

RNAscope® Tissue Profiling of Immuno-Oncology Checkpoint Controls in the Tumor Microenvironment

A Database of *In Situ* Expression Image Files and Image Analysis Quantification of Paired Immuno-Oncology Markers

Recent clinical successes with immune checkpoint blockade have provided promising immune-based therapeutic approaches for controlling malignancy. However, it is becoming increasingly apparent that the cellular composition and interactions between cells within the tumor microenvironment (TME) are extremely complex and varied. The RNAscope® technology is an advanced RNA *in situ* hybridization (ISH) assay that enables combinatorial visualization and quantification of cell-specific gene expression, targeting RNA sequences directly in tissues.

The R&D laboratory at ACD has examined the co-expression of multiple immune, checkpoint, and functional markers in 63 NSCLC tumor and non-tumor tissues using the RNAscope® technology and compiled these results into a comprehensive dataset consisting of 40x Aperio image files and digital image analysis for quantification of cellular expression.

- 49 RNAscope® 2.5 HD Duplex and Single-plex Assay datasets on 63 NSCLC tissue cores
- Aperio 40x full slide scans for all assay data
- High-resolution images representing the dynamic range of expression
- Quantitative image analysis for each TMA core available as:
 - Raw data
 - Tumor and stroma region of interest analysis
 - Analyzed expression with quantification of percentage of cells positive (%+) for each marker and percent dual-positive for marker pairs (%+, +)
 - H-scores for expression level of each marker across the TMA core
 - 3,840 tissue-marker expression measurements

RNAscope® 2.5 HD Duplex and Single-plex Assay Datasets Available:			
CCL2/CCR2	CCL2/CCR4	CCL22/CD163	CD44/CD274
CD45/CD274	CD8A/CD274	CD8A/IFNg	CD80/CD274
CSF1/CD274	CTLA4**	CTLA4/CD274	CTNNB1/CD274
CXCL10/CD68	FOSL1/PD-L1	FOXP3/CD274	GATA3**
HAVCR2/PDCD1	HAVCR2/CD274	ICOS/CD274	IDO1/CD274
IDO1/IFNg	IFNg/CD274	IL10/CD274	IL15/CD274
KRT19/CD274	LAG3**	LAG3/CD274	LAG3/FOXP3
LAG3/IL3RA	LAG3/PDCD1	MET**	MET/CD274
MET/EGFR	PAX8**	PDCD1/CD274	PDCD1LG2/CD274
TGFb1/CD274	TGFb1/COL11A1	TIGIT**	TIM3**
TNFRSF9/CD274	TNFRSF9/ICOS	TNFRSF18**	TNFRSF18/CD274
TNFRSF18/PDCD1	VTCN1/CD274		

*Additional data sets with partial results: 30 tissue cores only: CCL3/CCR1, CCL3/CCR4, CCL3/CCR5.
 ** Available as single-plex datasets using the RNAscope® 2.5 HD Assay-Brown.

We can provide these data and a set of high resolution images of representative staining for any or all duplex assays. If you are interested in purchasing this dataset, please contact sales@acdbio.com or visit us online for more information: acdbio.com/immunooncology

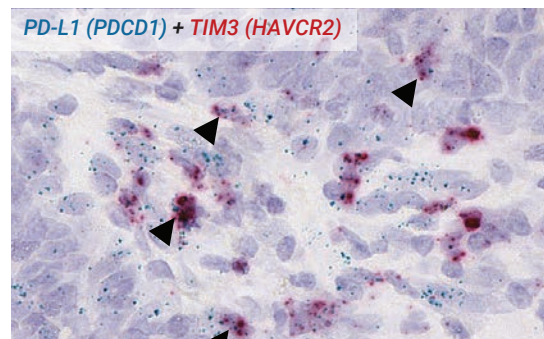
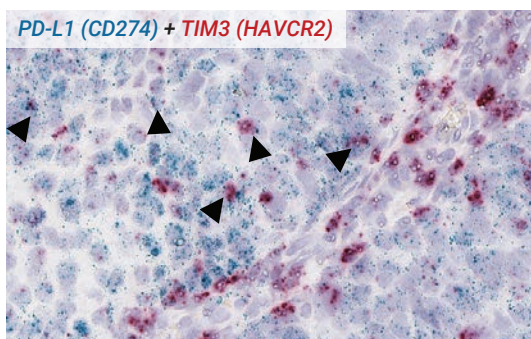


FIGURE 1. Co-expression of different checkpoint markers in the same cell. Single-cell analysis by the RNAscope® 2.5 HD Duplex assay revealed co-expression of TIM3 (red) with PD-L1 (green; left) or PD-1 (green; right) in the same cell (arrowheads), demonstrating the presence of multiple checkpoint molecules in the same cell.

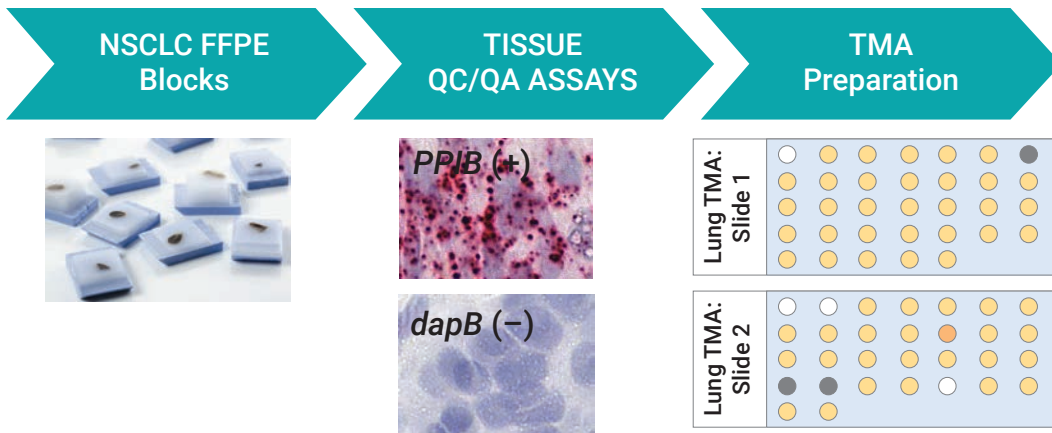


FIGURE 3. NSCLC Tissue Quality Control and TMA Construction. (A) Multiple FFPE tissue blocks from lung cancer patients were obtained and tested for RNA quality using RNAscope® PPIB positive control and bacterial *dapB* negative control probes. A set of 63 QC'd tissues with high quality RNA were selected. The TMA tissues included 56 NSCLC tumor tissues, 2 SCLC, 1 unknown primary metastasis to the lung, and 4 adjacent non-tumor (normal) tissues. The pathology of the 56 NSCLC samples was as follows: 26 squamous cell carcinoma, 22 adeno-carcinoma, and 8 mixed or unidentified. TMA cores size: 3mm.

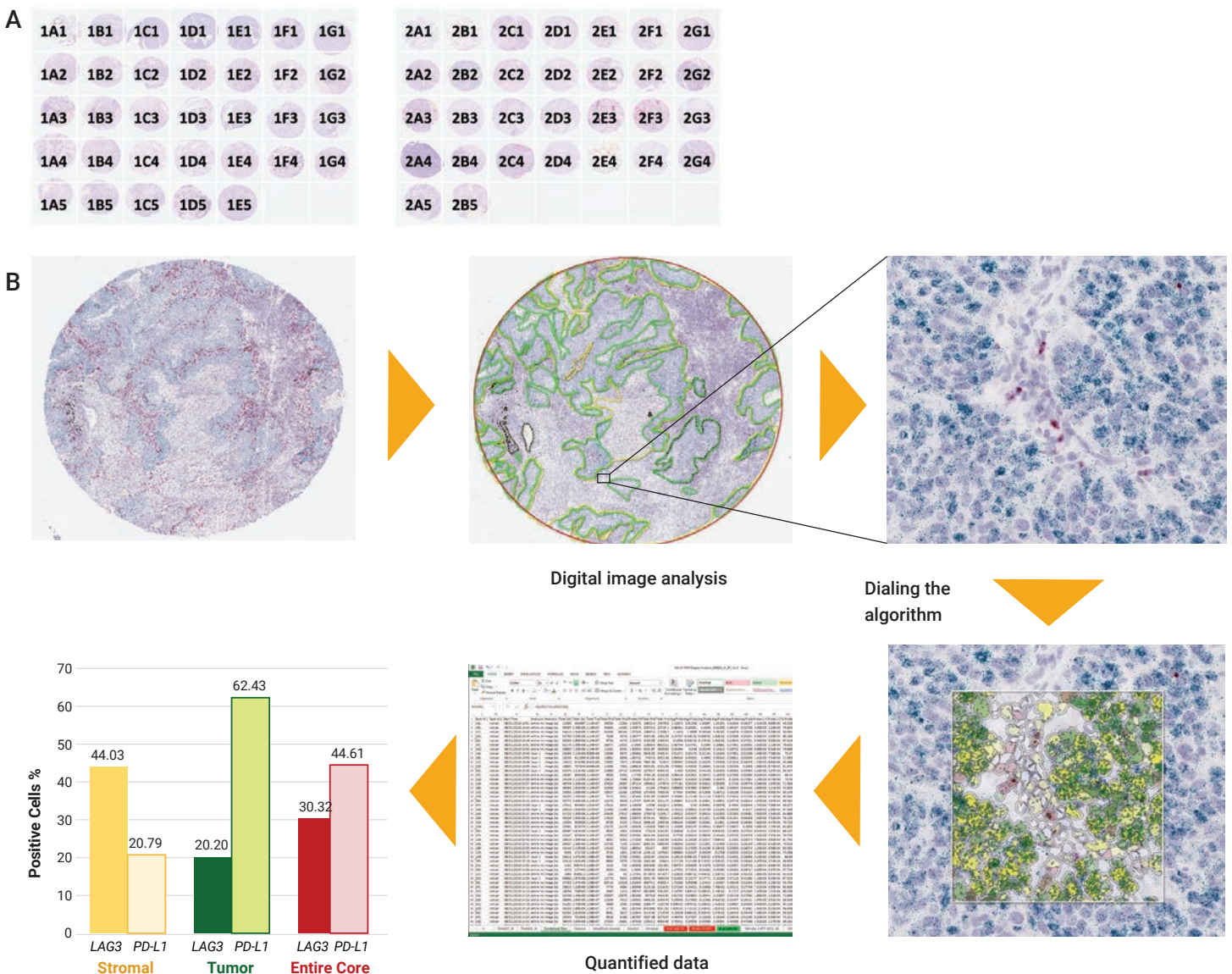


FIGURE 2. RNAscope® 2.5 HD Duplex Assay and quantitative analysis demonstrates a range of co-expression profiles among 63 NSCLC tissues. (A) Schematic depicting the assembly of the 63-core TMA. The 63 cores were divided into 2 TMAs, each of which contain at least 1 adjacent non-tumor core (1A1, 2A1, 2B1, 2E4). (B) Overview of the quantitative analysis workflow. Each core was analyzed using image analysis software for cell segmentation and marker staining; image analysis results were quantified as dots per cell, % positive cells, and H-scores.

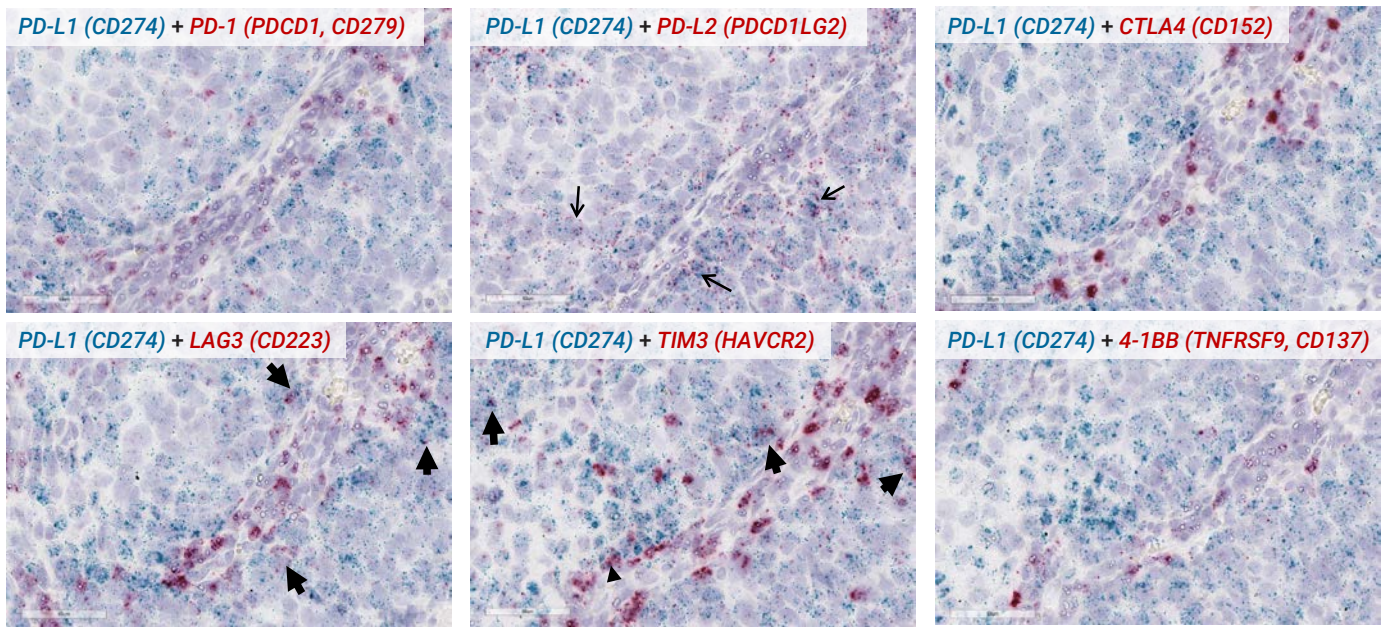
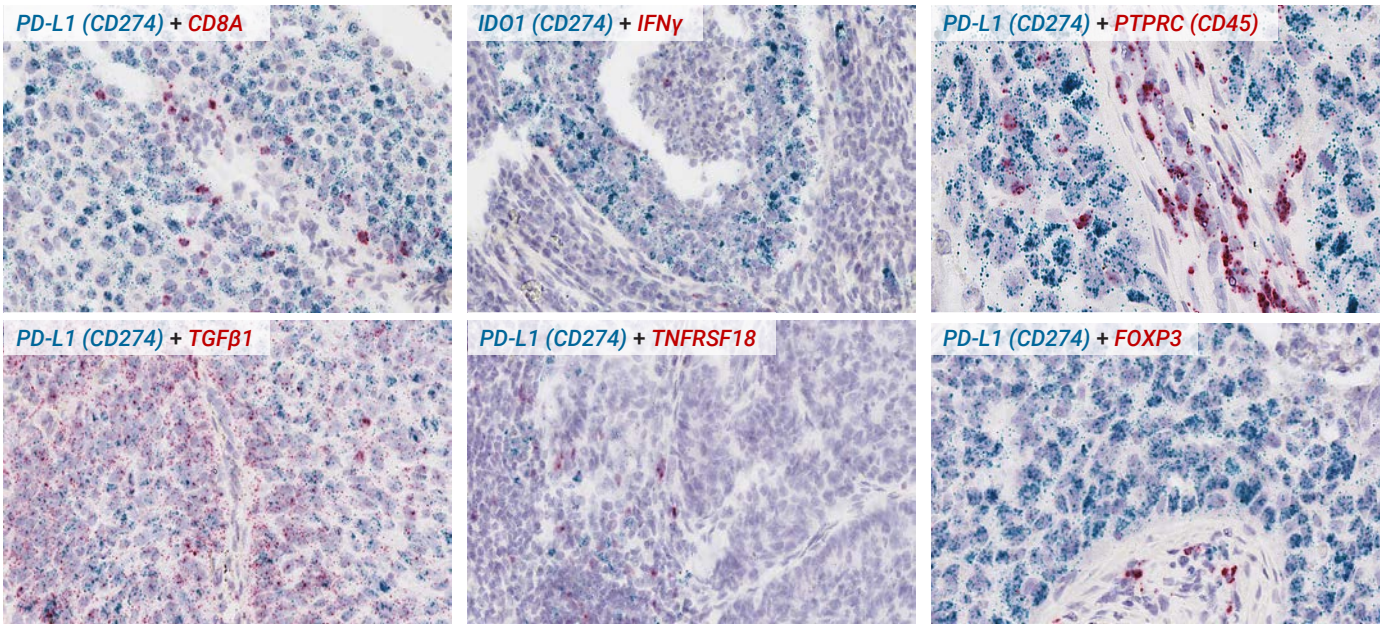
A**B**

FIGURE 4. Co-expression profiles of various immune checkpoint markers in the NSCLC tumor microenvironment. (A) The RNAscope® 2.5 HD Duplex assay was performed on serial sections from Core 2E2 to detect co-expression of *PD-L1* (green) and several immune checkpoint markers (red). *PD-L1* and *PD-L2* expression was detected in both tumor and stromal regions and co-expressed in the same cells (light arrows). *CTLA4*, *4-1-BB*, *PD-1*, *LAG3*, and *TIM3* expression was detected primarily in stromal regions. *LAG3* and *TIM3* were also detected to be co-expressed with *PD-L1* in some cells (dark arrows). (B) Representative images of additional immune checkpoint co-expression profiles examined in this dataset.

Materials and Methods	
Sample	63 NSCLC tissue cores divided onto 2 TMAs (26 squamous cell carcinoma, 22 adeno-carcinoma, 8 mixed/other, 2 small cell lung carcinoma, 1 unknown primary metastasis to the lung, and 4 adjacent non-tumor)
Kit	RNAscope® 2.5 HD Duplex Assay and RNAscope® 2.5 HD Single-plex Assay
Assay	49 RNAscope® duplex and single-plex assays (see table on page 1 for complete list)
Data Provided	<p>Aperio 40x full slide scans (all data)</p> <p>Raw image data analysis</p> <p>Image analysis and quantification on each TMA core (2 mm area):</p> <ul style="list-style-type: none"> Analyzed expression with quantification of percentage of cells positive (%+) for each marker in tumor and stroma regions of interest (ROI) Analyzed expression with quantification of percentage of cells dual-positive (%+,+) for each marker pair in tumor and stroma ROI H-scores for expression level of each marker in tumor and stroma ROI Representative mark-up images showing ROI on which image analysis quantification was performed <p>Project Report with representative images showing range of target expression at low and high magnification in tumor and stroma region, material and methods, tabulated control probe QC scores, Excel file format annotation of all tissues present on the TMA.</p>

** Refer to the terms and conditions for dataset at acdbio.com/termsandconditions

RNAscope Tissue Profiling in Ovarian Cancer Tumor Microenvironment

ACD has also examined the co-expression of multiple immune, checkpoint, and functional markers in ovarian tumor and non-tumor tissues using the RNAscope® technology and compiled these results into a comprehensive dataset consisting of 40x Aperio image files.

RNAscope® 2.5 HD Duplex and Single-plex Assay Datasets Available:

<i>CCL22/CD68</i>	<i>CD163/CCL22</i>	<i>CD274*</i>	<i>CD4/FOXP3</i>
<i>CD68/CXCL10</i>	<i>CD8/IFNγ</i>	<i>CTLA4*</i>	<i>CTLA4/PDCD1</i>
<i>GATA3*</i>	<i>LAG3*</i>	<i>LAG3/PDCD1</i>	<i>KRT19/PDCD1</i>
<i>KRT19/PTPRC</i>	<i>PAX8*</i>	<i>PDCD1*</i>	<i>PTPRC/PDCD1</i>
<i>TGFβ1/COL11A1</i>	<i>TIGIT*</i>	<i>TIGIT/PDCD1</i>	<i>TIM3*</i>
<i>TIM3/PDCD1</i>	<i>TNFRSF18*</i>		

* Available as single-plex datasets using the RNAscope® 2.5 HD Assay.

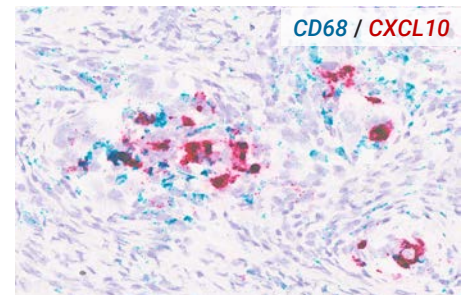
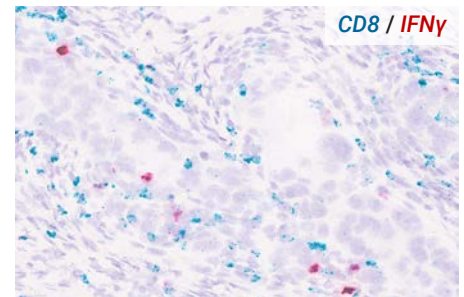


FIGURE 6. Visualization of lymphoid (top) and myeloid (bottom) immune infiltration in ovarian cancer with the RNAscope® 2.5 HD Duplex Assay.

Learn more about the RNAscope® immuno-oncology study at acdbio.com/immunooncology



Advanced Cell Diagnostics