PERSONALIZED THERAPY SELECTION:

Investigation of multiple immune-checkpoints

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 more efficiently guide the patient toward single or combination therapy.

INTRODUCTION

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 which 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 predictions toward a more accurate patient stratification strategy for single immune therapy or combination therapies (Figure 1).

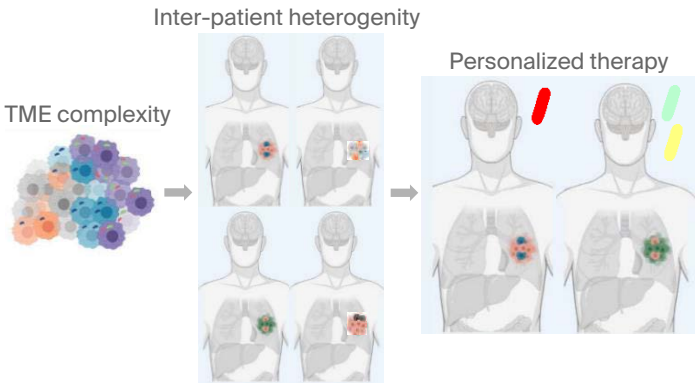


FIGURE 1. Investigation of the intra-tumor complexity allows for the understanding of the inter-patient heterogeneity and their stratification toward better immune therapy. (Extracted and adapted from El-Sayes N et al., 2021)

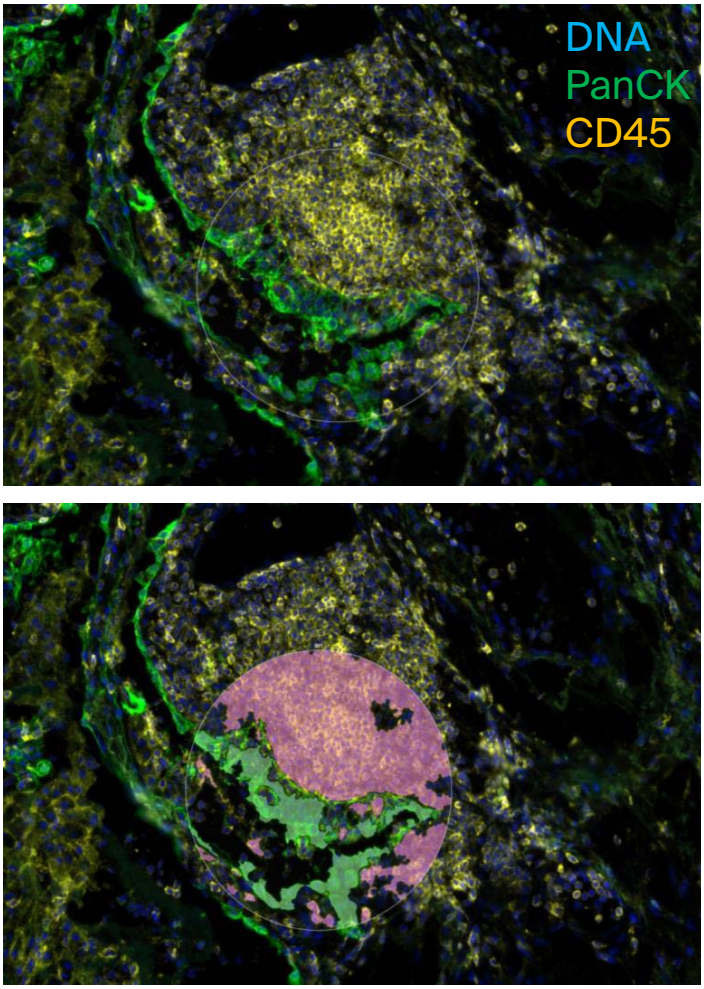
IMMUNE INFILTRATION

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 (Figure 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).

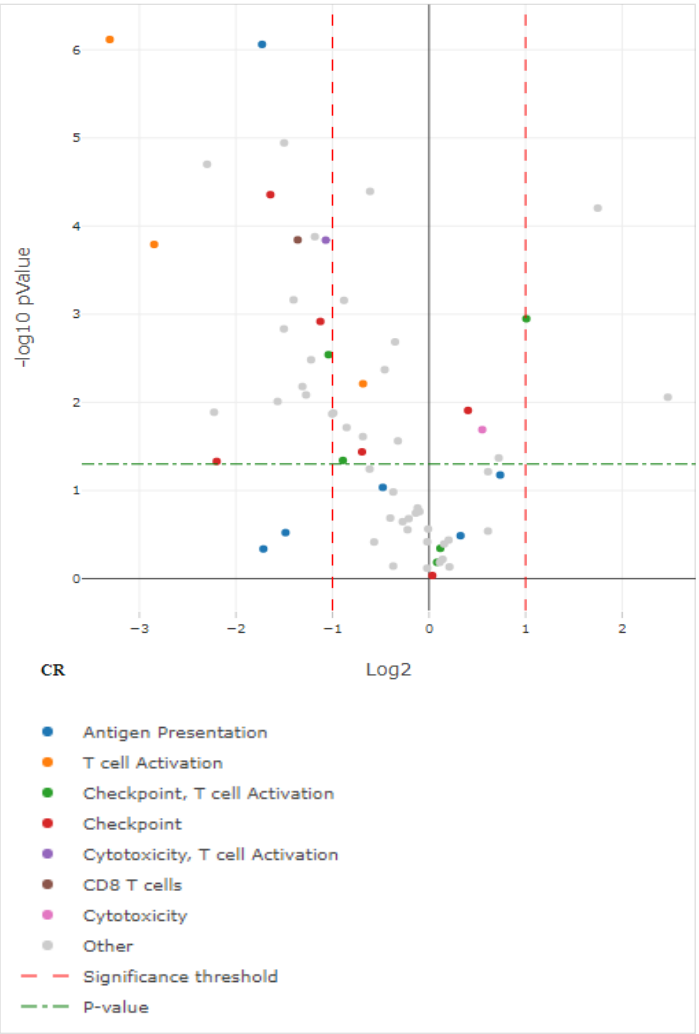


HOT PHENOTYPE CORRELATES WITH ICI RESPONSE

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.

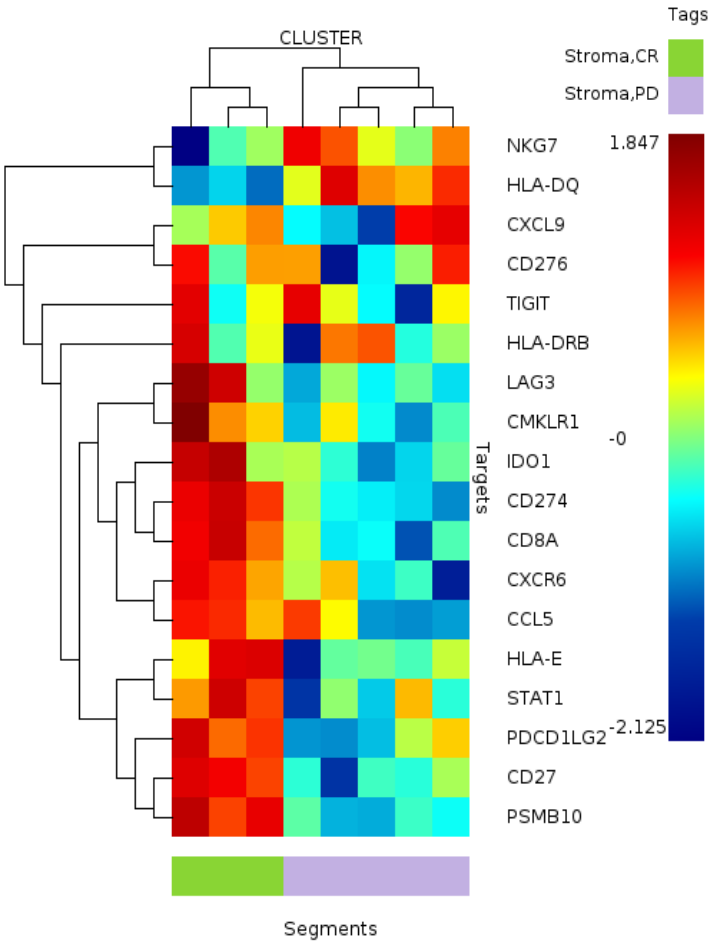
FIGURE 3. Volcano plot representing gene expression modulation with response to PD1 inhibitor. The highlighted genes correspond to genes involved in immune modulation and ICIs activity.



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 signature related to immune system activity for predictive response to anti PD1/PD-L1 therapy across several solid tumors, 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 (Figure 4).

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 (Figure 5). Significantly, the antigen presenting cells (APCs) inhibitory ligand CD86 and the tumor inhibitory ligand B7H3 (CD276) were overexpressed. Both markers drive inhibitory signals through their receptors, present on the surface of T lymphocytes (unknown for B7H3, CTLA4 for CD86).

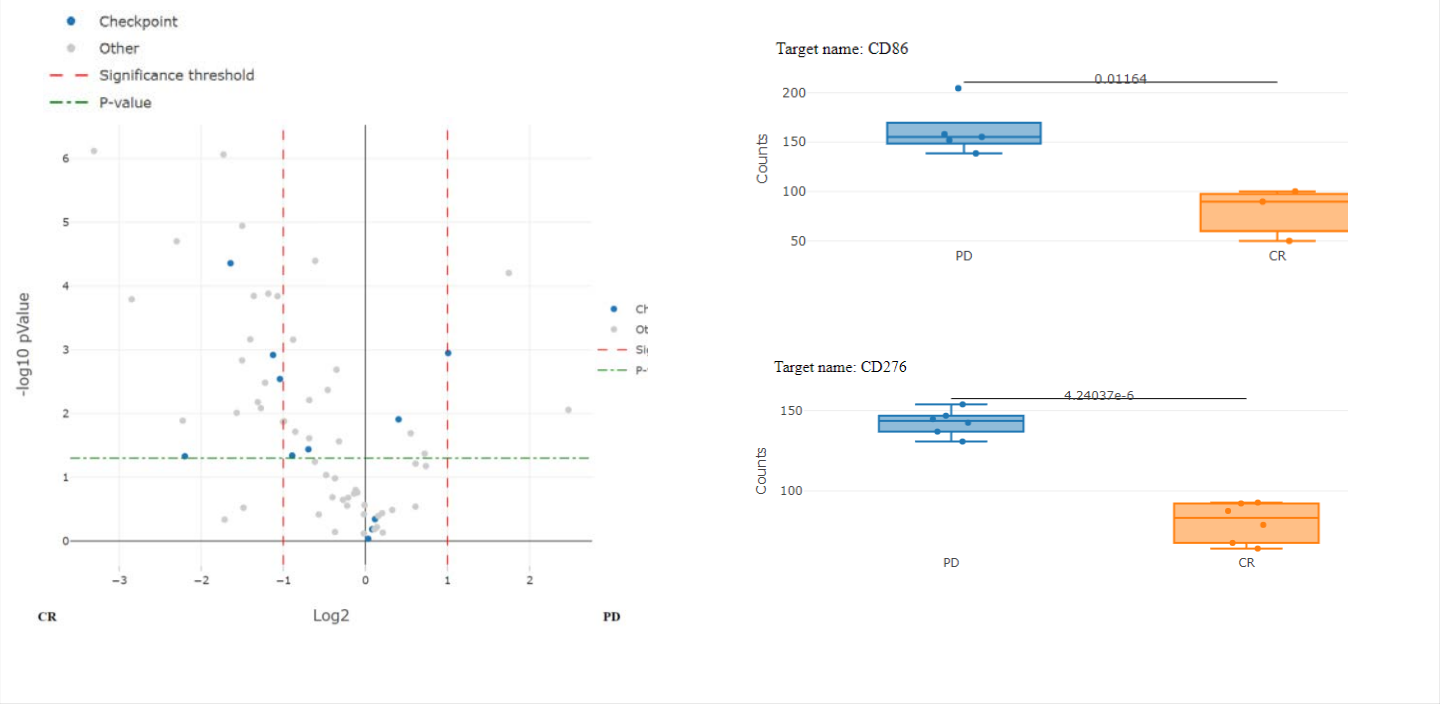


FIGURE 5. Distribution of the immune checkpoints with response to ICI and bar graph showing the significant overexpression of the ICI B-CD86 and the ICI CD276/B7H3.

TIGIT IN IMMUNE-CHECKPOINT TARGETING

The identification of several checkpoint receptors on cytotoxic cells showed the overexpression of three co-inhibitory ligands (Figure 6) in the sample that did not respond to ICI therapy. TIGIT in particular has a 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 correlates with the overexpression of other co-inhibitory receptors, such as CTLA4 and TIM-3 (HAVCR2). In this report, we have shown that antiPD1/PDL1 therapy would have been more efficient if combined with TIGIT, but also TIM-3 and CTLA4 therapy.

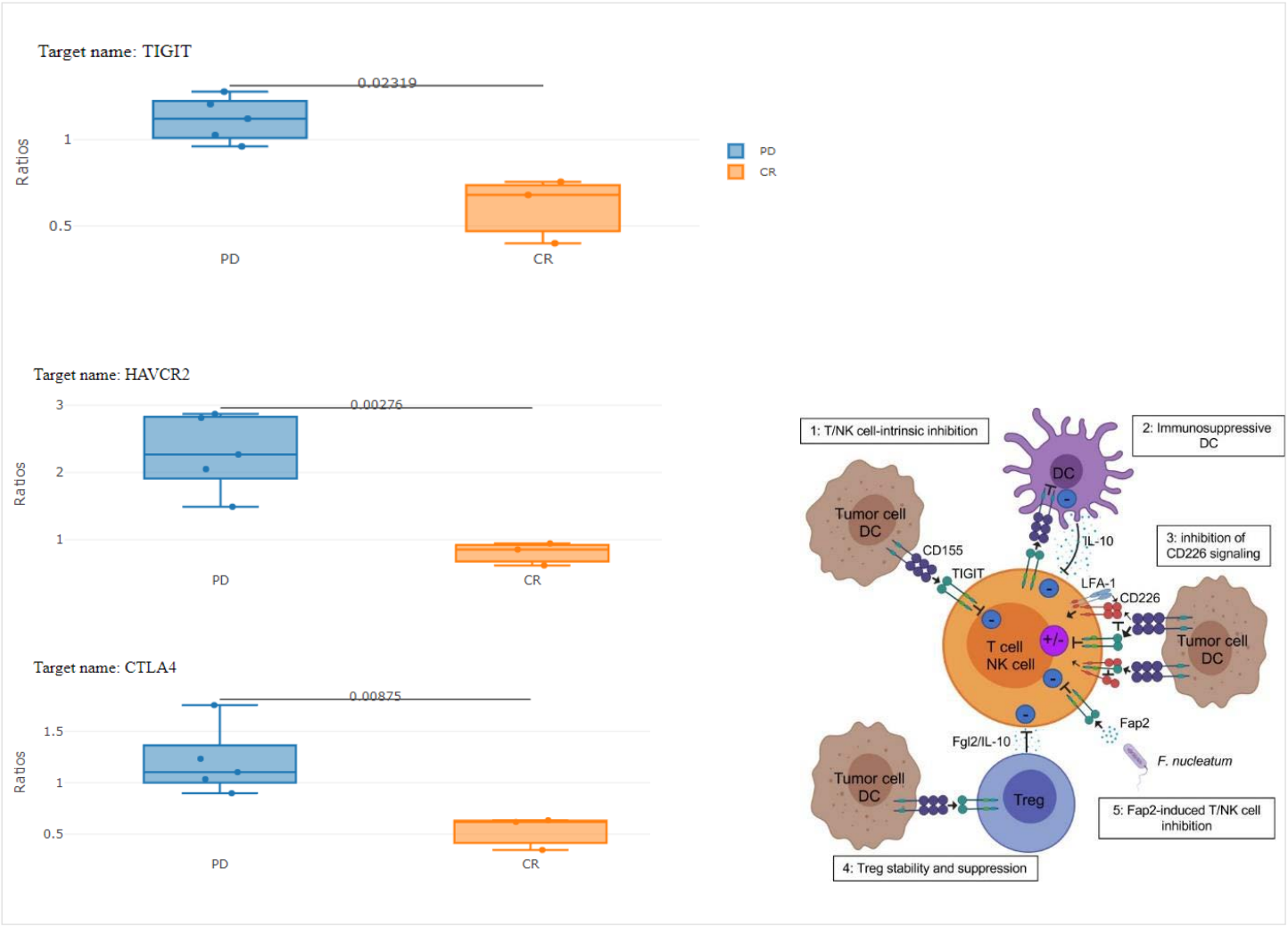


FIGURE 6. CD8a T cells co-inhibitory receptors expression. TIGIT receptor is significantly overexpressed by cytotoxic T cells in the PD TME patient. TIM-3 and C-CTLA4 are also significantly overexpressed in cytotoxic cells in the patient that did not respond to the therapy. Schematic of the multiple immunoinhibitory pathways driven by TIGIT on T lymphocytes, NK cells and Tregs (extracted from Chauvin JM et al. doi:10.1136/jitc-2020-000957).

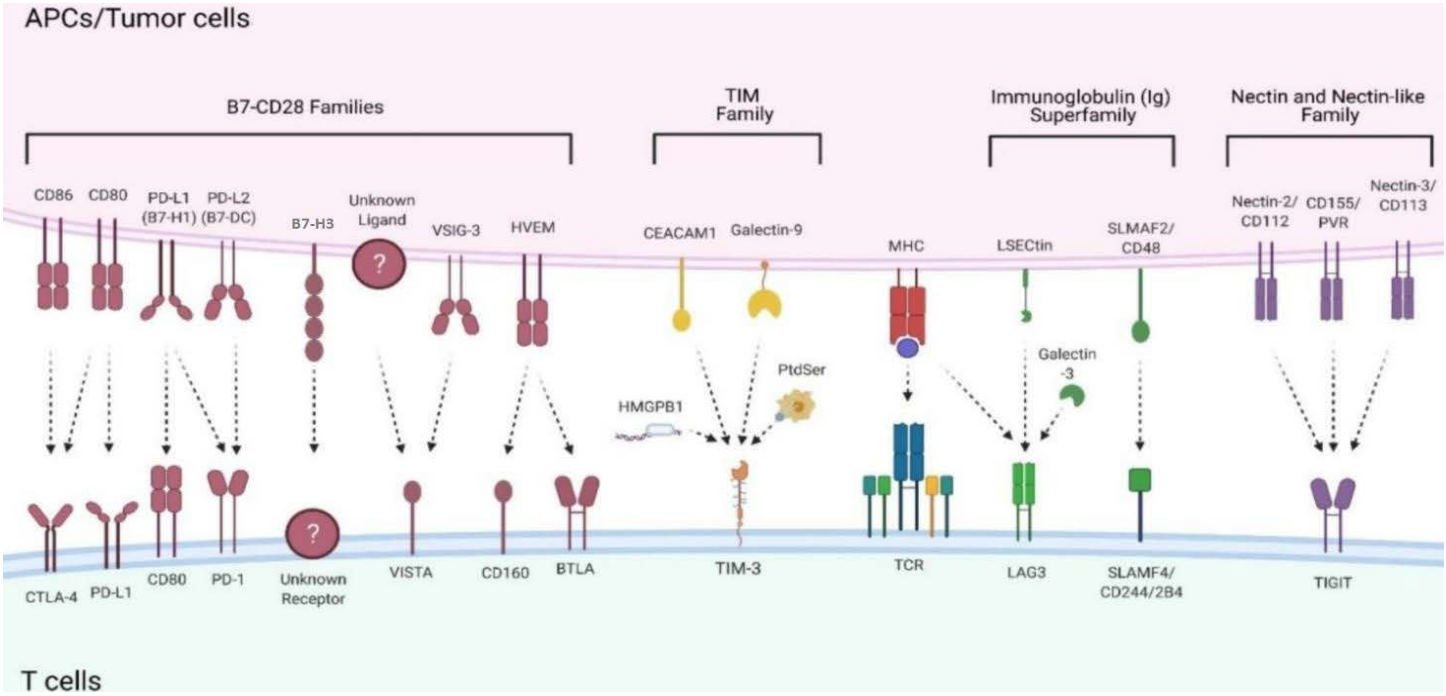


FIGURE 7. Immune checkpoints co-inhibitory ligands and receptors involved in immunosuppressive pathways in the TME and targeted by ICI therapies. (Extracted and adapted from Pansy K et al. doi:10.3390/ijms222413311)

CONCLUSION

Using multiplexed high-throughput analysis, several molecular actors involved in immune modulation pathways pertinent to the mechanism of ICI drug response and/or resistance were identified (Fig. 7). In order to better define the involved phenotype, these were classified as “hot” phenotypes with markers of T cells activation, drug targets presence and tumor inflammation signature. On the other hand, phenotypes involved in immune suppressive pathways defined as “cold” phenotypes allowed refinement of a better therapeutic strategy for the non-responder to monotherapy that would involve combination therapies.

These findings highlight the relevance of simultaneously investigating multiple biomarkers in the disease context to obtain an accurate picture of the immune contexture and then permit a comprehensive personalized strategy for immunotherapy selection.

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