Spatial distribution of B cells and lymphocyte clusters for the treatment of non-small cell lung cancer

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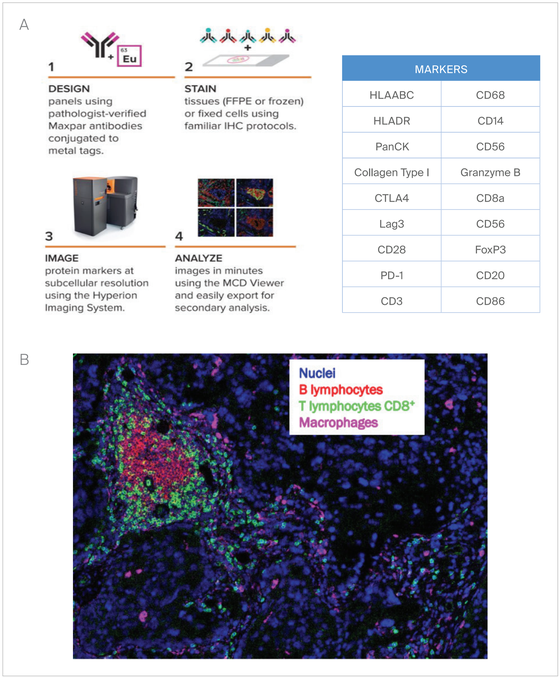
The presence of tertiary lymphoid structure (TLS) in tumor tissues has been reported to be a factor associated with a good prognosis in several types of cancers including non-small cell lung cancer (NSCLC). However, the relationship between TLS spatial organization and the treatment response remains unknown in NSCLC who received ant-PD-1 antibody. The purpose of this study was to evaluate the effect of the various stages of the spatial organization of the TLS from locally concentrated aggregates of immune cells, through clearly defined B cell follicles to mature follicles in NSCLC and its relationship with the tumor microenvironment on anti-PD1 treatment response.

METHOD

Frozen sections from retrospectively collected surgically resected NSCLC tumors treated with adjuvant pembrolizumab therapy were used. The TLS in tumor tissues was detected by high-plex imaging mass cytometry staining (Fig.1) and the difference in TLS spatial organization was compared to the features of the tumor microenvironment and the objective response rate of the patients.



MARKERS	
HLAABC	CD68
HLADR	CD14
PanCK	CD56
Collagen Type I	Granzyme B
CTLA4	CD8a
Lag3	CD56
CD28	FoxP3
PD-1	CD20
CD3	CD86

FIGURE 1. A- Imaging Mass Cytometry high-multiplex workflow. B- High-Plex Immunostaining.

REPEATABILITY

Here we present the results of validation test to verify the precision of the Hyperion (Fluidigm) IMC workflow, therefore the reproducibility of the data. Intra-variability measurement was performed by comparing the same region of interest (ROI) selected in the tissues (n=3). Those triplicates were then performed on different and different operators.

TARGET GROUP	METAL	TARGET NAME
Macrophage	159Tb	CD68
B lymphocytes	161Dy	CD20
T lymphocytes	162Dy	CD8a

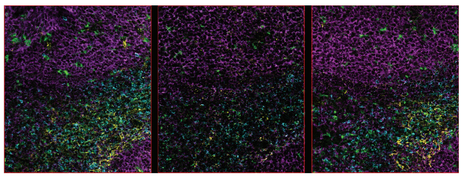


FIGURE 2. Visualization of inter-IMC staining. CD68 : Green, CD20 : Pink, CD8 : Cyan.

PERCENT CV	B CELLS	T CYTOTOXIC CELLS	MACROPHAGES
Intra-day 1	2.9	14.5	5.0
Intra-day 2	2.3	7.9	9.3
Intra-day 3	1.8	1.5	1.3
Inter-operator	2.5	12.9	12.1
Inter-day	2.5	14.8	3.2

TABLE 1. The coefficient of variation (CV) is a relative measure of the dispersion of data around the mean and it is calculated by dividing the standard deviation by the mean. This value is expressed in percentage (CV%).

CELL ANALYSIS WORKFLOW

Deep learning is used for cell segmentation 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, B cells and others according to the markers highlighted into the table in Figure 1.

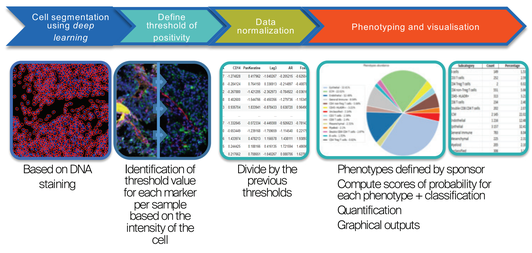


FIGURE 3. Automatized cell classification workflow.

RESULTS

TLS identified and characterized according to their spatial organization within or adjacent to the tumor showed that the presence of tumor-associated TLS (TA-TLS) correlated with favorable response to anti-PD-1 therapy. The abundance and the spatial distribution of B cells allowed a better definition of the correlation between B cell subsets with clinical outcomes showing that the heterogeneity in these TA-TLS influences the predictivity significance to anti-PD-1 therapy.

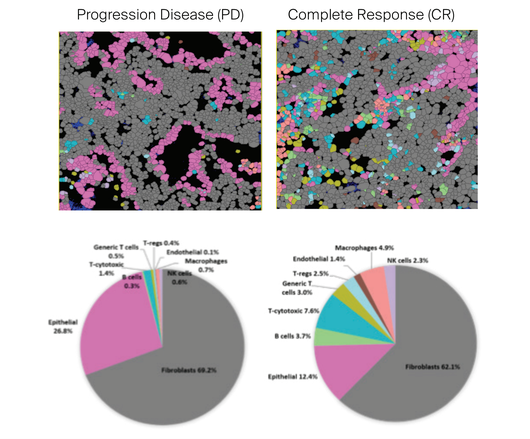


FIGURE 4. A-Immune cells infiltration within the tumor landscape in PD patient Left and CR patient right. Pie chart representing the relative percentage of immune cells present in the PD left and in CR tumor microenvironment right.

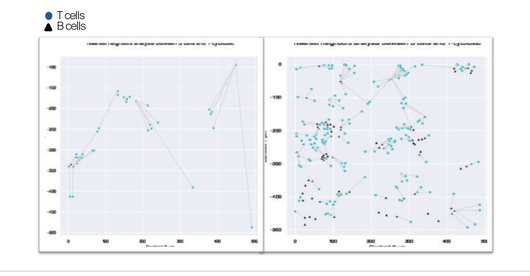


FIGURE 5. Nearest neighbor graphs representing spatial distribution of CD8+ T and B cells in PD tumor left and CR one right.

CONCLUSION

Identifying the phenotypic heterogeneity of intratumor B cells and their functional connection to CD8 T cell helps optimally guide the anti-PD-1 treatment strategy. This spatial mechanistic insight also provides an exciting opportunity for translation of B cell-based immunotherapies into clinics complementary to existing T cells centric strategies.

References

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