

Association of gene expression module biomarkers with clinical and therapeutic endpoints and their use with a universal companion diagnostic assay.

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Abstract:

Background: Gene expression patterns are increasingly capable of stratifying patients based on prognosis and response to therapy. Given the limited availability of sample tissue, however, it is not feasible to utilize every test for every patient, suggesting the need for a universal companion diagnostic assay that is informative with respect to multiple clinical and therapeutic endpoints. Key challenges are identification of appropriate gene expression biomarkers, translation of biomarkers to clinical assays, and development of reliable gene expression profiling of formalin-fixed clinical specimens. Here we describe a novel RT-PCR biomarker assay optimized for FFPE clinical samples that has broad prognostic and predictive potential.

Methods: A co-expression meta-analysis of 5,339 breast tumors from 56 microarray datasets identified highly co-expressed sets of genes (modules) across multiple datasets. Module biomarkers were tested for their ability to associate with prognostic and predictive targets in published datasets. In addition, each module was reduced from 10–1000 genes to 2–3 genes for use in companion diagnostic assays based on degree of co-expression across the meta-analysis, and validated against an independent panel of tumor samples.

Results: This study demonstrates that a single test utilizing multiple module biomarkers is informative with respect to standard parameters such as ER, PR and Her2, and in addition reproduces existing prognostic and predictive genomic signatures. Furthermore, we show that modules of 10–1000 genes can be represented by 2–3 genes for direct use in companion diagnostics development.

Conclusions: The molecular heterogeneity of breast cancer can be summarized by discrete gene expression modules that individually represent distinct biological programs, and that collectively can be represented by as few as 96 genes. Modules, together with outlier genes, allow for summation of the entire transcriptional program and provide a universal assay with broad application to companion diagnostics development.

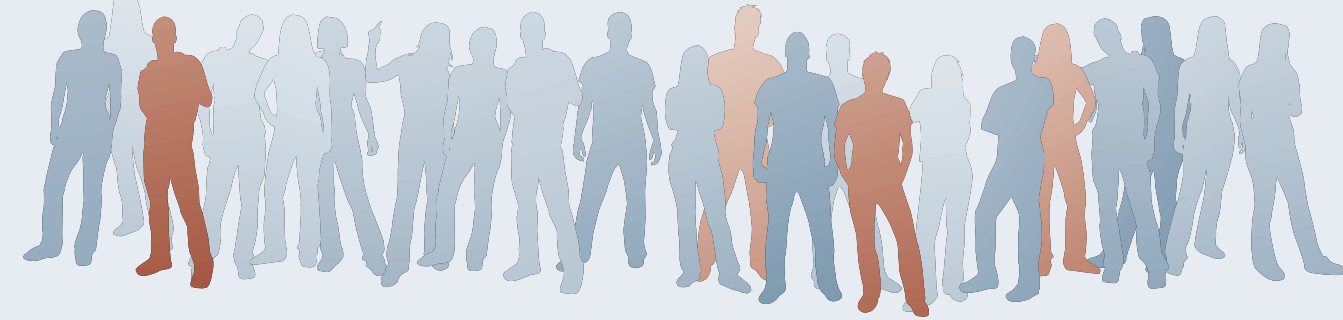
Introduction:

Companion Diagnostics Challenges and Solution

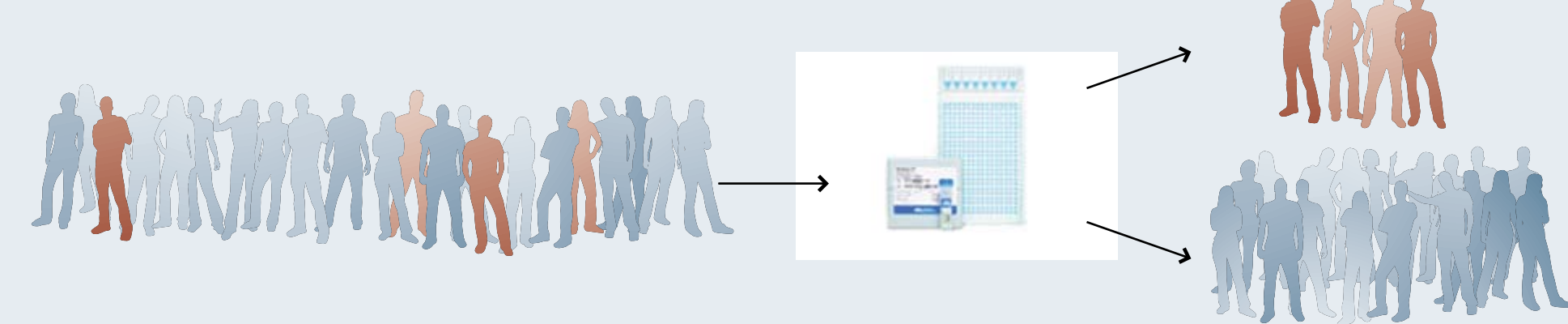
Current genomics technologies including microarrays and next-generation sequencing platforms are suitable for routine clinical testing, however in the current paradigm, every assay is custom designed for a single therapy. This model is prohibitive for clinical development due to the time, cost and risk involved in their development. We are developing a single assay with the ability to predict therapeutic responses for a wide variety of therapies and combinations and has been designed and validated with a streamlined regulatory process in mind. These assays enable the identification of patients that will respond to therapies in a definitive way that is not presently available by standard pathological analysis.

Background:

Response to cancer therapies may be limited to subset of the population.



Diagnostics are required to accurately stratify population and predict responder population.



Material and Methods:

A co-expression meta-analysis on 5,339 breast cancer samples from Oncomine identified highly co-expressed sets of genes (modules) across multiple breast cancer microarray datasets, with each module consisting on average of 450 genes (range: 11–962). These modules represented expected subclasses (e.g., basal, luminal A, luminal B), as well as additional subclasses (e.g., immune response, proliferation). The 96 gene panel which collectively predicted to identify each of the modules were selected by testing 384 candidates genes in FFPE-RT-PCR. The approach was tested on 65 FFPE samples with known histological parameters such as ER, PR, and HER2. The patterns of module expression in retrospective studies was matched in expected ways with prognosis and drug response.

Results:

Figure 1. Breast Cancer Module Development.

96 genes were selected whose gene expression correlated and consistently represented the associated module.

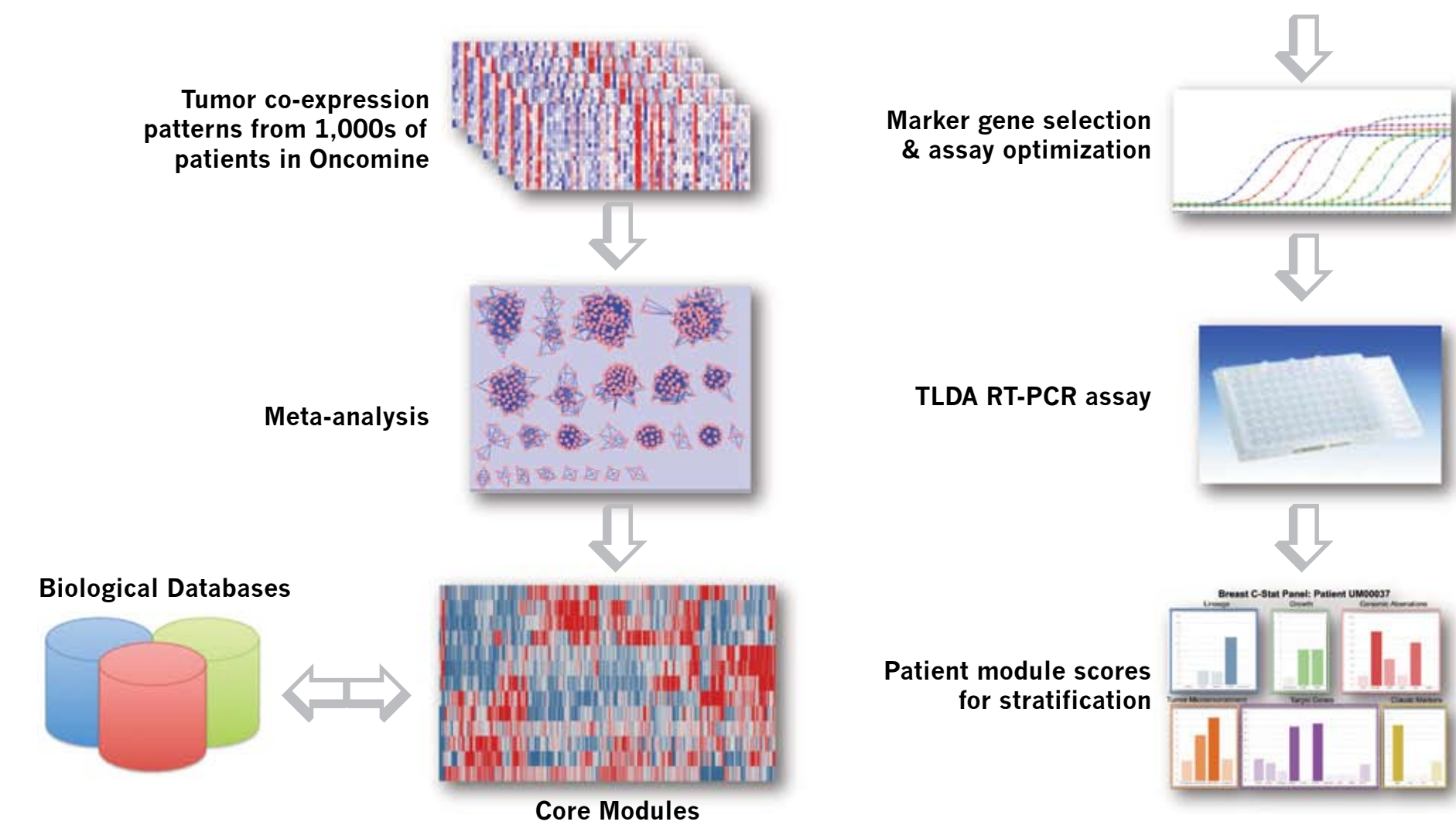


Figure 2. Breast Cancer Modules: Biological Variables.

21 physiological states represented by the companion diagnostic assay.

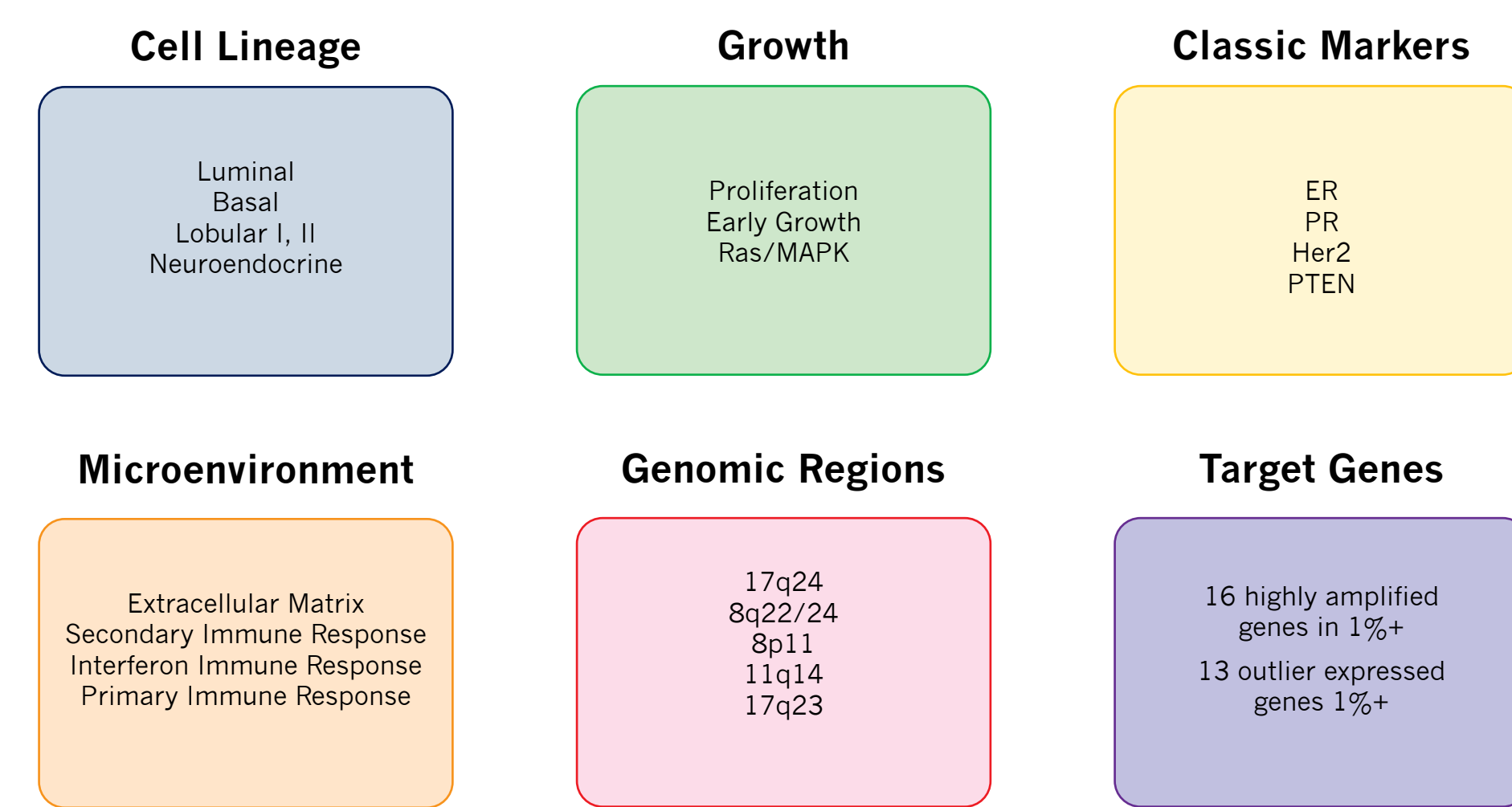


Figure 3. Breast Cancer Module Scoring.

Results for 2 patients showing diverse profiles.

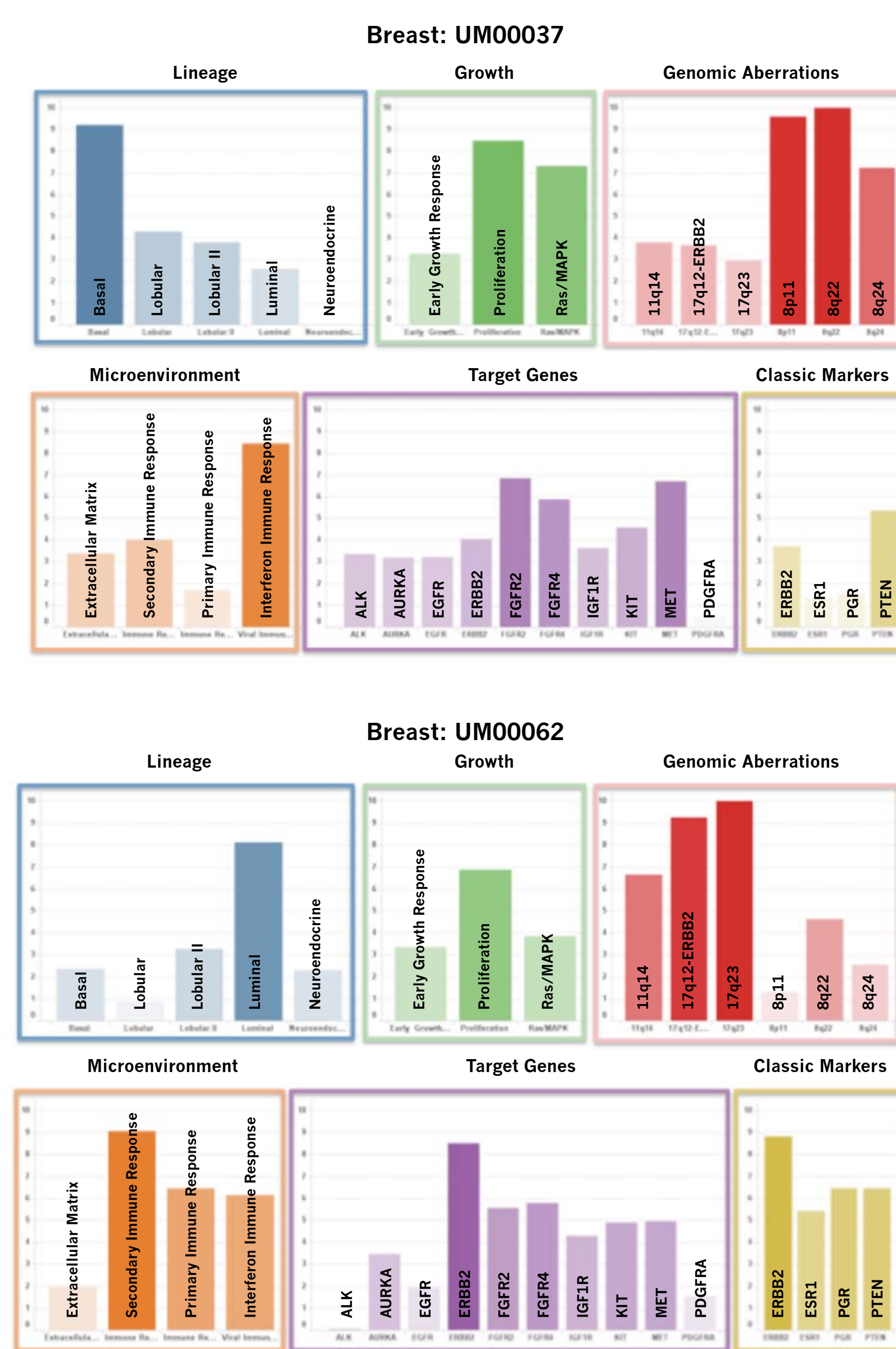


Figure 4. Module-based Molecular Stratification.

This assay recapitulates established molecular sub-classifications (basal, luminal A, luminal B, Her2, lobular) and also reveals substantial diversity within each sub-group (analysis of 65 patient cohort: University of Michigan.)

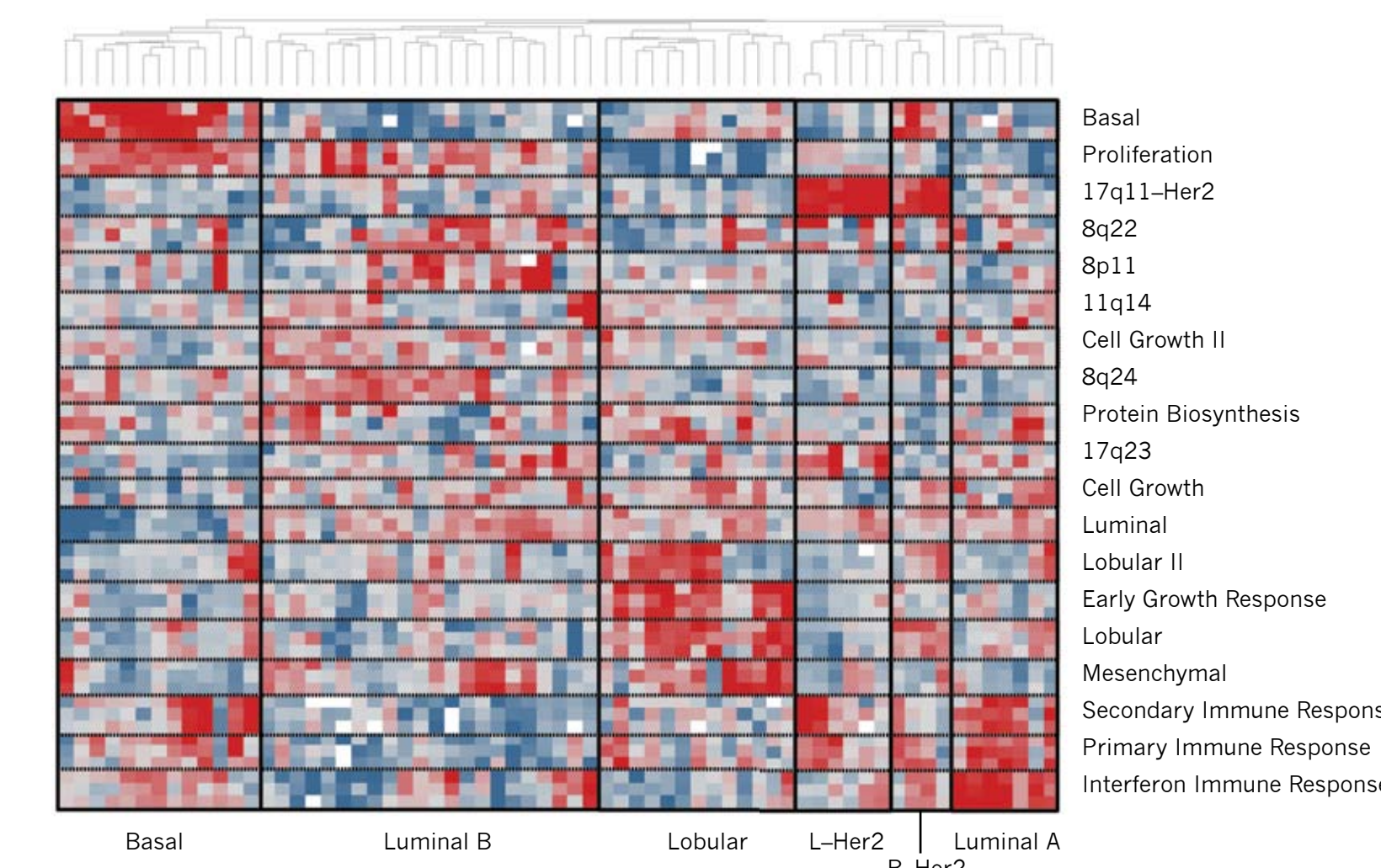


Figure 5. Breast Cancer Module Validation.

Module genes demonstrated expected gene-gene correlations and the genes also displayed correlations with expected clinical characteristics.

- ER+ tumors showed over-expression of luminal genes and under-expression of basal genes.
- ER-/Her2- tumors: over-expression of basal genes.
- Her2+ tumors: over-expression of 17q25 genes.
- ER-/Her2- tumors: high expression of proliferation genes
- ER+ tumors: variable expression.

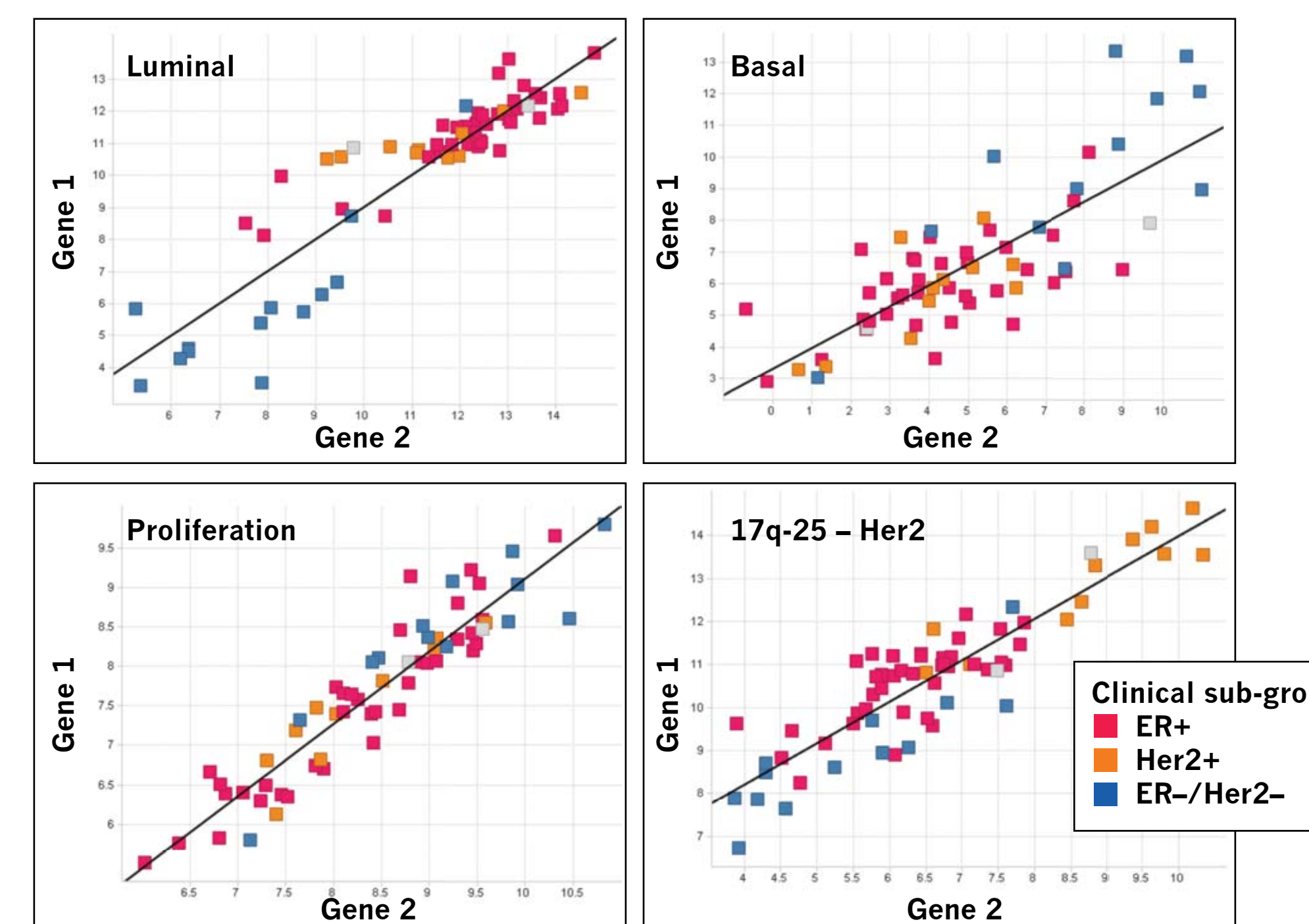
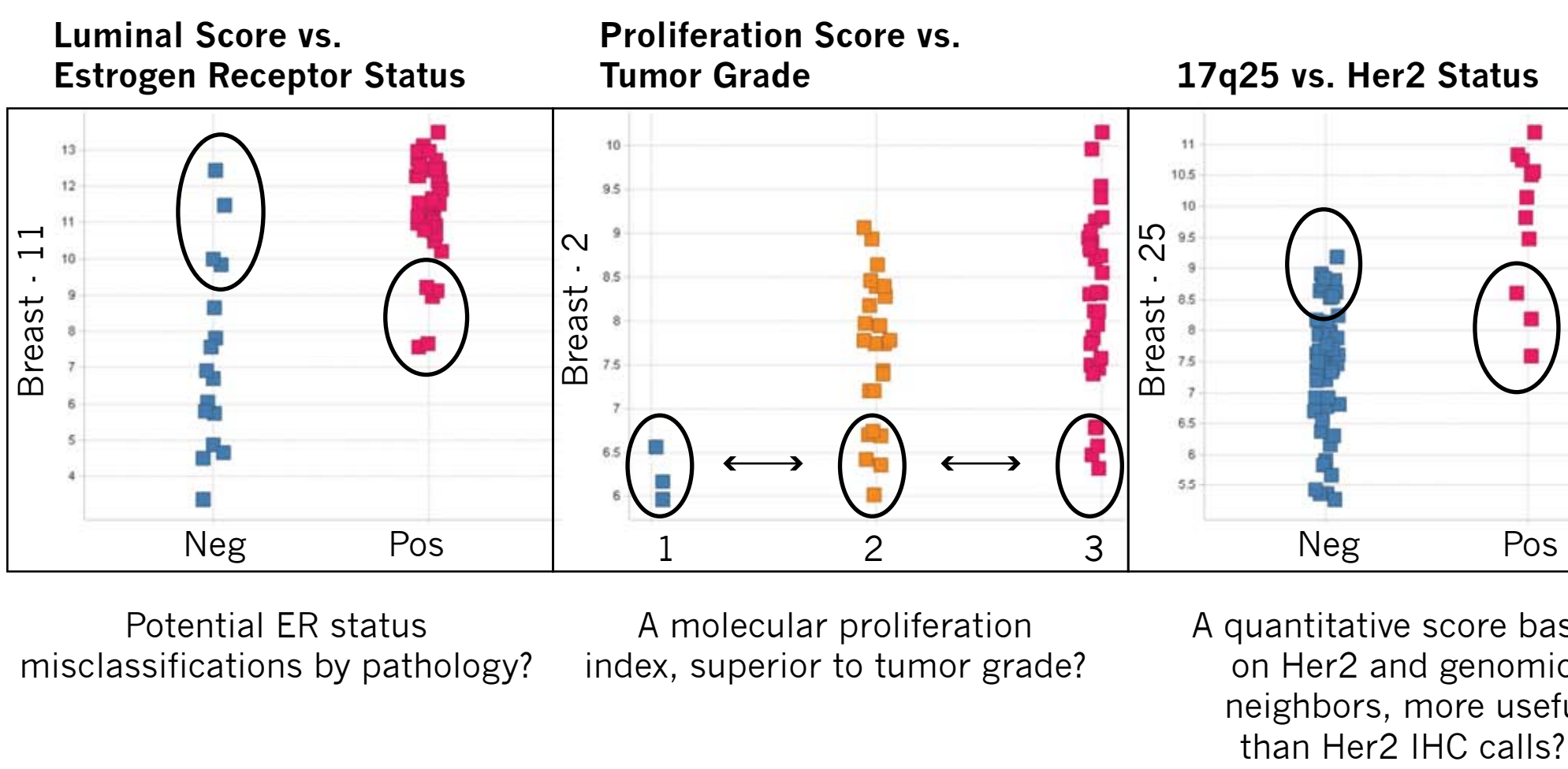


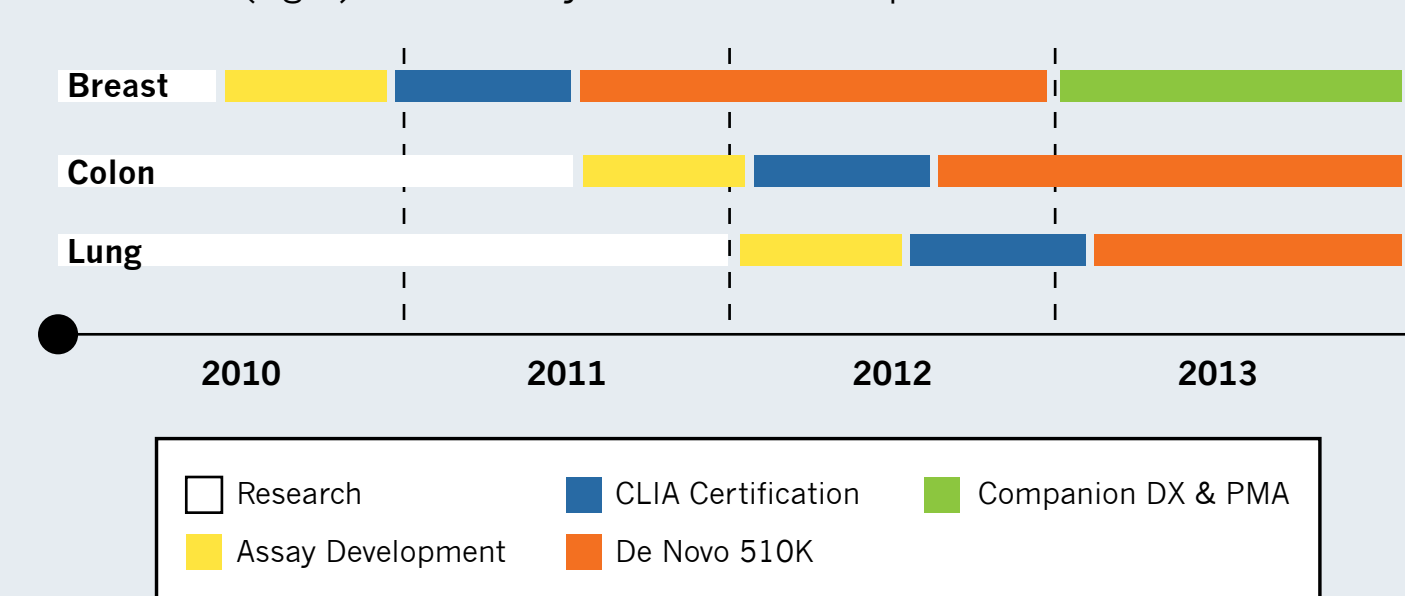
Figure 6. Module Score Clinical Trends.

Pathological analysis defines these patient's ER, proliferation and Her2 status, but module-based classification is more refined, but also sometimes discordant (see circled patients).



Assay Development

The table (right) shows assays in the research phase.



Type	Datasets	Tumors
Bladder	11	1,387
Brain	25	1,384
Gastric	4	384
Head & Neck	12	568
Leukemia	44	6,805
Liver	8	785
Lymphoma	20	2,325
Melanoma	9	343
Myeloma	11	1,731
Ovarian	14	1,469
Pancreas	8	225
Prostate	26	2,882
Renal	12	1,146
Sarcoma	14	1,065

Figure 7. Retrospective Validations.

Module scores computed from retrospective microarray studies. Modules tested for association with clinical and experimental endpoints.

- Overall survival: Long term clinical follow up for 287 patients. There are established prognosis associations apparent (bottom left), but additional stratification opportunities are uncovered (Van de Vijver MJ, et al. *N Engl J Med.* 2002). Module scores are quantitative (bottom right) and this quantitation results in refined stratification.
- Neoadjuvant chemotherapy response: analysis of EORTC clinical trial sub-study. There were 66 estrogen receptor negative patients in the study, 28 with complete pathological response and 38 with residual disease. Specific modules clearly trend with response.
- In vitro* sensitivity to MEK and PI3K inhibitors. When modules were ascribed to breast cancer cell lines tested with targeted agents the modules trended with sensitivity to MEK and PI3K inhibitors.

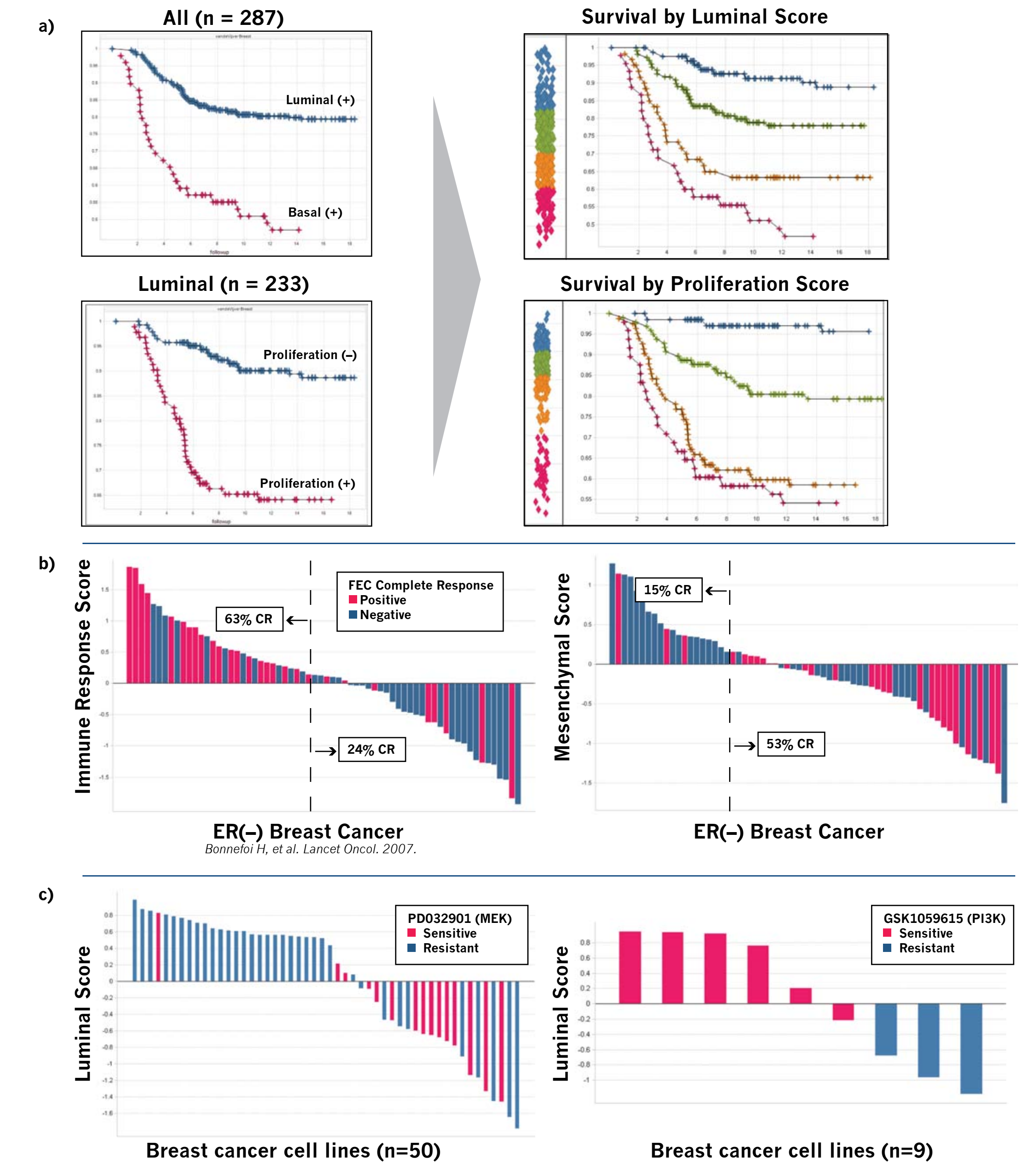
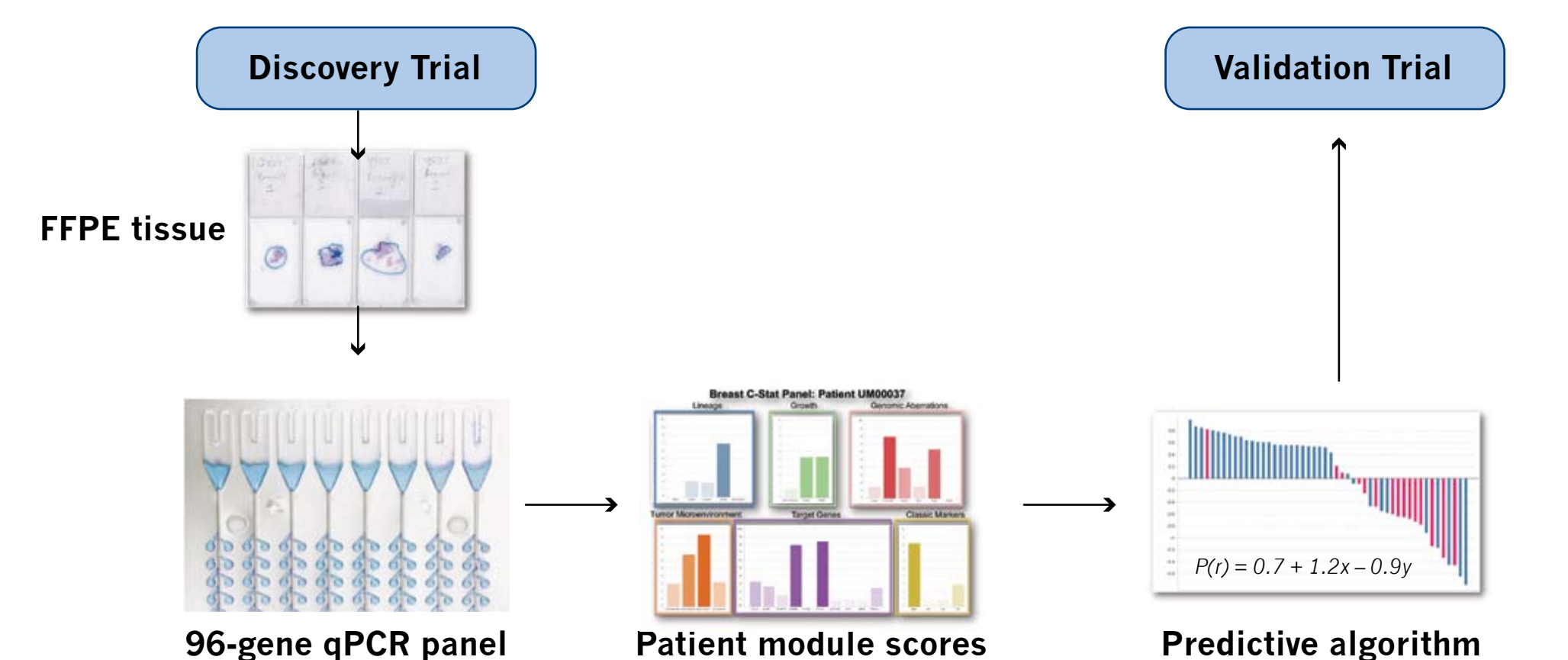


Figure 8. Validation of a Breast Cancer Module-based Predictive Biomarker.

Breast cancer module analysis provides a description of 21 physiological states that can be leveraged to predict a patient's outcome to any therapy targeting breast cancer.

- Report on standard histopathological parameters, such as ER, PR and Her2
- Focal genomic amplifications, such as Her2 and Myc, as well as focal expression events present subpopulations of patients
- Represent all of the major genetic factors that present within breast cancer.



Conclusions:

- Molecular heterogeneity of cancer can be reduced to small number of core modules
- Breast cancer modules provide a platform for patient stratification and responder enrichment
- Breast cancer modules ready to deploy throughout drug development process
- Companion diagnostic assay panels can be customized with specific response algorithms for candidate therapies
- Module-based tests have clear path to market as companion diagnostics