P2.214: Impact of Chemo-Immunotherapy on Circulating Monocyte Immunosuppressive Activity and Gene Expression in Advanced Non-Small Cell Lung Cancer

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INTRODUCTION

- Circulating monocytes (CM) may develop immunosuppressive activity, thereby promoting resistance to immune checkpoint inhibitors (ICIs), yet the impact of ICIs on CM function remains poorly defined.
- We have previously shown that ICIs downregulate cellular FLICE-inhibitory protein (c-FLIP), a critical regulator of CM-mediated immunosuppressive activity, in non-progressing non-small cell lung cancer (NSCLC) patients (1).
- This study aimed to characterize the effects of ICIs, alone or combined with chemotherapy, on CM immunosuppressive activity and transcriptional reprogramming in NSCLC patients.

METHODS

- Patients with advanced NSCLC treated with upfront ICIs, either as monotherapy (I) or combined with chemotherapy (CI), were prospectively enrolled at the University of Verona (**Figure 1**).
- Peripheral blood samples were collected at baseline (T0) and after 3 months (T1) (**Figure 1**).
- Patients were classified as responders (R; progression-free survival [PFS] ≥6 months) or non-responders (NR; PFS <6 months) (**Figure 1**).
- CM were isolated and co-cultured with anti-CD3/CD28-stimulated peripheral blood mononuclear cells from healthy donors to assess immunosuppressive activity by flow cytometry.
- Statistical analysis was performed using unpaired t tests ($p<0.05$).
- RNA sequencing was conducted using the TruSeq RNA Prep Kit v2 on a NovaSeq 6000 platform.
- The c-FLIP interactome was analyzed by liquid chromatography-mass spectrometry in nuclear and cytoplasmic fractions of FLIP-overexpressing THP-1 cells transfected with FLIP RNA.

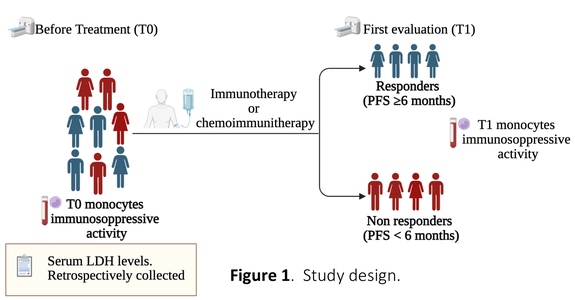


Figure 1. Study design.

RESULTS

Characteristics	Patients N= 40 (%)	Responders N = 21(%)	Non-responders N= 19(%)
Gender			
Male	20(50)	10(47.6)	10(52.6)
Female	20(50)	11(52.4)	9(47.4)
Age years, median (range)	67(38-81)	71(47-81)	66(38-80)
ECOG PS			
0	15(37.5)	11(52.4)	4(21.1)
1	19(47.5)	8(38.1)	11(57.9)
2	6(15)	2(9.5)	4(21.1)
Smoker			
Never	7(17.5)	4(19)	3(15.8)
Former	19(47.5)	11(52.4)	8(42.1)
Current	14(35)	6(28.6)	8(42.1)
Histology			
Adenocarcinoma	29(72.5)	16(76.2)	13(68.4)
Squamous Carcinoma	4(10)	1(4.8)	3(16.8)
Other	7(17.5)	4(19)	3(15.8)
PD-L1			
<1%	11(27.5)	9(42.9)	2(10.5)
>/=1%-<50%	28(70)	12(57.1)	16(84.2)
>/= 50%	1(2.5)	0(0)	
			1(5.3)
Stage			
III	0(100)	0(0)	0(0)
IV	40(100)	21(100)	19(100)
Metastatic site			
Lung and pleura	27(67.5)	15(71.4)	12(63.2)
Lymph nodes	32(80)	16(76.2)	16(84.2)
Bone	8(20)	2(9.5)	6(31.6)
Liver	5(12.5)	2(9.5)	3(15.8)
Brain	9(22.5)	4(19)	5(26.3)
Other	9(22.5)	4(19)	5(26.3)
Targetable Molecular alterations	11(27.5)	4(19)	7(36.8)
EGFR uncommon	1(9.1)	1(25)	0(0)
KRAS G12C	8(72.7)	3(75)	5(71.4)
MET ex14 skipping	1(9.1)	0(0)	1(14.3)
NA	1(9.1)	0(0)	1(14.3)

Table 1. Baseline clinical characteristics of patients treated with chemoimmunotherapy, assessable for T0 $CD3^+$ T-cell proliferation.

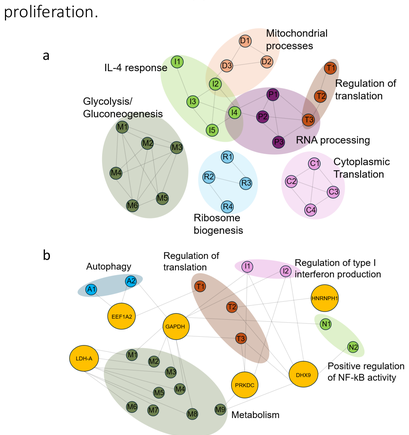


Figure 4. FLIP interactome in cytoplasmic (a) and nuclear (b) compartments.

- Between 2021 and 2025, 80 pts were enrolled. 57 were evaluable at baseline (T0), including 40 treated with CI (70.2%) and 17 with I (29.8%) (Table 1).
- Among CI-treated pts, 21 (52.5%) were responders (R) and 19 (47.5%) non-responders (NR).
- At T0, CM from R showed higher immunosuppressive activity than NR ($p=0.03$) (Figure 2).
- At T1, in CI-treated pts, CM from R displayed reduced immunosuppressive activity ($p=0.04$), whereas it increased in NR ($p=0.03$) (**Figure 2**).
- RNA-seq analysis showed that CI significantly reshaped the CM transcriptional profile in R (T1 vs T0, $p<0.05$), with 587 upregulated and 476 downregulated genes (**Figure 3**). Enrichment analysis highlighted modulation of lipid metabolism (MLXIPL, SPHK1, NR1D1) and cell differentiation pathways (FOSL2, NR4A1, MAFB).
- c-FLIP interactome analysis identified lactate dehydrogenase A (LDH-A) as an interacting protein in both nuclear and cytoplasmic compartments (**Figure 4**).
- Consistently, TCGA data associated high LDH-A expression with worse survival in lung adenocarcinoma ($p<0.0001$), and in our CI-treated cohort, R had lower baseline LDH levels than NR ($p=0.0071$) (**Figure 5**).

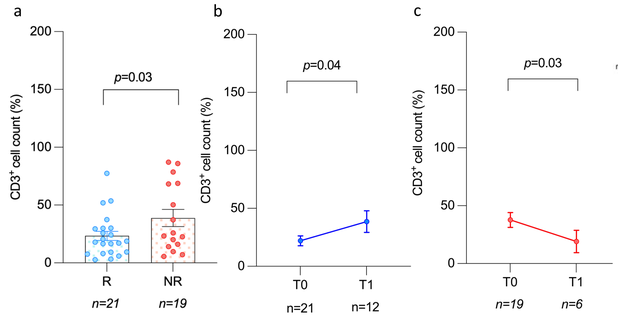


Figure 2. Baseline (T0) monocyte-mediated immunosuppression in the CI-treated population (a). Comparison of $CD3^+$ T-cell proliferation at baseline (T0) and post-treatment (T1) in R (b) and NR (c) patients treated with CI.

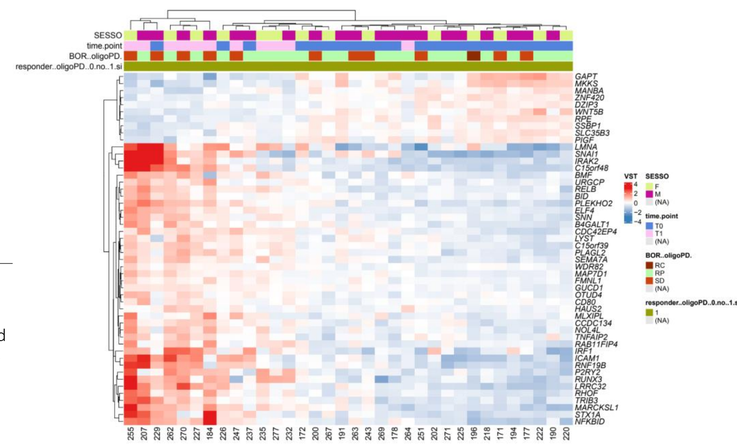


Figure 3. Transcriptional profile of monocytes from responder patients treated with chemoimmunotherapy

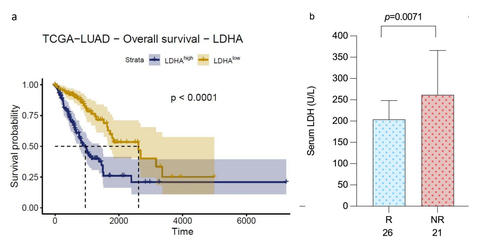


Figure 5. Overall survival according to LDH-A expression in the tumor microenvironment of lung adenocarcinoma patients by TCGA data (a). Baseline serum LDH levels in chemo-immunotherapy treated patients (b)

DISCUSSION AND CONCLUSION

- CI appears to modulate CM in NSCLC, reducing immunosuppressive activity and promoting transcriptional reprogramming associated with monocyte differentiation in responders.
- Additionally, supported by a potential biological link between c-FLIP and LDH-A, LDH may represent an easily accessible and immunologically relevant biomarker for patient selection.

REFERENCES & ACKNOWLEDGEMENTS

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