Gene expression signatures as predictors of chemotherapeutic response in breast cancer

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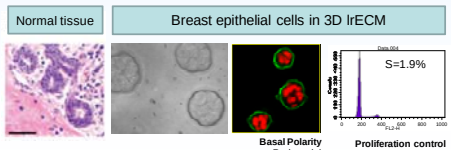
Background

Less than 10% of estrogen receptor–positive (ER+) breast tumors and 20–30% of estrogen receptor–negative (ER-) tumors respond to primary chemotherapy (preoperative; neoadjuvant) with disappearance of the primary tumor (pathological complete response; pCR).

Accurate identification of responders (sensitivity) would potentially:

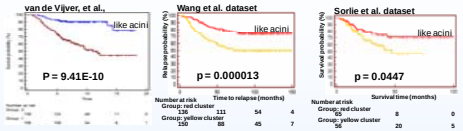
- **Tremendously benefit patients likely to respond because it would assure that these individuals would get timely treatment.**
- **Avoid ineffective treatment of insensitive tumors reducing stress for patients and health care costs.**
- **Increase survival rates by guiding therapy selection and allowing earlier treatment with the optimum therapy.**

BIOARRAY signature was selected by genome wide expression analysis of cultured breast epithelial cells in a physiologically relevant 3D model of laminin-rich extracellular matrix (lrECM).



Fournier et al. Cancer Res 2006

BIOARRAY signature accurately predicted breast cancer clinical outcome in 3 independent breast cancer datasets.



Kaplan Meier curves. ~700 patient biopsies analyzed.

Martin, et al., PLoS One, 2008

BIOARRAY signature was shown to be a strong independent factor to predict breast cancer clinical outcome. Multivariable proportional-hazards analysis of 10-year survival risk. Results were calculated using dataset of van de Vijver, et al., using overall survival as endpoint. Similar results were obtained using relapse as endpoint, BIOARRAY signature Hazard Ratio 3.3 (95% CI 2.0 to 5.3), p<0.0001.

	Hazard ratio (95% CI)[a]	P-value
Age (per 10 year increment)	0.62 (0.44 to 0.88)	0.008
Tumor diameter (per cm)	1.33 (1.04 to 1.69)	0.023
ER (positive vs negative)	0.55 (0.34 to 0.90)	0.018
Lymph node status (per positive node)	1.07 (0.96 to 1.20)	0.234
Chemotherapy	0.69 (0.38 to 1.26)	0.234
Mastectomy	1.05 (0.63 to 1.73)	0.864
BIOARRAY signature	**4.43 (2.32 to 8.46)**	**<0.00001**

Martin et al. PLoS One 2008

Objectives

- **Evaluate ability of BIOARRAY signature to predict response to chemotherapy in breast cancers.**
- **Compare BIOARRAY signature to other published predictive signatures.**

Methods

Datasets:

1)Hess KR, Anderson K, Symmans WF, et al. Journal of Clinical Oncology 24(26): 4236-44, 2006. Fine-needle aspirates from 133 patients with stage I-III breast cancer were obtained before neoadjuvant combination treatment and response was assessed after chemotherapy. In this dataset, estrogen receptor positive (ER+) patients (n= 73) were significantly less responsive to chemotherapy than estrogen receptor negative (ER-) patients (n=63) (p-value= 0.0000000199, Fisher's Exact test), as reported by Hess, et al.

2) Chang JC, Wooten EC, Tsimelzon A, Hilsenbeck SG et al. Lancet, 362:362-9, 2003. Core biopsies from 24 patients with locally advanced breast cancer were obtained before neoadjuvant docetaxel treatment (3-weekly Tx4 100 mg/m2) and response was assessed after chemotherapy.

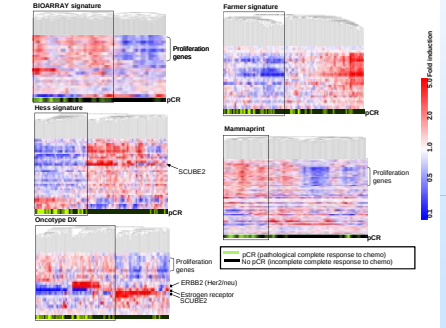
Statistical analysis: ***ROC analysis*** **are two-dimensional graphs in which the true positive rate (tp) is plotted on the Y axis and false positive rate (fp) is plotted on the X axis. Sensitivity: true positive rate (100=no false negatives). Specificity: true negative rate (100=no false positives).** ***Area under the curve (AUC)*** **is a summary statistic that combines the sensitivity and specificity measures into a single measure. AUC=1.0 is a perfect test.** ***Logistic regression*** **is used for prediction of the probability of occurrence of an event by fitting data to a logistic curve.**

Table 1. Comparison of five gene signatures

	Number of genes	Number of probe sets	Gene overlaps between: BIOARRAY	Mamma Print	Oncotype DX	Hess	Farmer
BIOARRAY	22	22	22	0	3	1	0
Mammaprint	67	70	0	67	1	2	1
Oncotype DX	21	21	3	1	21	1	0
Hess	31	26	1	2	1	31	0
Farmer	25	25	0	1	0	0	25

Results

Figure 1. Comparison of pCR prediction by five gene signatures using hierarchical clustering in dataset of Hess et al. Affymetrix cel files from 133 patients of Hess, et al., were preprocessed with GeneSpring software by RMA then normalized to the chip median and gene median. Cluster analyses used standard metric for patient dimension. Fold induction is shown. Boxes mark clusters that predict pCR.



Results

Table 2. ROC analysis comparing prediction of chemotherapy response by ER status and 5 signatures by clustering in dataset of Hess, et al.

	BIOARRAY	Oncotype DX	Hess signature	Tumor grade	ER status	Random 29 genes	Mammaprint	Farmer signature
Sample size	133	133	133	133	133	133	133	133
AUC	0.718	0.713	0.825	0.677	0.776	0.600	0.747	0.622
Sensitivity	**94.1**	91.2	88.2	85.3	79.4	70.6	67.6	61.8
Specificity	49.5	51.5	76.8	51.5	75.8	49.5	81.8	62.6
P-value	0.0001	0.0001	>0.0001	0.0001	0.0001	0.0834	0.0001	0.0125

Table 3. ROC analysis comparing prediction of chemotherapy response by ER status and 5 signatures by clustering by clustering in the dataset of Chang, et al.

	BIOARRAY	Oncotype DX	Farmer signature	Random 29 genes	Mammaprint	Hess signature	ER status
Sample size	24	24	24	24	24	24	24
AUC	0.900	0.811	0.722	0.55	0.644	0.500	0.567
Sensitivity	**100**	88.9	77.8	60.0	55.6	33.3	33.3
Specificity	80.00	73.3	66.7	50.0	73.3	66.7	80.0
P-value	0.0001	0.0001	0.0217	0.68	0.1724	1.000	0.507

Figure 2. BIOARRAY signature predicts response to chemotherapy using hierarchical clustering. Gene expression levels for the 22 genes of the BIOARRAY gene signature in the microarray dataset of Hess, et al., were organized by hierarchical clustering using a standard metric (GeneSpring). Significantly more patients from Cluster 1 responded to chemotherapy 29 of 78 (37%), than patients from Cluster 2, 3 of 55 (5%), (p=0.000011, Fisher's Exact Test). Responsive Cluster 1 also included more tumors of ERB+ (orange) and luminal-2 (purple) molecular classes, more high grade tumors, and more black (maroon) and Hispanic (pink) women than Non-Responsive Cluster 2.

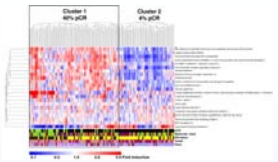
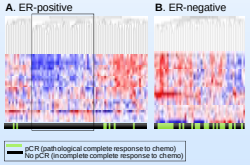


Figure 3. BIOARRAY signature predicts response in ER+ tumors using hierarchical clustering. A. Hierarchical clustering applied to ER-positive tumors from dataset of Hess, et al., shows the BIOARRAY signature predicts pCR with very high sensitivity. B. For ER-negative tumors, this model does not predict as well. Other models can be applied to predict pCR in ER-negative tumors using BIOARRAY signature. C. ROC statistics for these clustering results.



C. ROC statistics

	ER positive	ER negative
Sample size	82	51
Positive samples (pCR)	7	27
Negative samples (no pCR)	75	24
AUC	0.733	0.563
Sensitivity	**100**	**66.67**
Specificity	46.67	45.83
P-value	0.0001	0.3688

Results

Figure 4. BIOARRAY signature predicts pCR of a broad range of molecular classes of breast cancers using hierarchical clustering. This analysis used133 patients of Hess, et al. To determine molecular class of these patients, we compared gene expression patterns of each patient with the previously classified patients of Perou, C. M., T. Sorlie, et al. (Nature 406(6797): 747-752, 2000) using a Pearson correlation metric. Each Hess patient was then assigned the same class as that of the Perou patient to which it was most correlated. The genes used for this comparison included 35 reference genes that are highly differential between the molecular classes (Sieuwerts, A. M., J. Kraan, et al. Breast Cancer Res Treat 118(3): 455-468, 2009). The BIOARRAY signature predicted pCR with high specificity in luminal 1, luminal 2, and basal-like breast cancers. Alternative models will be applied to test prediction of pCR in ERB+ and normal-like tumors.

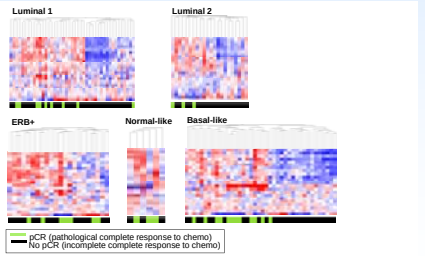
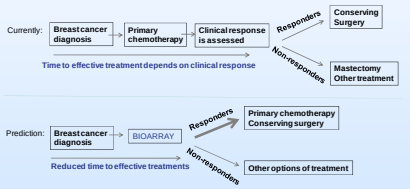


Table 4. ROC curve analysis comparing prediction of chemotherapy response clustering in different molecular classes of breast cancers.

	Luminal 2	Luminal 1	Basal-like	ERB+
Sample size	23	44	35	18
Positive samples (pCR)	3	9	12	5
Negative samples (no pCR)	20	35	23	13
AUC	0.775	0.687	0.721	0.531
Sensitivity (prediction of pCR)	100	89	83	60
Specificity (prediction of no pCR)	55	49	61	46
P-value	0.0001	0.0076	0.0039	0.83

BIOARRAY signature predicts breast tumors that are likely to respond to primary chemotherapy



Conclusions:

- **BIOARRAY has developed a 22-gene signature to provide, early in the care process, accurate and personalized information to predict response to primary chemotherapy.**
- **BIOARRAY 22-genes demonstrated excellent performance in terms of sensitivity (lowest false negatives). False negative would exclude a patient from chemotherapy when treatment would work.**

Future directions:

- **Validate the BIOIARRAY 22-gene signature's ability to predict response of ER+ tumors to primary chemotherapy in a prospective clinical study .**
- **Ongoing work is testing the BIOARRAY signature using different models for response prediction in ER-negative breast cancers. Preliminary results for logistic regression models have given AUC = 0.83 in ER- tumors from Hess et al (n=63).**