Microarray-based determination of ER, PR and HER2 receptor status: validation and comparison with IHC assessments

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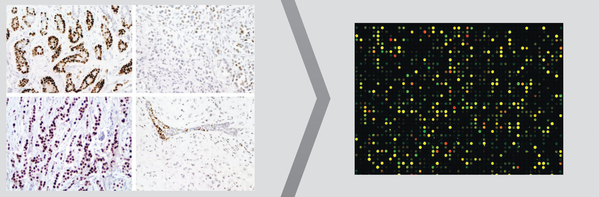
Background

In breast cancer patients the level of expression of estrogen receptor (ER), progesterone receptor (PR) and HER2 is predictive for prognosis and/or treatment response. However, differences in assessment methods and interpretation can substantially affect the accuracy and reproducibility of the results. Previously, we have determined the association between immunohistochemistry (IHC) and mRNA levels for ER, PR and HER2, and have confirmed the accuracy of microarray readout on >450 samples [*ref 1*].

In the current study we describe the use of this microarray based readout on prospectively collected samples. We compared these readouts with multiple IHC and fluorescent in situ hybridization (FISH) assessments generated in various hospitals and a CLIA-certified reference laboratory and developed a microarray based test called TargetPrint™.



Methods

Previously determined microarray thresholds for ER, PR and HER2, which have been validated on >450 samples (Figure 1 and 2) [*ref 1*], were used in this study. Gene expression data for ER, PR and HER2 were obtained by analysis of 100 breast tumors that have been collected prospectively within the RASTER study [*ref 2*]. Samples were analyzed using TargetPrint microarray and IHC assessment was performed (1) according to local standards of the hospital from where the sample originated, (2) by the central laboratory of the Netherlands Cancer Institute, and (3) at an independent reference laboratory using FDA-approved procedures and ASCO/CAP guidelines. A tumor was classified negative for ER and PR when 0% of tumor cells showed positive staining. HER2 IHC status was scored as 0, 1+, 2+ or 3+; a score of 3+ was considered positive. In case of 2+ samples, FISH was performed to assess final HER2 amplification status.

Results

Duplicate microarray readouts were highly reproducible (Pearson correlation 0.991) and resulted in 67, 61 and 39 percent positive samples for ER, PR and HER2, respectively. Comparison of microarray results with IHC (including FISH for HER2) performed at the three centers indicated highly similar results for receptor readout with a concordance of 92, 93 and 92% for ER; 84, 81 and 86% for PR; and 93, 95 and 94% for HER2 (Table 1). Overall misclassification rates between microarray and IHC readout were low for ER (0.08) and HER2 (0.06) and quite low for PR (0.14), and were comparable to the misclassification rates between the three IHC methods.

Microarray readout of ER and PR

Microarray readout of ER and PR has been validated on > 450 breast tumor samples. Gene expression measurements of ER and PR strongly correlated with central IHC (ER: 0.77, R2=0.60, P<0.0001; PR: 0.61, R2=0.37, P<0.0001). Concordance between central IHC and microarray was high for ER (93%, 95%CI: 91% to 95%) and moderately high for PR (83%, 95%CI: 80% to 86%)

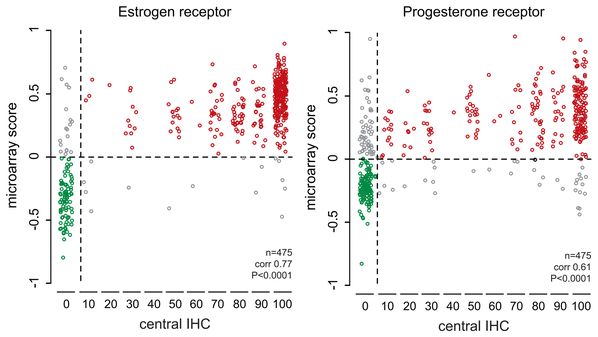


Figure 1

HER2 microarray readout

Microarray readout of HER2 has been validated on > 450 breast tumor samples. HER2 concordance was very high between central IHC and microarray readout (96%, 95%CI: 94% to 98%). Only 3 percent of the samples classified as HER2 negative by IHC were positive with microarray readout. Importantly, microarray readout accurately identified HER2 positive and HER2 negative samples within the IHC 2+ group, for which additional FISH analysis is currently required to determine the final HER2 status.

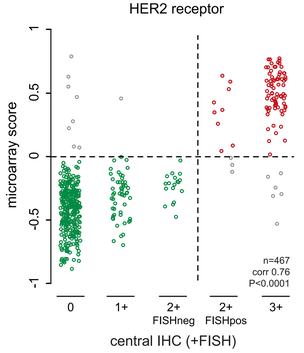
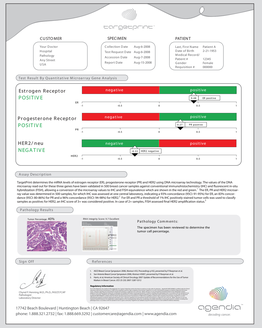


Figure 2

TargetPrint™, a diagnostic microarray

A diagnostic TargetPrint™ '8-pack' array is a single 1"x3" microarray slide with eight subarrays each containing ER, PR and HER2 probes and QC and normalization features. This allows simultaneous analysis of up to 8 samples.



High concordance with local, central and ASCO/CAP IHC

We compared the ER, PR and HER2 status of 100 selected breast tumor samples that were classified in duplicate by TargetPrintTM and by IHC according to (1) a local standard, (2) a central laboratory and (3) by an independent ASCO/CAP certified reference laboratory. Samples were pre-selected specifically for a relative high concordance between local and central IHC (>90% for ER and HER2) compared to typical interlaboratory IHC concordance (between 60 to 80%).

Misclassification rates between microarray and ASCO/CAP IHC readout were very low for ER (0.08) and HER2 (0.06) and low for PR (0.14), and were comparable to the misclassification rates between the three IHC methods

		ER		PR		HER2	
	n	conc	kappa	conc	kappa	conc	kappa
local IHC vs. central IHC	96	0.94	0.86	0.89	0.77	0.96	0.87
local IHC vs. reference IHC	87	0.99	0.97	0.90	0.79	0.94	0.88
local IHC vs. microarray	100	0.92	0.82	0.84	0.67	0.93	0.85
central IHC vs. reference IHC	84	0.94	0.87	0.94	0.88	0.95	0.89
central IHC vs. microarray	96	0.93	0.82	0.81	0.61	0.95	0.89
reference IHC vs. microarray	87	0.92	0.82	0.86	0.72	0.94	0.87
microarray vs. microarray	*88*	*1.00*	*1.00*	*0.97*	*0.93*	*1.00*	*0.98*

conc = concordance; kappa = Kappa score

Table 1

Discordant samples indicate improved classification by TargetPrint

Concordant classifications showed a higher survival rate for patients who were classified as Hormone receptor (HR) positive (ER or PR positive) compared to those who were HR negative (P=0.0001) Similarly, a lower survival rate was observed for patients who were classified as HER2 positive by both IHC and TargetPrint compared to those who were HER2 negative (P=0.001).

For discordant samples between IHC and TargetPrint, microarray based assessment was in better agreement with the concordant classification: HR microarray-negative/IHC-positive samples showed a poor survival rate and HR microarray-positive/IHC-negative showed a good survival rate, whereas HER2 microarray-negative/IHC-positive showed a good survial rate and microarray-positive/IHC-negative samples showed poorer survival.

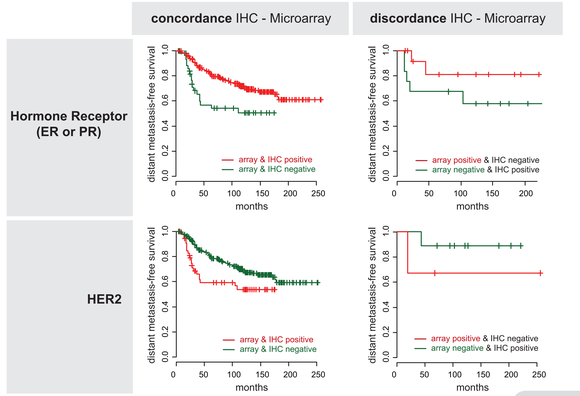


Figure 3

Conclusion

A microarray-based assessment of ER, PR and HER2 relative to mRNA levels gives results comparable to multiple IHC methods and FISH and provides an objective and more quantitative assessment of tumor receptor status than IHC alone. Using TargetPrint™ for microarray readouts for hormone and HER2 receptor gene expression in addition to standard IHC will improve molecular characterization of breast cancer tissue.

References

1. Roepman P, *et al.* Microarray-based readout of ER, PR, and HER2 expression in breast cancer tissue. *ASCO 2008 Breast Cancer Symp, abstract #33, Proceedings page 102.*

2. Bueno-de-Mesquita JM, *et al.* Use of the 70-gene signature to predict prognosis of patients with node-negative breast cancetr: a prospective community-based feasibility study (RASTER). *Lancet Oncol. 2007; 8 (12): p1079-87*