Challenges in HER2-positive Breast Cancer

By Mark D. Pegram, MD

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 *erb*B2 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 measureable effect on cell proliferation in HER2-negative cells.[1] Moreover, the reported synergism between trastuzumab and cytotoxic DNA damaging agents is also HER2-dependent.[2] Similarly, in xenograft models, only HER2-overexpressing human breast carcinoma xenografts showed in vivo response to trastuzumab.[3] 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.[4] 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.[5] 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* cohort randomized to receive trastuzumab. Trastuzumab did not improve efficacy for HER2 non-overexpressors.[6] This requirement for HER2 overabundance to predict response to HER2-targeted therapeutics in breast cancer is not restricted to HER2-directed humanized monocloncal antibodies, but also appears to be the case for small-molecule HER2 kinase inhibitors, such as lapatinib.[7] In a large prospective randomized phase III placebo-controlled trial comparing paclitaxel to paclitaxel plus lapatinib in the first-line MBC setting, only those subjects whose tumors were defined to be HER2-positive had a significant improvement in time to tumor progression (TTP; $p = 0.005$).[8] Among estrogen receptor (ER)-positive MBC patients, we recently reported results from a large (1,286 patients) prospective randomized placebo-controlled trial comparing letrozole plus placebo to letrozole plus lapatinib.[9] In the HER2-negative subgroup (952 patients), there was no increase in clinical response (CR), clinical benefit (CB), or progression-free survival (PFS), whereas in the HER2-positive subgroup (219 patients), all three endpoints were significantly superior with addition of lapatinib ($p = 0.021$, $p = 0.003$, and $p = 0.019$ for CR, CB, and PFS, respectively).[9] Taken together—and consistent with similar well-established paradigms from multiple other targeted agents in both solid and liquid tumors—all the available data from preclinical in vitro studies, preclinical in vivo models, and level 1 evidence from phase III clinical campaigns in the metastatic setting are remarkably consistent, indicating a robust relationship between aberrant HER2 amplification/overexpression and probability of response to more than one class of HER2-targeted therapeutic agent.

Is it possible to image this paradigm is any different in earlier stages (e.g., M0) of breast cancer? To the contrary, inasmuch as it is known that there must be a certain frequency of misclassified tumors in a given HER2-positive 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).[10-11] 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).[11] But perhaps this FISH-negative subset is composed predominantly of single-copy HER2 protein overexpressors. 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.[11] 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.[12] To test

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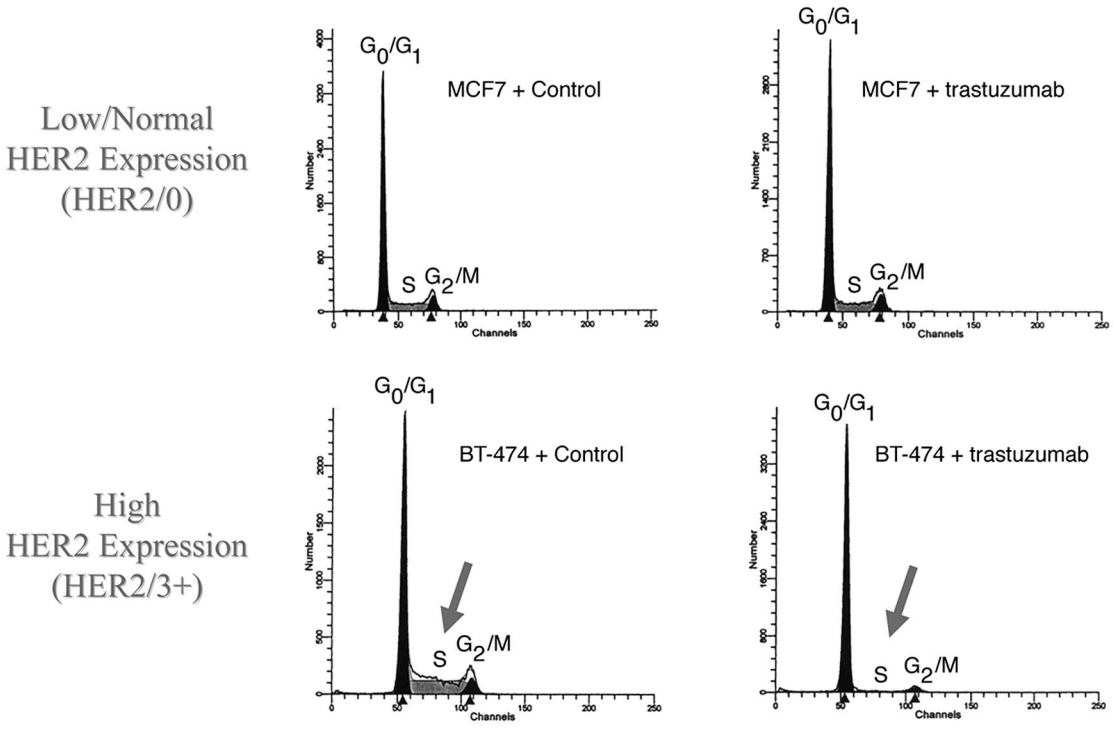

Fig 1. Trastuzumab blocks G1/S progression only in cells that overexpress HER2. There is no evidence of apoptosis. (Courtesy Gail Lewis Phillips,[1] Genentech, Inc., South San Francisco.)

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.

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-

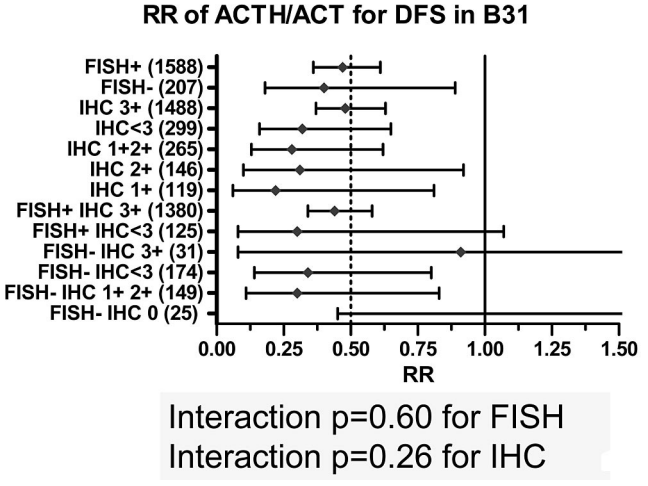

Fig 2. Relapse risk for various IHC and FISH subsets in NSABP B-31. Statistical test for interaction p = 0.60 for FISH; interaction p = 0.26 for IHC.

Abbreviations: RR, relapse risk; ACT, doxorubicin/cyclophosphamide → paclitaxel; ACTH, doxorubicin/cyclophosphamide → paclitaxel + trastuzumab; DFS, disease-free survival. (Courtesy Dr. Soon Paik, NSABP.[11])

ing sequence—once again confirming the original FISH observations. Finally, a blinded "round robin" exchange of 389 randomly sampled, HER2-normal (immunohistochemisty [IHC]-negative and FISH-negative) and HER2-positive breast tumors was performed among three central laboratories (North Central Cancer treatment group, Breast Cancer International Research Group, and NSABP) for confirmatory HER2 testing.[13] Independent reads were concordant across pathologists in IHC status in 351/381 (92%) cases and in FISH status in 343/373 (92%) cases. Similar to data obtained from NSABP B-31, among 1,888 patients enrolled in the N9831 adjuvant trastuzumab trial, 103 patients with HER2-"normal" tumors by central testing seemed to benefit from trastuzumab, but the published difference was not statistically significant (HR, 0.51; $p = 0.14$).[14]

Do these results sufficiently confirm the NSABP findings of benefit from adjuvant trastuzumab even in HER2-normal early breast cancers? Enough to justify the investment in cost, patient resources (3,260 patients), and potential toxicities associated with adjuvant trastuzumab? If not direct antiproliferative effects on HER2-positive tumor cells, what possible mechanism can explain a treatment effect observed in early-stage breast cancer, and not in metastatic disease?[1] Since all early breast cancers express at least some HER2 receptor (however low level), clinical investigators offer the possibility of immunologic effects of adjuvant trastuzumab in an occult micrometastasis disease setting, which may be too subtle to be appreciated in situations of bulk metastatic disease with high tumor burden. Indeed, measureable immunologic effects of trastuzumab have been observed in metastatic breast cancer cohorts. Musolino and colleagues published data in a trastuzumab-treated, HER2-positive MBC cohort in which polymorphisms in Fc gamma receptors were associated with time to tumor progression, indirectly suggesting an ADCC mechanism of action of trastuzumab.[15] Such effects have not yet been observed in adjuvant trastuzumab cohorts, although the number of events in these trials may yet be insufficient to expect difference in outcome based on Fcγ receptor genotypes.[16] Taylor and colleagues have reported induction of endogenous, polyclonal anti-HER2 serum antibodies in association with trastuzumab treatment, where antibody titer correlated with response rate.[17] In the same work, induction of HER2-specific cytotoxic T cells was also observed following trastuzumab treatment, suggesting induction of both humoral and cell-mediated immunologic responses by trastuzumab.

In summary, history teaches us that data based on unplanned, retrospective subset analysis should be viewed with circumspection. In interpreting these data, one should be mindful that the adjuvant trastuzumab trials were not placebo-controlled efforts; thus, there may be some potential for observer bias. Although laudable that the NSABP investigators went to extraordinary lengths to verify their HER2-classification of subjects enrolled in NSABP B-31, the fact remains that the alleged HER2-negative tumors were (at some point) classified by a local pathologist as being HER2-positive. On retesting, whenever discordance in HER2 results are encountered, because of intratumoral heterogeneity and potential bias introduced by repeat assays performed on different cuts through the same tumor, it simply may not be possible to discern which sample is in fact the "correct" result.[18] Moreover, the "round robin" reanalysis of adjuvant HER2 trial samples, though randomly selected, included only modest numbers of samples that may or may not be representative of the intent-to-treat populations, much less the HER2-normal subjects in particular. And although results from the N9831 trial also exhibit a trend for hazard ratio less than 1 in the HER2-normal subset, the results did not achieve statistical significance.[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 $p185^{HER2}$. 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 PI3kinase; 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 penetrance 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-

Table 1. Mechanisms of Trastuzumab and Lapatinib Resistance*

Mechanism of Resistance	Publications with Association to Trastuzumab Resistance	Publications with Association to Lapatinib Resistance	Clinical Evidence/Relevance
Expression of truncated HER2	Zabrecky et al[31]; Christianson et al[32]; Codony-Servat et al[33]; Lafky et al[34]; Spector[35]; Scaltriti et al[36]; Chen et al[37]	N/A	Ali 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]
Regulation of the stability of HER2 by HSP90 (decreases receptor turnover thereby potentiating HER2 signaling)	Chandarlapaty et al[44]	ND	ND
Steric hindrance/target occlusion: interaction between HER2 and trastuzumab becomes disrupted by overexpression of molecules such as MUC 4 and MUC1	Nagy et al[45]; Palyi-Krekk[46]; Price-Schiavi et al[47]; Fessler et al[48]; Workman et al[49]	N/A	ND
Altered downstream signaling affecting PTEN, P27 (kip1), and Akt activity	PTEN: Eichhorn et al[50]; P27 (kip1): Le et al[51]; Nahta et al[52]; Akt: Yakes et al[53]	Chan et al[54]; Chen et al[55]	Nagata et al[56]
Increased signaling from the ER in ER-positive HER2-overexpressing breast cancers	Xia et al[57]	Xia et al[57]	ND
Compensatory signaling: increased signaling from other receptor (Axl, IGFR, EGFR, MET, *p*-HER3), causing inhibition of HER family heterodimerization	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]	Axl: Liu et al[64]; *p*-HER3: Sergina et al[65]; Amin et al[66]	ND
Targeting of immune cells to HER2-positive tumor cells/Fc gamma polymorphisms and ADCC response	Junttila et al[67]; Koninki et al[68]	N/A	Musolino et al[69]; Tamura et al[70]
Inability to cross blood-brain barrier (intracerebral metastases)	Grossi et al[71]; Kinoshita et al[72]	N/A	Lin et al[73]; Lin et al[74]

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.

istent. Therefore, a significant challenge for clinical/translational investigators will be to (a) define who is clinically resistant compared with who is not (not a straightforward task in light of the demonstration of clinical activity of trastuzumab even beyond clinical tumor progression);[19] (b) seek which mechanism or mechanisms apply to a given individual with clinically resistant disease, however defined; and (c) exploit known mechanisms of resistance with targeted agents aimed at particular resistance factors or pathways. This is not at all a trivial set of tasks given the fact that for most of the known/suspected resistance targets, there are no validated assay reagents for accurate measurement of putative resistance factors in archival clinical tumor specimens. Moreover, in the case of acquired resistance, the expression of resistance factor(s) is likely to be a moving target that ideally might require repeated tumor sampling over time to even appreciate. And there is a limitation based on traditional clinical drug development paradigms, which often employ simplistic study designs like HER2-inhibitor plus or minus a particular resistance-targeting agent for all comers with resistant disease. Such designs will fail if the frequency of a particular resistance factor is low among myriad other possible resistance phenotypes in a given patient population under study, or worse yet, within the same patients.

This is all made even more complicated by the fact that we do not even know the natural history of HER2-postitive metastatic breast cancer in the adjuvant trastuzumab (or in some cases, adjuvant lapatinib) era. It seems likely that selection pressure exerted by HER2-targeting agents used in the adjuvant setting may well affect subsequent response to HER2-targeting agents at the time of local or distant relapse. Will first-line chemotherapy plus trastuzumab still be associated with a survival benefit in the era of adjuvant trastuzumab? Or would adding a second-generation HER2 antibody be more efficacious?[20-22] Or would switching to a HER2 kinase inhibitor-based strategy be more effective in the setting of progression after adjuvant trastuzumab?[7,9,23,24] Or, perhaps both an antibody plus a small-molecule kinase inhibitor given in combination?[7,25-27] Will any of these strategies remain relevant in a future era of antibody-drug conjugates?[28-30]

In summary, 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 $p185^{HER2}$ epitopes, and the combined inhibition of multiple signaling components and/or pathways (both vertical and horizontal inhibition). Ongoing studies will continue to evaluate the most promising approaches for overcoming mechanisms of resistance to HER2-targeted therapies.

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