Will Single-Time Tumor Profiling and a "Guilt by Association" Approach Allow Us to Outsmart HER2-Positive Breast Cancer?

□□*Commentary on Harris et al., p. 1198*

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Amplification of the *HER2/Neu* (*ERBB2*) gene, the second member of the epidermal growth factor receptor (ErbB) tyrosine kinase family, occurs in ~25% of invasive breast cancers and is associated with poor patient outcome (1). Trastuzumab (Herceptin), a humanized monoclonal IgG_1 that binds to the extracellular domain of the HER2 receptor, induces clinical responses in HER2-overexpressing breast cancers and prolongs patient survival when combined with chemotherapy (2–8). The randomized studies that showed the clinical benefit of trastuzumab enrolled mainly or exclusively patients with breast cancer that overexpress HER2 as measured by intense membrane staining in the majority of tumor cells with HER2 antibodies (3+ by immunohistochemistry) or excess copies of the *HER2* gene determined by fluorescence *in situ* hybridization. Patients with a single copy of the *HER2* gene (so called fluorescence *in situ* hybridization negative) or with 2+ HER2 protein by immunohistochemistry have not been shown to benefit from therapy with trastuzumab either alone or in combination with chemotherapy. For these reasons, trastuzumab is approved for use only in patients with HER2-overexpressing breast cancer as defined above. Therefore, HER2 overexpression by immunohistochemistry and/or fluorescence *in situ* hybridization is the biomarker predictive of good odds of response to treatment with the antibody. However, many patients with *HER2* gene–amplified metastatic breast cancers do not respond or eventually escape trastuzumab, suggesting both *de novo* and acquired mechanisms of therapeutic resistance.

Few studies have already reported or speculated on potential mechanisms of resistance to trastuzumab. For example, overexpression of the insulin-like growth factor (IGF)-I receptor or increased levels of IGF-I receptor/HER2 heterodimers (9, 10), which potently activate phosphatidylinositol-3 kinase and its downstream effector Akt, partially abrogate trastuzumab action when transfected into antibody-sensitive human breast cancer cells. Amplification of the phosphatidylinositol-3 kinase pathway as a result of loss or low levels of the phosphatase PTEN in primary tumors is also associated with lower odds of response to trastuzumab (11). High expression of epidermal growth factor receptor and ErbB ligands correlates with early escape from trastuzumab therapy (12). This is consistent with structural and cellular data using ErbB receptor ectodomains and different HER2 monoclonal antibodies, which show that trastuzumab is unable to block ligand-induced epidermal growth factor receptor/HER2 and HER2/HER3 heterodimers (13, 14). Finally, Anido et al. (15) reported the presence of HER2 C-terminal fragments which result from alternative translation initiation from methionines near the transmembrane domain of the full-length receptor molecule. These fragments are kinase-active but lack the trastuzumab binding epitope and, therefore, can potentially allow the cancer cell to escape antibody action. It should be emphasized that none of these studies used an open-ended unbiased approach with primary human tumors treated with trastuzumab to discover possible mechanisms of resistance. Despite these important leads, there is/are no biomarker(s) than can reliably predict the lack of benefit from trastuzumab which, in turn, can be used for subsequent clinical trial development and/or individual therapeutic decisions.

In this issue of *Clinical Cancer Research*, Harris et al. (16) report an unbiased transcriptional profiling approach to discover RNAs that predict resistance to therapy with trastuzumab and vinorelbine in patients with HER2-overexpressing, operable, early-stage breast cancer. Forty-eight patients received preoperative therapy with both drugs for 12 weeks. Eight of forty (20%) evaluable patients achieved a pathologic complete response, whereas only one patient progressed during therapy. Neither HER2 nor estrogen receptor status changed as a result of treatment. Only five (13%) patients experienced grade 1 cardiac dysfunction, which did not merit changes in dose or treatment schedule. Unsupervised analysis of gene expression arrays from pretherapy specimens was able to separate tumors that underwent pathologic complete response versus no clinical response, but no genes predictive of response were identified in the tumors that achieved pathologic complete response. However, the nonresponding tumors expressed higher levels of basal markers, such as p63, brother of CDO, and secreted frizzled protein 1 as well as growth factors, receptors, and signal transducers, including IGF-I, platelet-derived growth factor, hepatocyte growth factor, pleiotropin, c-Met, the leptin receptor, the regulatory subunit of phosphatidylinositol-3 kinase, p85, and microtubule-associated protein 2. Interestingly, hepatocyte growth factor/c-Met and leptin receptors have been reported to synergize and/or cross-talk with HER2 (17–19), suggesting a mechanistic basis for their association

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with resistance to trastuzumab. Ten of forty-six (22%) patients exhibited strong circumferential membrane staining with an IGF-I receptor antibody and were considered receptor positive. Response rate in this group was only 50% compared with 97% in the IGF-I receptor negative cases. This last finding, which is unrelated to the RNA profiling component of the study, adds to the circumstantial evidence associating IGF-I receptor signaling and resistance to trastuzumab and/or chemotherapy.

This study represents an important attempt at identifying biomarkers of response or resistance to anti-HER2 therapy. There are some strengths to the unbiased approach used by Harris et al. (16) in this trial that should apply to similar investigational study designs with trastuzumab or other novel therapies. First, the selected population was molecularly uniform (i.e., HER2-overexpressing breast cancers) and all patients received the same therapy. Second, pathologic complete response was used as a discriminator of the phenotype of interest, in this case drug sensitivity in those achieving pathologic complete response and drug resistance in those not achieving this clinical end point. Pathologic complete response after neoadjuvant therapy is strongly associated with longer term patient outcome (20) and arguably remains the best "predictor" of longer time to benefit from treatment in this setting.

It can be argued that 12 weeks of therapy might not be enough in some patients to see the full effect of trastuzumab. However, in a recent study, patients with locally advanced HER2-positive breast cancer were treated with weekly trastuzumab for 3 weeks before the addition of chemotherapy. Early tumor regressions were observed, and these correlated with evidence of increased tumor cell apoptosis but not inhibition of proliferation (21). With trastuzumab causing mainly tumor cell death in sensitive cancers, it is thus plausible that short durations of treatments are adequate for a clinical readout of drug action. Indeed, a 9-week course of adjuvant vinorelbine or docetaxel plus trastuzumab was recently shown to improve disease-free survival compared with chemotherapy alone in patients with breast cancer containing *HER2* gene amplification (8). Thus, the 12-week duration of therapy in the trial by Harris et al. (16) was probably appropriate to elicit the full effect of trastuzumab.

Unsupervised analysis of gene expression profiles from pretreatment specimens did not identify any predictors of pathologic complete response. Given the small size of the study cohort, this is not surprising. However, because the best predictor of response to neoadjuvant therapy is pathologic complete response itself, the value of an additional molecular predictor of response would be of questionable practical use, especially considering (*a*) the tolerability of this therapy and (*b*) that all patients with tumors that overexpress HER2, except few with a medical contraindication, will be treated with trastuzumab whether they exhibit a putative positive predictive biomarker or not.

A more clinically useful molecular signature would be one associated with *de novo* or acquired drug resistance. It is likely that correlation of a single-time observation in pretreatment specimens with clinical outcome will not be rigorous enough or completely informative for the following reasons. First, as a result of HER2 overexpression, these tumors exhibit a closely related pattern of gene expression (22) in which unsupervised analysis of differences in a small number of resistance-associated genes may not be detected. Second, a resistance-associated signature or biomarkers could be induced in response to treatment with trastuzumab and chemotherapy and only be detectable in posttreatment tumors. Of note, treated specimens were not profiled in the study under discussion here. Third, this negative predictive signature should be "enriched" in cancers that do not respond completely if it is in fact a true marker and/or a causal effector of resistance to treatment. Genes that remain overexpressed or whose level of expression increases in the post-therapy specimen are more likely to be at least in part "guilty" of the resistant phenotype than those that do not.

This approach will allow the selection of relevant RNAs that can be confirmed at the protein level with antibody-based methods. To differentiate markers of resistant tumors versus gene products that are causal to drug resistance, subsequent overexpression and RNA interference studies in HER2-overexpressing breast cancer cells will be required. A relevant example is the microtubule-associated protein tau, identified as a differentially expressed gene in mammary tumors treated with paclitaxel. Tumors that underwent pathologic complete response exhibited markedly lower levels of tau compared with nonresponsive tumors. RNA interference of tau expression increased sensitivity of breast cancer cells to paclitaxel but not epirubicin (23), suggesting that tau overexpression renders cancer cells less vulnerable to paclitaxel and, thus, can be exploited as a therapeutic target in combination with taxanes. Finally, the post-therapy specimens are necessary for documenting drug-induced inactivation of its molecular target. This is particularly true for novel signaling inhibitors, in which inactivation of the drug target is required for the clinical trial to be interpretable. Examples of this are pharmacodynamic trials with epidermal growth factor receptor and mammalian target of rapamycin inhibitors, in which the treated tumor section was used to document the inhibition of phosphorylated epidermal growth factor receptor and phosphorylated S6 *in situ*, respectively (24–26).

At this time, the identification of predictors of resistance in trials like the one discussed herein will likely generate hypothesis and information that can be used for exploratory clinical trials of combinations that include trastuzumab or other anti-HER2 agents. However, this information cannot be interpreted yet as having any utility for treatment decisions in individual patients. With the explosion of molecule-targeted drugs, the increasing number of neoadjuvant treatment studies with a genomic profiling component, as the one by Harris et al. (16), and the increasing power of technology capable of generating large information about either relevant genes or spurious leads to nowhere, it is important that we develop a consensus of rigorous criteria to prioritize the information generated by these studies. So, will a single-time profiling of tumors followed by a subsequent "guilt by association" approach allow us to outsmart and eventually cure HER2-positive breast cancer? Although I would not mind being proved wrong on this, my answer is no. The study by Harris et al. (16) is an important first step in a translational research process that should be sharpened to identify true markers and/or effectors of escape from anti-HER2 therapy. In turn, interfering with these mechanisms of escape should get us closer to the elimination of this type of breast cancer.

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