DEVELOP EFFECTIVE THERAPIES:

Delineation of cell subpopulations and cell-cell interactions to determine correlations between drug response and tumor microenvironment in early-stage lung cancer

SUMMARY

The tumor microenvironment (TME) is an integral player in cancer initiation, tumor progression, response and resistance to anti-cancer therapy. Understanding the complex interactions of tumor immune architecture has therefore become increasingly desirable to guide patient selection. Conventional studies that underestimate the potential value of the spatial architecture of the TME are unable to completely elucidate its complexity. To overcome these limitations, we used quantitative image analysis based on multiplexed immunohistochemistry and deep learning technologies to interrogate complex information from the tumor microenvironment and find predictive insights into treatment response for non-small cell lung cancer (NSCLC).

APPROACH

The dynamic interactions of cancer cells with their microenvironment consisting of stromal cells (cellular part) and extracellular matrix (ECM) components (non-cellular) is essential to stimulate the heterogeneity of cancer cell, clonal evolution and to increase the multidrug resistance ending in cancer cell progression and metastasis. Therefore, the understanding of the underlying cellular and molecular mechanisms governing the tumor microenvironment (TME) complexity can contribute to the development of efficient and safe therapeutic strategies to fight cancer.

In the present study, using high-multiplexed immunohistochemistry, we deciphered the TME of two resected NSCLC patients, treated with the anti-PD1 Pembrolizumab (Keytruda®) as immune checkpoint inhibitor (ICI) adjuvant therapy. The two patient's clinical characteristic presented in our white paper 5 were stained with the eighteen (18) markers presented in Table 1 to assess cellular heterogeneity in the TME.

These results highlighted the cell composition heterogeneity and the subsequent interactions to tumors cells between the patient that responded to the ICI therapy versus the one that progressed under it.

TABLE 1. Panel of markers

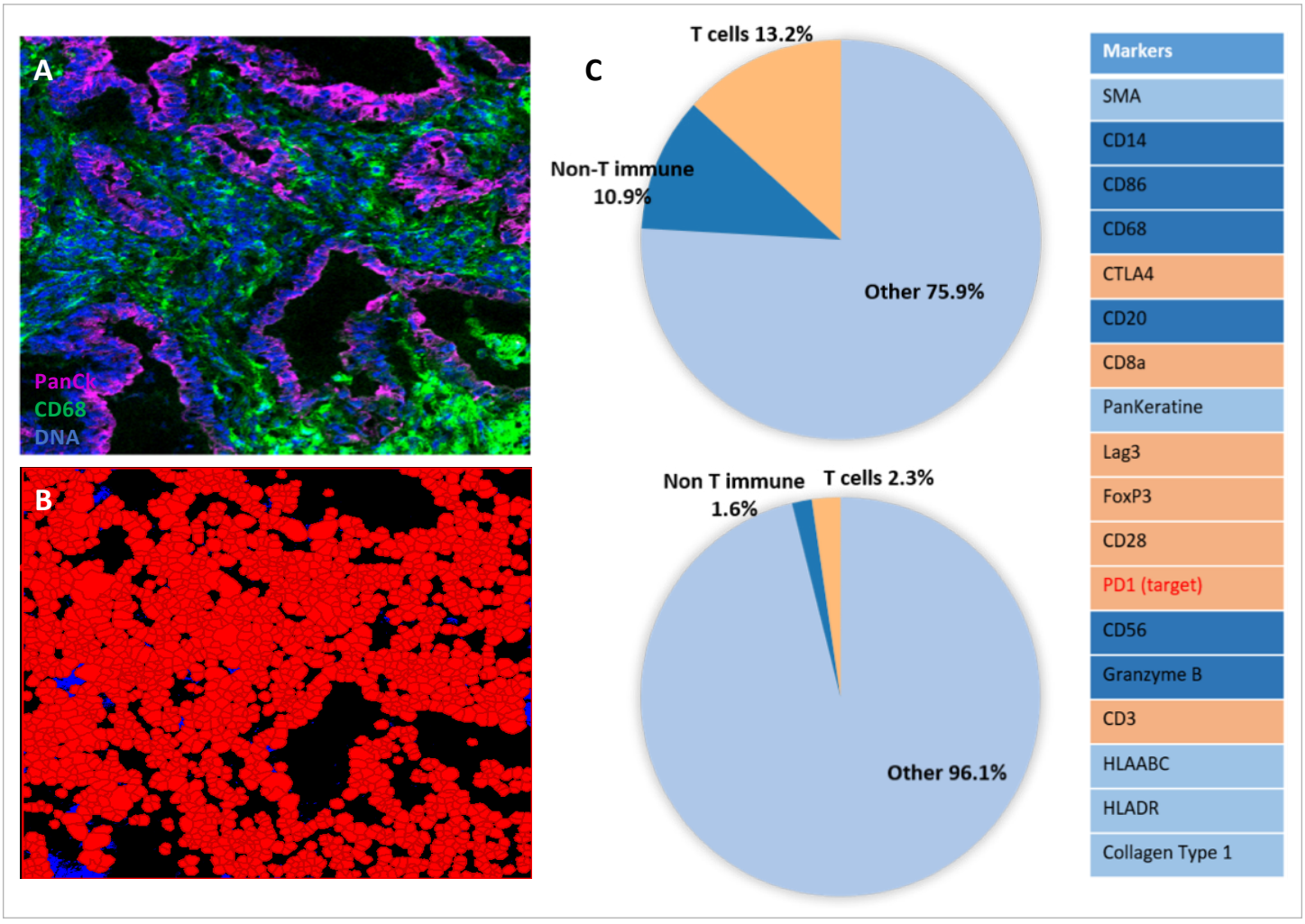
Markers	Phenotype
SMA	Endothelial cells
HLAABC HLADR PanKeratine	Epithelial cells
Collagen Type 1	Fibroblast
CTLA4 Lag3 CD28 PD1 CD3	Generic T lymphocytes
CD14 CD68	Macrophages
CD56 Granzyme B	NK cells
CD8a	T cytotoxic lymphocytes
FoxP3	T regulatory lymphocytes
CD20 CD86	B cells

TME EVALUATION SHOWED DIFFERENTIAL TUMOR/IMMUNE CELLS RATIO

To assess the TME complexity, one region of interest (ROI) was analyzed on each patient tissue sample (Fig.1A). ROIs were selected according to the presence of tumor and adjacent peritumoral stroma as defined by an expert pathologist.

Deep learning (StarDis QuPath extension) was used for cell segmentation (Fig.1B) according to the signal of cell intercalator tagged with natural Iridium (191 and 193 isotopes) that binds the DNA. Cells were then classified in categories: T cells, non-T immune cells, and others according to the markers highlighted into the table in Fig.1C. A significant difference in the cancer/immune cells ratio is seen between the two samples with the progression disease (PD) sample showing a low T cell infiltration corresponding to 2% of the total cell count; in contrast in the complete response (CR) sample the percentage of T cells increase up to 10% (Fig.1C).

FIGURE 1. A- High Plex Immunohistochemistry. B- Automatized cell segmentation according to DNA staining. C- Pie chart representing the complete response (CR) on top and progression disease (PD) TME cell composition.



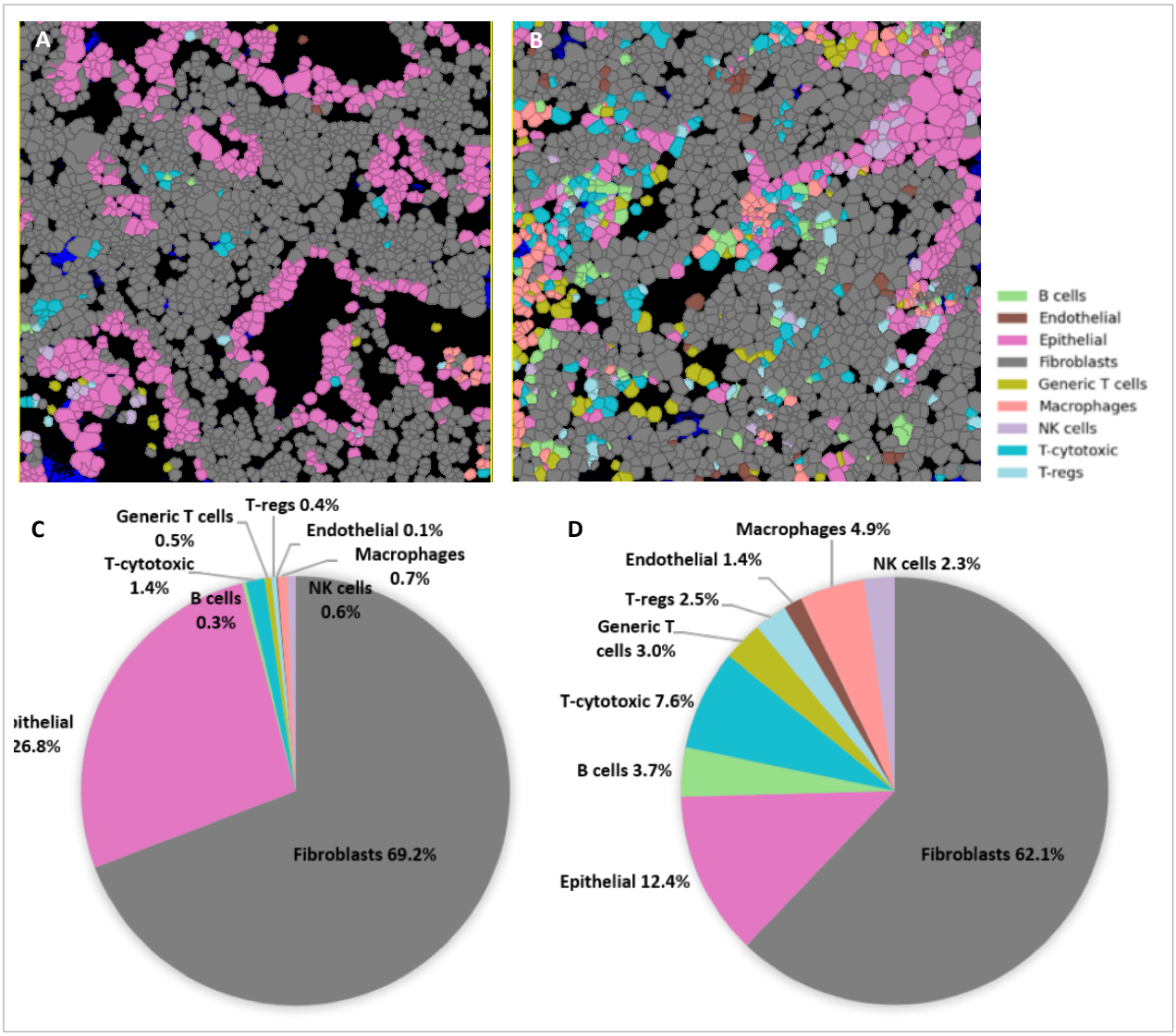
TME COMPLEXITY IMPLICATION IN DRUG EFFICACY

By associating the markers to more specific cell phenotypes (Fig. 2A and 2B), a more accurate picture of complexity and diversity between the two samples was seen. This finer analysis allowed us to highlight that each immune subpopulation tested was infiltrating in larger percentage the TME of the CR sample compared to the PD one.

Interestingly, in the CR sample the CD8+ T-cytotoxic lymphocytes, responsible for the anti-tumor activity, were the immune subset mostly represented (Fig. 2C and 2D).

This finding underscored the importance of the TME complexity governing the efficacy of the therapy.

FIGURE 2. A & B- Immune cells infiltration within the tumor landscape in PD patient Left and CR patient right. C & D-Pie chart representing the relative percentage of immune cells present in the PD left and in CR tumor microenvironment right. In blue, the cytotoxic T cells are the higher expressed immune subtypes in the CR sample. All the immune populations are present in higher percentage in the CR patient compared to the PD one.

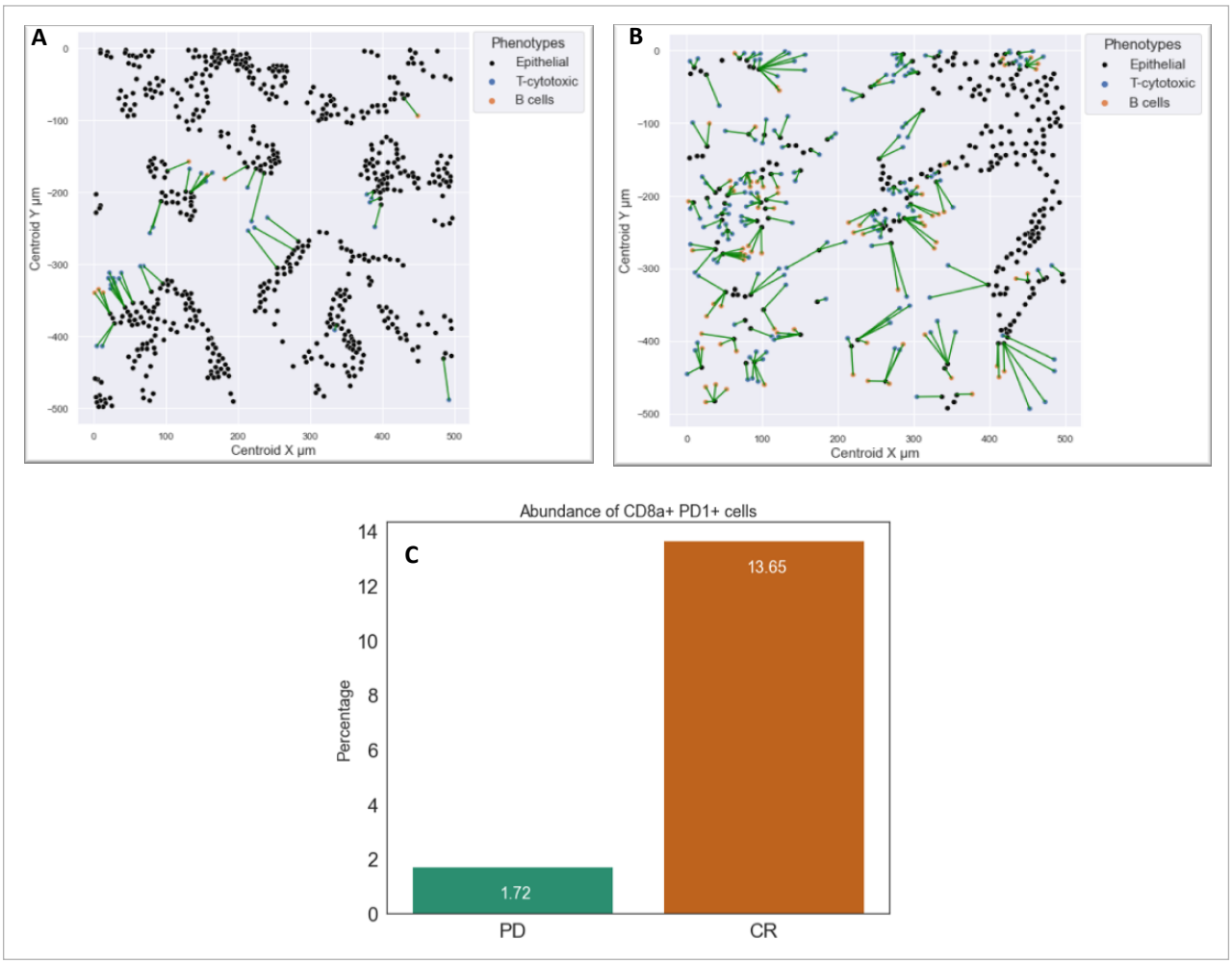


CELL-CELL INTERACTION IS INVOLVED IN PROGNOSIS

Spatial distribution of epithelial cancer cells relative to immune cells was studied in Fig. 3A & 3B. The nearest neighbor analysis showed that CD8+T lymphocytes were located at a minimum distance from tumor cells in the patient that responded to ICI therapy.

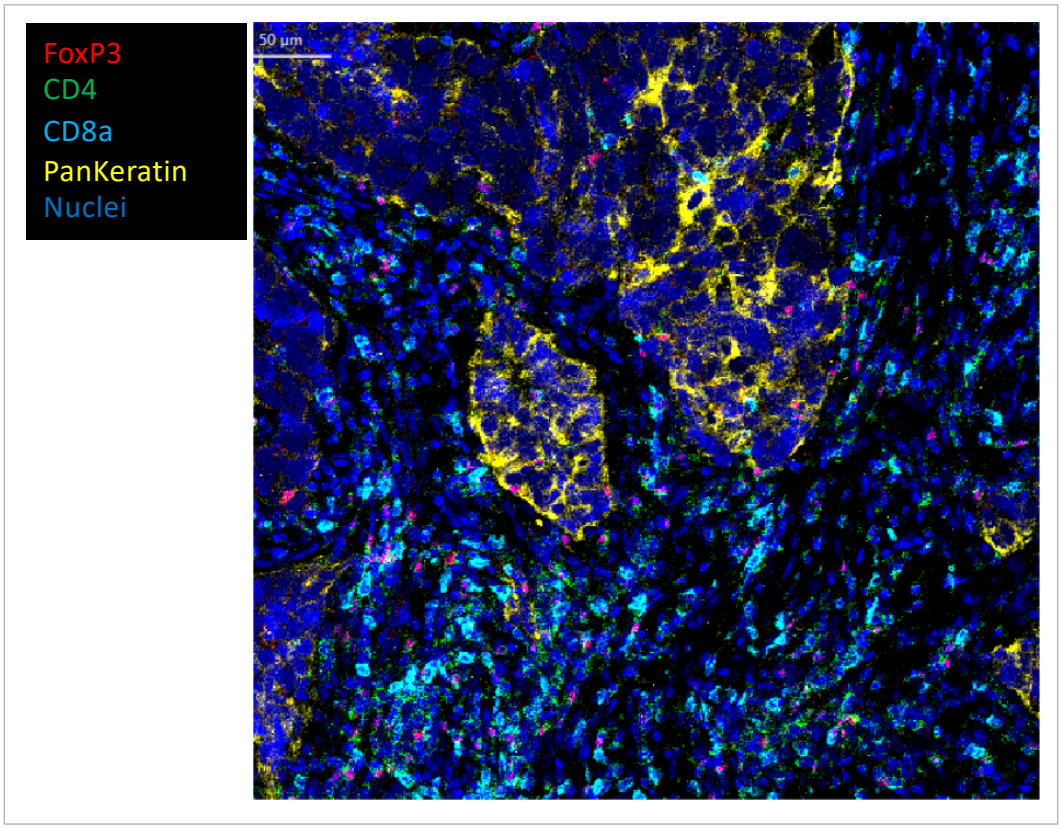
Furthermore the abundance of the drug target was found to be higher in the CR patient (Fig. 3C) demonstrating that in addition to the higher occurrence of the immune system activity in the responder patient, the same patient had the drug target highly expressed in the TME.

FIGURE 3. A & B- Nearest neighbor graphs representing spatial distribution of CD8+ T and B cells relative to epithelial cancer cells in PD tumor left and CR one right. C- Percentage of immune population co-expressing cytotoxicity activity marker and drug in the two patients relative to the entire TME cell composition.



CONCLUSION

Thanks to Aliri's platform, we have been able to identity the drug target in its spatial environment and the involvement of tumor cell interaction with the immune system in cancer prognosis. High-multiplexed proteomics analyses using the Hyperion imaging system help identify and analyze the tumor microenvironment heterogeneity of NSCLC to decipher the mechanism of ICI response for NSCLC patients. Overall, the cooperation between the TME composition, immune activation status and the spatial distribution of the drug target explained the response of the patient to ICI therapy highlighting the importance of deciphering TME molecular heterogeneity to evaluate drug efficacy.



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