Proteogenomic profiling of treatment-naïve breast cancer biopsies from patients receiving neoadjuvant chemotherapy

Eric Jaehnig[1], Matthew Holt[1], Jana Zecha[2], Matthew S. Glover[2], William Kelly[3], Barrett Nuttall[3], Bryant McCue[1], Ivan Marin[1], Stacey Carter[1], Elizabeth Bonefas[1], Logan Healy[1], George Miles[1], Meenakshi Anurag[1], Susan Hilsenbeck[1], Bing Zhang[1], Sonja Hess[2], Alastair Thompson[1]

[1]Baylor College of Medicine, Houston, TX; [2]Dynamic Omics, Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, MD; [3]Translational Medicine, Early Oncology, Oncology R&D, AstraZeneca, Gaithersburg, MD

Abstract

Background: Breast cancer is a heterogeneous disease, comprising approximately 70% hormone receptor-positive (HR+), 15% HER2-positive (HER2+), and 15% triple-negative breast cancer (TNBC) cases. As standard of care, HR+ tumors are treated with hormone therapy, often after surgery. However, chemotherapy may also be used before surgery (neoadjuvant setting). HER2+ breast cancer responds well (50-70% pathological Complete Response, pCR) to targeted anti-HER2 therapies. In contrast, TNBC is primarily treated with neoadjuvant chemotherapy, with only 30-50% achieving pCR. Identifying biomarkers that are predictive of pCR or that indicate alternative therapeutic strategies can help tailor therapy. Recent studies using proteogenomic data (DNA, RNA, protein, and phosphorylation site profiles from the same tumor) from clinical trials highlighted candidates including LIG1 loss in TNBC and GPRC5A/TPBG overexpression in HER2+ tumors as potential predictive biomarkers and therapeutic targets. This study applies proteogenomic profiling to pre-treatment biopsies from tumors treated with physician-selected neoadjuvant chemotherapy to further explore putative targets for precision oncology.

Methods: Microscaled proteogenomic profiling was conducted on 51 treatment-naive primary breast tumor core needle biopsies. Subtyping was determined via IHC: HER2+ (IHC 3+), HER2 Equivocal (IHC 2+), HR+ (ER or PR positive), or TNBC (negative for all three receptors). All received physician-selected neoadjuvant regimens, and pCR was evaluated after surgical resection. Multi-omics profiling of tumor sample from each biopsy included whole-exome sequencing (WES), RNA-seq, and mass spectrometry-based proteomics and phosphoproteomics. Both data-dependent acquisition with TMT-labeling (TMT) and data-independent acquisition (DIA) proteomics were used.

Results: Proteomic profiles were obtained for 16 HER2+, 17 HR+, and 18 TNBC patients. WES data were acquired for 50 patients; RNA-seq for 26. DIA quantified more total proteins/sites than TMT, though measurements for top variable proteins were highly correlated. To assess the potential for each proteomic data type to inform precision oncology, both were included in all subsequent analyses. The highest pCR rate (69%) was achieved in HER2+ tumors treated with dual antibody therapy whereas all non-pCR tumors were HR+, a known resistance marker, and 3 were HER2 equivocal with lower HER2 protein expression. Both the DIA and TMT data also confirmed significantly higher GPRC5A protein levels in non-pCR *vs.* pCR HER2+ tumors. HR+, HER2- tumors had the lowest pCR (12.5%), but two pCR cases co-treated with pembrolizumab showed high expression of cell cycle and immune proteins. TNBC showed 35% (6/17) pCR overall, improving to 56% (5/9) when treatment only including pembrolizumab-treated patients. Non-pCR tumors had lower cell cycle/immune and higher epithelial-mesenchymal transition protein levels than pCR tumors. Detailed proteogenomic profiles for the FDA-approved drug targets, TNBC-specific kinases, and cell membrane proteins enriched in the context of non-pCR TNBC cases are presented.

Conclusion: This study demonstrates the feasibility of proteogenomic profiling from clinical breast tumor biopsies and highlights mechanisms of anti-HER2 resistance, potential factors underlying exceptional chemotherapy response in ER+ cancers, and candidate alternative therapeutic targets for non-pCR TNBC, offering a template for future precision oncology efforts. However, the current cohort was not well powered, and further validation is needed in independent cohorts, along with further mechanistic studies in preclinical models.

Overview of Dataset

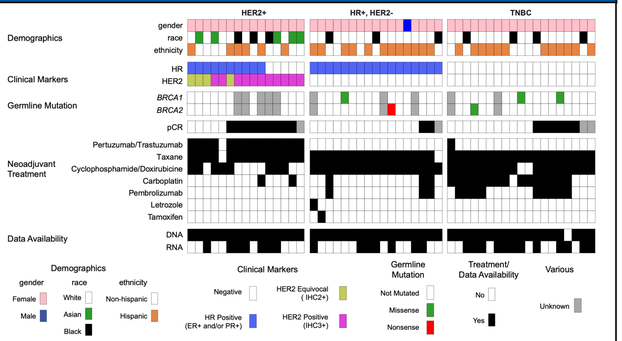


Figure 1: Sample map shows meta data and genomic data availability for 51 pre-treatment core needle biopsies obtained in the clinical setting. Pathological Complete Response (pCR) to neodjuvant chemotherapy in combination with additional indicated treatment regimens was determined at time of surgery. Samples for which whole-exome (DNA) and RNA sequencing data is available as well as those with germline BRCA mutations are indicated. Proteomic and phosphoproteomic data was obtained for all biopsies.

Genomic Landscape

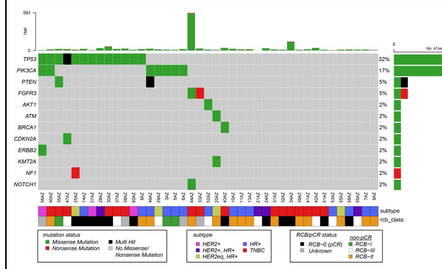


Figure 2: Oncoprint showing tumor mutation burden (TMB) and mutation status in each sample for genes frequently mutated in breast cancer. RCB: residual cancer burden

Proteomics Overview

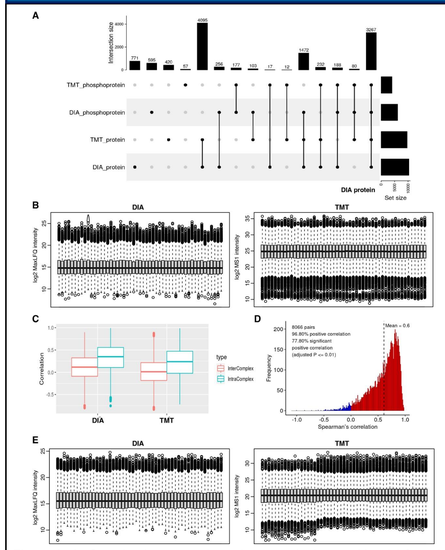


Figure 3: A. Gene level coverage in each proteomic dataset. Material from the same biopsies was used for TMT and DIA proteomics and phosphoproteomics. **B.** Similar distributions were observed for proteins in all samples in both the DIA (left) and TMT (right) datasets. **C.** Pairs of proteins from the same complex show higher correlation than pairs from different complexes in both datasets. **D.** Histogram of correlations between DIA and TMT for each protein. **E.** Similar distributions were observed for phospho-sites in all samples for both DIA (left) and TMT (right).

Proteogenomic Assessment of Clinical Markers

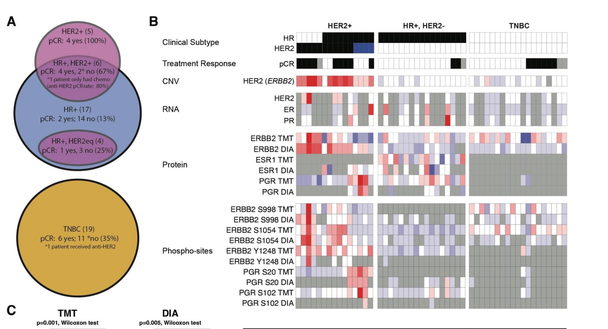


Figure 4: A. All HER2+, HR- tumors achieved pCR whereas all non-pCR tumors were also HR+, and worst response rate for anti-HER2 was observed among HER2 equivocal samples, which also showed lower levels for gene amplification and expression **(B). C.** GPRC5A, recently identified as a biomarker elevated in non-pCR (Jaehnig, et al., *Cell Rep. Med.*, 2025), is significantly higher in non-pCR HER2+ tumors in this dataset.

Cell Cycle and Immune Protein Enrichment in HR+ pCR

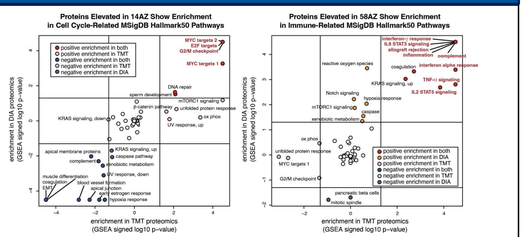


Figure 5: Compared to set of non-pCR HR+, HER2- tumors (n=14), the two pCR tumors showed enrichment for higher levels of cell cycle (left) and immune (right) pathways from MSigDB.

EMT Proteins are Elevated in non-pCR TNBC

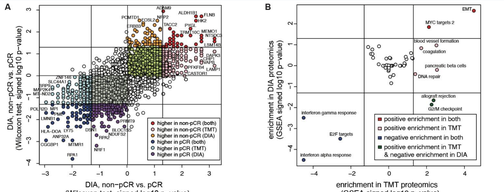


Figure 6: Comparison of proteins **(A)** and MSigDB Hallmark pathways **(B)** that are differential between non-pCR (n=11) and pCR (n=6) in TNBC in DIA (y-axis) and TMT data (x-axis).

Key Point

Both DIA and TMT proteomics of primary breast tumor biopsies reveal pathways associated with pCR to neoadjuvant therapy and nominate alternative therapeutic targets for non-pCR cases, but further validation in independent cohorts is needed.

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Proteogenomic Assessment of Potential Therapeutic Targets with Elevated Levels in non-pCR TNBC

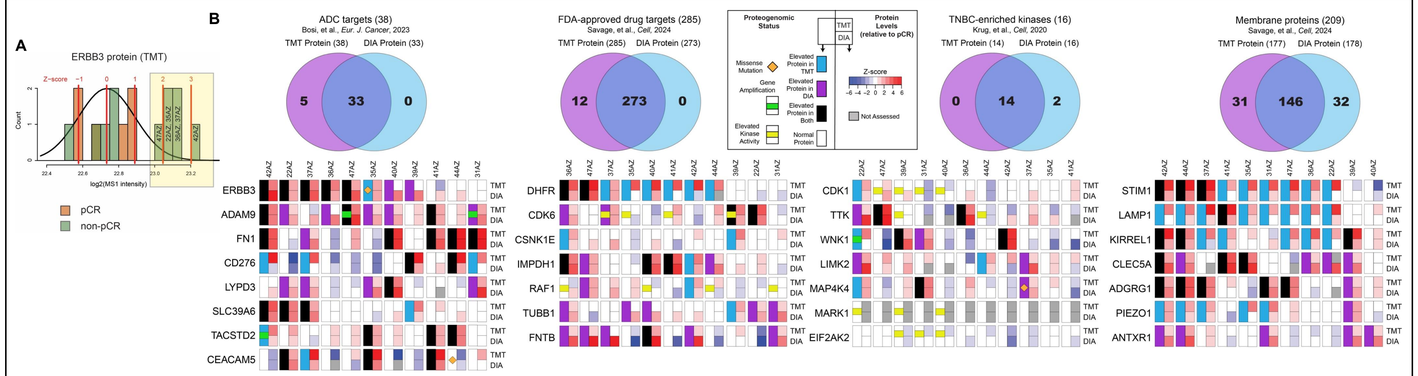


Figure 7: A. Identification of non-pCR tumors with elevated protein or kinase activity inferred from substrate phospho-site levels for potential therapeutic targets using the set of pCR tumors as the baseline. **B.** Oncoprints summarize proteogenomic data for candidate therapeutic targets from four categories (antibody-drug conjugate (ADC) targets approved or in development, FDA-approved drug targets, kinases enriched in TNBC, and membrane proteins). Each non-pCR tumor is included as a column.

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