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Use of estrogen receptor (ER) expression by quantitative RT-PCR to identify an ER-negative subgroup by IHC who might benefit from hormonal therapy

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Introduction: Immunohistochemistry (IHC) is a method of choice to establish ER status for the optimal indication of hormonal therapy. ER (*ESR1*) mRNA expression as measured with RT-PCR and DNA microarrays has been reported previously to correlate with IHC results, but the concordance is not complete.

Methods: High-throughput RT-PCR assays were used by the Genomic Health laboratory to quantify expression of 384 genes (including those from Oncotype DX[®]) on paraffin-embedded core biopsies of 85 patients with locally advanced breast cancer. All pts had an IHC evaluation of ER (1D5 DAKO, Carpinteria, CA). The cut-off for ER status by RT-PCR was defined at the 6.5 threshold value as in previous studies. We also used all genes excluding *ESR1* to build a multi-gene predictor of ER status on samples with full agreement between IHC and *ESR1* expression and applied them to predict ER status in cases with disagreement. Several significance thresholds and classification methods were performed to reduce the risk of spurious results. We also evaluated a M.D. Anderson dataset of 133 patients with mRNA expression measured with Affymetrix chips (JCO 24: 4236, 2006). All analyses were done using BRB-ArrayTools and R packages.

Results: ER status according to *ESR1* expression and IHC was strongly associated (Spearman's correlation coefficients 0.77; κ 0.85; CI: 0.74-0.97). In 7% of cases there was a disagreement. Using *ESR1* as a reference, the IHC assessment included 6% false positive (FP) and 9% false negative (FN) cases. All false negative tumors were predicted as ER-positive by the genomic classifiers even when different thresholds and methods were used with individual classifiers. Of interest, all false positive cases had very high Recurrence Scores (76-92) and 2 false negative had a low RS according to Oncotype DX[®]. Using the same criteria with the M.D. Anderson dataset, the disagreement between IHC and *ESR1* mRNA expression was 6% (4% FP, 10% FN), and 4 of the 5 false negative tumors were confirmed as positive by ER genomic status.

Conclusions: *ESR1* expression by RT-PCR using Oncotype DX[®] or cDNA microarrays identifies a subgroup of tumors with ER negative status by IHC who might benefit from hormonal treatment.