Analysis of Multiplexed Immunotherapy Targets and Secreted Ligands Using Computational Tumor Microenvironment Profiling

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Abstract

As the number and diversity of cellular and signaling pathway therapeutic targets in immuno-oncology increases, more therapy combinations are aimed at multiple cell types in the tumor microenvironment (TME). To develop rational combinations of therapies that will be effective in any individual patient, multivariate biomarker assays will be required to understand each patient's specific tumor and immune profile. Understanding the mechanistic effects of immunotherapy requires identifying the tissue context of direct immunotherapy targets and downstream effector molecules such as secreted cytokines. Although inflammatory response and gene expression of the total tumor can be ascertained through several methods, the spatial arrangements and proximity of cells and colocalization of biomarkers can only be determined with tissue-based assays.

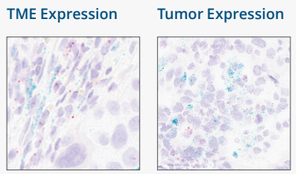
Duplex chromogenic in situ hybridization (CISH) assays using the RNAscope® platform allow for the direct visualization of gene expression of messenger RNA targets for multiple cell types and biomarkers. Computational Tissue Analysis (cTA®) was used to quantify cellular spatial distributions, including regional density, proximity, and biomarker colocalization, in both the tumor nests and surrounding stroma that would be challenging to quantify by visual inspection of duplex RNAscope assays in non–small cell lung cancer (NSCLC) tissue microarrays (TMAs). Oftentimes neither immune effector cells nor the therapeutic target is sufficient in isolation to understand blocked antitumor immune responses and therefore the potential efficacy of a single or combination therapeutic strategy. Multiplex RNAscope assays and cTA in combination allow for the assessment of end points that measure spatial relationships between cell types expressing certain biomarkers. Because these are performed in the context of the tissue microarchitecture within and across multiple tissue sections, they are a significant advancement over traditional tissue-based manual methods.

Methods

CCL2/CCR2 Duplex

C-C chemokine ligand 2 (CCL2), when bound to its C-C chemokine receptor 2 (CCR2) receptor, is thought to recruit immune cell activity and may have protumorigenic properties.

CCL2 (green) and CCR2 (red) were found to be expressed in both tumor cells and the TME.

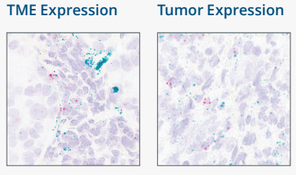


CCL2/CCR2
RNAscope Assay

IDO1/IFNy Duplex

Interferon gamma (IFNy) may function as an antitumor immune factor; however, it is thought to upregulate indoleamine 2, 3-dioxygenase 1 (IDO1). This pairing may allow for tumor cell escape from immune detection.

IDO1 (green) and IFNy (red) were found to be expressed in both tumor cells and the TME



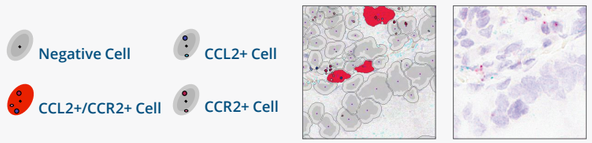
IDO1/IFNy
RNAscope Assay

ACD RNAscope Quantification Using cTA

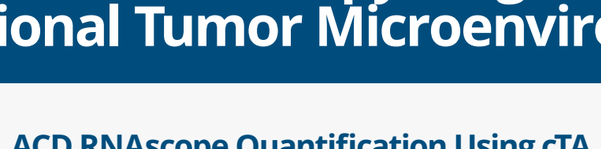
The quantification of multiplex ACD RNAscope CISH using cTA allows for both separate and combined measurements of multiple biomarkers across the full tissue section.

- The algorithm identifies cells and CISH dots for each biomarker separately.
- The whole-slide digital image is overlaid with a cTA markup to visually denote single and dual positivity from the data collected, and individual cell data are recorded in a database.

CCL2/CCR2

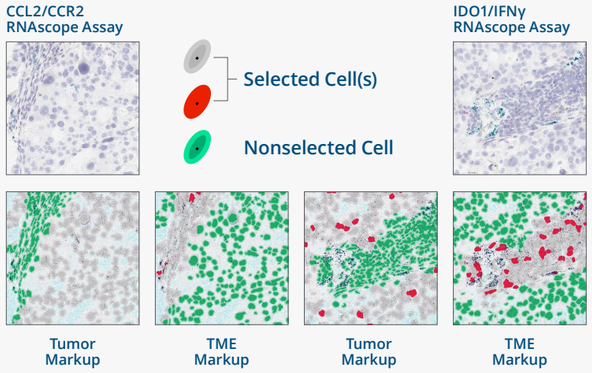


IDO1/IFNy



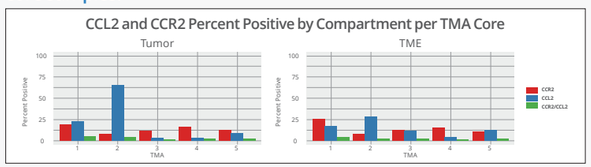
Biopsy Tissue Compartmentalization

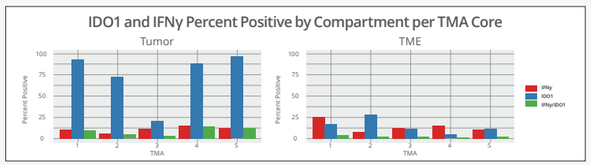
Cells are automatically separated into the tumor and TME using cTA, allowing for the quantification of RNAscope expression in each tissue compartment separately.



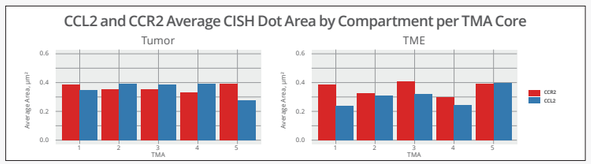
Quantification in Tumor vs TME Using RNAscope and cTA

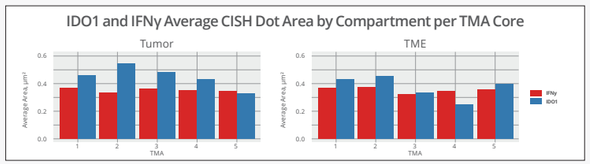
cTA quantification demonstrates differential biomarker expression using the RNAscope duplexes analyzed across multiple NSCLC TMA core samples.





Individual and Dual Cell Positivity in the Tumor and TME Tissue Compartments Across Samples





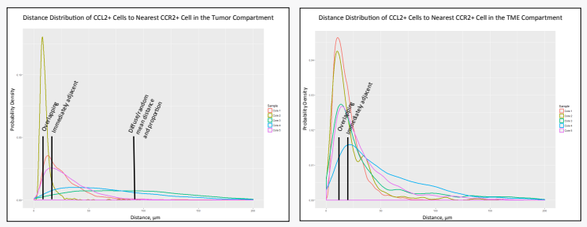
The Average CISH Dot Area Per Marker in the Tumor and TME Tissue Compartments Across Samples

Spatial Distance Measurements Using RNAscope and cTA

cTA demonstrates the differential proximity for CCL2 and CCR2 in the tumor and TME.

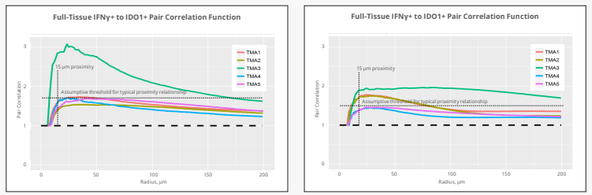
The distance from a CCL2+ cell to the nearest CCR2+ cell was measure in both the tumor and TME compartments.

- Histograms plot the probability density (ie, number) of CCL2+ cells at a given distance (µm) from the nearest CCR2+ cell.
- Samples showed differential proximity between CCL2 and CCR2 in the tumor compartment, and most samples showed strong proximity or overlapping CCL2 and CCR2 in the TME.



Spatial Clustering Measurements Using RNAscope and cTA

cTA-derived cell-to-cell distance measurements describe spatial clustering phenotypes for CCL2/CCR2 and IFNg/IDO1 proximity.



- The majority of samples demonstrate a nonrandom distribution for a CCL2/CCR2 proximity of < 25 mm.
- One sample shows a stronger CCL2/CCR2 clustering than the remaining samples.
- There is consistent variability in the strength of the IFNy/IDO1 proximity relationships.
- The differential strength of the pair correlation function (PCF) value suggests varied clustering of IFNy and IDO1 between samples.
- PCF interpretation:
  - PCF value < 1 indicates that the measured value occurs less than the event happening randomly.
  - PCF value = 1 indicates that the measured is consistent with randomness.
  - PCF value > 1 indicates that the measured value is greater than the probability of the event happening randomly and may suggest clustering.

Conclusions

The use of cTA in multiplex RNAscope assays allows for:

- Reporting of additional end points not available with traditional immunohistochemistry testing.
- Quantification of individual and dual positive cells in separate tissue compartments.
- Spatial measurements to investigate proximity interactions between cells expressing different biomarkers to represent unique biological phenotypes.

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