Evaluation of Immune Checkpoint Marker Co-expression Profiles in the Tumor Microenvironment

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Background

Recent clinical successes with immune checkpoint blockades have provided promising immune-based therapeutic approaches for controlling malignancy. While therapeutic antibodies against CTLA-4 and PD-1/PD-L1 have resulted in potent and durable clinical responses in many patients, there still remains an urgent need to develop biomarker based assays to identify patients who may benefit from these approaches while avoiding toxicity and without compromising efficacy. While gene and protein expression of immune cell and checkpoint markers can be obtained through traditional techniques, insight regarding spatial and cell-specific expression within the tumor microenvironment is lacking, particularly in regards to secreted proteins with key immune functions. RNAscope® is an advanced *in situ* hybridization assay that allows for the visualization of single-cell gene expression, targeting mRNA sequences directly in tissues. The probes designed for this approach permit the detection of secreted markers, including cytokines and chemokines.

Design

In this investigation of 60 archived formalin-fixed paraffin embedded (FFPE) non-small cell lung cancer (NSCLC) biopsies, expression profiles of immune checkpoint markers and immune functional molecules were evaluated in the tissue environment by RNAscope® ISH assay (Figure 1). Each of the tumors displayed distinct co-expression patterns of immune checkpoint markers as well as varying levels of cytokines (including IFNγ and TGFβ1) and chemokines, and immune cell markers.

Tissue microarray (TMA) preparation and RNAscope analysis: FFPE tissues of 60 Lung carcinoma samples were assembled as TMAs as shown in Figure 2. Serial sections of slides were subjected to gene expression analysis by applying manual RNAscope 2.5 HD duplex assay (Figure 1).

Immunohistochemistry (IHC) analysis of PD-L1: FFPE tissue sections of TMAs were assays for the expression of PD-L1 protein using two antibody clones by NeoGenomics Laboratories.

Imaging and quantitation: Images were acquired using a Leica Biosystems Aperio AT2 Digital Pathology Slide Scanner and quantified using quantified by the HALO™ imaging analysis algorithm (Indica Labs).

Figure 1. RNAscope® technology and workflow

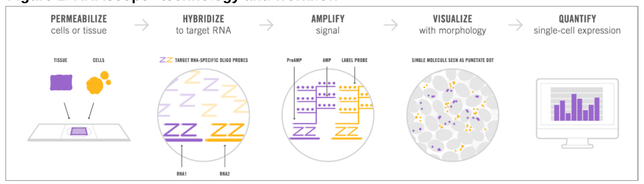
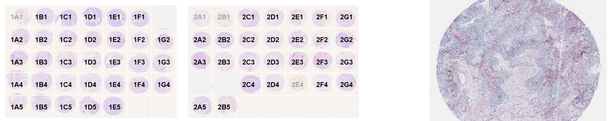
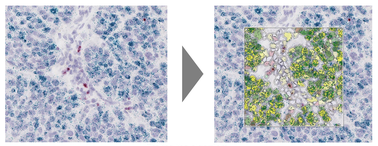


Figure 2. Human NSCLC TMA schematic and HALO analysis



60 core TMA: 56 tumor cores and 4 normal tissues (Normal: **1A1**, **2A1**, **2B1**, **2E4**)



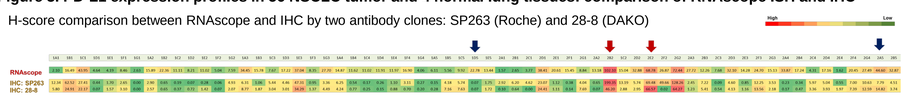
Dialing the algorithm

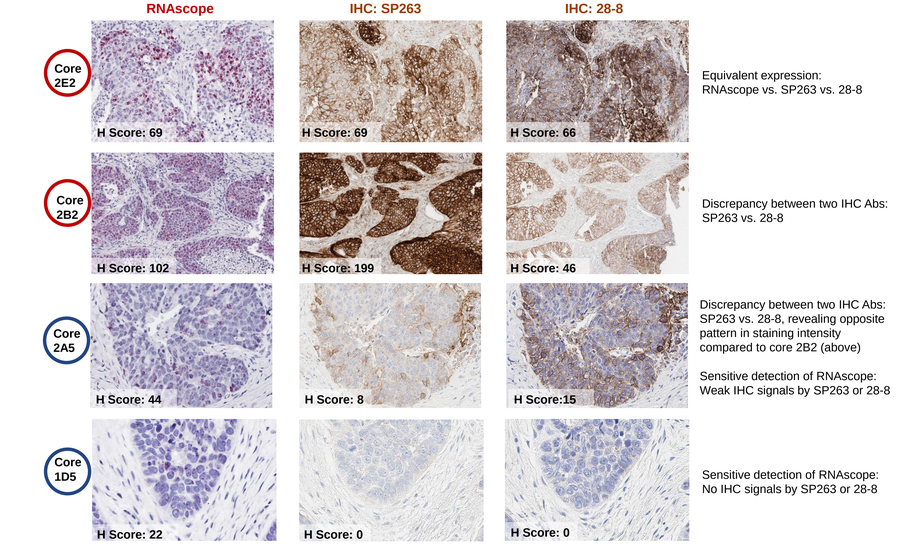
Quantified data

Results

Figure 3. PD-L1 expression profiles in 56 NSCLC tumor and 4 normal lung tissues: comparison of RNAscope ISH and IHC

H-score comparison between RNAscope and IHC by two antibody clones: SP263 (Roche) and 28-8 (DAKO)





Equivalent expression: RNAscope vs. SP263 vs. 28-8

Discrepancy between two IHC Abs: SP263 vs. 28-8

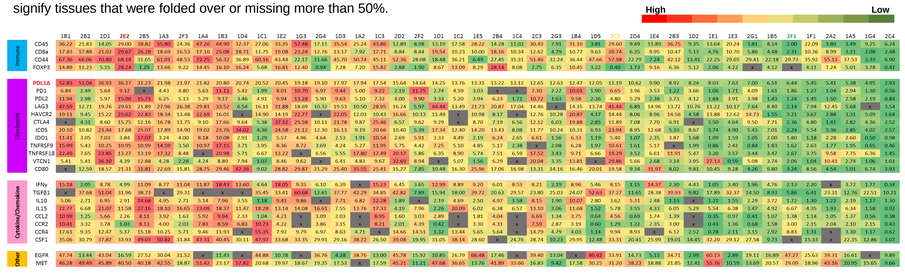
Discrepancy between two IHC Abs: SP263 vs. 28-8, revealing opposite pattern in staining intensity compared to core 2B2 (above)

Sensitive detection of RNAscope: Weak IHC signals by SP263 or 28-8

Sensitive detection of RNAscope: No IHC signals by SP263 or 28-8

Figure 4. Heat map of selected TMA cores representing percent positive cells expressing respective markers: immune lineage, checkpoint-related, chemokine/cytokine, or tumor markers.

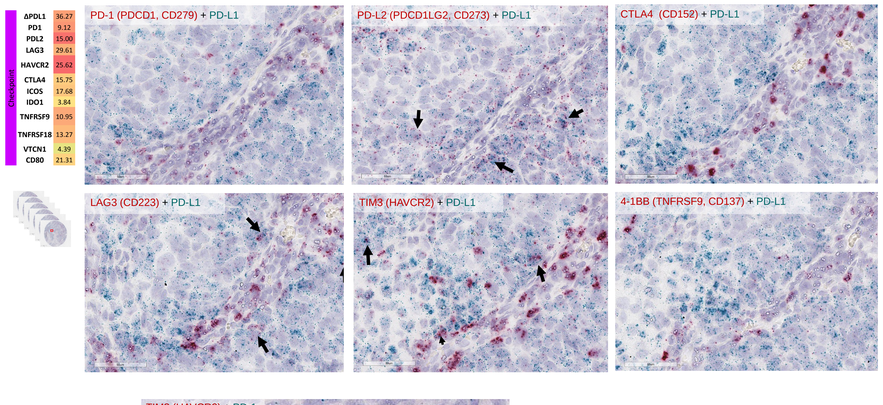
Data sorted based on %PDL1 positive cells. PDL1 values represent average from 18 separate assays. Cores shaded in grey signify tissues that were folded over or missing more than 50%.

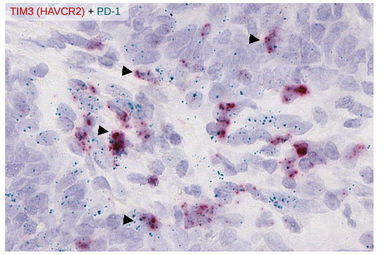


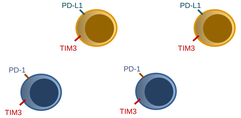
Results

Figure 5. Co-expression profiles of immune checkpoint markers visualized in a tumor microenvironment (core 2E2)

-Expression profiles from serial sections in the selected tumor region







Co-expression profiles expose the presence of multiple checkpoint molecules in the same cell.

Conclusions

In this study, the expression profiles of immune checkpoint and functional markers were evaluated in the tumor microenvironment of 60 NSCLC FFPE tissues by RNAscope®.

- **The immune infiltration in the tumor microenvironment, revealed by RNAscope ISH, is readily quantifiable in addition to visual characterization of specific target RNA detection within the morphological context.**
- **A large proportion of NSCLC cases (>80%) were identified as PD-L1 positive by RNAscope® ISH assay. In comparison with IHC, the RNAscope® assay revealed a dynamic range of PD-L1 expression with higher sensitivity, and the assay readily quantifiable by image analysis software.**
- **Detection of co-expression profiles of multiple checkpoint markers revealed a unique yet heterogeneous pattern of expression in different tumor tissues. This information may reveal potential insight into combination therapies targeted against different checkpoint pathways.**