Co-detection of PD1/PD-L1 interaction, immune cell markers and cytokine mRNAs using RNAscope™ Multiomic LS assay is important for understanding anti-PD-L1 treatment response in muscle invasive bladder cancer patients

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INTRODUCTION

- Network of protein-protein interactions (PPIs) and the underlying stoichiometry both within a tissue and with the immune repertoire is adversely impacted in many diseases, including cancer. While screening technologies to predict and map the protein interactome are advancing our understanding of intercellular/intracellular PPIs, no orthogonal technologies exist that can validate inferred PPIs and add context using known mRNA/protein markers.
- Technology that uses modifications to RNAscope™ - a validation technology known for its high sensitivity and specificity – has been developed to visualize PPIs, proteins, and mRNA *in situ* on the same tissue section.
- The assay was deployed on samples from patients with localized, muscle-invasive bladder cancer who received neoadjuvant anti-PD-L1 checkpoint inhibitor therapy prior to planned surgical cystectomy on a clinical trial (NCT02451423). To characterize tumor immune microenvironment of these patients, both responders and non-responders to the treatment, PD-1/PD-L1 interactions were assessed using a panel of targets to include cell phenotyping protein markers for both immune and tumor cells and mRNA markers for effector molecules. We visualized PD-1/PD-L1 interactions, and changes in cellular interactions between cytotoxic T cells or regulatory T cells and CK+ tumor cells before and after immunotherapy treatment.

METHOD

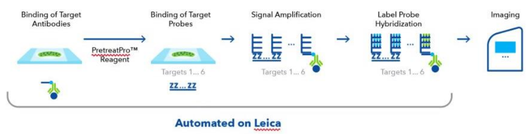
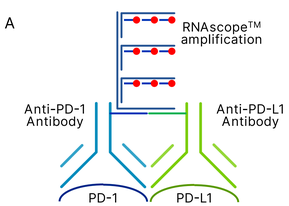


Figure 1. RNAscope Multiomic LS Assay workflow. FFPE sections are first pretreated, followed by application of conjugated antibodies and target RNA specific probes. RNA transcripts and protein-protein proximity/interaction appear as punctate dots.

A



B

Target	Marker Type
PD1-PDL1	Protein proximity
CD3e	Protein
CD4	Protein
CD68	Protein
CD8a	Protein
FoxP3	Protein
PanCK	Protein
GZMK	RNA
GZMB	RNA

Figure 2. (A) Schematic for detection of PD-1/PD-L1 interaction using oligonucleotide-conjugated primary antibodies targeting PD-1 and PD-L1. (B) List of target markers detected on the patient samples.

1. RNAscope Multiomic LS assay visualizes tumor-immune microenvironment surrounding PD-1/PD-L1 interaction on bladder cancer tissues

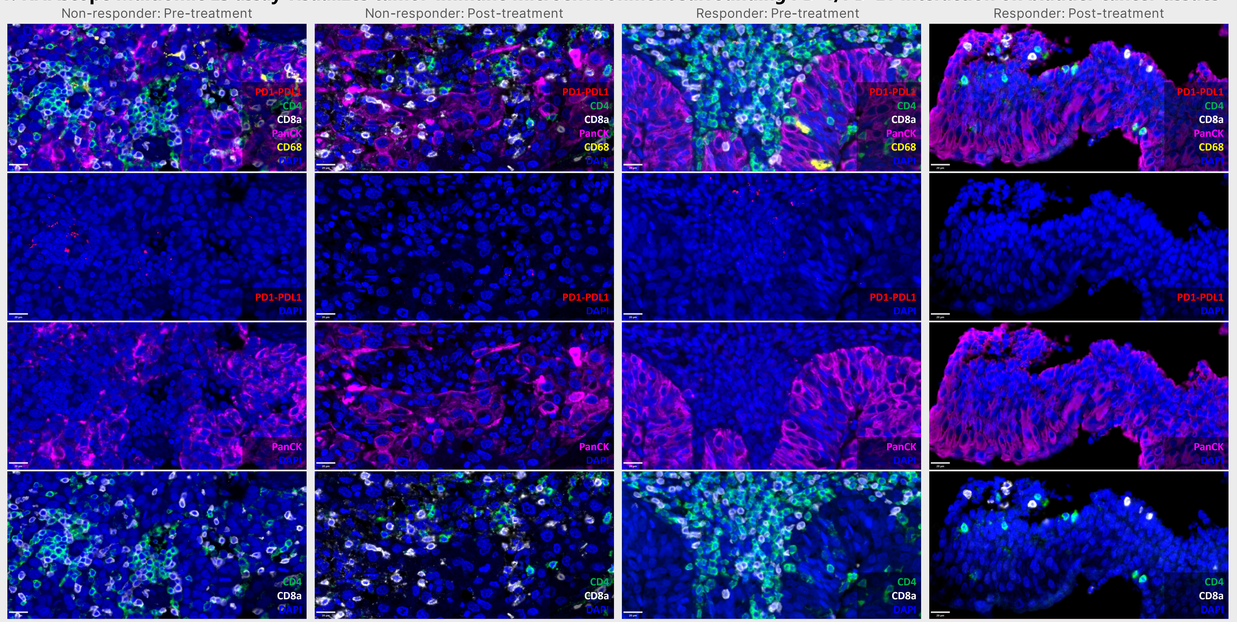


Figure 3. Co-detection of PD-1/PD-L1 interaction, immune cell and tumor markers using RNAscope Multiomic LS assay.

2. Understanding the spatial context of PD1-PDL1 interaction is important

Response to anti-PD-L1	Dosage	%PD-1/PD-L1⁺ cells (pre/post)
Non-responder #1	3	0.23% / 0.14% ↓
Non-responder #2	2	0.081% / 0.011% ↓
Non-responder #3	2	0.054% / 0.16% ↑
Responder #1	2	0.32% / 0.10% ↓
Responder #2	2	0.097% / 0.62% ↑

Table 1. Percentage of PD-1/PD-L1⁺ cells out of total cells did not have correlation to treatment response without spatial context.

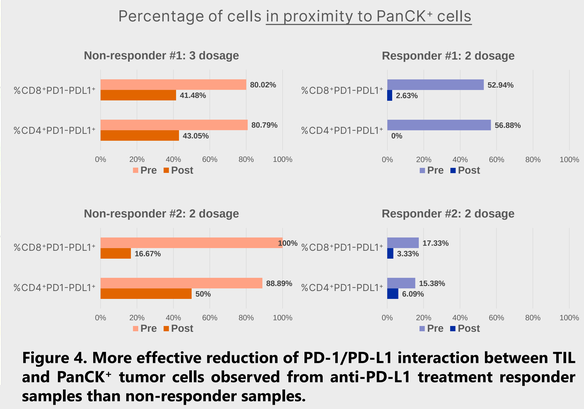


Figure 4. More effective reduction of PD-1/PD-L1 interaction between TIL and PanCK⁺ tumor cells observed from anti-PD-L1 treatment responder samples than non-responder samples.

3. Changes in regulatory and cytotoxic T cell distribution with anti-PD-L1 treatment

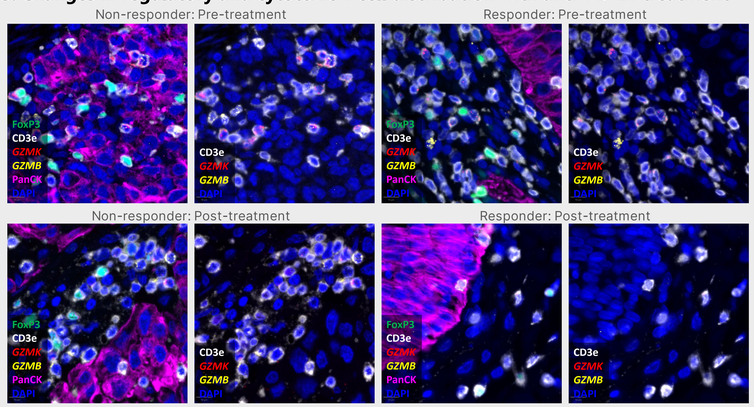


Figure 5. Protein (CD3e, FoxP3, PanCK) and RNA (*GZMB*, *GZMK*) markers for regulatory T cells and cytotoxic T cells were visualized using RNAscope Multiomic LS assay.

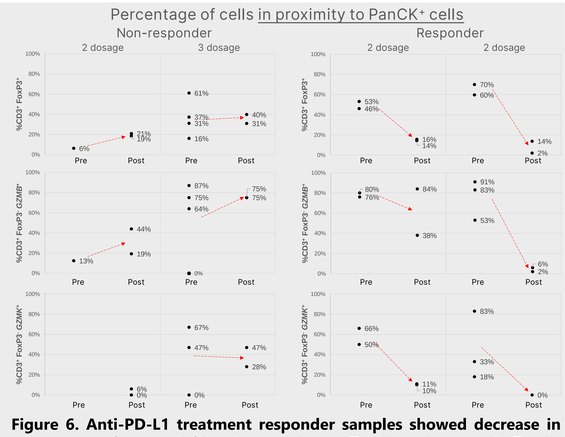


Figure 6. Anti-PD-L1 treatment responder samples showed decrease in percentage of tumor-infiltrating regulatory T cells and cytotoxic T cells expressing granzyme B (*GZMB*) or granzyme K (*GZMK*).

SUMMARY

- This fully-automated assay on BOND RX enables simultaneous detection of 1 protein-protein proximity/interaction and up to 5 targets of RNA and individual protein on a single FFPE tissue section.
- PD-1/PD-L1 interaction is visualized at high resolution with oligonucleotide-conjugated primary antibodies for PD1 and PD-L1 using RNAscope™ Multiomic LS assay on human bladder cancer patient tissues.
- PD-1/PD-L1 interaction evaluated along with spatial multiomics is essential to derive context around anti-PD-L1 treatment response.

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