Feasibility of a machine learning-based peripheral blood immunoprofiling platform to stratify patients with early-stage triple-negative breast cancer (eTNBC) by neoadjuvant therapy response

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Introduction

- Tissue-based biomarkers for predicting breast cancer outcomes are limited by sampling bias and tumor heterogeneity[1,2]. **Blood-based immunoprofiling** offers a promising alternative[3].
- A machine learning-based platform using **multiparameter flow cytometry** (MFC) analysis of **peripheral blood mononuclear cells** (PBMCs) previously identified 5 immunotypes: G1-Naïve (Naïve T cell enriched), G2-Primed (Memory CD4+ T cell enriched), G3-Progressive (DC/NK/Memory CD8+ T cell enriched), G4-Chronic (Effector/Exhausted CD8+ T cell enriched), G5-Suppressive (Myeloid/MDSC enriched), with potential associations with treatment outcomes[4].
- We aimed to assess whether applying this platform to serial PBMC samples from patients (pts) with **eTNBC** treated with **neoadjuvant therapy** (NAT) (1: chemotherapy [ChT]; 2: ChT+immunotherapy [IO]) could stratify pts into response groups.

Materials and Methods

- Pts with eTNBC, available pathological response information, and PBMCs at both baseline (treatment-naive) and on-NAT (weeks 4–7) were identified from the **prospective DFCI Multicenter TNBC registry**.
- PBMCs were stained with 10 custom antibody panels[4] (average 14 antibodies/panel) and analyzed by MFC.
- A regression model calculated immune signature scores (ISSs; 0: least aligned, 10: most aligned), assigning samples to one of 5 immunotypes based on maximum ISS. Associations with pathologic complete response (pCR, yes/no) were assessed by Fisher's exact test, Mann-Whitney U test (p<.05), and ROC-AUC.

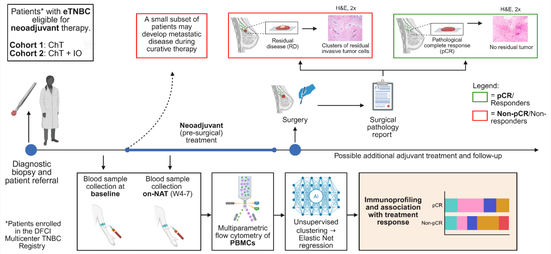


Figure 1. Study design.

Results: Quality Control (QC)

- **49 baseline** and **46 on-NAT** PBMC samples passed QC (median viability 88%), with all major immune cell families well represented and stable across time points.

Results: Patient population by baseline immunotype

Baseline immunotype Characteristic	Overall (N=49)	G1 Naive (N=10)	G2 Primed (N=9)	G3 Progressive (N=16)	G4 Chronic (N=8)	G5 Suppressive (N=6)	p-value
Age (diagnosis)							
Mean (SD)	50.7 (13.4)	50.3 (10.8)	57.0 (12.3)	43.0 (10.4)	59.5 (15.6)	50.6 (14.7)	**0.023**
Median [Min, Max]	51.0 [28.6, 87.7]	51.4 [32.3, 65.5]	58.4 [42.3, 82.9]	41.7 [28.6, 61.2]	58.5 [32.8, 87.7]	51.7 [33.3, 73.5]	
Race							
African American	4 (8.2%)	1 (10.0%)	1 (11.1%)	1 (6.3%)	1 (12.5%)	0 (0.0%)	0.824
Asian/Pacific Islander	2 (4.1%)	1 (10.0%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Caucasian	40 (81.6%)	8 (80.0%)	7 (77.8%)	13 (81.3%)	6 (75.0%)	6 (100.0%)	
Unknown	3 (6.1%)	0 (0.0%)	0 (0.0%)	2 (12.5%)	1 (12.5%)	0 (0.0%)	
Ethnicity							
Non-Spanish/ non-Hispanic	47 (95.9%)	10 (100.0%)	9 (100.0%)	15 (93.8%)	7 (87.5%)	6 (100.0%)	0.608
Spanish/Hispanic	2 (4.1%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	1 (12.5%)	0 (0.0%)	
Clinical stage (Prognostic)							
Stage IA	1 (2.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0.606
Stage IB	6 (12.2%)	0 (0.0%)	2 (22.2%)	3 (18.8%)	1 (12.5%)	0 (0.0%)	
Stage IIB	22 (44.9%)	5 (50.0%)	3 (33.3%)	4 (25.0%)	5 (62.5%)	5 (83.3%)	
Stage IIIA	7 (14.3%)	2 (20.0%)	0 (0.0%)	3 (18.8%)	1 (12.5%)	1 (16.7%)	
Stage IIIB	9 (18.4%)	3 (30.0%)	3 (33.3%)	3 (18.8%)	0 (0.0%)	0 (0.0%)	
Stage IIIC	4 (8.2%)	0 (0.0%)	1 (11.1%)	2 (12.5%)	1 (12.5%)	0 (0.0%)	
Histologic grade							
G2	4 (8.2%)	1 (10.0%)	1 (11.1%)	0 (0.0%)	1 (12.5%)	1 (16.7%)	0.672
G3	45 (91.8%)	9 (90.0%)	8 (88.9%)	16 (100.0%)	7 (87.5%)	5 (83.3%)	
Neoadjuvant therapy type							
ChT	27 (55.1%)	2 (20.0%)	4 (44.4%)	9 (56.3%)	7 (87.5%)	5 (83.3%)	**0.030**
ChT + IO	22 (44.9%)	8 (80.0%)	5 (55.6%)	7 (43.8%)	1 (12.5%)	1 (16.7%)	
Path. response							
pCR	25 (51.0%)	6 (60.0%)	1 (11.1%)	10 (62.5%)	4 (50.0%)	4 (66.7%)	0.112
Non-pCR	24 (49.0%)	4 (40.0%)	8 (88.9%)	6 (37.5%)	4 (50.0%)	2 (33.3%)	
RCB category							
RCB-0 (pCR)	25 (51.0%)	6 (60.0%)	1 (11.1%)	10 (62.5%)	4 (50.0%)	4 (66.7%)	0.327
RCB-I	3 (6.1%)	1 (10.0%)	0 (0.0%)	1 (6.3%)	1 (12.5%)	0 (0.0%)	
RCB-II	14 (28.6%)	2 (20.0%)	7 (77.8%)	3 (18.8%)	1 (12.5%)	1 (16.7%)	
RCB-III	5 (10.2%)	1 (10.0%)	1 (11.1%)	1 (6.3%)	1 (12.5%)	1 (16.7%)	
Unknown	2 (4.1%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	1 (12.5%)	0 (0.0%)	

Results: Immunotypes (max ISS) were not significantly associated with NAT response (pCR vs non-pCR) in either cohort (a), but they differed by therapy type (b)

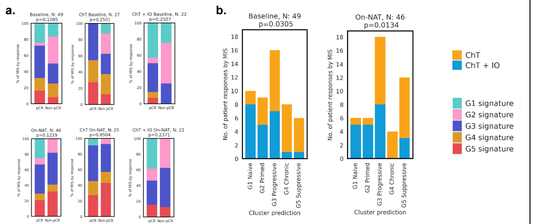


Figure 2. Panel a. Immunotype distribution by treatment response (pCR vs non-pCR) at baseline (*top*) and on-NAT (*bottom*) for the overall cohort (*left*), chemotherapy-only subgroup (*middle*), and chemoimmunotherapy subgroup (*right*) (Fisher's exact test). **Panel b**. Immunotype distribution by therapy type is shown at baseline (*left*) and on-NAT (*right*) (Fisher's exact test).

Results: The immunotype distribution shifted from baseline to on-NAT in a higher proportion of pts in the ChT+IO group (76%) compared with the ChT group (39%)

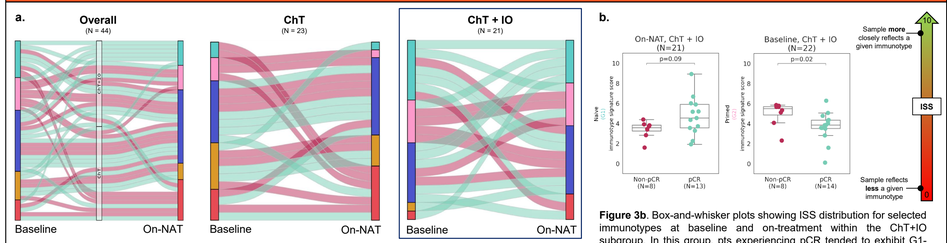


Figure 3a. Sankey diagrams showing immunotype transitions from baseline (*left axis*) to on-NAT (*right axis*). The left plot represents the overall cohort, the middle plot the neoadjuvant chemotherapy subgroup, and the right plot the neoadjuvant chemoimmunotherapy subgroup. Aqua-green lines indicate pts experiencing pCR, and red lines indicate non-pCR pts. Side bars denote individual immunotypes.

Figure 3b. Box-and-whisker plots showing ISS distribution for selected immunotypes at baseline and on-treatment within the ChT+IO subgroup. In this group, pts experiencing pCR tended to exhibit G1-Naïve on-NAT, regardless of baseline immunotype, whereas non-pCR pts more often showed G2-Primed at baseline. Consistently, ISS was modestly associated with response for G1-Naïve on-NAT (p = 0.09) and G2-Primed at baseline (p = 0.02).

Exploratory (1a): Considering all ISSs per sample, immune scores were not independent predictors of treatment response

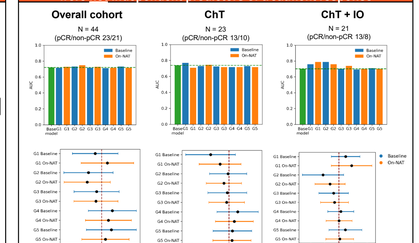


Figure 4. Univariable logistic regression model assessing association between clinicopathologic variables, all ISSs at baseline and on-NAT, and treatment response (pCR vs non-pCR).

Exploratory (1b): The inclusion of immunotype scores with clinical variables improved predictive performance in the training set

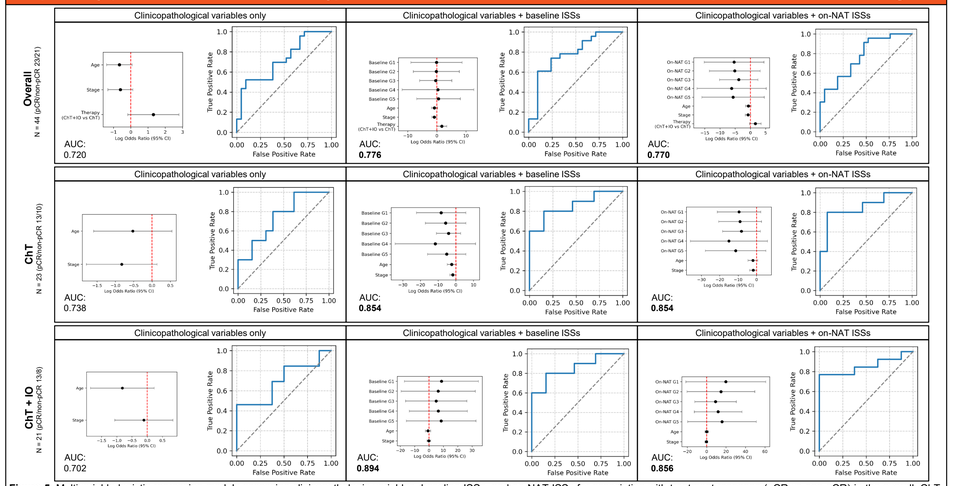


Figure 5. Multivariable logistic regression models assessing clinicopathologic variables, baseline ISSs, and on-NAT ISSs for association with treatment response (pCR vs non-pCR) in the overall, ChT, and ChT+IO groups (*training set*). **Leave-one-out cross validation (LOOCV) suggests the current dataset may be too small to train a generalizable model** (AUC~0.6-0.5, *not shown*).

Exploratory (2): CD8+ T-cell subsets predominated in pCR, CD4+ subsets were more frequent in non-pCR

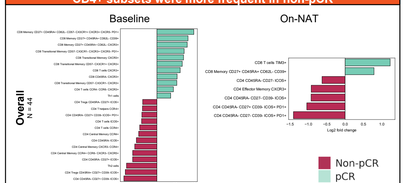


Figure 6. Differential immune cell population analysis in the overall cohort, highlighting subsets enriched in pts with pCR or non-pCR ($p < 0.05$; minor populations filtered). $CD8^+$ T-cell subsets predominated in pCR, whereas $CD4^+$ subsets were more frequent in non-pCR pts at both timepoints. This pattern was consistent across ChT and ChT+IO subgroups (*not shown*).

Exploratory (3): Baseline Th1/Th2 ratio may differentiate between pCR and non-pCR.

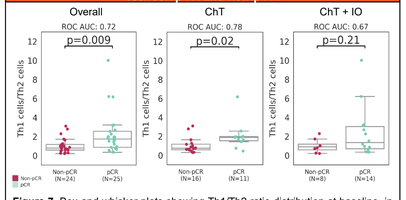


Figure 7. Box-and-whisker plots showing Th1/Th2 ratio distribution at baseline, in the overall cohort, ChT and ChT + IO subgroups. MWU test, no correction.

Conclusions

- Immunotype **distribution varied** by **treatment type** and **age**, and **evolved** during therapy, particularly in the ChT+IO group.
- **Peripheral blood immunotyping** alone **did not predict pCR**, but its integration with clinicopathological variables improved predictive performance (*training set*). The **current dataset** might be **too small** to train a generalizable model (LOOCV AUC ~0.6-0.5).
- Blood-based immunotyping and immune dynamics may help inform NAT benefit and warrants **further investigation**.

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References

1. Bianchini G, et al. Nat Rev Clin Oncol 2016 Nov;13(11):674-690.
2. Sankar K, et al. Biomark Res. 2022 May 18;10(1):32.
3. Nixon AB, et al. J Immunother Cancer. 2019 Nov 27;7(1):325.
4. Dyikanov D. et al. Cancer Cell. 2024 May 13;42(5):759-779.e12.

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