Prognostic value of the immune and metabolic profile in the response to neoadjuvant treatment with ICIs in triple-negative breast cancer patients (TNBC)

Serrano-García, L[a]*; Quintas, G[b]; Pérez-Álvarez de Lara[a], M; Moret[a], A; Portero-Hernández, M[a]; Cejalvo JM[c]; Iranzo, V[d]; Sanchez-García, J[a]; Marín, S[a]; Llombart-Cussac, A[a]; Fernández-Murga, ML[a]*.

[a]*Clinical and Molecular Oncology Department, Arnau de Vilanova Hospital, Valencia, Spain.*

[b]*Health and Biomedicine Department, Leitat Technological Center, Barcelona, Spain.*

[C] *Medical Oncology Department, Hospital Clínico Universitario, Valencia - Biomedical Research Institute INCLIVA, Spain*

[d] *Medical Oncology Department, Hospital General Universitario, Valencia, Spain.*

***** luchiserga@gmail.com **, malefermu67@gmail.com**

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BACKGROUND:

Immune checkpoint inhibitors (ICIs) are showing promising results in TNBC, but there is a percentage of patients who do not respond to therapy. Knowing the cellular and molecular components that influence the response to treatment can provide a better approach of this disease. **The aim of this study is to investigate, through non-invasive methods, cellular and metabolic immune profiles that allow us to predict which patients will respond to treatment.**

MATERIAL AND METHODS:

Twenty-nine patients with TNBC treated with ICIs (pembrolizumab) and chemotherapy in neoadjuvant setting were included, and blood samples were taken before treatment (baseline), after 3 weeks (3W), and prior to surgery (Preop). Demographic and clinical data were collected (Table 1). Treatment response was evaluated histopathologically after surgery based on whether a pathological complete response was obtained or not, classified as responders (R) and non-responders (NR) respectively. Immunophenotype panels (lymphocyte populations and Myeloid Derived Supressor Cells (MDSCs)) were performed using flow cytometry in fresh blood. Metabolic profiling was analyzed in serum using UPLC-MS/MS and a DataBase-guided dynamic Data Dependent Acquisition (dynamic-DDA) for MS/MS fragmentation to improve the ratio of fragmented metabolites and the annotation ratio.

The study was approved by the institutional ethics committee of Arnau de Vilanova Hospital (Ref. IMNOTCH/2021).

RESULTS:

Demographics:

Variables	Patients, N=29
Age (years)	
Median (range)	50 (32-78)
Menopause state	
Pre	19 (65.5%)
Post	10(34.5%)
Comorbidities	
Hypothyroidism	2 (6,9%)
Dyslipidemia	2 (6,9%)
Others (Migraine, miocarditis, arterial hypertension, allergy, thalassemia)	6 (31,6 %)
Tumor stage at diagnosis	
II	13 (44.8%)
III	16 (55.2%)
Nods at diagnosis	
N0	12 (44.1%)
N1	12 (41,4%)
N2	3 (10,3%)
N3	2 (6,9%)
Point of treatment	
Active treatment	11 (38%)
End of treatment	18 (62%)
Response at the end of treatment	**N=18**
Responding	12 (61,1%)
Non-responding	6 (38.9%)
Adverse events	
Hematological toxicity	4 (22,2%)
Transaminitis	1 (5,6%)
Diarrhoea	1 (5,6%)

Table 1: Demograhic and clinical features of patients included

Cytomics:

To date, 18 of the 29 patients have completed treatment and surgery with a result of 12 responders and 6 non-responders. For all patients, neoadjuvant treatment produced a significant and progressive increase over time in the CD3+ T lymphocyte population, specifically the CD8+ or cytotoxic T lymphocyte subpopulation (p=0,006) and Natural Killer T (NKT) lymphocytes (p=0,007). This increase was 2.2-fold and 1.8-fold respectively when comparing the Preop percentage with respect to the baseline percentage (Figure 1). MDSCs populations did not show significant changes with treatment for all patients (Figure 2), although a tendency towards a decrease in the population of total, granulocytic and early MDSCs (Figure 2A, 2C and 2D) was observed after the administration of ICIs.

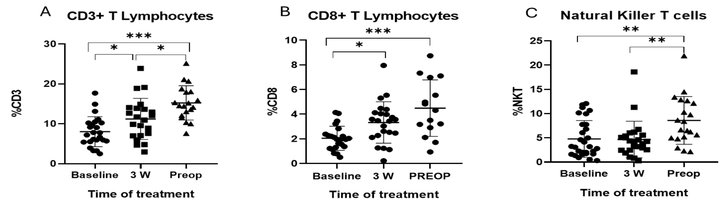

Figure 1: Changes in lymphocytary populations during treatment with ICIs: CD3+ T Lymphocytes (A), Cytotoxic T Lymphocytes (B) and Natural Killer T Lymphocytes (C)

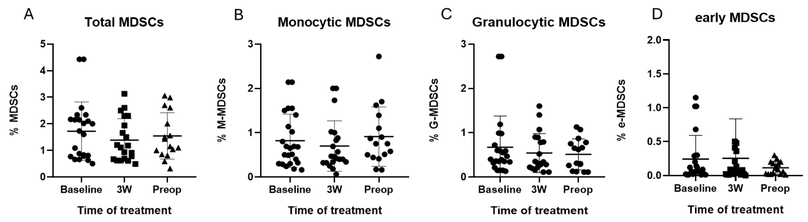

Figure 2: Changes in MDSCs populations during tratment with ICIs: Total MDSCs (A), Monocytic MDSCs (B) Granulocytic MDSCs (C) and early MDSCs (D).

When classifying patients into R vs NR, we observed that R had a significant 2.5-fold increase in CD8+ T cells and 2.6-fold increase in NKTs (p<0,001), both populations associated with antitumor immunity. However, in NR patients we observed a slight increase that was not statistically significant(Figure 3). NR patients showed higher levels than R of all the MDSCs subpopulations, associated with immunosuppression, at all sample times. This difference was statistically significant for total MDSCs (3W; p=0.04 and preop; p=0.02) and mainly in granulocytic MDSCs (G-MDSCs) at 3W (NR 2,8 times higher than R) (p=0.03) (Figure 4).

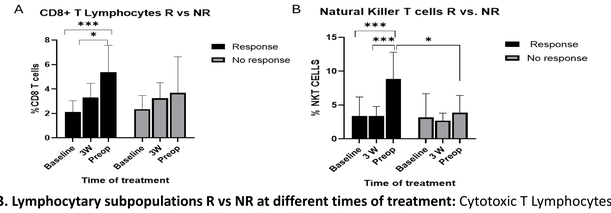

Figure 3. Lymphocytary subpopulations R vs NR at different times of treatment: Cytotoxic T Lymphocytes (A) and Natural Killer T Lymphocytes (B)

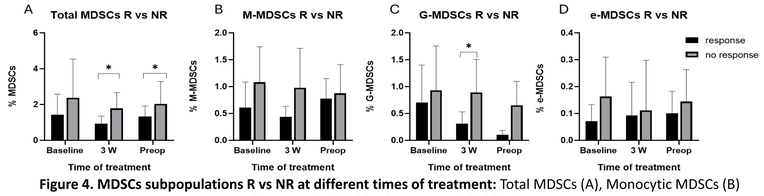

Figure 4. MDSCs subpopulations R vs NR at different times of treatment: Total MDSCs (A), Monocytic MDSCs (B) Granulocytic MDSCs (C) and early MDSCs (D).

Metabolomics:

The analysis of the metabolic profiles using MS/MS and functional analysis identified significant alterations in the Purine, Pyrimidine, and Tryptophan metabolism pathways between R and NR groups at baseline (p<0.05) (Figure 5). At 3 weeks of treatment, a distinct set of altered pathways was observed, including Lysine degradation, Inositol phosphate metabolism and Tyrosine metabolism (p<0.05) (Figure 6).

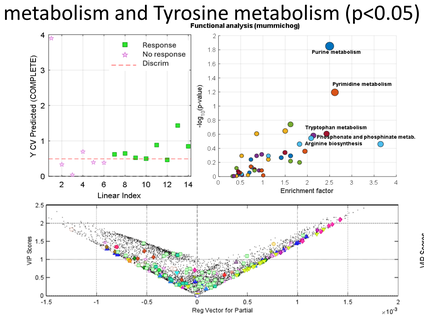

Figure 4. Metabolomic analysis R vs NR at baseline timepoint. XT (14 X 5383). PLSA, CV:LOO Autoscaled data, LVs:2. Functional analysis m/z 10 ppm. KEGG Homo sapiens.

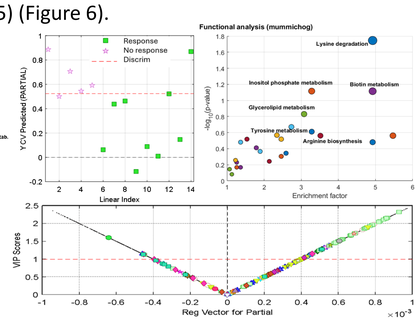

Figure 5. Metabolomic analysis CR vs PR at 3 weeks timepoint. XT (14 X 5383). PLSA, CV:LOO Autoscaled data, LVs:1. Functional analysis m/z 10 ppm. KEGG Homo sapiens.

At baseline, we observed that pathways linked to nucleic acid metabolism (purines and pyrimidines) have altered metabolites associated with R group (p<0.01). On the other hand, alterations in metabolites of the tryptophan pathway are associated with the NR group (p<0.001). At 3 weeks, the profile changes and alterations appear in the Lysine degradation, Inositol phosphate and Tyrosine metabolism pathways and are associated with NR patients (p<0.001) (Table 2).

Number of altered metabolites	Pathway	Timepoint sample	Test groups	Average NR/R ratios	P value
12	Purine Metabolism	Baseline	NR vs R	0.7	P<0.01
7	Pyrimidine metabolism	Baseline	NR vs R	0.7	P<0.01
11	Tryptophan metabolism	Baseline	NR vs R	1.3	P<0.001
3	Lysine degradation	3W	NR vs R	1.2	P<0.001
3	Inositol phosphate metabolism	3W	NR vs R	1,1	P<0.001
2	Tyrosine metabolism	3W	NR vs R	1.7	P<0.001

Table 2: Association between affected metabolites and R vs NR groups. Responders: NR/R<1; No-responders: NR/R>1

CONCLUSIONS:

- Treatment with ICIs is capable of triggering activation of the immune system in patients. This effect was most evident in patients responding to treatment.
- Non-responders have activated mechanisms of immunosuppression, such as MDSCs.
- The NR group has alterations in metabolites of the tryptophan, inositol phosphate, tyrosine and lysine degradation pathways, while in the R group, the alterations appear in metabolites associated with the metabolism of purines and pyrimidines (nucleic acid metabolism).
- The changes observed between R and NR may allow us to establish personalized patterns that predict the effectiveness of ICIs therapy in TNBC and may aid in clinical decision-making.
- The study is in an open phase, 11 patients are still under treatment, and we do not know the response to the therapy. We are currently continuing to recruit patients to reinforce the significance of these trends.