Molecular Evaluation of Residual Disease Following Neoadjuvant Chemotherapy in Triple Negative Breast Cancer CALGB 40603 (Alliance)

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Trial and Sample Overview

CALGB 40603 (Alliance) evaluated the effects of adding carboplatin or bevacizumab to a neoadjuvant chemotherapy regimen of weekly paclitaxel, followed by dose-dense doxorubicin and cyclophosphamide, in patients with stage II/III TNBC. Trial results (PMID: 25092775) and analyses of pre-treatment RNA and DNA sequencing were previously published (PMID: 35044810, PMID: 40057511).

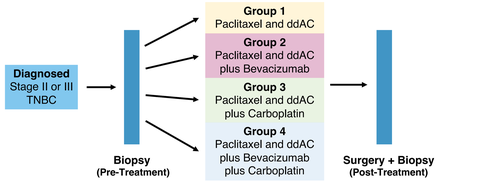


Figure 1: Trial design of CALGB 40603, with collection of pre-treatment and post-treatment samples for histology, and RNA and DNA extraction for RNA and DNA sequencing.

Sample Overview

Of 340 patients with pre-treatment RNAseq samples, 162 (47.6%) also had matched post-treatment samples; 92/162 (56.8%) achieved a pathological complete response (pCR) and 70/162 (43.2%) had residual disease (RD) at surgery.

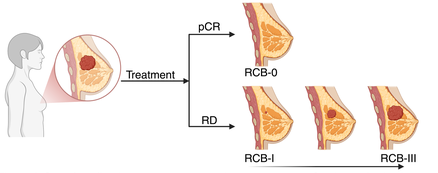


Figure 2: Sample collection pre-treatment and post-treatment of 162 matched pairs

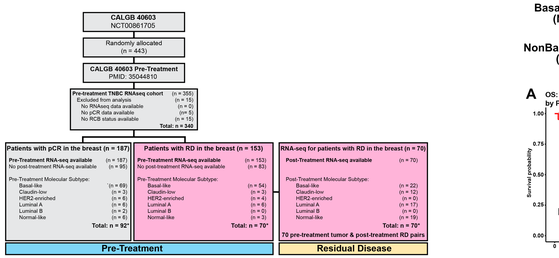


Figure 3: CONSORT diagram of available samples from CALGB 40603 for RNAseq and survival analysis, including 70 matched pairs of pre-treatment tumors and post-treatment RD.

Molecular Characteristics of Matching Pre-treatment Tumors and Post-Treatment RD

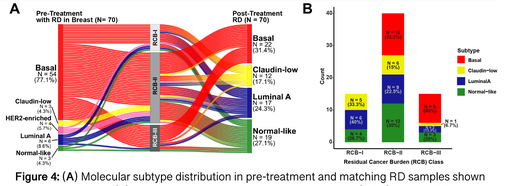


Figure 4: (**A**) Molecular subtype distribution in pre-treatment and matching RD samples shown as Sankey plot, and (**B**) molecular subtype by residual cancer burden (RCB) class of RD.

Survival by Proliferation and Molecular Subtype

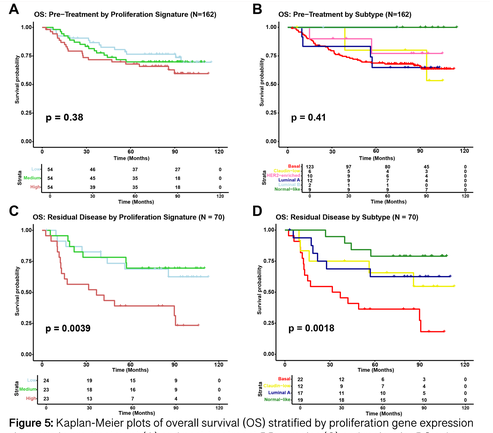


Figure 5: Kaplan-Meier plots of overall survival (OS) stratified by proliferation gene expression signature in pre-treatment (**A**) and post-treatment RD samples (**C**) and molecular BC subtype in pre-treatment (**B**) and post-treatment RD samples (**D**).

Sample Overview by Basal-Molecular Subtype



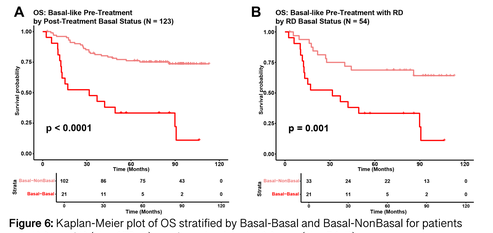


Figure 6: Kaplan-Meier plot of OS stratified by Basal-Basal and Basal-NonBasal for patients with RD or pCR (N = 123, **A**) and for patients with RD only (N = 54, **B**).

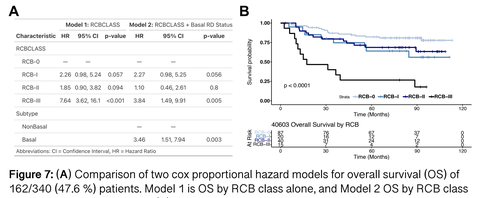


Figure 7: (**A**) Comparison of two cox proportional hazard models for overall survival (OS) of 162/340 (47.6 %) patients. Model 1 is OS by RCB class alone, and Model 2 OS by RCB class adjusted for Basal RD status. (**B**) Kaplan-Meier plot of 162 patients with matching post-treatment samples, stratified by RCB.

Supervised Analysis to Identify Molecular Features Associated with Poor Outcome Pre-Treatment

Patients with Basal-like RD have a significantly poorer outcome than patients with NonBasal RD. We used supervised analyses and Gene Set Enrichment Analysis (GSEA) to identify which molecular features are different in pre-treatment tumors of patients with Basal-Basal disease.

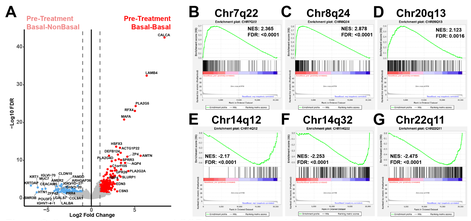


Figure 8: Supervised analyses and GSEA between Basal-Basal and Basal-NonBasal pre-treatment tumors using edgeR based differential expression analysis (**A**) and GSEA (**B**).

DNA Sequencing Analysis of Basal-like Pre-Treatment Tumors

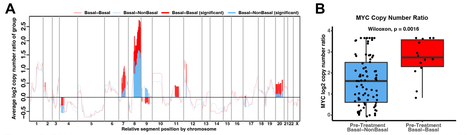


Figure 9: DNA-based log2 copy number ratio scores of predefined chromosomal segments (**A**) and the MYC gene (**B**) between Basal-Basal (red, N = 14) and Basal-NonBasal (blue, N= 82) pre-treatment tumors.

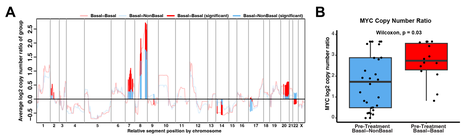


Figure 10: DNA-based log2 copy number ratio scores of pre-defined chromosomal segments (**A**) and the MYC gene (**B**) between Basal-Basal (red, N = 14) and Basal-NonBasal (blue, N= 30) pre-treatment tumors, limited to the pre-treatment Basals with RD (N = 54).

Supervised Analysis Using Gene Expression Signatures

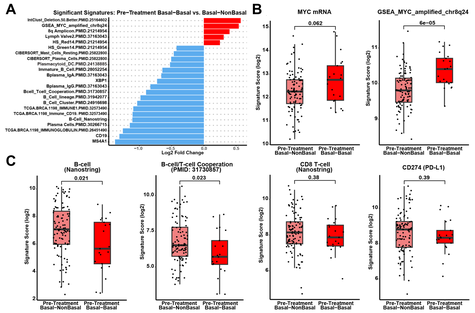


Figure 11: SAMR-based supervised analysis using 957 gene signatures , reflective of BC biology (PMID: 25109877) comparing Basal-Basal vs. Basal-NonBasal pre-treatment tumors. (**A**) Top differentially expressed signatures—higher (red) and lower (blue) expressed in Basal-Basal tumors. (**B**) Box plots of select signatures higher and (**C**) lower expressed in Basal-Basal tumors.

Supervised Analysis Between Pre- and Post-Treatment Basal-Basal Samples

Patients with Basal-like RD have a significantly poorer outcome than those with NonBasal RD. We used supervised analyses and GSEA to identify which molecular features are different in post-treatment Basal-Basal RD compared to their matching pre-treatment Basal-Basal tumors.

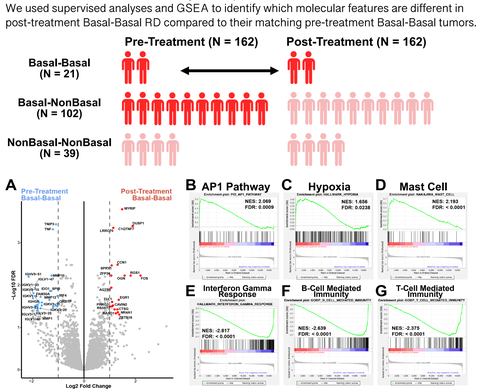


Figure 12: Supervised analyses and GSEA between Basal-Basal pre-treatment and Basal-Basal RD tumors using edgeR based differential expression analysis (**A**) and GSEA (**B**).

Supervised Analysis Using Gene Expression Signatures

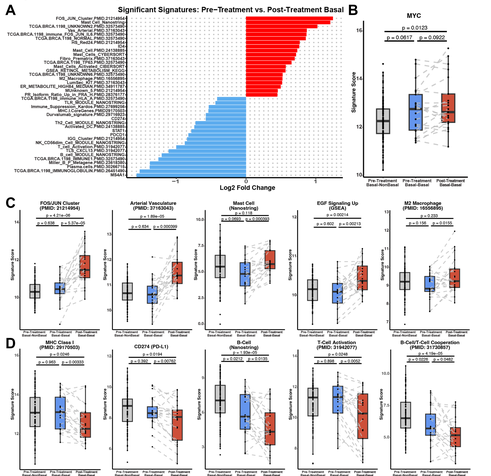


Figure 13: Supervised SAMR analysis with 957 gene signatures (PMID: 25109877) comparing matched Basal-Basal pre- and post-treatment samples. (**A**) Top differentially expressed signatures—higher (red) and lower (blue) in post-treatment Basal-Basal RD, including MYC (**B**). Box plots of select higher (**C**) and lower (**D**) signatures in post-treatment Basal-Basal RD.

Contact

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Survival Predictors in Pre-Treatment Samples Compared to Residual Disease

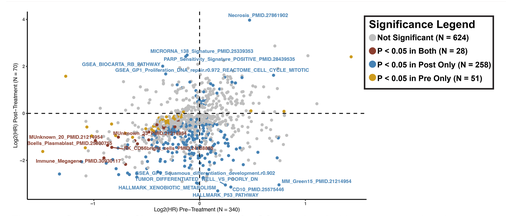


Figure 14: Comparison of hazard ratios (HRs) for OS associated with gene expression signatures (N = 957) in pre-treatment and RD samples. Pre-treatment HRs are on x-axis, and RD HRs on the y-axis. Colored points indicate significant overlapping or unique gene signatures.

ADC Treatment in Basal-like PDX Tumors

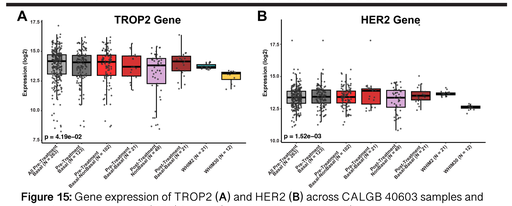


Figure 15: Gene expression of TROP2 (**A**) and HER2 (**B**) across CALGB 40603 samples and patient derived xenograft (PDX) tumors (i.e., WHIM2 and WHIM30).

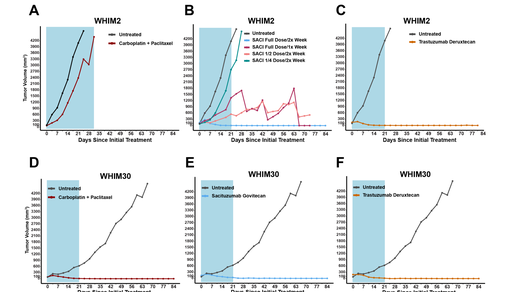


Figure 16: Treatment of PDX models, WHIM2 (**A-C**) and WHIM30 (**D-F**), with carboplatin + paclitaxel, sacituzumab govitecan (SACI), and trastuzumab deruxtecan (T-DXd), respectively.

Conclusions

- Patients with Basal-like RD have poorest survival outcomes.
- Basal-Basal pre-treatment tumors express low levels of B-Cell signatures and have higher Chr8q amplifications (including MYC).
- Basal-Basal RD shows dysfunctional immune response and immunodepleted gene expression.
- Pre-treatment and RD survival predictors are different.
- Standard chemo-refractory Basal-like PDX tumors are sensitive to ADCs.

Acknowledgements

PEROU LAB

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