Optimal selection of endocrine therapy for women with estrogen receptor–positive breast cancer will require biomarkers with better positive predictive value than the current immunohistochemical assay. There has been great interest to employ genomic technology to identify the subset of women with estrogen receptor–positive breast cancers who benefit from hormonal agents, and from those studies we consistently realize the importance of study design for discovery and validation.

Translation of a predictive biomarker into a diagnostic test requires well-designed clinical trials for proof of benefit. The most common design is to empirically discover biomarkers from archival tumor samples with annotated survival after monotherapy and to then measure the predictive accuracy in an independent cohort. This conveniently uses existing samples and known clinical outcome but also has obstacles relating to sample size, unseen bias, availability and usability of archival samples, and translation of technology from a broad discovery platform to a focused test. The clinical context of treatment, however, presents a potentially greater challenge. Adjuvant hormonal therapy is administered over a period of years and may then be switched to a different hormonal agent. Treatment often follows adjuvant chemotherapy, making it difficult to determine the survival benefit from different treatments. Those who receive only hormonal therapy usually have early-stage breast cancer; therefore, adjuvant studies cannot distinguish treatment responders from those who would have survived from surgery alone. Finally, because survival differences between estrogen receptor–positive and estrogen receptor–negative breast cancers are time dependent, the duration of study follow-up affects the results. Whereas neoadjuvant studies do circumvent the uncertainties of follow-up, the primary end point of pathologic complete response is infrequent and it is difficult to translate a prediction of clinical response after a few months into the adjuvant setting. The development of a HOXB13/IL17BR gene expression ratio illustrates the complexity of developing a predictive biomarker for hormonal therapy.

The ratio of HOXB13/IL17BR gene expression was first derived from microarrays of 60 frozen samples of estrogen receptor–positive breast cancer based on their association with distant relapse after adjuvant tamoxifen therapy. In the original microarray data, this two-gene ratio was independently associated with distant relapse-free survival (odds ratio, 7.3; 95% confidence interval, 2.1-26.3) in a multivariate model that included other prognostic factors (1). The investigators also developed a real-time reverse transcription-PCR assay to measure the expression of both genes relative to a housekeeping gene and showed that the reverse transcription-PCR assay provided equivalent results in both frozen and formalin-fixed paraffin-embedded tissue samples. A pilot validation was then conducted in 20 selected formalin-fixed paraffin-embedded samples (10 patients relapsed, 10 did not) and yielded promising results (1). That set the scene for a larger study [presented in part by Sgroi et al. at American Society of Clinical Oncology 2004 (2) and published in full by Goetz et al. (3) in this issue] in which the two genes were measured using reverse transcription-PCR in 206 archival primary tumor samples from a completed multicenter clinical trial. The ratio of HOXB13/IL17BR expression was compared with survival after adjuvant tamoxifen treatment (3).

In the interim, there has been a dialogue of published letters and reports about this two-gene ratio in estrogen receptor–positive breast cancer. A letter from the authors clarified that 19 of the 20 formalin-fixed paraffin-embedded samples were from lymph node–negative breast cancers (4). Meanwhile, a different group tested the ratio using reverse transcription-PCR of HOXB13, IL17BR, and a housekeeping gene, but in 58 frozen samples and using primers that recognize a different region of each gene transcript (5). The ratio of HOXB13/IL17BR expression had no significant association with distant relapse in this second study (5). Seventy-seven percent of the patients in the second study, however, had node-positive breast cancer. The accompanying editorial discussed in detail that sample size, study bias, different assay methods, and lack of independent validation limit the ability to reliably interpret such phase II diagnostic studies (6). A third study from Jansen et al. (7, 8) tested the ratio of HOXB13/IL17BR gene expression from microarrays of 112 frozen primary estrogen receptor–positive breast cancer samples (nodal status not stated) and reported a significant association with objective response (versus progressive disease) when the women had relapsed and were treated with first-line tamoxifen therapy (area under the curve, 0.612; P = 0.04). These studies were small, used different assays, and addressed different stages of breast cancer. In addition, the biological relevance of this two-gene expression ratio from the original primary tumor sample in patients with relapsed disease is unknown (1, 7–9).

The current study is not seen as an independent validation of the original two-gene ratio test because the assay and the methods of interpretation were changed substantially to accommodate archival formalin-fixed paraffin-embedded...
samples. The investigators used different PCR primer sequences to recognize HOXB13 and IL17BR for this current study to identify sequence near the poly(A) tail (3’ end) where reverse transcription reaction begins (3). That reduces the amount of target sequence lost due to transcript degradation or formalin fixation in archival samples. In this study, a control gene was not measured and the direct ratio of expression was recorded. Consequently, the ratio values are different from the previous study; therefore, new thresholds were optimized according to nodal status because those distributions barely overlapped. To account for the prior optimization of thresholds in the same data, the investigators calculated Faraggi and Simon cross-validation estimates of odds from Cox regression analyses of survival. Quoting results from the multivariate analysis after cross-validation, the ratio of HOXB13/IL17BR expression in 130 node-negative samples identified a significant association with overall survival (odds ratio, 2.01; 95% confidence interval, 1.02-3.99; \( P = 0.045 \)), but not with disease-free survival or relapse-free survival. It seems likely that this two-gene ratio has an association with survival in tamoxifen-treated women with node-negative breast cancer, but independent validation using an identical assay and thresholds is still needed to confirm and estimate the effect of HOXB13/IL17BR ratio on survival. Even then, we cannot learn from adjuvant treatment whether the two-gene ratio test has prognostic, tamoxifen-predictive, or combined power in node-negative breast cancer.

The ratio of HOXB13/IL17BR expression was higher in 76 node-positive women and had no significant association with survival (3). This might confirm the negative result reported by Reid et al. (5), but unfortunately, the gene expression values from the two studies are not comparable. Higher values in node-positive tumors might occur if the ratio of HOXB13/IL17BR expression increases during disease progression. If the ratio is strongly prognostic, however, then tumors with higher values might be more frequently node positive. Conversely, a strong predictive test for tamoxifen response should have more power to separate tamoxifen responders from nonresponders in a node-positive cohort who are at otherwise greater risk of relapse if they do not obtain any benefit from the adjuvant tamoxifen therapy.

The current generation of genomic assays for tamoxifen sensitivity all contain a combination of predictive and prognostic information. Ideally, we could assess prognosis and endocrine sensitivity independently and report those results separately to guide distinct clinical decisions. It is difficult, however, to dissect endocrine sensitivity from inherent prognosis because estrogen receptor expression and the state of tumor differentiation are related. A spectrum of differentiation among estrogen receptor–positive breast cancers is illustrated by the genomic tests that combine the measurements of gene expression related to estrogen receptor, HER2, and proliferation. An alternative strategy is to measure the transcriptional output from estrogen receptor activity to assay endocrine sensitivity. Epigenomic and proteomic strategies are also being developed. Time will eventually tell which of these strategies were successfully translated into clinically robust tests.

One has to be concerned that the impetus to adapt genomic (and soon proteomic) assays for archival formalin-fixed paraffin-embedded samples to conduct validation studies from older trials leads to an unnecessary compromise of the potential sensitivity and accuracy of assay measurements. A further concern is that we would then be locked by regulation into only using that adapted assay for prospective clinical testing. It is unrealistic to expect that optimal molecular measurements should always be obtained from formalin-fixed paraffin-embedded tissue blocks. Demand for molecular diagnostic tests to guide specific treatment decisions will continue to increase, and we are obliged to reconsider our methods of handling clinical tissue samples. It is long accepted that to characterize lymphoma, one must obtain extra samples specifically for molecular testing. We should learn to do likewise to accurately assess prognosis and select treatment for women with invasive breast cancer.

References
Genomic Testing for Sensitivity of Breast Cancer to Hormonal Therapy

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