RNAscope ASSAY:

Single cell transcriptomics for drug target discovery

SUMMARY

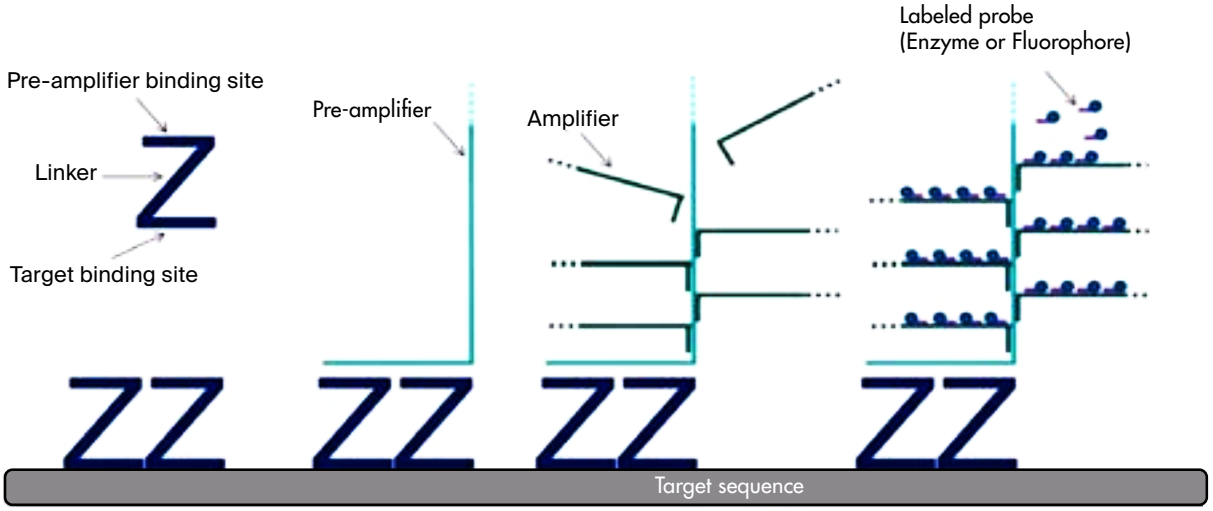
In the context of biomarker/drug target discovery, profiling tissue transcriptome in its spatial organization is key because the tissue function relies on the precise spatial organization of cells. This is especially relevant within a complex and heterogeneous tissue such as a tumor, where a complex interplay between the tumor cells and their environment shapes patients' fates. Therefore, it can be crucial and time sensitive to obtain information about the single-cell transcriptome. The application of RNAscope™ assay, which is a spatial single-cell transcriptomic technique based on *in-situ* hybridization, allows us to evaluate the presence of transcripts within spatial context and in a single-cell. In this perspective, we highlight the use of this technique to identify immune drug targets in a non-small-cell lung cancer (NSCLC) sample with the aim to predict response to immunotherapy.

APPROACH

Here we give an overview of RNAscope Multiplex Fluorescent assay (Figure 1). The workflow that reports biomarkers at the single-cell level starts with the step-wise in-situ hybridization of a whole scaffold of probes.

This system allows for a better specificity compared to a single probe. Several amplifier molecules further bind to the scaffold, and finally, detectable molecules such as enzymes or fluorophores hybridize this tree structure allowing signals detection.

FIGURE 1. RNAscope™ workflow (ACDBio)



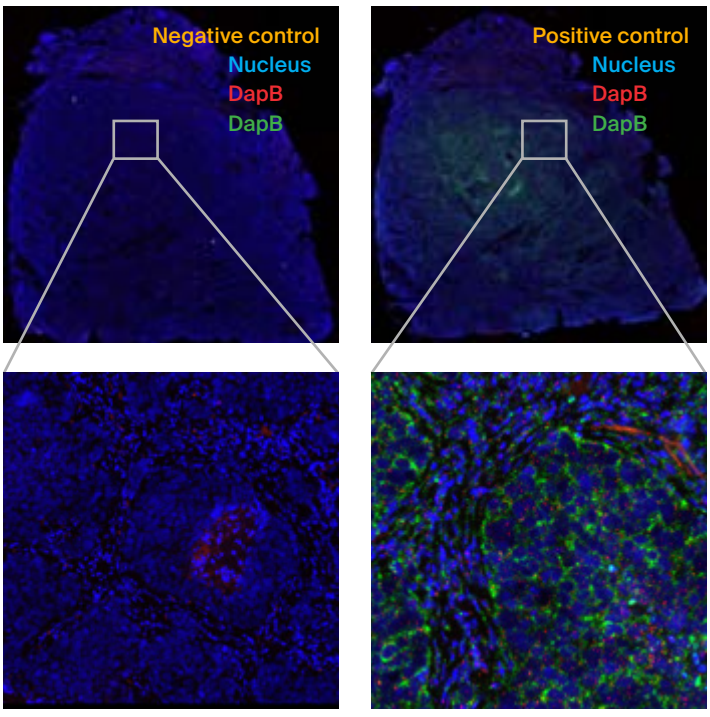
CASE ILLUSTRATION STUDY

In the present study, we investigate the presence of the PD-L1 drug target transcript and the Granzyme B (GZMB) biomarker of clinical outcome transcript in a FFPE NSCLC cancer tissue further treated with Pembrolizumab that progressed on therapy.

Quality control

Control probes to validate the hybridization technique were used. Two positive probes were then used to identify mRNAs that encode the RNA polymerase II subunit A (POLR2A) and Ubiquitin C (UBC), two proteins that are expected to be detected in most of the cells of the tissue. As negative control, two probes that encode different isoforms of a bacterial protein: L-2,3-dihydrodipicolinate reductase (DAPB) were used. As shown in Figure 2, no cells were stained with DapB probes. Non-specific signal was detected and was easily distinguishable from the typical punctuate RNAscope signal obtained in the positive control (Figure 2) where the signal for UBC was higher than the signal for POLR2A as expected. Results indicate that mRNAs of interest in this preserved FFPE tissue can be robustly evaluated by this technique.

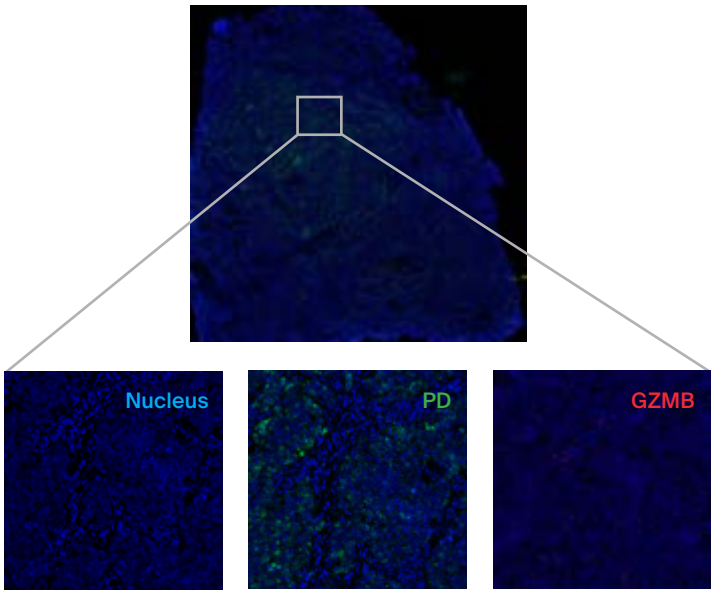
FIGURE 2. RNAscope signal obtained with negative (left) and positive (right) controls



Heterogenous distribution of transcripts

PD-L1 and GZMB mRNAs staining are reported in Figure 3. Difference in expression levels were seen between the two probes across the tumor with PD-L1 staining mostly located in the tumoral area and Granzyme B in stromal area of the NSCLC tissue. This is consistent with the expected expression of PD-L1 on tumor epithelial cells and Granzyme B on T cells. This heterogeneity in the signal distribution is specific to the analyzed tissue and correspond to intra-tissue cells metabolism heterogeneity between cell types. It also informs on the suitability of looking at those probes as patient selection markers.

FIGURE 3. Differential distribution of GZMB and PD-L1 RNAs in NSCLC tissue section



Quantification

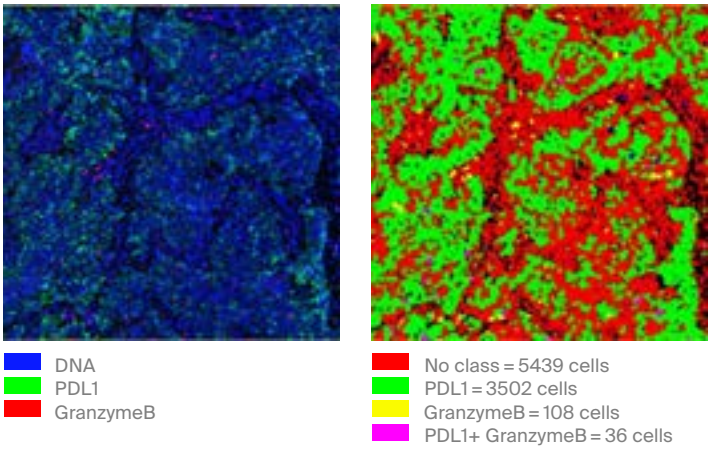
Quantifications of PD-L1 and GZMB were performed using QuPath. One region of interest (ROI) in the tumor compartment was analyzed for both targets' expression. Results are expressed either as percentage of cell expressing the target displayed as a pie chart or as average number of spots per cell displayed as a box-and-whisker plot (min, max) with medians indicated by the horizontal bar (Figure 4). 60% of the cells in the analyzed region of interest of the tissue were double-negative. Among the positive cells, 3% were GZMB$^+$, 96% were PD-L1$^+$, 1% were double-positive. The range of PD-L1 expression in tumor epithelial cells was quite variable but in most cases was above 10 spots per-cell. The translation of this mRNA expression to a clinically relevant cutoff or threshold opens the doors to new investigations to understand the cause of patient resistance to immunotherapies.

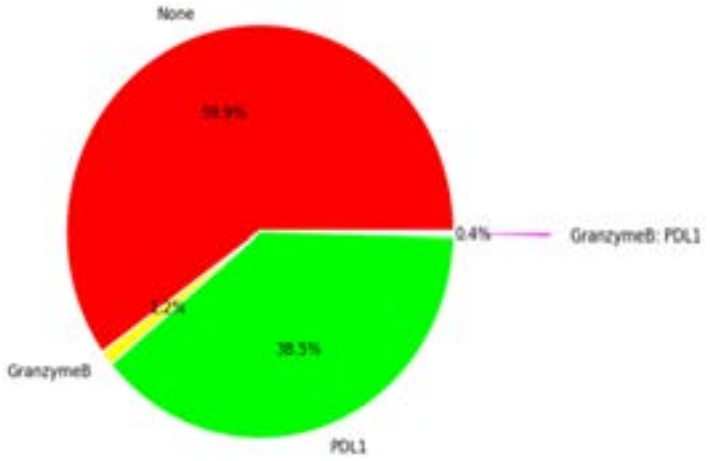
Applications

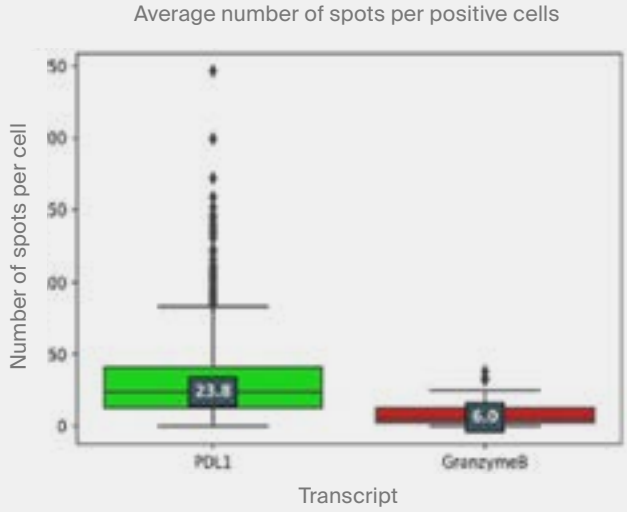
In-situ analysis of RNA in single cells at single molecule sensitivity in clinical specimens is a valuable tool in the era of personalized medicine, offering a realistic alternative for measuring drug target expression. Here we present the use of RNAscope for the detection of RNA targets in the tumor microenvironment (TME) that are involved in tumor immunology and immunotherapy. Detecting RNA biomarker expression at the single-cell level while preserving spatial information is critical to understanding cellular organization and cell-to-cell interactions in the cancer-immunity cycle. Specifically, this detection aids in:

- Localization of specific immune cell types (i.e., cytotoxic lymphocytes and regulatory T cells) in the TME
- Determining spatial relationships between different cell types in the TME
- Characterization of secreted proteins (i.e., cytokines and chemokines)
- Evaluation of immune function in TME beyond enumeration of tumor infiltrating lymphocytes

FIGURE 4. Quantification of RNAscope signals for both PD-L1 and Granzyme B targets







CONCLUSION

Leveraging biomarkers and drug targets early on in discovery can help bring life-saving therapies to market with speed. Gaining access to spatial insights and data by profiling tissue transcriptome in its spatial organization can allow you to understand how the tissue function relies on the precise spatial organization of cells. In this specific example, we were able to showcase this capability in regard to a complex and heterogeneous tissue such as a tumor.