ADVANCING NOVEL THERAPIES:

Investigation of multiple immune-checkpoints in the context of the disease for personalized therapy selection

SUMMARY

PD1 blockade through monoclonal antibody-based therapy has revolutionized the immunotherapeutic approach against solid tumors, however only a small number of patients benefits from this treatment due to the lack of accurate methodology including immunohistochemistry to guide patient that could respond to this immune checkpoint inhibitor therapy. Understanding the tumor microenvironment (TME) complexity with a single biomarker is not accurate enough to predict the interaction of the drug in its site of action and, therefore, its effectiveness. Here, we present a strategy in which the TME is deeply investigated at the molecular level in order to guide more efficiently the patient toward single or combination therapy.

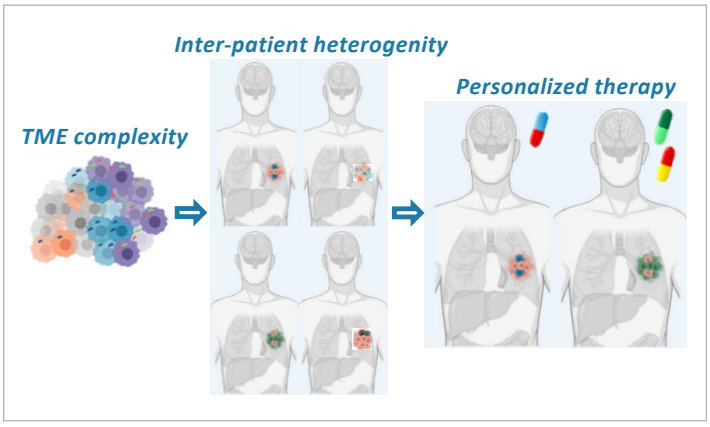
APPROACH

The tumors immune evasive pathway programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) is the pharmaceutical target of immune checkpoint inhibitor (ICI) compounds, such as the anti-PD1 monoclonal antibody Pembrolizumab (Keytruda®). To guide patients that could benefit from this therapy, the gold standard is based on PD-L1 quantification by immunohistostaining on patient's tumor samples derived before therapy. However, the evaluation of a single biomarker does not reflect the complexity of the biological system changing, that contributes to TME heterogeneity.

In this study we performed a deep biomarkers spatial profiling on two fresh frozen baseline non-small cell lung cancer (NSCLC) tissue samples from patient further treated with Pembrolizumab, a PD1 inhibitor, as adjuvant therapy (patient's clinical characteristic are presented in our white paper #5). We characterized the modulation of gene expression with a focus on the immune cell population in specific regions of interest on the tissue of the patient that responded to PD1 inhibitor (CR) versus the one that did not respond (PD).

This methodology allowed us to identify all the players in the TME responsible for the fate of the treatment response and therefore make prediction toward a more accurate patient stratification strategy for single immune therapy or combination therapies (Fig. 1).

FIGURE 1. Investigation of the intra-tumor complexity allow for the understanding of the inter-patient heterogeneity and their stratification toward better immune therapy. (Extracted and adapted from El-Sayes N et al., 2021)



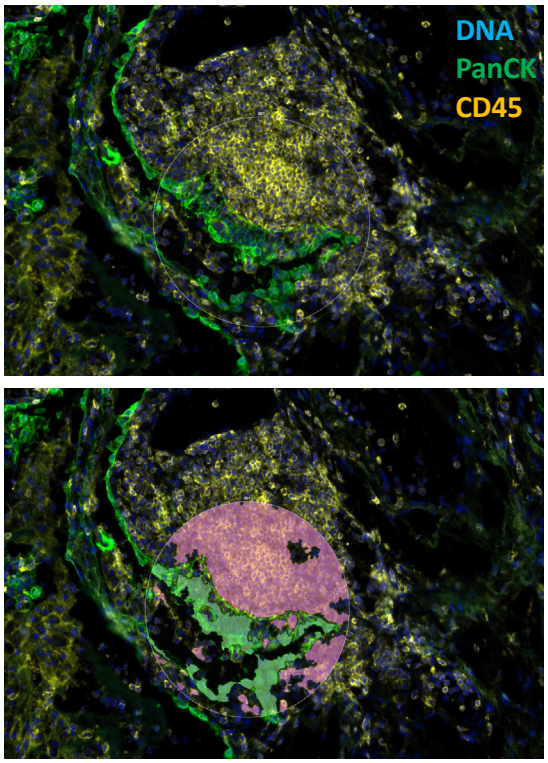
IMMUNE INFILTRATION

To understand the relative distribution and interaction of stromal and tumor compartments within the TME, we stained both samples with fluorescently labeled antibodies directed to CD45 marker for the immune compartment and Pan Cytokeratin for the tumor region.

By applying GeoMX® DSP control center automatic segmentation we defined two areas of illumination (AOIs) in each sample(Fig. 2):

- Tumor (PanCK+, CD45-) in pink
- Stroma (PanCK-, CD45+) in green

FIGURE 2. Regions of acquisition segmentation In green the tumor region and in red the immune infiltrate (stroma).

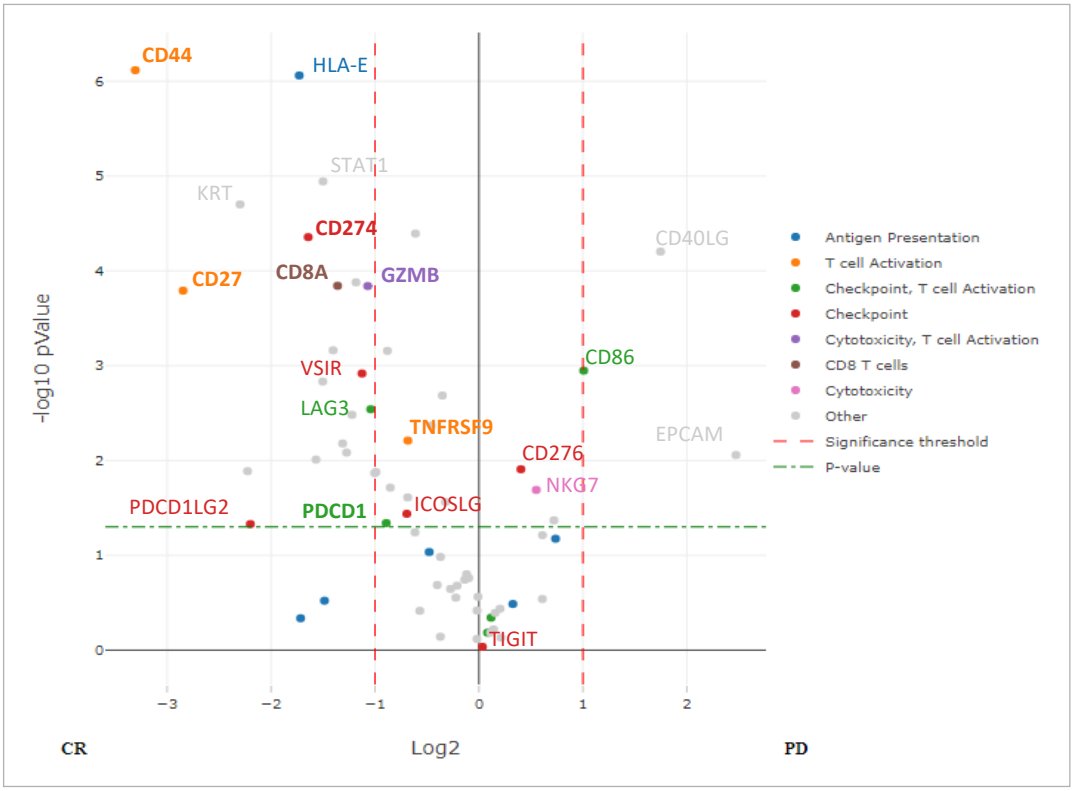


HOT PHENOTYPE CORRELATES WITH ICI RESPONSE

We then investigated the spatial transcriptome signature of cells composing the stroma compartment by assaying it with the I/O RNA panel (Nanostring®) and analyzed the data with the GeoMx® DSP analysis suite.

The volcano plot in Fig. 3 shows the modulation of molecular marker expression in response to PD1 inhibitor. In the CR patient, a higher expression of the drug targets, PD1 (PDCD1) and PD-L1 (CD274) was found. In addition, the CR sample presented with a “hot” phenotype, characterized by genes related to T lymphocytes cytotoxicity (GZMB, CD8a) and activation (CD44, CD27, TNFRS9). Those findings showed that the TME of the CR sample was characterized by molecular actors involved in anti-tumor activity rescue after PD1/P-L1 inhibition.

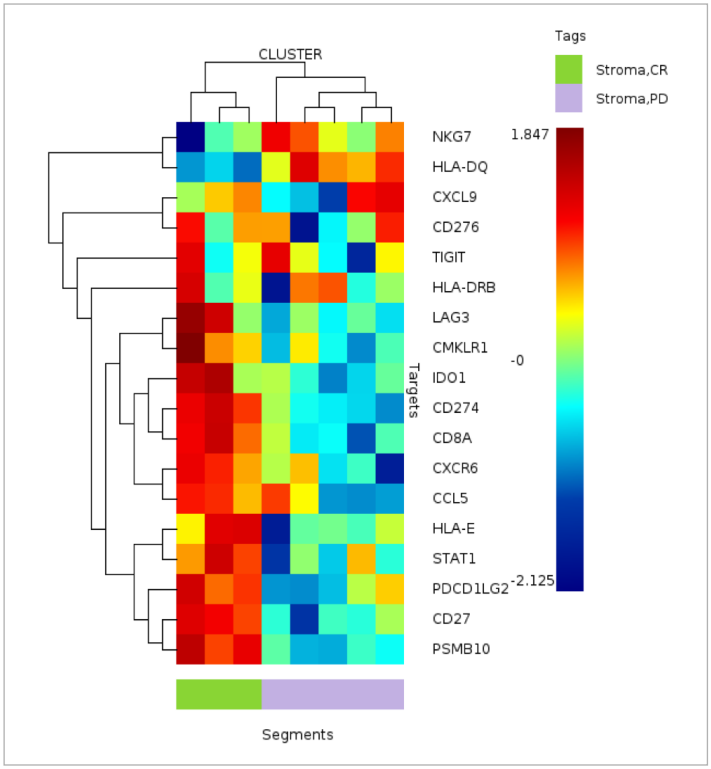
FIGURE 3. Volcano plot representing gene expression modulation with response to PD1 inhibitor. The highlighted genes correspond to genes involved in immune modulation and ICIs activity.



T-CELL-INFLAMED GENE-EXPRESSION PROFILE HAS A HIGH PREDICITIVE VALUE

Investigation of the T cell inflamed gene expression profile (GEP), a clinically validated 18-gene signatures related to immune system activity for predictive response to anti PD1/PD-L1 therapy across several solid tumor showed increase expression of genes related to IFN-γ signaling, cytotoxic effector molecules, antigen presentation and T cell active cytokines in the patient that responded to ICI therapy (Fig. 4). This confirm the high predictivity value of this 18 genes signature in the context of solid tumor.

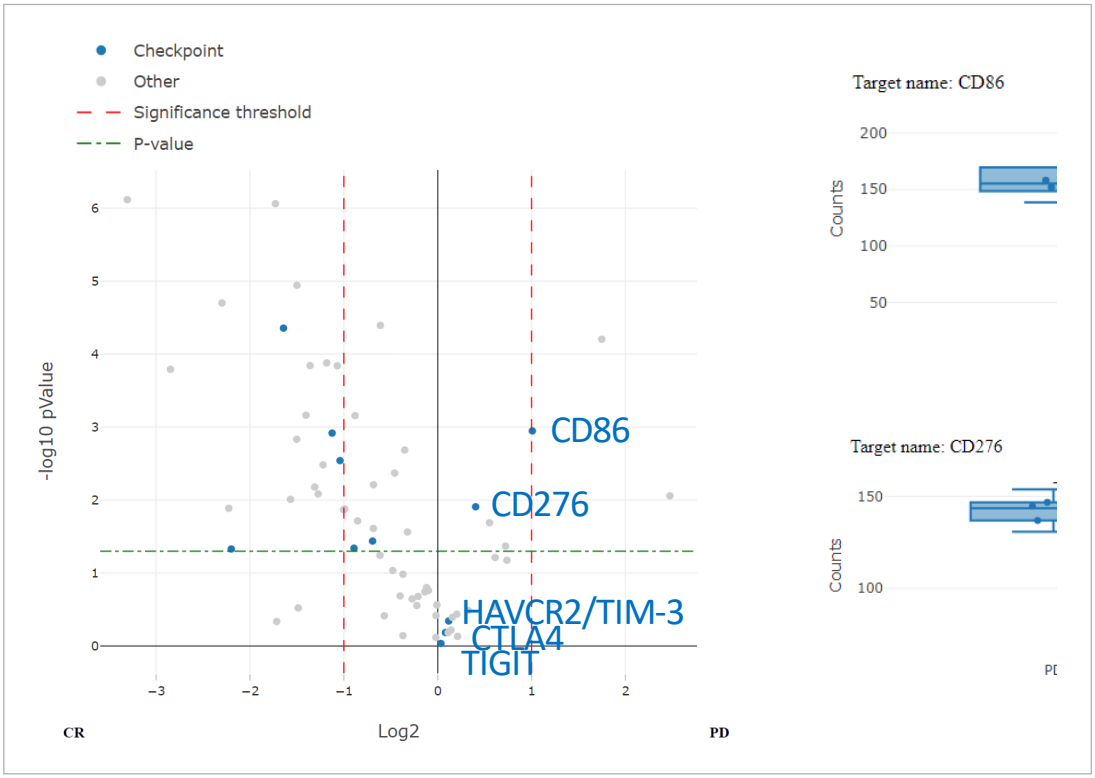
FIGURE 4. Unsupervised clustering heatmap of the T cell-inflamed GEP 18 genes in ICI responder versus the non-responder.



IMMUNE SUPPRESSIVE PATHWAYS REDUCE ICI ACTIVITY

PD tumor sample was characterized by an overexpression of different immune checkpoints (Fig. 5). Significantly, the antigen presenting cells (APCs) inhibitory ligand CD86 and the tumor inhibitory ligand B7H3 (CD276) were overexpressed. Both markers drive inhibitory signals trough their receptors, present on the surface of T lymphocytes (unknown for B7H3, CTLA4 for CD86).

FIGURE 5. Distribution of the immune checkpoints with response to ICI and Bar graph showing the significant overexpression of the ICI B-CD86 and the ICI CD276/B7H3.



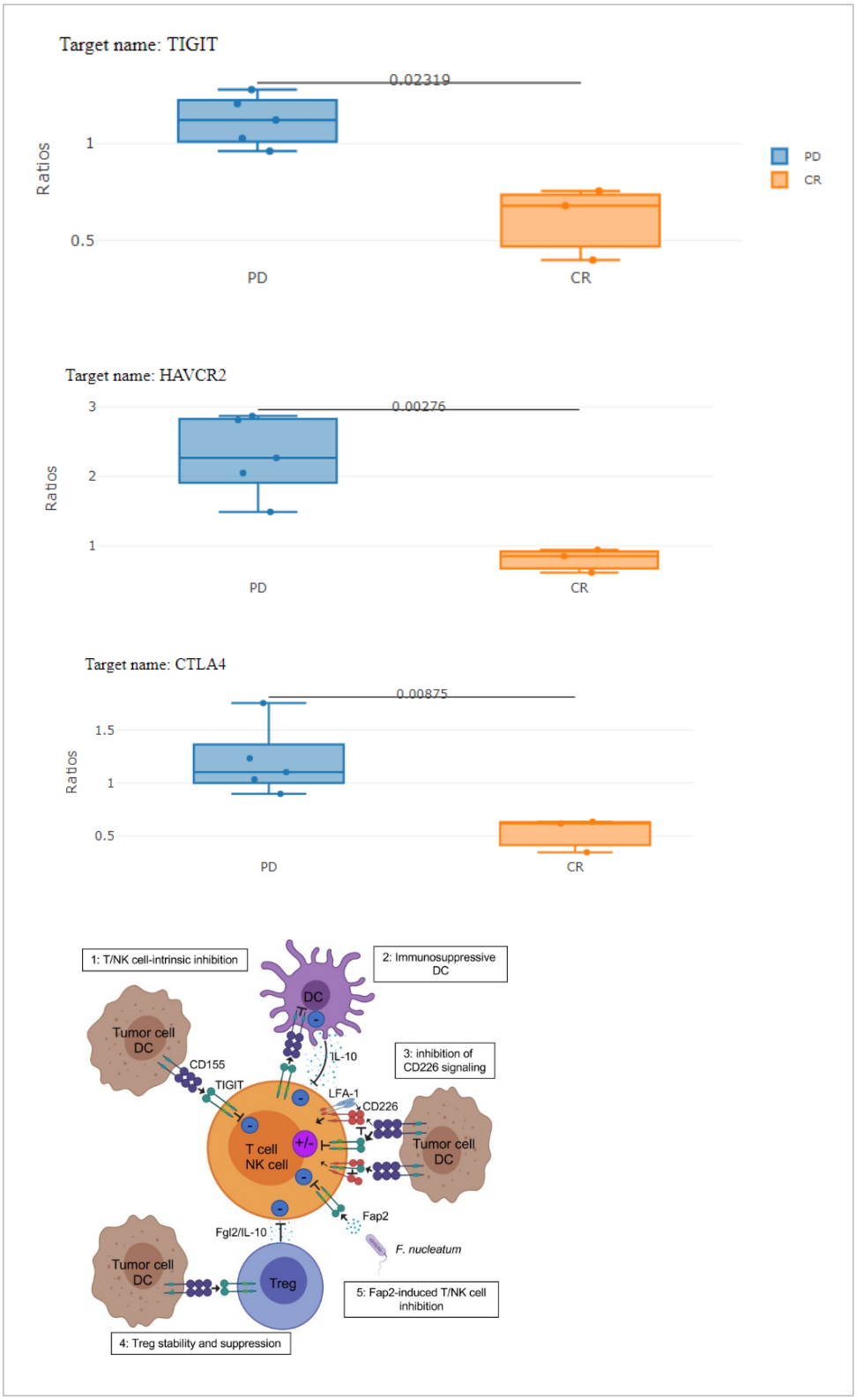
TIGIT IN IMMUNE-CHECKPOINT TARGETING

Ratio between checkpoint receptors and CD8a was calculated to showcase the specific phenotype of the engaged T cells with response to ICI.

Even thought the cytotoxic T cells were less infiltrated in the TME of the patient who did not respond to the therapy, they were significantly overexpressing three co-inhibitory ligands (Fig. 6). TIGIT in particular has an high clinical relevance due to its multiple activity not only on T cells, but also on natural killer and regulatory T cells, all involved in immune suppression pathways. Its overexpression often correlate with the overexpression of other co-inhibitory receptors, such as CTLA4 and TIM-3 (HAVCR2).

It have been demonstrated that antiPD1/PDL1 therapy is more efficient if in combination with TIGIT, but also TIM-3 and CTLA4, blocking therapy, even in anti PD1 resistant tumor models.

FIGURE 6. T cells co-inhibitory receptors overexpression relative to the cytotoxicity marker CD8a expression represented in bar graph. TIGIT receptor is significantly overexpressed by cytotoxic T cells that infiltrate the PD TME. Its presence on T cells, NKs and Tregs surfaces leads to multiple inhibitory pathways. TIM-3 is a co-inhibitory receptors which co-regulated dysfunctional T cells in cancer. C-CTLA4 is B7H3 receptor present on CD8a+ T cells and the first target of ICI approved by FDA for cancer therapy. D-Multiple immunoinhibitory pathways drived by TIGIT on T lymphocytes, NK cells and Tregs (extracted from Chauvin JM et al. doi:10.1136/jitc-2020-000957).

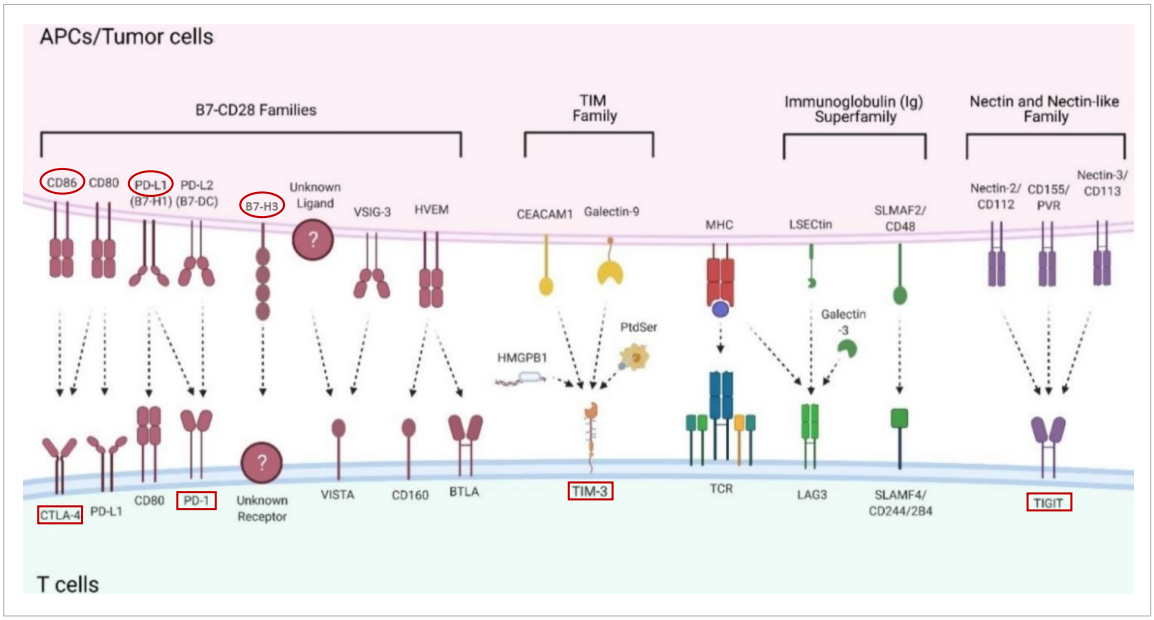


CONCLUSION

Using multiplexed high throughput analysis we have been able, with a single technique, to investigate at molecular level several actors involved in pathways of immune modulation that relate with mechanism of drug response and resistance (Fig. 7). With a wider set of probes to investigate the spatial transcriptome, we better defined the overall pre-treatment phenotype the render the tumor suitable for therapy effectiveness: «hot» phenotype, markers of T cells activation, drug targets presence, tumor inflammation signature. The same data allowed us to define the features that justify the ineffectiveness of the anti-PD1 single therapy in the patient with progression disease: the presence of several pathways that lead to immune suppression.

These findings highlight the relevance of investigating more biomarkers involved in the pathways that relate to immune suppression, to obtain a comprehensive strategy for personalized immune therapy, or combination of immunotherapies, selection. Our results suggest that, for the patient with progression disease, it would have been necessary to resort to a combined ICIs therapy, more aimed to the complete inhibition of all the immunosuppressive pathways and the resumption of the immune system activity against the tumor.

FIGURE 7. Immune checkpoints Co-inhibitory ligands and receptors involved in immunosuppressive pathways in the TME. Those are targeted by immune checkpoints inhibitor therapies and are involved in mechanism of drug resistance. Highlighted in red the ones that we have been able to investigate. (Extracted and adapted from Pansy K et al. doi:10.3390/ijms222413311).



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