



#3003 A novel prognostic immunohistochemical biomarker panel for estrogen receptor expressing breast cancer.

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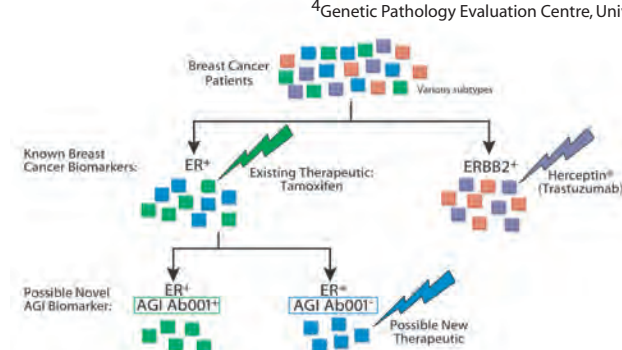


Background: Gene expression signatures have been identified that distinguish biologically and clinically significant breast tumor diversity. We have used gene expression data to target the production of hundreds of novel antibody reagents to explore whether a panel of targeted immunohistochemistry reagents can distinguish similar tumor diversity and identify patients at increased risk of poor outcome.

Methods: Eighty novel and seven commercially available antisera were used to stain tissue arrays containing a retrospective breast cancer cohort from the Comprehensive Cancer Center of Huntsville (550 patients). Cox proportional hazard analysis was used to identify a recurrence score algorithm that uses only five antisera to predict risk of recurrence in estrogen receptor-positive, lymph node-negative patients. To validate this model and antibody panel, we then tested the prognostic association of this prospectively defined algorithm by staining two independent tissue array cohorts, with linked clinical follow-up data.

Results: In both validation cohorts, the Kaplan-Meier estimates of recurrence confirmed that the Cox model distinguished estrogen receptor-positive patients with poor outcomes. In a cohort assembled at the British Columbia Cancer Agency (440 patients total) the five antisera algorithm identified ER+ patients with a high risk of recurrence that showed an overall 5 year survival rate of 56% compared to 74% for moderate and 89% for good (p=0.003). In the Cleveland Clinic Foundation Cohort (292 patients total), the Cox model identified ER+ 'bad' patients with a five year recurrence rate of 51% compared to 40% for patients classified as either moderate or good (p=0.0022). Although both cohorts were underpowered to test the association of the prognosticator with ER+ lymph node negative patients, in a multivariable analysis the calculated risk of recurrence was independent of stage, grade and lymph node status.

Conclusion: A panel of five antibodies can significantly improve upon traditional prognosticators in predicting outcome for estrogen receptor positive breast cancer patients. Additional retrospective and prospective studies focused on early stage patients are indicated for further validation.



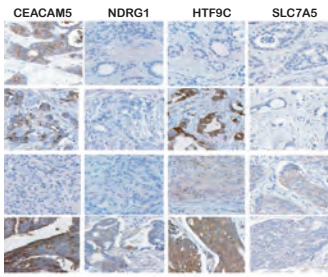
This cartoon depicts breast cancer as a number of different disease types that can be distinguished by either by gene expression patterns or by immunohistochemical staining with AGI's "Antibody Panel of Diversity". Our hypothesis is that disease types distinguished by differential gene and protein expression may exhibit diverse response to treatment and/or clinical course. By identifying associations between disease type and response to therapy, we can identify biomarkers of therapeutic response as well as targets for directed therapeutic development.

Material and Methods: Tissue and tissue arrays: The 'discovery' cohort was assembled by identifying patients seen at the Comprehensive Cancer Institute of Huntsville (CCIH) between 1989 and 2002. For the breast studies, the validation cohorts were assembled at Vancouver General Hospital (BCCA) and the Cleveland Clinic Foundation (CCF) respectively. Staining datasets include only those samples from the cohort that gave interpretable data for all markers in the diagnostic antibody set. All protocols, including identification of patients, assembling of paraffin blocks, screening of blocks for excess material, and collection of clinical information by chart review were approved by the appropriate Institutional Review Boards.

Antibody generation and antisera screening: Targets for antibody production were selected on the basis of gene expression patterns in breast cancer and other tumor types. Novel polyclonal anti-peptide antisera and commercially available antisera were screened by staining tissue arrays containing diverse normal and tumor tissue specimens. Each antibody exhibiting potentially useful discrimination amongst breast and/or lung cancer patients were included in the clinical studies. Each case was assigned a semi-quantitative score that reflected the presence or absence of tumor tissue and the relative strength of the stain (score: 0=tumor present-no stain, 1=no information, 2=tumor present-weak stain, 3=tumor present-strong stain).

Model building for prognostication: The log-rank test was used to identify the subset of reagents that were associated with significant differences in recurrence in the discovery cohorts (p<0.10). Models predicting recurrence were built using this subset of reagents using cox proportional hazard analysis and by binning recurrence values to the classes 'good', 'moderate', 'bad'. Ten-fold cross validation indicated that these models were not likely overfit to the discovery datasets.

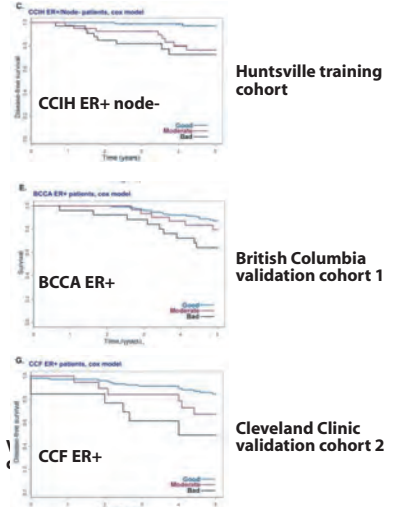
Novel Mammostrat™ antisera



Mammostrat™ Antibodies

SLC7A5 CEACAM5 NDRG1 HTF9C p53

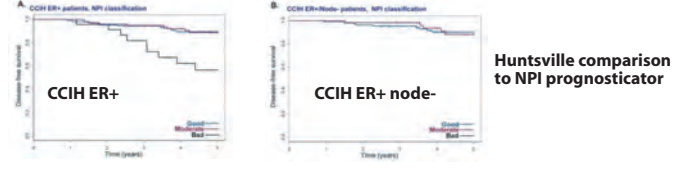
Marker	Model	HR	95% CI
NCCA ER+ node-	cox	0.00033	2.46 (1.49 to 4.06)
	node	0.005	4.33 (1.87 to 10.05)
	stage	0.0037	1.9 (1.22 to 2.96)
CCF ER+ node-	cox	0.00075	2.46 (1.46 to 4.15)
	node	0.0048	3.88 (1.51 to 9.95)
	stage	0.00014	3.51 (2.28 to 5.42)
NCCA ER+ node+	cox	0.0046	3.346 (1.68 to 6.67)
	stage	0.89	1.071 (0.4 to 2.86)
	grade	0.19	0.584 (0.26 to 1.34)
CCF ER+ node+	cox CV	0.089	2.568 (0.85 to 7.79)
	stage	0.85	1.119 (0.37 to 3.42)
	grade	0.58	0.796 (0.35 to 1.81)
NCCA ER+ node-	cox	0.00028	2.94 (1.62 to 5.32)
	stage	0.45	1.37 (0.6 to 3.14)
	grade	0.72	0.868 (0.39 to 1.92)



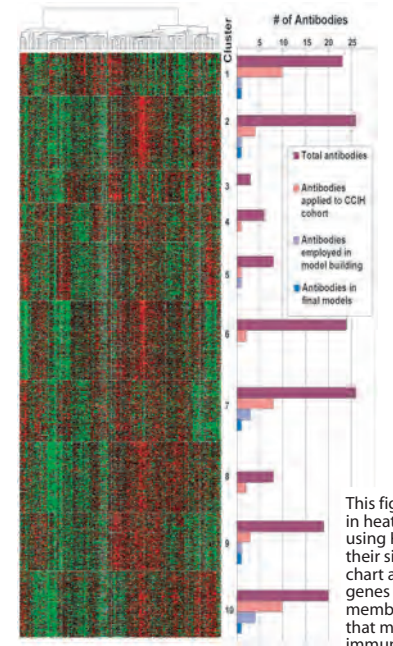
Cohort Characteristics

	CCIH	CCF	BCCA
Total patients	488	299	344
ER+	208	208	273
ER+ node negative	159	137	103
ER+	341	87	71
Tumor size			
T1	242	167	164
T2	173	98	114
T3	71	19	66
T4	12	13	47
Unknown	12	6	102
Age			
<60	143	74	104
60-70	319	225	240
>70	126	100	140
Her2 status			
Negative (0 to 1+)	278	263	314
Positive (2 to 3+)	68	34	127
Unknown	20	12	102
NPI			
Good	320	106	104
Moderate	150	106	104
Bad	55	106	104
Unknown	112	106	102
Node status			
N0	264	170	200
N1	159	48	101
N2	9	24	101
N3	0	12	46
Unknown	55	26	61
Stage			
I	100	100	104
II	230	109	104
III	37	34	104
IV	9	6	104
Unknown	6	127	102
Grade			
I	56	45	104
II	166	109	104
III	145	83	104
Unknown	121	68	102
Recurrence	122	87	104
Deaths	55	28	58
Used in cox model	150	107	101

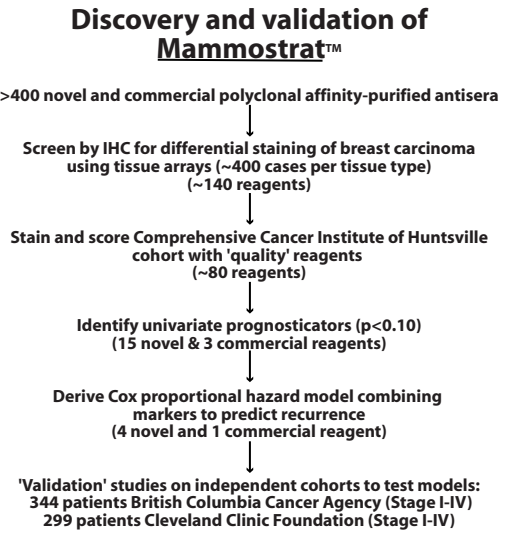
Nottingham Prognostic Index



Immunohistochemistry reagents mapped to breast cancer gene expression data



This figure depicts the gene expression data from Sorlie et al. 2003 in heat-chart form. The gene expression data has been organized using K-means clustering which places genes into bins based upon their similarities in expression to one another. Adjacent to the heat chart are bar graphs depicting numbers of antibodies targeted to genes within each bin. The genes that encode the mature panel members are distributed widely amongst the various bins. Note that many genes were targeted in order to identify robust immunohistochemistry reagents for inclusion in the "Panel of Diversity".



Summary: These studies began by generating and sorting through hundreds of novel and commercially available immunohistochemistry reagents targeted by gene expression data to identify the most useful reagents for distinguishing biologic diversity in carcinoma. This process resulted in the identification of "Panels of Diversity" tailored to tissue type that distinguish carcinoma diversity. "Discovery" studies to identify prognosticators were performed by staining a breast cancer cohort in collaboration with the CCIH. Cox proportional hazard models were constructed to combine markers into sets to best predict outcome. A five antisera model which uses antisera targeted to p53 and four additional biomarkers (SLC7A5, NDRG1, CEACAM5, HTF9C) was selected for ER+ node negative breast cancer. Validation of this prognostic test was performed by prospectively defining and then staining two independent cohorts at the BCCA and CCF. The association of this prognostic assay with clinical outcome was validated in both cohorts.

Conclusions: 1. Staining data using a panel of five immunohistochemistry reagents combined by a Cox proportional hazard model can predict outcome in ER+ breast cancer patients. This prognostic test was validated in two independent ER+ cohorts.

2. Monoclonal antibodies are now available for all five markers used in this prognostic test.

3. Clinical studies in ER+ node negative populations are planned to further explore the clinical utility of this test.

Inquiries for collaborative opportunities should be directed to DTR at dross@applied-genomics.com or RS at rseitz@applied-genomics.com

References: Sorlie, T., Perou, C et al., Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. PNAS. 2001 Sep 11;98(19):10869-74.