

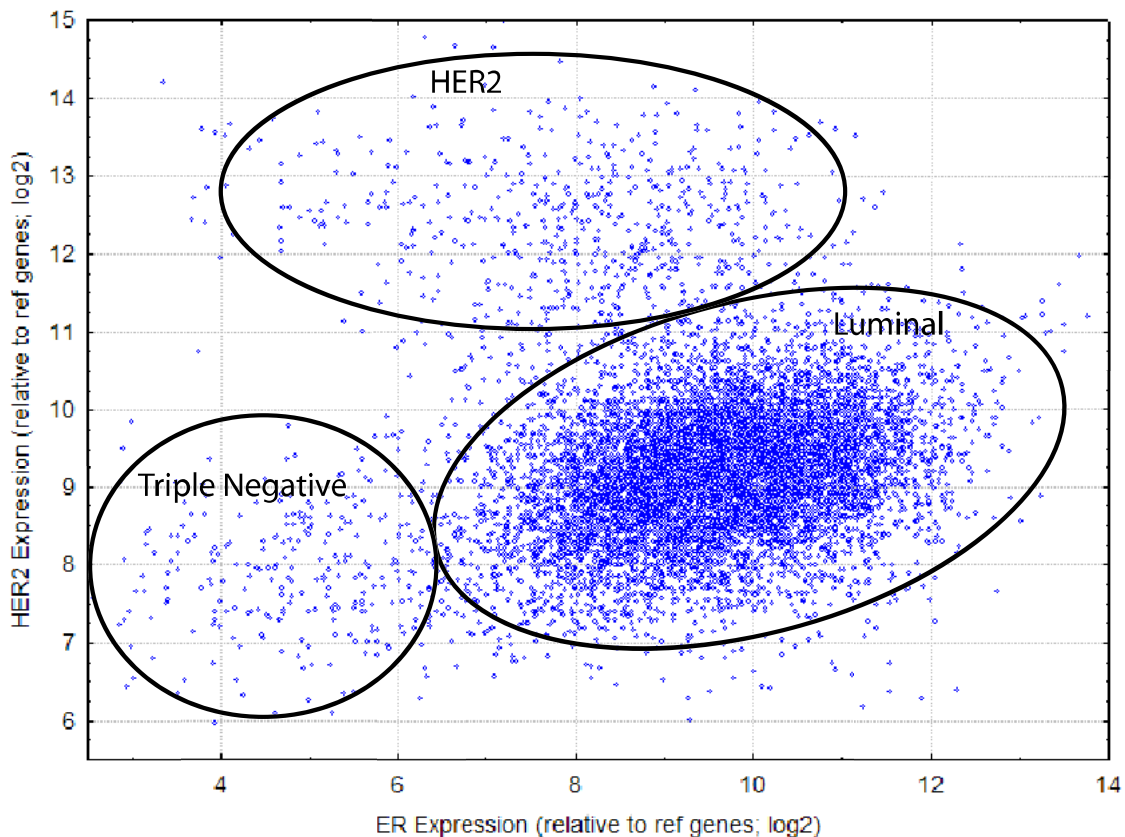
Subtypes of breast cancer defined by standardized quantitative RT-PCR analysis of 10,618 tumors

Steven Shak, MD, Frederick L Baehner, MD, Gary Palmer, MD MBA MPH, Julie T Ballard, BS, Joffre Baker, PhD, and Drew Watson, PhD. Medical, Genomic Health, Redwood City, CA, United States, 94063.

Background: A number of different approaches have been suggested to subtype breast cancer based on analysis of gene expression. To identify the subtypes revealed by a standardized quantitative expression assay, we examined RT-PCR analysis of the 21 *Oncotype DX*TM genes in a large number of tumors.

Material and Methods: All tumor specimens successfully examined in the Genomic Health laboratory from January 2004 through March 2006 were included. Quantitative expression for each gene was measured by the pre-specified 21-gene *Oncotype DX* assay on a scale from 0 to 15 (relative to reference genes), where a one-unit increment is associated with a 2-fold change in expression. Reproducibility is typically within 0.4 units for each gene. The provisional cutoffs of 6.5 for ER positivity and 11.5 for HER2 positivity were derived from three prior IHC correlation studies.

Results: There were 10,618 tumors. As expected for patients for whom *Oncotype DX* was appropriate, the vast majority had quantitative expression of ER typical of ER-positive breast cancer: 10,230 tumors had ER ≥ 6.5 (of which 4.3% had HER2 ≥ 11.5) and 388 tumors had ER < 6.5 (of which 22.2% had HER2 ≥ 11.5). A plot of the relationship between quantitative expression of ER and HER2 shows a wide dynamic range ($> 3,000$ fold) for both ER and HER2.



The data in this cohort are consistent with three major subtypes of breast cancer: ER+HER2- or luminal, HER2+, and ER-PR-HER2- or triple negative (rarely was PR expression high in cases with low ER). Of note, detailed analysis of the ER+HER2- subtype did not find that there were two categories of luminal breast cancer based on the expression of any combination of the 21 genes. Indeed, there was great heterogeneity of quantitative expression in each of the three major subtypes, and a continuous range of expression within each subtype. Finally, the rarity of high quantitative ER expression in HER2+ tumors is consistent with previous reports that indicate that HER2+ breast cancer is less responsive to tamoxifen.

Discussion: The data for this cohort of 10,618 tumors are consistent with the presence of three major breast cancer subtypes: luminal, HER2 positive, and triple negative, with a continuous range of expression within each subtype. These results emphasize the importance of standardized quantitative measurement of gene expression.