PS2-09-17 Comprehensive Peripheral Immune Profiling in Triple Negative Breast Cancer Links Immune Cell Dynamics to Therapeutic Response and Immune-related Adverse Events: Insight from the IRIS Study

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Abstract

Background: Triple-negative breast cancer (TNBC) is the most aggressive subtype with limited targeted options. Immune checkpoint inhibitors (ICIs) have reshaped treatment approaches in both metastatic and early-stage TNBC. In the neoadjuvant setting, ICIs combined with chemotherapy improve pathological complete response (pCR) rates over chemotherapy alone. Despite their benefits, ICIs pose risks of immune-related adverse events (irAEs), requiring careful patient selection and monitoring. Comprehending the immune context may be fundamental for tailoring therapy and predicting outcomes in TNBC.

Methods: Between March 2021 and April 2025, peripheral blood samples were collected at baseline from patients with TNBC treated with ICIs and enrolled in the translational, prospective and multicentre (12 Italian sites) IRIS study investigating circulating immune cells as predictive or prognostic biomarkers. In 60 patients (49 in the neoadjuvant and 11 in the metastatic setting), immunophenotype was performed by flow cytometry (FACS) to characterize circulating B cells, T cells, dendritic cells (DC), and Natural Kiler cells (NK) by a manual gating strategy. To capture immune cell heterogeneity, high-dimensional analysis was applied, including t-distributed stochastic neighbour embedding (t-SNE) for dimensionality reduction and FlowSOM for unsupervised clustering. For consistency, datasets were down sampled to 6000 T cells and 1600 B cells per sample, excluding sample below this cut-off (FlowJoTMv10). Statistical analysis included Mann-Whitney U test, univariate and multivariate Cox models for time to first irAE (TTirAE), defined as the interval between the first ICI cycle and onset of irAE (any grade), and Logistic regression (R software v.4.4.3).

Results: Focusing on TTirAE (No irAEs: n = 34; irAEs: n = 13), a higher baseline percentage of peripheral plasmacytoid dendritic cells (pDCs) was associated with a lower risk of irAEs (HR = 0.89; 95% CI: 0.80 - 0.99; p = 0.040), whereas increased frequencies of CM Th1 cells were linked to a higher risk (HR = 2.10; 95% CI: 1.05 - 4.20; p = 0.036). These associations remained statistically significant after adjusting for age at diagnosis and treatment setting. Considering only patients with early-stage TNBC, baseline circulating immune profiling revealed distinct differences between patients achieving pCR (n = 29) and those with residual disease (RD, n = 11). Patients with pCR showed higher percentages of dysfunctional CD4+CD28-Tregs (p = 0.014) and NKT cells (p = 0.038), whereas those with RD had increased frequencies of mature plasma cells (p = 0.015) and CM Tc1 cells (p = 0.043). Although not statistically significant, higher baseline frequency of CD8+ naive T cells showed a trend toward a positive association with pCR (OR = 1.05; 95% CI: 1.00-1.11; p = 0.068). In contrast, CM Tc1 cells, plasma cells, and transitional marginal zone-like B cells were negatively associated with pCR (OR < 1.0; 0.070 < p < 0.080).

Conclusion: Our preliminary data highlight the potential utility of peripheral immunophenotyping in TNBC as a non-invasive tool to identify predictive biomarkers and guide patient stratification in immunotherapy-based approaches.

Introduction

Triple-negative breast cancer (TNBC) is an aggressive disease with limited targeted options[1]. Immune checkpoint inhibitors (ICIs) have improved outcomes across both early-stage and metastatic settings, yet clinical benefit remains heterogeneous[2,3]. Moreover, ICIs can trigger immune-related adverse events (irAEs), underscoring the need for reliable biomarkers to identify patients most likely to benefit[4]. A deeper understanding of the immune context in TNBC may help balance efficacy and toxicity, guiding more precise immunotherapy strategies.

Material and Methods

From March 2021 to April 2025, baseline peripheral blood samples were collected from patients with TNBC treated with ICIs and enrolled in the multicentre, prospective IRIS study (12 Italian sites).

- **Flow cytometry and gating:** Immunophenotyping was performed by flow cytometry to characterize circulating B, T, dendritic (DC), and natural killer (NK) cells through a manual gating strategy defining major immune subsets.
- **High-dimensional analysis:** To further resolve cellular heterogeneity, *t*-distributed stochastic neighbour embedding (*t*-SNE) was applied for dimensionality reduction, followed by FlowSOM clustering to identify finer subpopulations. For consistency, datasets were down sampled to 6000 T and 1600 B cells per sample, excluding samples below this threshold (FlowJoTMv10).
- **Statistical analysis:** Mann-Whitney U test, multivariate and univariate Cox models, Kaplan-Meier curves and logistic regression were performed using R (v4.4.3).

Cohort Description

A total of 60 patients with TNBC treated with ICIs were analysed. Median age at diagnosis was 54 years (range 29–78).

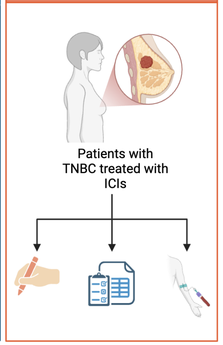
The majority received therapy in the neoadjuvant setting (82%), while 18% were treated for metastatic disease. Most tumours presented as cT2 (48%) and were clinically node-negative (45%). Pembrolizumab represented the predominant ICI used (82%), with atezolizumab administered in 12%. irAEs were observed in 25% of patients, including 15% experiencing grade 3/4 toxicity. Over a median follow-up of 1.26 years (IQR 0.50–1.66), recurrence or progression was observed in 8% of patients (Tab.1).

Table 1. *Baseline demographics and clinical characteristics are summarized as counts and percentages. Recurrence/progression and patient status represent follow-up outcomes. NA indicates missing data.*

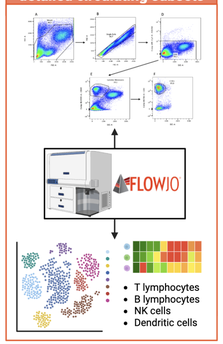
Patient Characteristics		N	%
Age at diagnosis	<54	30	50.00
	≥54	28	46.60
	NA	2	3.40
Treatment setting	Neoadjuvant (N)	49	81.70
	Metastatic (M)	11	18.30
cT	1	12	20.00
	2	29	48.30
	3	5	8.30
	4	2	3.40
	x	2	3.40
	NA	10	16.60
cN	0	27	45.00
	1	16	26.70
	2	3	5.00
	3	4	6.70
	NA	10	16.60
Type of immunotherapy	Atezolizumab	7	11.60
	Pembrolizumab	49	81.70
	NA	4	6.70
irAEs	No	46	75.00
	Yes	14	25.00
irAEs Grade	G1/G2	6	10.00
	G3/G4	9	15.00
Recurrence/ Progression	No	51	85.00
	Yes	5	8.30
	NA	4	6.70
Patient status	Alive	56	93.30
	Dead	3	5.00
	NA	1	1.70

Study Flowchart

1 Patients provided informed consent, and peripheral blood with clinical data was collected at baseline (T0)



2 Immune profiling combined manual gating, t-SNE mapping and FlowSOM clustering to identify detailed circulating subsets



3 Statistical analyses were performed using appropriate tests



- **Mann-Whitney U test** for group differences
- **Cox models** and **Kaplan-Meier** for time-to-firs irAE (TTirAE: time from first ICI cycle to the onset of any grade irAE)
- **Logistic regression** for pCR associations

Results

Analysis of TTirAE (irAEs n = 13; no irAEs n = 34): Patients with **higher baseline percentage of peripheral plasmacytoid dendritic cells (pDCs)** had a **lower risk of developing irAEs** (HR 0.89; 95% CI 0.80–0.99; p = 0.040; Fig.1A). In contrast, **central memory Th1 (CM Th1) cells enrichment** was linked with **increased risk of toxicity** (HR 2.10; 95% CI 1.05–4.20; p = 0.036; Fig.1B). Both associations remained significant after adjustment for age and treatment setting (neoadjuvant vs metastatic) (Tab.2).

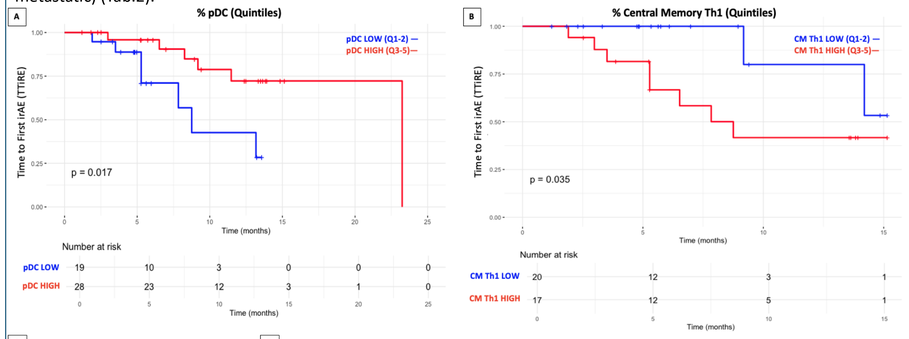


Table 2. Multivariate Cox models adjusted for age at diagnosis and treatment setting

	MULTIVARIATE COX MODEL adjusted for age (<54 vs ≥54) and treatment setting (N vs M)		
Immune Subset	**n (event)**	**HR (95% CIs)**	**p**
pDC (HLA-DR+CD123-CD11c+)	47 (13)	1.88 (0.78 – 0.99)	0.046
CM Th1 (CD3+CD4+CD28+CD45RA-CD45RO+CD197+CXCR3+)	37 (10)	2.40 (1.08 – 0.99)	0.031

Figure 1. *Kaplan–Meier curves for time to immune-related adverse events (TTirAE): **A)** Patients stratified by baseline pDC percentages. The higher-pDC group shows a clearly lower cumulative incidence of irAEs over follow-up, consistent with the protective association observed in Cox analysis; **B)** Patients stratified by CM Th1 cell frequencies. The higher-CM Th1 group exhibits earlier and more frequent irAEs, in line with the increased risk identified in multivariable models.*

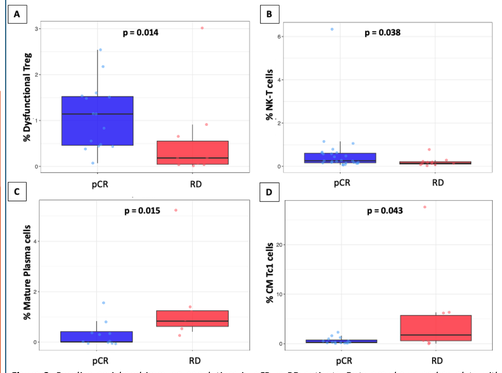


Figure 2. *Baseline peripheral immune populations in pCR vs RD patients. Data are shown as box plots with individual points; statistical differences tested with t he Mann-Whitney test. **A)** Dysfunctional Tregs **B)** NKT cells **C)** Mature plasma cells **D)** CM Tc1 cells.*

Analysis on Early-stage TNBC cohort: Baseline circulating immune profiles differed between patients later achieving pCR (n = 29) and those with residual disease (RD, n = 11) (Tab.3). Patients with **pCR** showed **higher percentages of dysfunctional Tregs** (p = 0.014; Fig.2A) and **NK-T cells** (p = 0.038; Fig.2B). In contrast, **RD** patients had increased frequencies of **mature plasma cells** (p = 0.015; Fig.2C) and **central memory Tc1 (CM Tc1) cells** (p = 0.043; Fig.2D).

Immune Subset	pCR group		RD group		p-value
	median	IQR	median	IQR	
Dysfunctional Treg (CD3+CD4+CD28-CD127-CD25++)	1.14	1.06	0.18	0.50	0.014
Mature Plasma cells (CD19+CD20-CD38-CD27+HLA-DR+)	0.00	0.41	0.83	0.62	0.015
NK-T cells (CD3+CD56+)	0.26	0.45	0.14	0.11	0.038
CM Tc1 cells (CD8+CD28+CD45RA-CD45RO+CD197+CXCR3+)	0.29	0.59	1.75	5.16	0.043

Table 3. *Baseline immune cell frequencies in pCR vs RD patients, reported as median and IQR. Group differences were assessed using the Mann–Whitney test.*

Patients reaching **pCR showed a tendency toward elevated baseline CD8+ naive T cells** (OR = 1.05; p = 0.068; Tab.4). In contrast, **CM Tc1, plasma cells, and transitional marginal zone-like B cells appeared to relate inversely to pCR** (OR < 1.0; 0.070 < p < 0.080; Tab.4).

Immune Subset	OR	95% CIs	p-value
CD8 Naive (CD3+CD8+CCR7+CD45RA+)	1.05	1.00 – 1.11	0.068
CM Tc1 cells (CD3+CD8+CD28+CD45RA-CD45RO+CD197+CXCR3+)	0.46	0.15 – 0.87	0.072
Plasma cells (CD19+CD20+CD38++)	0.81	0.62 – 1.01	0.076
Transitional Marginal Zone-like B cells (CD19+CD20+IgM+IgD+CD38+CD27+HLA-DR++CD24+)	0.01	0.00 – 0.49	0.080
Mature Plasma cells (CD19+CD20-CD38-CD27+HLA-DR+)	0.12	0.01 - 0.79	0.083

Table 4. *Univariate logistic regression models evaluating the association between baseline immune subsets and the probability of achieving pCR . Results are reported as odds ratios (OR), 95% confidence intervals (CI), and p-values. OR >1 indicates higher odds of pCR per unit increase of the immune subset, whereas OR <1 indicates lower odds.*

Conclusions

Baseline immune composition shows distinct associations with both irAEs and ICIs response in TNBC

Our preliminary data support the potential utility of peripheral immunophenotyping as a non-invasive tool to predict outcomes and guide more personalized immunotherapy-based approaches in TNBC.

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