

## 28<sup>th</sup> Annual San Antonio Breast Cancer Symposium—Abstract #310

### Measurement of gene expression using tissue microarray cores of paraffin embedded breast cancer tissue.

Joffre Baker, Janine Salter, Mei-Lan Liu, James R Hackett, Maureen Cronin, Steven Shak, Mitch Dowsett, Genomic Health, Inc., Redwood City, CA United States, Department of Biochemistry, Royal Marsden Hospital, London, UK

**Introduction.** High throughput RT-PCR technology can be used to profile gene expression in fixed paraffin-embedded (FPE) tumor specimens and predict distant recurrence (Paik et al. NEJM [2004]). However, in many studies, limited amounts of FPE tissue are available, for example as 600  $\mu\text{m}$  cores in the form of tissue microarrays (TMA). We conducted a feasibility study to determine first, whether the standard 21 gene Oncotype DX assay can be obtained with such limited FPE tissue, and second, whether RNA amplification prior to RT-PCR can be successfully performed to maximize the number of additional genes that can be correlated with prognosis and treatment benefit.

**Methods.** Single cores (600  $\mu\text{m}$  diameter,  $\sim 0.2$  mm long) were taken from 8 tumor blocks prepared from excisions of invasive breast cancer from patients in the IMPACT trial. RNA was extracted and total RNA content was determined using the RiboGreen fluorescence method. The standard Oncotype DX 21 gene assay was performed on 375 ng of unamplified RNA. In addition, 50 ng of RNA was amplified, and then analyzed by the Oncotype DX assay. The RT-PCR results using unamplified and amplified RNA were compared.

**Results.** Sufficient total RNA ( $> 375$  ng) was obtained in all 8 specimens (mean RNA yield 1066 ng, range 510 – 3276 ng) to run the standard Oncotype DX assay without pre-amplifying RNA. Gene expression profiles in all 8 specimens for the 21 gene Oncotype DX assay on unamplified RNA had strong signals and met all criteria for successful RT-PCR. As has been observed in all other breast cancer studies, the range of expression of genes is very large (e.g., 1000-fold range in quantitative expression of ER). Profiles for 3 of the samples were typical of ER negative tumors and profiles for 5 of the samples were typical of ER positive tumors. RNA amplification yielded a 100-200 fold increase in total RNA. Overall, profiles and Recurrence Scores obtained using amplified RNA were similar to those obtained with unamplified RNA. Amplified RNA can be used for screening studies, but further work will be required to provide high fidelity. We are currently assessing the phenotype of these samples by conventional means to correlate with the quantitative RT-PCR expression results.

**Conclusion:** Gene expression profiling from very small FPE specimens taken for TMA construction is feasible using RT-PCR.