

Expression of the 21 genes in the Recurrence Score assay and prediction of clinical benefit from tamoxifen in NSABP study B-14 and chemotherapy in NSABP study B-20

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Background: Two NSABP studies have been conducted to evaluate the 21 gene Recurrence Score (RS) assay as a predictor of distant recurrence in patients with node negative, estrogen receptor positive breast cancer who were treated with tamoxifen alone. The study of 233 patients in the tamoxifen only arm of NSABP study B-20 was one of 3 studies conducted to select the 21 gene panel and define the Recurrence Score algorithm. The large independent study of 668 tamoxifen-treated NSABP B-14 patients validated that the Recurrence Score accurately predicts the likelihood of distant recurrence. To determine whether the Recurrence Score assay is predictive of tamoxifen or chemotherapy benefit, we conducted two new studies of the Oncotype DXTM 21 gene assay.

Subjects and Methods: 1) The placebo arm patients in NSABP B-14 were studied to determine whether the genes in the Recurrence Score assay predict pure prognosis, responsiveness to hormonal therapy, or both. 2) The patients in the tamoxifen plus chemotherapy arms of NSABP B-20 were studied to determine whether expression of the genes in the Recurrence Score assay predict pure prognosis, responsiveness to chemotherapy, or both. Patients were eligible if tumor blocks were available and contained at least 5% invasive cancer. Based on tumor cellularity, RNA was extracted from three 10 micron sections without macrodissection or from macrodissected tumor from six 10 micron sections (when tumor area was less than 50% of the section). Expression was quantified by RT-PCR for 16 cancer-related genes (ER, PGR, BCL2, SCUBE2, Ki-67, MYBL2, Survivin, Cyclin B1, STK15, HER2, GRB7, Stromelysin 3, Cathepsin L2, GSTM1, BAG1, and CD68) and 5 reference genes (beta-actin, GAPDH, GUS, RPLPO, and TFRC).

Results: Study B-14. There were 645 evaluable patients (355 randomized to placebo and 290 randomized to tamoxifen). Results from regression analysis indicate that the 21 gene Recurrence Score assay quantifies the likelihood of distant recurrence in node negative, ER + breast cancer because it captures both prognosis and response to tamoxifen. Not all ER + patients benefited equally from tamoxifen. The largest benefits of tamoxifen were observed with high quantitative ER (and generally low RS). Smaller benefits were observed with low

quantitative ER (and generally high RS). The tests for interaction between tamoxifen treatment and both RS and quantitative ER were statistically significant ($p < 0.05$). Study B-20. There were 651 evaluable patients (227 randomized to tamoxifen and 434 randomized to tamoxifen plus either CMF or MF). Patients with tumors that had high Recurrence Scores ($RS \geq 31$) had a large absolute benefit of chemotherapy (with an absolute increase in DRFS at 10 years of $27.6 \pm 8.0\%$, mean \pm SE). Patients with tumors that had low Recurrence Scores ($RS < 18$) derived minimal, if any, benefit from chemotherapy (with an absolute increase in DRFS at 10 years of $-1.1 \pm 2.2\%$, mean \pm SE). The test for interaction between chemotherapy treatment and RS was statistically significant ($p < 0.05$).

Conclusion: The Oncotype DX Recurrence Score assay not only quantifies the likelihood of breast cancer recurrence in women with node negative, estrogen receptor positive breast cancer, it also predicts the magnitude of chemotherapy benefit.