PS2-10-14_Blood-Based Metabolomic Profiling and Transcriptomic Analysis of Circulating CD3⁺ T Cells Reveal Associations with Treatment Response and Immune Activation in Early Breast Cancer (eBC)

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#1967_Abstract

Background: Pathological complete response (pCR) is a strong predictor of long-term outcomes in early breast cancer (eBC), establishing neoadjuvant chemotherapy (NACT) as the standard of care for aggressive BC subtypes. Recently, targeting host and tumor metabolism has emerged as a promising approach to boost antitumor immunity and improve NACT efficacy. However, the specific metabolic biomarkers involved, and their immunological effects remain poorly defined.
Methods: We performed untargeted metabolomics on baseline plasma from 84 eBC patients undergoing NACT at our institution between 2021–2023 (22% luminal, 36% HER2+, 42% triple-negative; 48% achieved pCR). Data were analysed using univariate (t-test), multivariate (PLS-DA), and pathway enrichment approaches. In a subset of 49 patients (58%), bulk RNA was extracted from sorted CD3+ circulating T cells for gene expression analysis. Gene Ontology enrichment analysis was used to characterize upregulated pathways in T cells.
Results: Pathway enrichment analysis of metabolomic data identified alpha-linolenic acid (ALA, omega-3) and linoleic acid (LA, omega-6) metabolism as top pathways associated with pCR and residual disease, respectively. Specifically, the VIP score analysis showed that docosahexaenoic acid (DHA, a long-chain omega-3 derived from ALA) and dihomo-gamma-linolenic acid (DGLA, a downstream omega-6 derived from LA) has the strongest association with pCR and RD respectively. Additional top ranked metabolites at VIP analysis included palmitoleic acid (POA, an omega-7 lipokine with immunomodulatory role) and conjugated linoleic acid (CLA, an omega-6 known to counteract the inflammatory effects of classical non-conjugated omega-6). We developed an "Omega Signature" tracking the balance between anti-inflammatory omega-3/6/7 and pro-inflammatory omega-6 as POA + [(DHA + CLA)/DGLA]. This signature was significantly associated with pCR in a multivariate logistic regression model adjusted for age and BMI (OR 1.53, 95% CI 1.33–1.88, p < 0.0001). To assess whether the Omega Signature reflects T cells polarization, we performed transcriptomic profiling of circulating T lymphocytes in 49/84 patients. Patients with high Omega Signature (above the median) showed upregulation of gene pathways involved in T cell activation and adaptive immune responses compared to those with low scores.
Conclusion: Our findings reveal that a specific circulating fatty acid profile seems to be associated with treatment response and immune activation in eBC. This signature reflects a favorable balance of immunomodulatory lipids and may serve as a non-invasive biomarker to identify patients more likely to achieve pCR. Correlation analyses with tissue metabolomics and gene expression data are ongoing will be presented at the Congress.

Introduction

Pathological complete response (pCR) is a strong predictor of long-term outcomes in early breast cancer (eBC), establishing neoadjuvant chemotherapy (NACT) as the standard of care for aggressive subtypes. In recent years, targeting host and tumor metabolism has emerged as a promising strategy to enhance antitumor immunity and improve NACT efficacy. However, the specific metabolic biomarkers and their immunological implications remain poorly characterized.

Methods and Materials

- **Patients and Samples:**

Baseline plasma was collected from 84 early breast cancer (eBC) patients treated with NACT at our institution (2021–2023).
Molecular subtypes: 22% luminal, 36% HER2-positive, 42% triple-negative. 48% of patients achieved pathological complete response (pCR).

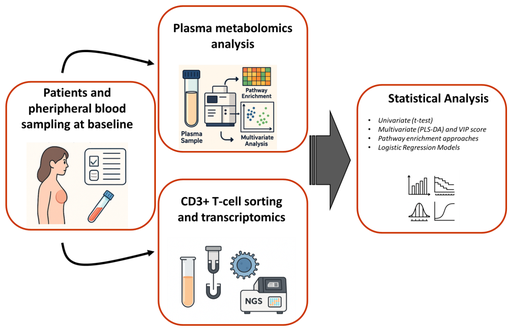
- **Metabolomics:**

Untargeted metabolomics was performed on plasma samples. Data were analyzed using univariate (t-test) and multivariate (PLS-DA) methods, followed by pathway enrichment analysis.

- **T-cell Gene Expression:**

In a subset of 49 patients (58%), CD3⁺ circulating T cells were sorted using magnetic beads, and bulk RNA was extracted for gene expression profiling. Differential gene expression between *Omega Signature* high *vs* low groups was evaluated by Significance Analysis of Microarrays (SAM).

Study Workflow



Patients Characteristics

Characteristics		N	%
Age at diagnosis	> 52	44	53.00%
	≥ 52	39	47.00%
BMI	> 25	48	57.80%
	≥ 25	35	42.20%
cT	T1	10	12.10%
	T1b	1	1.20%
	T2	41	49.40%
	T2m	6	7.20%
	T3	19	22.90%
	T3m	2	2.40%
	T4	2	2.40%
	T4b	1	1.20%
	T4d	1	1.20%
cN	N0	41	49.40%
	N1	27	32.50%
	N2	9	10.80%
	N3	5	6.10%
	N3b	1	1.20%
Subtype	Luminal	18	21.70%
	HER2	30	36.10%
	TNBC	35	42.20%
pCR	Yes	40	48.20%
	No	43	51.80%

Table 1. Summary of baseline characteristics of the 84 patients enrolled, including demographic variables (age, BMI), clinical staging (cT and cN), molecular subtypes (Luminal, HER2+, TNBC), and treatment outcomes (pCR). Values are presented as absolute numbers and percentages.

AIM

To identify metabolic signatures associated with response to NACT and to explore their immunometabolic correlates in circulating T cells from eBC patients.

Results

1 Metabolomic Approaches to Identify Response-Related Pathways

Univariate (t-test), multivariate (PLS-DA), and pathway enrichment analyses consistently identified alpha-linolenic acid (ALA, **omega-3**) and linoleic acid (LA, **omega-6**) metabolism as key pathways influencing treatment response.

Fig.1a: Top 25 metabolic pathways distinguishing pCR from RD. Dot size = enrichment ratio; color = p-value.

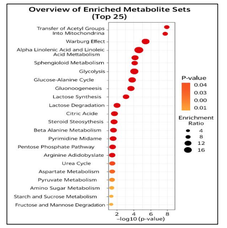
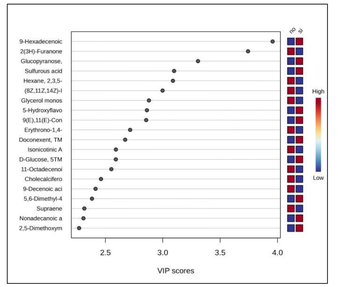


Fig. 1b: Top metabolites by VIP score from PLS-DA separating pCR and RD.



VIP scoring revealed that **DHA** (a long chain omega-3 derived from ALA) was enriched in patients achieving pCR, while icosatrienoic acid (GLDA, a downstream omega-6 derived from LA) was associated with Residual Disease (RD).

2 Development of an Omega-Based Metabolic Signature

We developed an "***Omega Signature***" capturing the balance between immunomodulatory (**POA, DHA, CLA**) and proinflammatory (GLDA) fatty acids.

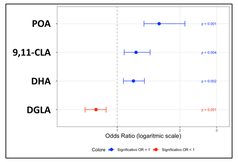


Figure 2. *Forest plot of odds ratios for fatty-acid predictors of pCR. Blue = OR > 1; red = OR < 1. Error bars show 95% CIs and p-values are displayed.*

Table 2. Key Fatty Acids Included in the Omega Signature

Fatty Acid	Class	Key Features
Palmitoleic Acid (POA)	MUFA (ω-7)	Lipokine with immune-regulatory functions
9,11-Conjugated Linoleic Acid (9,11-CLA)	PUFA (ω-6)	Enable to inhibit classical ω-6 conversion to pro-inflammatory prostaglandins
Docosahexaenoic Acid (DHA)	PUFA (ω-3)	Established immunomodulatory properties
Dihomo-γ-linolenic Acid (DGLA)	PUFA (ω-6)	Enable to compete with ω-3 for synthesis and to produce small amounts of arachidonic acid

Omega Signature

$$\text{POA} + \frac{\text{DHA} + 9{,}11\text{-CLA}}{\text{DGLA}}$$

3 The *Omega Signature* is significantly associated with pCR

Patients were stratified into high and low Omega Signature groups (median split). Patients with high Omega Signature achieved a pCR in 73% of cases (30/41), compared with only 24% in the low signature group (10/42) [p < 0.001]. Logistic regression analysis showed significant association between the Omega Signature (continuous variable) and pCR (OR: 1.53; 95% CI: 1.33-1.88, *p*<0.0001).

4 Omega Signature and T-Cell Transcriptional Profiling

To evaluate the immune relevance of the *Omega Signature*, we profiled circulating T cells in 49 patients. High-signature patients showed upregulated pathways linked to T-cell activation and adaptive immunity.

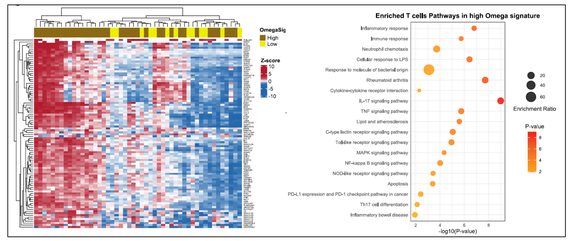


Fig. 3. Heatmap and pathway enrichment analysis of CD3⁺ T-cell transcriptomes comparing high vs. low Omega Signature. High Omega Signature patients show upregulation of inflammatory, cytokine, and T-cell activation pathways.

Conclusions

Our findings indicate that a distinct circulating fatty-acid profile is associated with both treatment response and immune activation in eBC. This *Omega Signature* reflects a favorable balance of immunomodulatory PUFAs and MUFA, supporting its potential as a non-invasive biomarker for predicting pCR.

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