CIRCULATING DETERMINANTS OF RESPONSE TO IMMUNOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER (TNBC)

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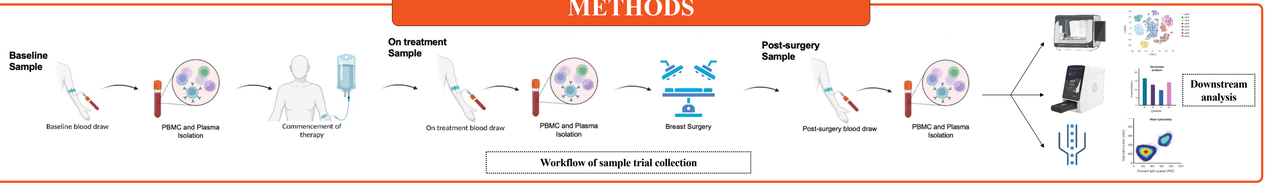
INTRODUCTION

- **TNBC** represents approximately **20%** of all **breast cancers** and is an **aggressive subtype** with **poor prognosis**, high tumor mutational burden, extensive immune infiltration, and paucity of molecular targets.
- The **KEYNOTE-522** trial established **neoadjuvant pembrolizumab + chemotherapy** as the standard of care for **early-stage TNBC**, significantly **improving pathological complete response (pCR)** rates.
- Patients who achieve a **pCR** have significantly **higher long-term survival rates**. Conversely, a **lack of pCR** is a marker of **high-risk disease and poor outcomes**.
- **~30-45%** do **not achieve** a **pCR**, and the biological mechanisms that determine response remain unclear.
- This study aimed to identify **circulating predictors of pCR** in TNBC receiving neoadjuvant chemo-immunotherapy.

HYPOTHESIS

Pretreatment differences in peripheral blood immune compartment could help predict TNBC patients most likely to achieve a pCR

METHODS



RESULTS

Table 1. Baseline characteristics of all patients enrolled in the study

No significant differences in baseline characteristics were observed between responders and non-responders.

Characteristic	Treatment Response: Overall N = 46[1]	Non-responder N = 21[1]	Responder N = 25[1]	p-value[2]
Age at Diagnosis, years	51.0 (39.0, 60.0)	52.0 (39.0, 62.0)	49.0 (40.0, 57.0)	0.5
Sex				
Female	46 (100%)	21 (100%)	25 (100%)	
Race/Ethnicity				0.3
Asian	2 (4.3%)	2 (9.5%)	0 (0%)	
Black or African American	20 (43%)	10 (48%)	10 (40%)	
Hispanic or Latino	1 (2.2%)	0 (0%)	1 (4.0%)	
White	23 (50%)	9 (43%)	14 (56%)	
Stage at Diagnosis				0.11
II	11 (24%)	6 (29%)	5 (21%)	
III	31 (69%)	12 (57%)	19 (79%)	
IV	3 (6.7%)	3 (14%)	0 (0%)	
Missing	1	0	1	
BMI Category				0.6
Underweight	0 (0%)	0 (0%)	0 (0%)	
Normal	13 (28%)	7 (33%)	6 (24%)	
Overweight	16 (35%)	8 (38%)	8 (32%)	
Obese	17 (37%)	6 (29%)	11 (44%)	
Elixhauser Comorbidity Score	3.0 (2.0, 13.0)	3.0 (2.0, 13.0)	6.0 (2.0, 13.0)	0.8
Missing	2	0	2	
BRCA Mutation				
BRCA-1	5 (10.8%)	0	5 (20%)	0.05
BRCA-2	1 (2.1%)	1 (4.8%)	0	
No mutation	38 (82.6%)	20 (85.2%)	18 (72%)	
Declined testing	2	0	2	
Has Single-Cell Data	18 (39%)	7 (33%)	11 (44%)	0.5
Has Proteomics Data	28 (61%)	12 (57%)	16 (64%)	0.6
Has Flow Cytometry Data	29 (63%)	15 (71%)	14 (56%)	0.3

[1]Median (Q1, Q3); n (%)

[2]Wilcoxon rank sum test; Fisher's exact test; Pearson's Chi-squared test

Figure 2. Overlap of patients across the study methods

All patients who underwent single-cell sequencing also underwent proteomic analysis. The largest overlap was observed between HPFC and proteomics (n = 25).

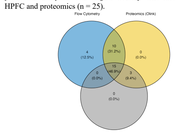


Figure 3. Kaplan-Meier survival curve

Responders demonstrated a statistically significant survival advantage relative to non-responders.

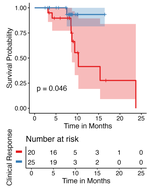


Figure 4. (A) UMAP depicting the distribution of cell types; (B) cell type distribution by group and timepoint

Cell types were clustered based on transcriptional similarities and annotated according to characteristic marker expression. A higher proportion of CD4+ T cells was observed, and the overall cell type distribution was consistent across groups and timepoints.

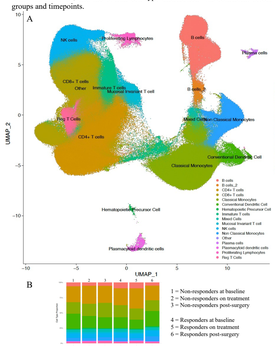


Figure 5. Cell communication at each of the study timepoints

Responders exhibited increased and stronger intercellular communication at baseline and during treatment, whereas non-responders showed the strongest communication post-surgery.

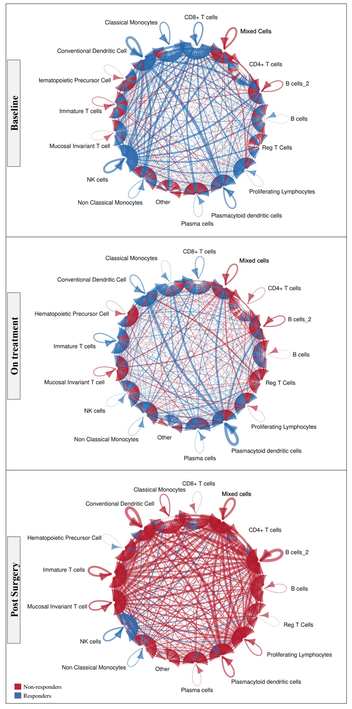


Table 2. Changes in cell population proportions across treatment timepoints

Key differences between responders and non-responders were observed in baseline B cells and classical monocytes, and in baseline-to-post-surgery changes in $CD4^+$, $CD8^+$, NK, classical monocytes, and regulatory T cells, which showed opposite trends between groups.

Cell Type	Responders: Baseline	Responders: On treatment	Responders: Post-surgery	Non-responders: Baseline	Non-responders: On treatment	Non-responders: Post-surgery
B cells	9.40%	9.50%	5.10%	6.30%	5.50%	5.60%
B cells_2	0.80%	0.80%	0.60%	0.50%	0.50%	0.60%
CD4+ T cells	26.90%	32.20%	23.30%	25.30%	28.80%	27.10%
Mixed cells	0.50%	0.60%	0.60%	0.60%	0.70%	0.60%
CD8+ T cells	19.60%	18.10%	17.40%	18.20%	19.70%	23.50%
Classical Monocytes	17.90%	14.90%	25.10%	24.60%	19.50%	18.00%
Conventional Dendritic Cell	1.40%	1.50%	1.60%	1.60%	1.80%	1.20%
Hematopoietic Precursor Cell	0.10%	0.10%	0.10%	0.20%	0.20%	0.10%
Immature T cells	4.50%	4.70%	4.00%	3.50%	5.00%	4.60%
Mucosal Invariant T cell	1.90%	1.90%	1.50%	2.40%	2.50%	2.40%
NK cells	8.00%	7.60%	9.30%	7.50%	7.60%	6.40%
Non Classical Monocytes	5.80%	3.80%	8.10%	5.20%	4.10%	5.60%
Other	0.20%	0.10%	0.10%	0.10%	0.10%	0.10%
Plasma cells	0.20%	0.30%	0.20%	0.40%	0.20%	0.30%
Plasmacytoid dendritic cells	0.50%	0.50%	0.60%	0.60%	0.80%	0.50%
Proliferating Lymphocytes	0.40%	1.00%	0.70%	0.50%	0.80%	0.80%
Reg T Cells	1.90%	2.40%	1.70%	2.00%	2.10%	2.70%

Figure 6. Pathway enrichment analysis

Responders exhibited a higher baseline interferon state, primarily driven by lymphocytes, alongside a myeloid-driven TNFα/NFκB inflammatory response during treatment. In contrast, non-responders mounted an ineffective interferon response with TNFα/NFκB downregulation.

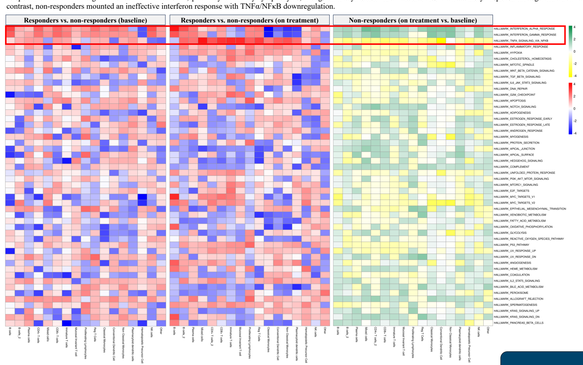


Figure 7. UMAPs from Flow Cytometry analysis at baseline

The plots highlight differences in cell distribution between groups, where each color represents a distinct cell population defined by marker expression within the corresponding gate.

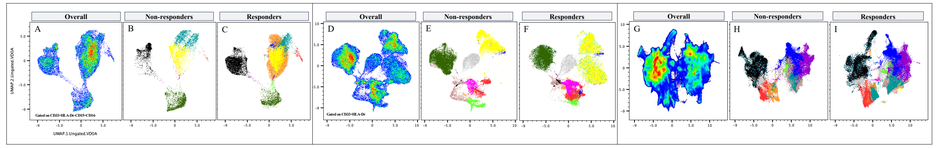


Figure 8. Dynamic proteomic changes (x-axis: log2 fold change)

Red dots indicate $p < 0.05$. Responders exhibited more pronounced and dynamic proteomic changes across all panels.

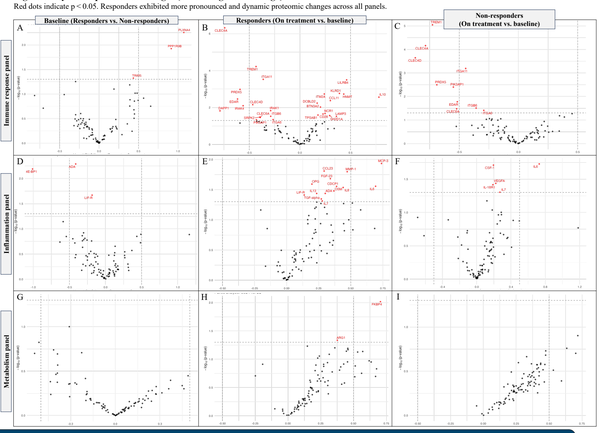


Table 3. Correspondence of HPFC markers with cell types, functions, and proportions in responders vs. non-responders

Panels (Fig 7)	Markers	Cell Type	% responders	% non-responders
A-C	CD15+CD16-CD86+CD80+	Immature activated neutrophils	50.4	2.1
	CD15+CD16+CD163+CX3CR1+	Mature neutrophils with an immunosuppressive phenotype	6	23.8
D-F	CD15+CD16+PD-L1+VISTA+	Immunoregulatory myeloid cells	43.1	22.2
G-I	CD57+LAG3+TIGIT+TIM3+	Exhausted T-cells	1.5	8.7

Table 4. Role, actions, and impact on T-cells of neutrophils by phenotype/marker expression

	Immature Activated Neutrophils	Mature Immunosuppressive Neutrophils
Primary Role	Anti-tumor	Pro-tumor
Key Actions	Direct tumor killing, T-cell recruitment, inflammation	T-cell suppression, immune evasion
Impact on T-cells	Helps activate and recruit	Inhibits

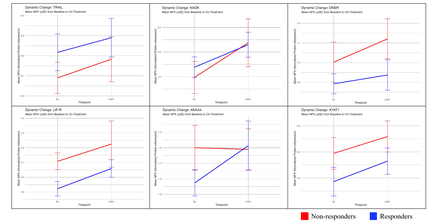
Table 5. Potential baseline proteomic predictors of response and non-response identified using a Machine Learning (ML) approach

The ML approach used LASSO and Random Forest models trained with 5-fold cross-validation. Proteins consistently identified as top predictors by both models were included in the final results. The predictive direction (response vs. non-response) for each protein was determined by its LASSO coefficient.

Potential predictors of pCR	Potential predictors of non-response
TRAIL (TNF-related apoptosis-inducing ligand)	DNER (Delta/Notch-like EGF-Related Receptor)
NADK (NAD Kinase)	LIF-R (Leukemia Inhibitory Factor Receptor)
	ANXA4 (Annexin A4)
	KYAT1 (Kynurenine Aminotransferase 1)

Figure 9. Dynamic changes from baseline to on-treatment in the proteins identified by the ML approach (Table 5)

The x-axis represents the normalized protein expression (NPX), and the y-axis indicates timepoints, where BL denotes baseline and C3D1 denotes on-treatment.



CONCLUSIONS

Response to immunotherapy in TNBC may be largely dictated by baseline immune status. Patients with pCR showed active T-cell reserves and a pro-immune proteomic profile enabling effective anti-tumor responses, whereas residual disease was linked to a pre-existing immune-suppressive state with suppressive myeloid cells and factors causing T-cell dysfunctional activity

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