et al(^{73}); Lin et al(^{74})</td>
</tr>
</tbody>
</table>

Abbreviations: HER2, human epidermal growth factor receptor 2; N/A, not applicable; HSP90, heat shock protein 90; ND, not done; PTEN, phosphatase and tensin homolog; ER, estrogen receptor; IGFR, insulin-like growth factor receptor 1; EGFR, epidermal growth factor receptor; ADCC, antibody-dependent cell-mediated cytotoxicity.

* Table summarizes published mechanisms of resistance to trastuzumab and lapatinib and highlights clinical evidence amassed to date for hypothesized mechanisms.
REFERENCES


34. Lafky JM, Wilken JA, Baron AT, et al. The clinical implications of the HER2 ectodomain is a pervandatable activable process that is inhibited by the tissue inhibitor of metalloproteases-1 in breast cancer. Cancer Res. 1999;59:1196-1201.


