

Use of Macrodissection in Multi-Gene RNA Analysis of Fixed Paraffin-Embedded Tumor Tissue

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Background: We examined predictors of recurrence in the NSABP B-20 study using a new RT-PCR assay (Onco^{type} DX) designed to quantitate small fragments of RNA in fixed paraffin-embedded tumor tissue. Here we report the pathologic eligibility criteria and the results using macrodissection in protocol-defined cases.

Design: Pts in the NSABP B-20 study (node-negative, ER+ pts enrolled 1988 to 1993) treated with tamoxifen 20 mg/day were eligible if blocks were available and, upon review, contained at least 5% invasive cancer. RNA was extracted from three 10 μ sections without dissection or by grossly dissecting the neoplastic tissue and non-tumor tissue from six 10 μ sections (when tumor area was less than 70% of the section and amenable to macrodissection). Expression was quantified for 185 cancer-related genes and 5 reference genes using RT-PCR in a 384 well format.

Results: Tumor was available in 277 clinically eligible pts. In 33 pts, there was no invasive cancer or <5% invasive cancer. In 31 pts, macrodissection was performed and RT-PCR analysis was performed separately on the neoplastic tissue and on the non-tumor tissue. In 213 pts, sufficient tumor was present and no macrodissection was performed. Adequate RT-PCR profiles were obtained in 28 of 31 macrodissected cases and in 206 of 213 undissected cases. As expected, gene expression in the neoplastic tissue and the non-tumor tissue in the 28 cases showed marked differences. For example, stromelysin 3, pS2, and c20orf expression was more than 4-fold higher and cytokeratin 14 and integrin 7 expression was more than 2-fold lower in the neoplastic tissue compared to the non-tumor tissue. Gene expression in the neoplastic tissue from the macrodissected cases was similar to gene expression in the whole sections. The median difference in the calculated Recurrence Scores (based on 16 cancer-related genes; 0-100 unit scale) was 10 units between the neoplastic tissue and non-tumor tissue and 3 units between the neoplastic tissue and the whole section. The similarity between the results in the neoplastic tissue and the whole section may reflect increased metabolic activity of neoplastic tissue. Notably, RNA yield as a function of area was 3 — 5 fold higher in the neoplastic tissue than in the non-tumor tissue.

Conclusions: Overall, RT-PCR analysis of RNA was successful in 242 of 252 tumor blocks from the NSABP B-20 study. Macrodissection was performed to enrich for tumor tissue in a protocol-defined subset of 31 patients.