value at the bedside and at the societal level. The risk of not being proactive will be a widening of the gap in outcomes between those patients with and without the resources to access these innovations that can improve the quality and length of their lives.

References


Beyond a doubt, trastuzumab works. In women with early-stage breast cancer, adjuvant use of the anti-HER2 monoclonal antibody trastuzumab reduces recurrence risk by half when added to standard chemotherapy (1). In fact, clinical experience suggests that data from randomized trials in the adjuvant setting may underestimate the real-world benefits. The success of trastuzumab for early-stage disease is so dramatic that many clinicians sense that the incidence of recurrence of HER2-positive breast cancer is plummeting, disappearing faster than the trials might have suggested.

But who really benefits from trastuzumab? It’s a question that might seem like asking, “Who’s buried in Grant’s tomb?” Since the first report more than 25 years ago that HER2 overexpression is an adverse prognostic factor in breast cancer (2), it has been an article of faith that the sine qua non for anti-HER2 treatments must be HER2 itself. Surely then, the importance of trastuzumab must have something to do with HER2. But in what ways, precisely? Does trastuzumab lower recurrence risk in all cases of HER2-overexpressing breast cancer across the board by 50%? Is there a subgroup of HER2-expressing tumors that are particularly sensitive to trastuzumab therapy? Is there a subgroup that is resistant? Based on experience with other novel, targeted agents, it seems unlikely that all patients derive similar benefit from trastuzumab.

Meanwhile, a small but notable number of patients develop disease recurrence despite trastuzumab-based therapy. Paradoxically, although clinically “resistant,” such tumors still retain sensitivity to ongoing anti-HER2 treatment (3). A biomarker to identify those patients who are not likely to benefit from trastuzumab would be clinically useful, allowing patients to move in other therapeutic directions. Similarly, a marker that pegged tumors as exquisitely sensitive to anti-HER2 drugs might enable treatment without the

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Trastuzumab: Qui Bono?

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blunt instrument of chemotherapy or define patients most suited for novel targeted or immunological approaches centering on HER2 expression. A predictive biomarker could be particularly important in the adjuvant setting where one cannot measure tumor response to assess the effectiveness of a therapy in an individual patient.

Alas, identifying a robust clinical or molecular predictor of adjuvant trastuzumab benefit has proven challenging. Trastuzumab benefit arises regardless of patient age, tumor size, nodal status, estrogen receptor (ER) status, grade, or flavor of chemotherapy. Retrospective analyses of tumor samples from the major adjuvant studies of trastuzumab have failed to demonstrate a single biomarker or biomarker signature that can identify a subset of patients who do not benefit from trastuzumab therapy (4,5). Even the most obvious candidate biomarker for a predictor of trastuzumab benefit, HER2 itself, has proven to be surprisingly ambiguous. All of the adjuvant trials selected patients by virtue of HER2 expression, requiring tumors to be either 3+ by immunohistochemistry (IHC) or to have evidence for HER2/neu gene amplification by fluorescence in situ hybridization (FISH). These criteria have become the benchmarks for defining tumors as HER2 positive (6). In retrospect, investigators have sought to define how much HER2 expression is critical for adjuvant trastuzumab benefit. Neither quantitative measurement of HER2/centromere 17 ratio (ie, FISH ratio) nor chromosome 17 copy number predicts benefit of adjuvant trastuzumab (7); soluble HER2 levels also do not (8). Primary archival tumor samples from the NSABP B-31 and NCCTG N9831 adjuvant trastuzumab studies were reviewed in a central research lab for HER2 status, and it was discovered that 5% to 10% of tumors in each zumab studies were reviewed in a central research lab for HER2 ples from the NSABP B-31 and NCCTG N9831 adjuvant trastu-

The development of this eight-gene classifier is a substantial technical achievement. It was derived from a high-quality randomized dataset, it was able to distinguish a population of patients that does not appear to benefit from trastuzumab in an independent dataset, and the statistical strength of its discriminatory ability appears robust; the formal test of interaction between the classifier and trastuzumab benefit is highly statistically significant ($P < .001$). However, several aspects of this classifier raise concerns about its potential clinical usefulness. One is that the interaction between classifier score and trastuzumab benefit is quite complex and leads to a discontinuous distribution of patients into the three benefit groups. This complexity makes establishing optimal cutoffs difficult—nearly impossible, in fact—and if applied arbitrarily could potentially lead to misclassification of patients and thus inappropriate treatment recommendations. Validating the classifier in additional independent trial datasets, preferably with a sensitivity analysis for alternate cutoff points, is needed before considering clinical use of the assay.

In an ambitious attempt to address the need for a predictor of trastuzumab benefit, Pogue-Geile et al., as reported in an article in this issue of the Journal (10), obtained gene expression data from more than 1500 primary tumor samples from the NSABP B-31 study, which had randomized patients with early-stage, node-positive, HER2+ cancers to anthracycline- and taxane-based chemotherapy alone or with 1 year of trastuzumab (1). Pogue-Geile et al. (10) cast a broad net to capture predictive markers in samples from a discovery cohort of 588 of the patients, but selecting genes for a predictive model using an unbiased approach based purely on the interaction between gene and treatment arm proved difficult. In short, it couldn’t be done. So the investigators instead developed a model using a total of eight genes associated with expression of either the ER (ESR1, NAT1, GATA3, CA12, IGF1R) or the HER2 (ERBB2, c17orf37, GRB7) amplicon. Clinicians may recognize several (ESR1, ERBB2, GRB7) because they form part of the 21-gene recurrence score captured in the OncotypeDX assay. GATA-3 is associated with a luminal A breast cancer subtype. After building the model, the investigators then followed good research practice and applied it to an independent cohort of 991 patients from the same trial.

In this validation cohort, the eight-gene model stratified tumors into 3 subsets with different clinical outcomes. A distinct subset (called Group 3 by the investigators) of approximately 45% of the patients with tumors characterized by low- or absent levels of ER and high-level HER2 expression was associated with an extraordinary benefit from trastuzumab (hazard ratio [HR] = 0.28; > 70% risk reduction). This group might account for the rapid disappearance of HER2-positive metastatic recurrence from our clinics. The actual risk reduction is huge in a group of tumors that would be expected to recur relatively early (first 5 years) after diagnosis (11). By contrast, among the 10% of cases classified as Group 1, there was no apparent benefit from trastuzumab (HR = 1.58). Interestingly, this subset had tumors characterized by intermediate-level but clear HER2 (ERBB2) expression and very high-level ER (ESR1) expression. In between were the Group 2 patients, who derived moderate benefit (HR = 0.60) from trastuzumab. Tumors here clustered at lower HER2 expression—including cancers with no HER2 overexpression—and with variable degrees of ESR1 expression.

It is intriguing, however, to explore what the classifier’s gene/outcome interaction reveals about the underlying biology of HER2-positive breast cancer and the benefit from anti-HER2 treatment with trastuzumab. The first observation is that nearly 90% of tumors are likely to benefit from the addition of trastuzumab therapy. That percentage is far higher than one might have guessed from response rates in studies of anti-HER2 monoclonal antibody treatments, either alone with trastuzumab (12) or in combination with pertuzumab (13). Such high sensitivity rates are concordant with emerging data showing remarkably high rates of clinical and complete pathological response when anti-HER2 antibodies are combined with chemotherapy (14). The observation that high expression levels of ER-associated genes are associated with lack of benefit of trastuzumab dovetails with several lines of evidence suggesting that ER signaling may be both a mediator of trastuzumab resistance and a marker of exquisite sensitivity to endocrine treatments that might render additional therapy unnecessary (15). Preclinical studies demonstrate that the ER can activate signaling pathways downstream of HER2 and drive the expression of several antiapoptotic proteins (16,17). Consistent with this finding, multiple neoadjuvant clinical trials of HER2-directed therapy demonstrate consistently lower rates of pathological complete
responses in ER-positive, HER2-positive cancers compared with ER-negative, HER2-positive cancers (18–20), a finding that fits closely with the observations of the Group 1 and Group 3 tumors in the current report (10).

The Pogue-Geile et al. study also showed that tumors with low HER2 gene expression (including those found to be HER2 negative by centrally tested IHC and FISH) still derive substantial benefit from trastuzumab. Although consistent with a prior report derived from this same sample set using the same general type of assay (HER2 mRNA expression) (9), the result is nonetheless counterintuitive and important. It is interesting, however, that in the Pogue-Geile et al. study (10) a substantial proportion of the tumors with low HER2 expression levels that benefit from trastuzumab have moderate to high levels of ER (ESR1) expression. Thus, their data would suggest that cancers with low HER2 expression and moderate/high ER expression benefit from trastuzumab, whereas those with intermediate levels of HER2 and high ER expression do not benefit. If this finding is validated, it points to a very complex relationship between HER2, ER, and trastuzumab sensitivity. Whether cancers with low HER2 expression (scored as 1+ or 2+ by IHC, but FISH negative) benefit from trastuzumab is now being definitively evaluated in a large randomized study, NSABP B-47, which looms as a critical test for where to use trastuzumab in HER2-negative breast cancer.

All of this begs the question: Why has it been so difficult to develop a marker for trastuzumab benefit, or any other anti-HER2 therapy? Trastuzumab is a highly targeted (ie, specific) therapy aimed at a pathway that many consider relatively well understood. The fact that it has been challenging to develop a predictive biomarker may be at least in part because the mechanism of action of trastuzumab is multifaceted (eg, inhibition of HER2 signaling, inhibition of extracellular domain shedding, activation of antibody-dependent cellular cytotoxicity, potentiation of chemotherapy action) and the mechanism(s) important for the clinical effectiveness of trastuzumab are unclear. Certainly if immune-mediated effector functions play a dominant role in the clinical benefits of trastuzumab, then additional studies focusing on host factors need to be undertaken. Recent data from a preoperative sample set suggest that genes involved in modulating immune effects are predictive of benefit of HER2-targeted therapy (21). It is also possible that the mechanisms may differ in early- and late-stage breast cancer. In retrospective studies of women with metastatic breast cancer, there has been no compelling signal of benefit from anti-HER2 treatments outside of cases of HER2-overexpressing disease (22–24).

At present, clinicians should rely on established markers of HER2 expression for selecting patients for adjuvant trastuzumab or neoadjuvant pertuzumab-trastuzumab therapy. Those markers include IHC expression at the 3+ level or FISH ratio of 2 or greater (25). Patients with IHC 1+ or 2+ but FISH less than 2 tumors should be encouraged to participate in the NSABP B-47 trial. Although it seems obvious that trastuzumab would work in HER2-overexpressing breast cancer, the data in this issue of the Journal (10) offer the intriguing possibility that there is more to the story. Aficionados of Groucho Marx know that the “Who’s buried in Grant’s tomb?” question was a throw-away to make everyone a winner on his TV show, “You Bet Your Life.” But purists have noted that there is more to that story, too; in fact, no one is buried in Grant’s tomb. Ulysses S. Grant and his wife are entombed, above ground, in the monument (26). When it comes to HER2 expression and trastuzumab benefit, maybe we haven’t yet heard the secret word.

References
Phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (PI3KCA) is encoded by the PIK3CA gene and catalyzes the synthesis of phosphatidylinositol-3, 4, 5-triphosphate (PIP3). PIP3 is important for growth factor receptor tyrosine kinase signaling, such as for EGFR (epithelial growth factor receptor) or VEGFR (vascular endothelial growth factor receptor). PIP3 plays an important role in AKT (protein kinase B)/mTOR (mammalian target of rapamycin) activation, which is critical for cell growth and protein synthesis, which are important for carcinogenesis and cancer progression. PI3KCA activity is regulated by PTEN (phosphatase and tensin homolog), and loss of PTEN has been tested for its predictive and prognostic significance; however, it has not been validated (1).

PIK3CA mutations are found in a large number of different malignancies, such as breast, stomach, liver, lung, and colorectal cancer (CRC) (2). In CRC the frequency of PIK3CA mutations is approximately 10–30% (3,4). In 50% of PIK3CA mutated tumors, concomitant KRAS exon 3 mutations exist in about 50% (5). Activating mutations of PIK3CA are predominantly found in codon 9 and codon 20 (4), and there is evidence that the negative predictive value of PIK3CA mutations may be limited to codon 20 mutations (1) or to tumors that are mutant to both codon 9 and 20 locations (6). PI3KCA signaling is triggered through the phosphorylated intracellular domain of receptor tyrosine kinases or by RAS (rat sarcoma) oncogenes. Interestingly, another activator of PI3KCA is estrogen signaling, which may explain the sex-associated prognosis reported by Majek and colleagues (7). Embedded into such an interconnected and redundant network, the prognostic value of isolated PIK3CA mutations is difficult to assess. In addition, the tumor heterogeneity of CRCs will show that tumors contain subpopulations of PIK3CA-mutated cells. This complexity may explain why prognostic value could only be demonstrated in tumors without BRAF and RAS mutations (4,6). PIK3CA mutations seem to be subordinate in comparison with RAS or RAF mutations. Because of a recent publication showing a survival benefit for patients with PIK3CA-mutant CRC who are taking aspirin (8,9), PIK3CA mutations may have more attention. Because RAS-mutated tumors display a higher expression of COX2 (cyclo-oxygenase-2) and PI3KCA is downstream of RAS, it is speculated that PIK3CA-mutant tumors may also have a higher expression of COX2 (10), which may explain the positive effect of aspirin use. Furthermore CRC tumors with higher COX2 expression have been shown in a mouse model to be more likely to metastasize into the liver (11), suggesting that PI3KCA may be predictive in a molecular defined subgroup of CRC.

In CRC, PIK3CA mutations are associated with prognosis when tested in large (n = 1170) cohorts and adjusted for BRAF and KRAS mutations (6). However PIK3CA’s role as a predictive marker for anti-EGFR targeted antibodies such as cetuximab and panitumumab is still unclear (12), but this may be because of the lack of control groups, small sample size, and no adjustment for RAS and RAF mutations or PTEN expression.

Ogino and colleagues (13) investigated the role of PIK3CA mutations in codon 9 and 20 on the outcome of stage III Union