Microarray-Based Determination of Estrogen Receptor, Progesterone Receptor, and HER2 Receptor Status in Breast Cancer

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Abstract

Purpose: The level of estrogen receptor (ER), progesterone receptor (PR), and HER2 aids in the determination of prognosis and treatment of breast cancer. Immunohistochemistry is currently the predominant method for assessment, but differences in methods and interpretation can substantially affect the accuracy, resulting in misclassification. Here, we investigated the association of microarray-based mRNA expression levels compared with immunohistochemistry.

Experimental Design: Microarray mRNA quantification of ER, PR, and HER2 was done by the developed TargetPrint test and compared with immunohistochemical assessment for breast tumors from 636 patients. Immunohistochemistry was done in a central laboratory and in an independent reference laboratory according to American Society of Clinical Oncology/College of American Pathologists guidelines for 100 cases. For HER2 immunohistochemistry 2+ cases, additional chromogenic in situ hybridization (CISH) was used to determine the final status.

Results: ER concordance between microarray and central immunohistochemistry was 93% [95% confidence interval (95% CI), 91-95%]. Only 4% of immunohistochemistry-positive samples were classified negative using microarray, whereas 18% of immunohistochemistry-negative samples showed a positive microarray ER status. Concordance for PR was 83% (95% CI, 80-86%) and 96% of all samples showed an identical classification of HER2 status by microarray and immunohistochemistry/CISH (95% CI, 94-98%). Nine percent of immunohistochemistry HER2-positive samples showed a negative microarray classification. Detailed review of 11 cases with discordant classifications by American Society of Clinical Oncology/College of American Pathologists and central immunohistochemistry indicated that microarray assessment was likely to add additional information in 5 cases.

Conclusion: Microarray-based readout of ER, PR, and HER2 shows a high concordance with immunohistochemistry/CISH and provides an additional, objective, and quantitative assessment of tumor receptor status in breast cancer. (Clin Cancer Res 2009;15(22):7003–11)

Breast cancer is the most common malignancy in women. The level of estrogen receptor (ER) and progesterone receptor (PR) is prognostic in early-stage breast cancer patients and predictive for response to tamoxifen and other hormonal therapies (1–4). HER2 overexpression and/or amplification is an additional prognostic molecular marker (5) indicated for selection of patients for trastuzumab or other HER2-targeted therapies (6, 7). Accordingly, primary breast carcinomas should be tested for these three molecular markers for optimal diagnosis and treatment selection for the patient.

Immunohistochemistry is currently the predominant method for determination of ER, PR, and HER2 status (8), often with additional in situ hybridization assays to clarify HER2 immunohistochemical results (9). Despite its long-term use, immunohistochemical assays have not been standardized...
Translational Relevance

In this study, we have examined the association of the expression of the estrogen receptor (ER), progesterone receptor (PR), and HER2 genes as measured by microarray analysis with the expression of the proteins encoded by these genes as determined by immunohistochemistry. We show that there is a very high correlation between the expression of these genes at the mRNA level and the expression of the proteins as quantified by immunohistochemistry. These findings are relevant to clinical practice, as determination of ER, PR, and HER2 plays a very important role in guiding systemic therapy in individual breast cancer patients. Because it is known that there is marked variability in the outcome of the determination of ER, PR, and HER2 among pathology laboratories, with relatively high false-positive and false-negative testing rates, microarray-based assessment of these genes could be used for the determination of ER, PR, and HER2 status.

Materials and Methods

Tumor samples. Previously determined microarray gene expression measurements of ER, PR, and HER2 in 295 breast cancer samples (24) were used for training and primary validation of microarray readout (cohort 1, Table 1). One hundred samples were randomly assigned as training samples and the remaining 195 samples were used for validation. Two additional cohorts (cohorts 2 and 3, Table 1) were selected for validation of microarray readout. Cohort 2 consisted of early-stage breast cancer patients. One hundred samples were randomly assigned as training and primary validation of microarray readout measurements of ER, PR, and HER2 in 295 breast cancer samples (24) of the 636 samples that were used for this study, we could successfully determine ER/PR and HER2 status using both immunohistochemistry and microarray for 575 and 567 samples, respectively.

Immunohistochemistry and CISH/FISH. For all specimens, ER, PR, and HER2 status were assessed by analyzing the expression of these proteins using immunohistochemistry. Tissue microarrays were constructed from paraffin blocks with breast tumor samples that belong to patient cohorts 1 and 2 by a manual tissue arrayer (Beecher Instruments). Core tissue biopsies of 600 μm cores were taken from each individual paraffin-embedded tumor and arrayed in triplicate in a new paraffin block. Serial sections of 3 μm were cut from the tissue microarray blocks, deparaffinized in xylene, and hydrated in a graded series of alcohol. Immunohistochemistry of all samples was centrally performed by the pathology laboratory of the Netherlands Cancer Institute using the Lab Vision Immunohistochemical Autostainer with primary antibodies against ER-α (1D5 + 6F11, dilution 1:50; Neomarkers, Lab Vision), PR (R-1, dilution 1:500; Klinpath), and HER2 (cohorts 1 and 2: antibody 3B5, dilution 1:3,000; cohorts 2 and 3: antibody SP3, dilution 1:25; ref. 29). Immunohistochemical analysis of ER, PR, and HER2 for cohort 3 was done on individual paraffin-embedded tumor slides at the originating hospital according to local standards (28), at a central laboratory (Netherlands Cancer Institute), and at an independent College of American Pathologists (CAP)–accredited U.S. reference laboratory using Food and Drug Administration–approved procedures and American Society of Clinical Oncology (ASCO)/CAP guidelines (Table 1).

Central laboratory scoring of the immunohistochemical staining was done by an experienced pathologist (M.J.V.). ER and PR were determined based on the percentage of tumor cells showing positive nuclear staining and were considered positive if nuclear staining was present in ≥10% of the cells according to Dutch guidelines.7 HER2 expression was scored as follows: 0 for no staining at all or membrane staining in <10% of the tumor cells, 1+ for a faint/barely perceptible partial membrane staining in ≥10% of the tumor cells, 2+ for weak to moderate complete membrane staining in ≥10% of the tumor cells, and 3+ for strong complete membrane staining in ≥10%. HER2 was considered positive if the score was ≥3+. HER2 status was evaluated according to commonly accepted pathologic guidelines (30). A tumor was considered to be HER2-positive when a score of 3+ was found and negative when a score of 0 or 1+ was observed. Tumors with a score of 2+ were evaluated by CISH; tumors with HER2 gene amplification (>6 spots per tumor cells) were scored as HER2-positive and all other tumors as HER2-negative.

ASCO/CAP reference scoring was done consistent with good clinical practice guidelines in a good laboratory practice–approved laboratory. Receptor testing was done using Ventana ultraView Universal DAB kit (Ventana Medical Systems), Vision Biosystems (Novocasta) antibodies for ER (clone 6F11) and PR (clone 16), and DAKO Herceptest for HER2, all of which are Food and Drug Administration cleared for in vitro diagnostic use. ER and PR results are reported as positive or negative, and % positive in 10% increments and stain intensity are given. Any positive staining of tumor nuclei is considered a positive result, but cases with minimal staining are reported as ≤5% staining. HER2 immunohistochemistry is reported as 0, 1, 2, 3+ according to CAP guidelines (membrane staining only). Samples with a score of 2+ were evaluated by FISH and reported as positive for HER2/CEP17 > 1.80.

For 536 of the 636 samples reported in this study central immunohistochemistry was done using tissue microarray (cohort 1, n = 295 and cohort 2, n = 241). ER and PR status could successfully be assessed by tissue microarray immunohistochemistry for 89% of the samples and HER2 immunohistochemistry/CISH could successfully be performed for 88% of all tissue microarray cases (Table 1). The 100 samples within approximately two-thirds hormone-positive samples and one-third HER2-positive samples. For all cohorts, tumor samples were assessed histologically to confirm invasive ductal carcinoma or invasive lobular carcinoma and the presence of sufficient tumor cells. Of the 636 samples that were used for this study, we could successfully determine ER/PR and HER2 status using both immunohistochemistry and microarray for 575 and 567 samples, respectively.
cohort 3 were selected based on a successful local immunohistochemical assessment (and selected to include approximately two-thirds hormone-positive samples and one-third HER2-positive samples) and were subsequently scored for immunohistochemistry by a central and an ASCO/CAP laboratory using whole sections instead of tissue microarray. Success rates of central and CAP immunohistochemistry were 96% and 87% for ER/PR scoring and 96% and 85% for HER2 assessment, respectively. The main cause of failure of central and CAP immunohistochemistry was to the unavailability of paraffin-embedded samples (local, central, and ASCO/CAP) and TargetPrint microarrays (Table 1). Microarray readout of ER, PR, and HER2 by TargetPrint.

**Table 1.** Overview of sample sets used for development of ER, PR, and HER2 readout by immunohistochemistry (local, central, and ASCO/CAP) and TargetPrint microarrays

<table>
<thead>
<tr>
<th>Total</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>295</td>
<td>241</td>
<td>100</td>
<td>636</td>
</tr>
<tr>
<td>ER</td>
<td>Immunohistochemistry</td>
<td>Tissue microarray</td>
<td>Whole sections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>286</td>
<td>193</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>ASCO/CAP</td>
<td>-</td>
<td>-</td>
<td>87</td>
</tr>
<tr>
<td>TargetPrint</td>
<td>295</td>
<td>241</td>
<td>100</td>
<td>636</td>
</tr>
<tr>
<td>Train</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Validation</td>
<td>186</td>
<td>193</td>
<td>96</td>
<td>475</td>
</tr>
<tr>
<td>PR</td>
<td>Immunohistochemistry</td>
<td>Tissue microarray</td>
<td>Whole sections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local</td>
<td>-</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>286</td>
<td>193</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>ASCO/CAP</td>
<td>-</td>
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<td>TargetPrint</td>
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<tr>
<td>Train</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Validation</td>
<td>186</td>
<td>193</td>
<td>96</td>
<td>475</td>
</tr>
<tr>
<td>HER2</td>
<td>Immunohistochemistry/CISH</td>
<td>Tissue microarray</td>
<td>Whole sections</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Local</td>
<td>-</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Central</td>
<td>273</td>
<td>198</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>ASCO/CAP</td>
<td>-</td>
<td>-</td>
<td>85</td>
</tr>
<tr>
<td>TargetPrint</td>
<td>295</td>
<td>241</td>
<td>100</td>
<td>636</td>
</tr>
<tr>
<td>Train</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Validation</td>
<td>173</td>
<td>198</td>
<td>96</td>
<td>467</td>
</tr>
</tbody>
</table>

**NOTE:** "Immunohistochemistry tissue" indicates the type of tissue that was used for immunohistochemical assessment: tissue microarrays or whole sections.

Statistical analysis. Statistical analysis was done in R/Bioconductor. Correlation of continuous microarray readout with immunohistochemical assessment was determined using Pearson correlation and linear fit models. Agreement measurements between binary microarray and immunohistochemical classifications were based on two-way contingency table analysis and included overall concordance, positive agreement defined as the number of samples classified positive by both immunohistochemistry and microarray divided by the number of positive samples using immunohistochemistry, negative agreement, and Cohen’s κ coefficient scores (33). All measurements were associated with 95% confidence intervals (95% CI). Statistical tests were considered significant for \( P < 0.05 \).

Results

ER, PR, and HER2 microarray readout of 100 training samples selected from cohort 1 were compared with central immunohistochemical determination. Optimal microarray thresholds for ER, PR, and HER2 with the lowest discordance to immunohistochemistry/CISH were determined and classified 76% and 63% of the samples as ER-positive and PR-positive, respectively, and 22% as HER2-positive (Fig. 1). Compared with immunohistochemistry, microarray readout showed a concordance of 88% (95% CI, 81-92%) for ER, 85% (95% CI, 77-91%) for PR, and 95% (95% CI, 89-97%) for HER2.

Microarray readout was validated on the remaining samples within cohort 1 and cohorts 2 and 3 for which the corresponding receptor status could successfully be determined by immunohistochemistry/CISH as well as by microarray (\( n = 475 \) for ER and PR and \( n = 467 \) for HER2; Table 1). Microarray measurements correlated strongly with central immunohistochemical determination (\( r = 0.77, P < 0.0001 \), Fig. 2A; PR: \( r = 0.61, P < 0.0001 \), Fig. 2B; and HER2: \( r = 0.76, P < 0.0001 \), Fig. 2C) with a significant difference in readout between immunohistochemistry-negative and immunohistochemistry-positive samples.
samples (ER: -0.232 versus 0.414, P < 0.0001; PR: -0.084 versus 0.314, P < 0.0001; and HER2: -0.372 versus 0.473, P < 0.0001). Thus, 79%, 64%, and 22% of the validation samples were classified positive for ER, PR, and HER2 by microarray, respectively.

Concordance between microarray readout by TargetPrint and central immunohistochemistry was high (Table 2). ER assessment showed a concordance of 93%, with a \( \kappa \) score of 0.79 (95% CI, 0.72-0.85). Positive agreement was 95% (95% CI, 94-96%) and negative agreement was 85% (95% CI, 80-90%). Only 15 of 373 (4%) samples that were scored ER-positive by immunohistochemistry were negative using microarray, whereas, interestingly, 18 of 102 (18%) samples classified negative by immunohistochemistry showed a positive microarray ER status. PR assessment showed a concordance of 83% (95% CI, 80-86%) with a \( \kappa \) score of 0.65 (95% CI, 0.58-0.71; Table 2). About one-quarter (26%) of immunohistochemistry PR-negative samples showed positive microarray readout. Concordance between microarray and immunohistochemistry for HER2 was very high (96%; 95% CI, 94-98%) with a \( \kappa \) of 0.88 (Table 2), a positive agreement of 90% (95% CI, 84-93%), and an agreement for HER2-negative cases of 98% (95% CI, 97-99%). Three percent of the samples classified as HER2-negative by immunohistochemistry were scored positive by microarray readout. On the other hand, 9% of samples that were classified positive for HER2 by immunohistochemistry had a negative readout by microarray. Interestingly, within HER2 immunohistochemistry 2+ cases, microarray identified HER2 CISH-positive and HER2 CISH-negative samples in a high proportion of cases (accuracy of 87%, \( \kappa = 0.72 \); Supplementary Data 1).

Next, we compared the ER, PR, and HER2 status of cohort 3 samples (Table 1) that were classified in duplicate by TargetPrint microarrays and in triplicate by immunohistochemistry according to local, central, and ASCO/CAP standards. Samples were preselected for a relatively high concordance between local and central immunohistochemistry (\( > 90\% \) for ER and HER2). Duplicate microarray analysis showed a very high concordance (Table 3). Comparison of microarray results with immunohistochemistry (including CISH for HER2) performed according to
the three different standards indicated similar concordances of 92%, 93%, and 92% for ER; 84%, 81%, and 86% for PR; and 93%, 95%, and 94% for HER2 (Table 3). Discordance between microarray and ASCO/CAP immunohistochemistry was very low for ER (8%) and HER2 (6%) and low for PR (14%) and was comparable with the misclassification rate among the three immunohistochemical laboratories (local, central, and ASCO/CAP).

ASCO/CAP reference immunohistochemistry used a threshold of 1% positive tumor cells compared with the 10% threshold for positive staining on which the TargetPrint readout was developed. However, none of the observed discordance in cohort 3 was due to the use of different criteria for immunohistochemical determination, as none of the samples scored by ASCO/CAP showed a staining between 1% and 10%.

We further investigated if the discordance between microarray and central immunohistochemistry of all analyzed samples in cohorts 1, 2, and 3 could be explained by the difference in the cutoff for positive versus negative (1% and 10% positivity). The 18 ER discordant samples that were classified positive by microarray readout but negative by immunohistochemistry using a cutoff of 10% (18 of 376 samples) were all scored by immunohistochemistry as 0%. Therefore, this discordance could not be explained by a difference in ER immunohistochemical threshold. Of the 53 samples that were scored positive for PR by microarray but negative by immunohistochemistry >10%, 10 samples showed staining between 1% and 5% and could be reported positive for immunohistochemistry with a threshold of 1%, thereby lowering the false-positive rate for PR from 18% to 14%.

Finally, we checked whether the discordance rate between immunohistochemistry and microarray could partially be explained by the differences in immunohistochemical determination using tissue microarrays (cohorts 1 and 2) versus using whole sections for antibody staining (cohort 3). Concordance rates of tissue microarray versus microarray and whole sections versus microarray were high with 0.93 (353/379) and 0.95 (318/379) for ER classification and 0.84 (318/379) and 0.80 (77/96) for PR classification, respectively, indicating that use of tissue microarray or whole sections had no effect on the observed discordance.

To take a closer look at the discrepancies between microarray readout and immunohistochemistry and to investigate which

Table 2. Contingency table of microarray readout by microarray and central immunohistochemistry of ER, PR, and HER2 on all validation samples

<table>
<thead>
<tr>
<th></th>
<th>Central immunohistochemistry +</th>
<th>Central immunohistochemistry -</th>
<th>Total</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER Microarray +</td>
<td>358</td>
<td>18</td>
<td>376</td>
<td>Con    93%</td>
</tr>
<tr>
<td>ER Microarray -</td>
<td>15</td>
<td>84</td>
<td>99</td>
<td>κ       0.79</td>
</tr>
<tr>
<td>PR Microarray +</td>
<td>247</td>
<td>53</td>
<td>300</td>
<td>Con    83%</td>
</tr>
<tr>
<td>PR Microarray -</td>
<td>26</td>
<td>149</td>
<td>175</td>
<td>κ       0.65</td>
</tr>
<tr>
<td>HER2 Microarray +</td>
<td>85</td>
<td>10</td>
<td>95</td>
<td>Con    96%</td>
</tr>
<tr>
<td>HER2 Microarray -</td>
<td>8</td>
<td>364</td>
<td>372</td>
<td>κ       0.88</td>
</tr>
</tbody>
</table>
method gave more reproducible results, we performed a
detailed second review of several discordant samples between
TargetPrint and central immunohistochemistry. For this analysis,
we restricted ourselves to discordance within cohort 3 as immu-
nohistochemistry for this cohort was based on complete tumor
slides (tissue microarray were used for cohorts 1 and 2) and be-
cause these samples were also assessed by an independent
ASCO/CAP-certified reference laboratory. Sample review was
based on three additional determinations of ER and HER2: a
new readout by TargetPrint microarray, an assessment by the
ASCO/CAP reference laboratory using new tissue sections, and
also by a blindly performed rescoring of the centrally performed
immunohistochemistry, which, if the result of the rescoring
remained discordant, was restained and again rescored blindly.
Eleven discordant cases were analyzed, 6 with mismatching ER
and 5 with mismatching HER2 readouts (Table 4).
Reassessment of all selected samples by microarray indicated a
similar result for all samples and suggested reliable detection
of ER and HER2 RNA levels within the tumor samples. Review of
the six ER discordant cases based on ASCO/CAP and central
immunohistochemistry indicated that, for two samples (#26
and #30), the original immunohistochemical determination
was likely inaccurate as immunohistochemical review of both
indicated a different outcome (although sample #26 remained
borderline ER-positive). Secondary review of one sample (#92)
showed no clear result as the ER remained around the borderline
(0%, 5%, and 10%). For the remaining three samples (#57, #74,
and #92), immunohistochemistry and microarray remained dis-
cordant. Of the five selected HER2 discordant cases, three sam-
ples (#48, #62, and #94) remained discordant between
microarray and immunohistochemistry/FISH. Interestingly, al-
though sample #94 remained discordant after review, it showed
borderline microarray negativity (-0.07 and -0.03) and border-
line central immunohistochemistry positivity (immunohisto-
chemistry 2+, CISH-positive). HER2 review of samples #27 and
#30 indicated that the original assessment (HER2-negative)
might have generated false-negative results. However, for these
samples, either the microarray readout (sample #30) or the im-
munohistochemistry/FISH assessment (sample #27) remained
questionable. Although the number of samples used for the de-
tailed review was limited, this analysis indicates that microarray
readout provides additional information regarding ER and
HER2 status in about one-half (5 of 11) of the discordant
cases. Interestingly, it also suggests that microarray as well as

<table>
<thead>
<tr>
<th>Sample</th>
<th>Original Central immunohistochemistry</th>
<th>Central CISH</th>
<th>Microarray Microarray</th>
</tr>
</thead>
<tbody>
<tr>
<td>#26 ER</td>
<td>60%</td>
<td>-0.23</td>
<td>-0.24</td>
</tr>
<tr>
<td>#30 ER</td>
<td>0%</td>
<td>0.62</td>
<td>0.60</td>
</tr>
<tr>
<td>#57 ER</td>
<td>0%</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>#62 ER</td>
<td>100%</td>
<td>-0.47</td>
<td>-0.44</td>
</tr>
<tr>
<td>#74 ER</td>
<td>50%</td>
<td>-0.41</td>
<td>-0.41</td>
</tr>
<tr>
<td>#92 ER</td>
<td>10%</td>
<td>-0.20</td>
<td>-0.20</td>
</tr>
<tr>
<td>#27 HER2</td>
<td>1+</td>
<td>0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>#30 HER2</td>
<td>0</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>#48 HER2</td>
<td>2+ Positive</td>
<td>-0.12</td>
<td>-0.19</td>
</tr>
<tr>
<td>#62 HER2</td>
<td>0</td>
<td>0.53</td>
<td>0.57</td>
</tr>
<tr>
<td>#94 HER2</td>
<td>2+ Positive</td>
<td>-0.07</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

NOTE: Assessments resulting in a positive receptor status are shown in bold.

Table 4. Review of 11 discordant cases with mismatching ER or HER2 microarray and central immunohistochemistry readout by additional microarray readout (review 1), by independent CAP immunohistochemistry/FISH assessment (review 2), and by restaining and/or rescoring of centrally performed immunohistochemistry (review 3)
immunohistochemistry-based determinations might be correct for discordant cases, as both techniques generally result in reproducible outcomes.

**Discussion**

In this study, we investigated the use of microarray-based readout of three biomarkers that are crucial for treatment decisions in breast cancer, that is, ER, PR, and HER2 (1, 2, 5, 7). Currently, immunohistochemistry is the standard for determination of ER, PR, and HER2 status (34). However, despite the long practice of immunohistochemistry, procedural inconsistency remains high in clinical settings, leading to intralaboratory and interlaboratory variation and to high false-negative (for ER and PR) and false-positive (for HER2) classifications (18, 19). Although the ASCO/CAP guidelines for HER2 assessment have recently been published (22) and those for ER/PR testing are in development, the inconsistency in immunohistochemical determination recommends an additional, reliable, testing method to assess hormone and HER2 receptor status.

Here, we present another method for determination of ER, PR, and HER2 levels in breast cancer that is based on microarray analysis. Microarray-based readout shows a high concordance with multiple immunohistochemistry as well as CISH/FISH assessments, performed according to ASCO/CAP standards, and is based on controlled and objective quantification of receptor mRNA levels. TargetPrint microarray analysis is performed centrally in a highly qualified and Clinical Laboratory Improvement Amendments-certified microarray laboratory (31) and uses fresh (stored in a RNA-stabilizing solution) or frozen tumor tissue from which high-quality RNA can be obtained. The microarray assay has been proven to give reliable quantification of ER, PR, and HER2 levels for samples with sufficient (≥50%) tumor cells.

The high level of concordance between microarray readout and immunohistochemistry/CISH reported here (93% for ER and 96% for HER2) indicates that microarray-based readout is a reliable addition to current techniques. Our findings are in agreement with a study by Gong and colleagues, who investigated the use of Affymetrix microarrays for quantification of ERα and HER2 mRNA levels and reported an overlap of 92% with ER immunohistochemical measurement and 90% with HER2 immunohistochemistry/CISH assessment (35). Interestingly, a recent study by Badve and colleagues reported a similar lower concordance between PR mRNA levels and immunohistochemistry (36) as observed in our study. As their study used quantitative reverse transcription-PCR for PR detection instead of microarray, the lower PR concordance might not be caused by technical difficulties in one technique (e.g., low sensitivity) but could reflect real differences between RNA and protein levels. The possible occurrence of a tumor subgroup that does not express protein despite the presence of mRNA transcripts is supported by our finding that, for ER and PR, we observed a higher proportion of cases that were immunohistochemistry-positive/microarray-positive (18% and 26%) than immunohistochemistry-positive/microarray-negative (4% and 9%).

In this study, a tumor sample was classified positive by immunohistochemistry for ER and PR when at least 10% of tumor cells showed positive staining. Consensus regarding the accurate cutoff for ER and PR positivity is considered to be the ≥10% threshold (37). However, Harvey and colleagues reported good outcomes after adjuvant tamoxifen treatment for tumors when a lower number of cells stained positive (8). Additional support for the use of a 1% cutoff was provided by the study of Viale and colleagues who indicated a better prognosis using this threshold and some degree of endocrine responsiveness for lower staining tumors (18). We have investigated whether TargetPrint readout also validates using a cutoff of 1% and found no difference in performance for ER when a 1% threshold was used instead of a 10% threshold and a minor increase (+4%) in performance of PR using a 1% threshold for immunohistochemistry positivity. Although ER/PR-positive cases present on the same tissue microarray can be considered as positive controls, a limitation of the immunohistochemical assessment of ER and PR in this study is that we did not separately record ER and PR staining in normal epithelial cells of adjacent breast tissue in cases where the breast carcinoma was ER-negative and/or PR-negative.

Currently, there remains a controversy about the relationship of measured ER/PR immunohistochemical levels and the ER/PR protein present in the tumor cells. Whereas older ligand-binding assays indicate a continuum of values, formalin-fixed, paraffin-embedded–based immunohistochemistry generally shows a bimodal distribution instead of a broad range of values among ER-positive patients (38). In line with this controversy, the immunohistochemical methods reported in this study classified the great majority of breast tumor samples with either 0% or 100% ER positivity. Interestingly, mRNA levels measured by microarray (this study and ref. 35) or by quantitative PCR (36) show a large dynamic range within the immunohistochemistry 0% and 100% groups. The same holds true for HER2 determination in which immunohistochemistry 0 and immunohistochemistry 3+ samples show a range of HER2 gene expression levels. These observations indicate a continuum of mRNA levels of ER, PR, and HER2 within positively and negatively stained samples by immunohistochemistry. Such quantitative mRNA-based measurement, in addition to the immunohistochemical assessment, may provide clinically important prognostic and predictive information.

An important issue in studies comparing different methods for the assessment of ER, PR, and HER2 is the gold standard to which the test results are compared. The ER, PR, and HER2 status is mainly assessed to predict tumor responsiveness to hormonal therapy and to anti-HER2 therapy; however, responsiveness to these treatments can only be clinically evaluated in neoadjuvant treatment or treatment of metastatic disease. For the patients in our study (and in most other comparable studies), information on treatment response is not available. We can therefore only speculate on what the most likely “true” ER, PR, and HER2 status is for cases where there is a discrepancy between the microarray test result and immunohistochemistry (for HER2 combined with CISH/FISH).

An additional potential complication of these comparisons is that both immunohistochemistry and microarray rely on the presence of protein or mRNA, but neither assay determines whether that protein or mRNA is capable of making functional receptor proteins. As such, both methods have an inherent

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* R. Bender, personal communication.
uncertainty in predicting whether a tumor is truly positive for functional ER, PR, or HER2 protein. Efforts have been made to circumvent this problem by developing assays to measure ER functionality (39). The development of additional gene signatures that reflect ER/PR positivity (40, 41) may provide another method to test for ER functionality in tumor cells. It may also be worthwhile to ask if such target gene signatures for ER as well as for HER2 are concordant with immunohistochemistry and microarray readout of ER and HER2 activity in breast cancer.

It appears that an important benefit of microarray readout will be to further develop methods to reduce false-positive and false-negative results. In this study, only 4% of all immunohistochemistry ER-positive samples were classified negative by microarray of which about one-half showed <50% positively stained cells. In contrast, 18% of immunohistochemistry ER-negative samples were classified positive by microarray. For PR, this number increases to about one in every four immunohistochemistry-negative samples. This relatively large group of immunohistochemistry classified ER/PR-negative patients might potentially benefit from hormone-targeted therapies. For HER2, very few (3%) of the samples that were classified negative by immunohistochemistry showed a positive microarray readout. However, ∼10% of the immunohistochemistry/CISH HER2-positive samples were negative by microarray readout. This group of samples might represent patients that are proven immunohistochemistry HER2-positive but who may not respond to HER2-targeted therapies such as trastuzumab (42). A detailed review of discordant cases suggests a real difference between mRNA expression and measured protein levels for the majority of the these particular tumor specimens in which multiple immunohistochemical assessments remained discordant with duplicate microarray readouts. Conclusions as to which method gives the most valuable readout with respect to patient outcome and treatment response remain to be determined in future studies that include treatment response information for hormone receptor–based treatment (e.g., tamoxifen and ER/PR status) and HER2-targeted therapy (e.g., trastuzumab).

We suspect that the principal clinical benefit of microarray readout will be to resolve the discordance between current methods for HER2 (e.g., CISH versus immunohistochemistry), to reduce the number of false-negative classifications for hormone receptor status and false-positive readouts of HER2 status, and to further evaluate tumors that are classified as ER-negative/PR-positive, tumors classified as ER-positive/PR-negative, and tumors classified as triple-negative (ER, PR, and HER2 negative). The developed microarray-based readout of ER, PR, and HER2 provides an objective and quantitative assessment of tumor receptor status in breast cancer. Use of microarray receptor readout in addition to current techniques may improve molecular classification of breast cancer and provide an additional rationale for hormonal and HER2-targeted therapies and clinical trial design. The MINDACT trial currently under way at >70 centers across Europe may help to answer some of these questions as both immunohistochemistry and microarray data on ER, PR, and HER2 are being obtained prospectively and correlated with the results of adjuvant endocrine therapy in over one-half of the 6,000 patients being enrolled. These results will go a long way to helping establish whether immunohistochemistry or microarray expression results correlate better with treatment outcomes.

Disclosure of Potential Conflicts of Interest

P. Roepman, O. Krijgsman, R. Bender, and A.M. Glas are employees of Agenda.

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