8P - Pre-treatment blood gene expression changes associated with durable clinical benefit in metastatic non-small-cell lung cancer with high PD-L1 expression receiving first-line pembrolizumab

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Background:

Metastatic non-small-cell lung cancers (NSCLC) with PD-L1 tumor proportion score (TPS) ≥ 50% are immune reactive neoplasms with sensitivity to first-line monotherapy using the PD-1 inhibitor pembrolizumab but approximately 20% patients suffer from immune-related adverse effects (irAE)[1].

Furthermore, most patients do not respond or turn out to be refractory, and PD-L1 protein expression on tumor or immune cells was found to have limited utility as a predictive biomarker of clinical outcome[2], making the need for further biomarkers more pressing.

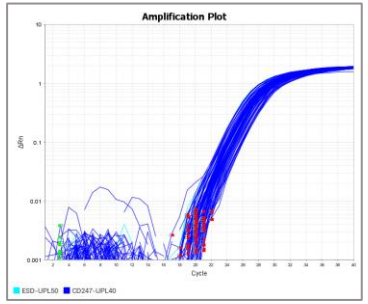
Here, we examine the expression of 13 genes of special immunologic interest in stage IV NSCLC receiving first-line pembrolizumab monotherapy.

Baseline characteristics:

	Rapid progression (PFS < 3 months) (n = 17)	Durable benefit (PFS > 6 months) (n = 27)
Age	68 ± 12	67 ± 11
Sex		
Female	8	12
Male	9	15
Smoking status		
Never	4	3
Former	8	15
Current	5	9
ECOG		
0	5	14
1	12	13
PD-L1 (%)	75 ± 13	82 ± 14
NSCLC Histology		
ADC	10	21
SCC	4	5
other (NOS, LCNEC9)	3	1

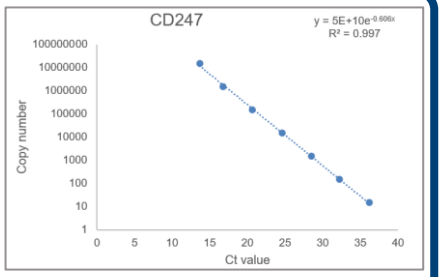
Methods:

Gene expression of 13 genes of special immunologic interest: PD1, FOXP3, CD247, PRF1, GZMB, TBX21, MKI67, HLA-DRA, FAS, FASLG, GATA3, RORγt, IFNγ was quantified in absolute terms using real-time PCR (RT-PCR) from reverse-transcribed whole blood RNA.



Sample amplification curves for CD247 (dark blue) and ESD (light blue) as internal plate calibrator

RNA was extracted using the PAXgene Blood RNA Kit (PreAnalytiX), and reverse transcribed using the Transcriptor First Strand cDNA Synthesis Kit (Roche). RT-PCR was then performed using the standard curve method on the QuantStudio™ 5 Real-Time PCR Instrument (Applied Biosystems) in technical triplicates using PowerTrack™ SYBR Green Master Mix (Thermo Fisher Scientific). Ct values were calculated with the QuantStudio™ Design & Analysis Software v1.3.1 (Thermo Fisher Scientific). The copy numbers were then calculated using the standard curve, for which an 8-step 10-fold dilution of plasmid DNA generated with CloneJET PCR Cloning Kit (Thermo Fisher Scientific) was used.



Standard curve for CD247 in a semi-logarithmic plot

Finally, patient groups were compared using unpaired t-tests. To examine gene expression independently of lymphocyte abundance in blood, copy numbers were divided by the percentage of lymphocytes among leukocytes.

Conclusions:

- Newly diagnosed patients with stage IV NSCLC and PD-L1 ≥ 50% who experience durable clinical benefit under pembrolizumab monotherapy have detectable changes in peripheral blood immune cell subsets and gene expression profile.
- Durable clinical benefit is associated with higher absolute counts and percentages of lymphocytes, as well as with a lower neutrophil-to-lymphocyte ratio.
- It is also associated with a higher expression of PD-1, and a relative downregulation of FOXP3 and TBX21 in the peripheral blood prior to therapy start.
- Ongoing work aims to validate these findings in larger patient cohorts, evaluate the deregulation of other genes, explore longitudinal changes under treatment, and analyze the potential clinical utility in other NSCLC patient subsets.

Results:

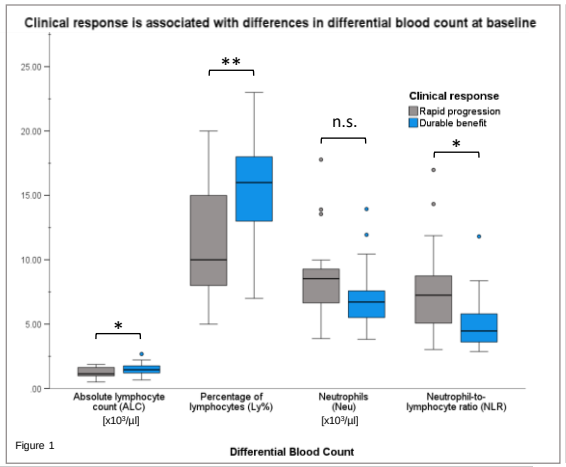


Figure 1

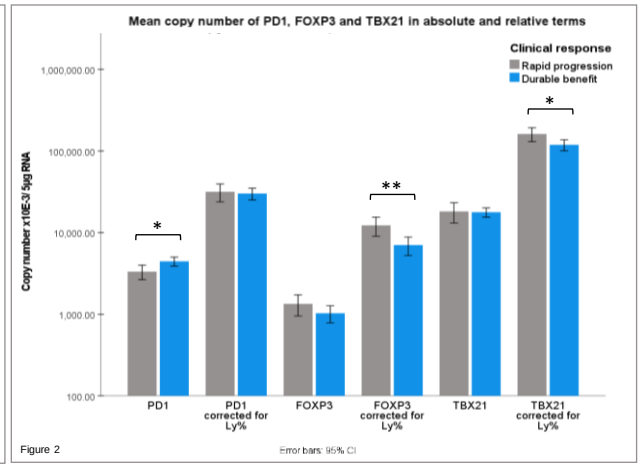


Figure 2

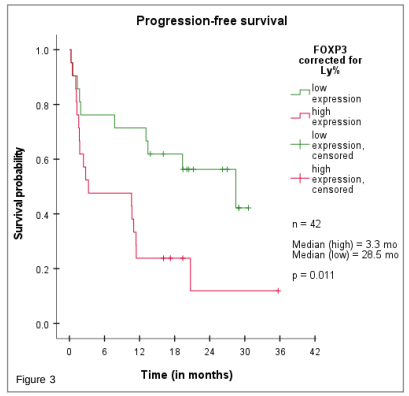


Figure 3

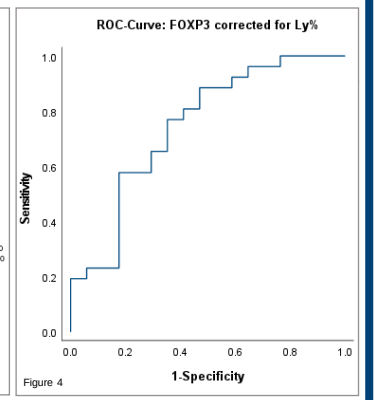


Figure 4

n.s. = not significant, p >0.5; * <0.5; ** <0.01; *** <0.001; CI = Confidence interval; AUC = area under the curve

References:

1. Daniello, L., Elshiaty, M., Bozorgmehr, F., Kuon, J., Kazdal, D., Schindler, H., Shah, R., Volckmar, A. L., Lusky, F., Diekmann, L., Liersch, S., Faehling, M., Muley, T., Kriegsmann, M., Benesova, K., Stenzinger, A., Thomas, M., & Christopoulos, P. (2021). Therapeutic and Prognostic Implications of Immune-Related Adverse Events in Advanced Non-Small-Cell Lung Cancer. Frontiers in oncology, 11, 703893. https://doi.org/10.3389/fonc.2021.703893
2. Davis, A. A., & Patel, V. G.. (2019). The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. Journal for Immunotherapy of Cancer, 7(1). https://doi.org/10.1186/s40425-019-0768-9

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No conflicts of interest to declare.