

Genomic comparison of paired primary breast carcinomas and macrometastatic lymph node metastases using quantitative RT-PCR by *Oncotype DX*: Assessment of the Recurrence Score and quantitative single genes

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Background: NCCN guidelines now include consideration of the 21 gene RT-PCR assay in node negative, hormone sensitive breast cancers greater than 0.6 cm. Recent data suggests a potential role for testing of node positive (N1), hormone sensitive patients as well. Currently, no data exists on testing of metastatic lymph nodes. We sought to establish the feasibility of *Oncotype DX*[®] testing in the metastatic lymph nodes and, furthermore, to evaluate the genomic concordance between the primary tumor and the nodal deposit. These results may further our understanding of tumor heterogeneity and biological sensitivity in the process of lymph node metastasis and ultimately systemic metastases.

Methods: We examined the formalin fixed paraffin embedded tumor tissues (FPET) from 100 breast cancer patients from our institution's pathology database with available paired primary tumor and macrometastatic nodal deposits by *Oncotype DX* testing. Inclusion criteria included patients with macrometastatic lymph node disease, hormone receptor positive, HER2 negative primary breast cancer. The testing laboratory was blinded from the clinical outcomes data available on these patients. All FPET samples had H&E slides made which were reviewed by board certified surgical breast pathologists to determine if there was sufficient invasive tumor and to direct dissection of all lymph node samples. The Recurrence Scores and quantitative single gene values from the paired samples will be examined descriptively with scatterplots and Pearson correlation coefficients and reported at the meeting.

Results: All 100 paired specimens were sent for *Oncotype DX* testing. 24 samples were found during standard H&E review to not have sufficient tumor for the assay. Of the 176 samples created for RNA extraction 173 samples had sufficient RNA for the *Oncotype DX* assay: 85 lymph node and 88 primary breast carcinoma samples. Recurrence Scores and quantitative single gene values from the paired primary breast carcinoma and lymph node samples will be examined descriptively and reported at the meeting.

Conclusion: Previous comparisons of paired primary and metastatic samples have used immunohistochemistry and FISH which are susceptible to variability in preanalytic variability such as delay to fixation, choice of fixative and duration in fixative. This study, using quantitative RT-PCR, will be one of the largest comparisons of tumor biology in paired samples yet reported in the era of genomic subtyping and may have implications for systemic adjuvant treatment.