Multiplex IHC for Immuno-oncology: A Flexible Approach to Characterize the Tumor Microenvironment

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Background

In immuno-oncology (I/O), limited tissue availability underscores the need for precise characterization of the tumor microenvironment (TME). Comprehensive phenotyping of immune cell populations and novel biomarkers is critical for understanding pro- and anti-tumor dynamics and guiding immunotherapy strategies. As such, multiplex immunohistochemistry (mIHC) offers a powerful approach to achieve this.

Method

Panel Development and Staining Platforms

Five mIHC panels were optimized using two automated staining platforms: Leica Bond Rx and Roche Discovery Ultra. Detection chemistries included Akoya Opals for fluorescence-based panels and Roche Chromogens for chromogenic assays. Marker combinations were selected to enable quantification of immune subsets, functional states, and tumor-immune interactions.

Tissue Cohort

Panels were applied to normal and tumor tissues from lung, colon, and pancreas. For each tissue type:

- 2 healthy donor blocks
- 5 tumor samples

ROI Selection and Mapping

A board-certified pathologist identified four regions of interest (ROIs) per block on hematoxylin-and-eosin (H&E)–stained slides. These ROIs were precisely mapped to the corresponding multiplex panel slides to ensure consistent evaluation across samples.

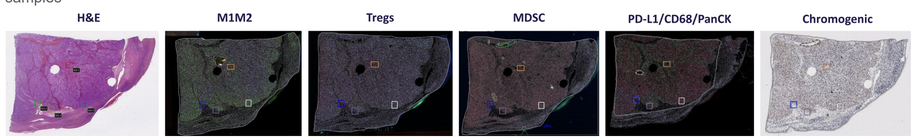

Fig 1. ROI Mapping on ovary cancer sample

Results: Multiplex IHC Panels and Associated Phenotypes in a Colon Tumor Sample

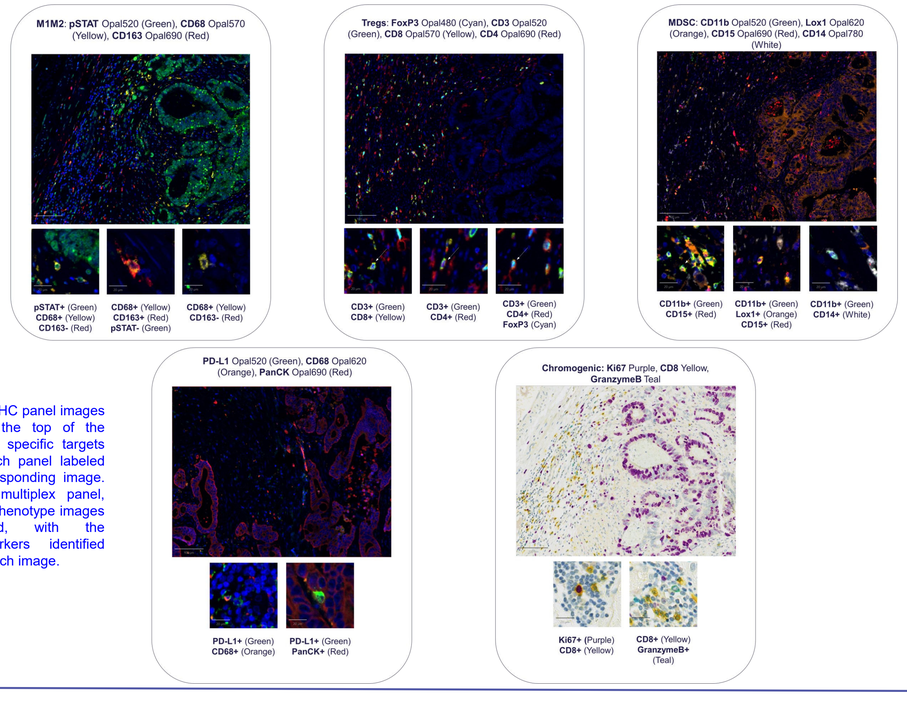

Fig 2. Multiplex IHC panel images are shown at the top of the layout, with the specific targets included for each panel labeled above the corresponding image. Beneath each multiplex panel, the associated phenotype images are presented, with the phenotype markers identified directly below each image.

Method: Image Acquisition and Analysis

Images were acquired and processed using an internally validated workflow that does not require spectral deconvolution. Quantification was performed using HALO® (Indica Labs). Metrics extracted:

- Percentage of positive cells
- Cell density (cells/mm²)
- Phenotypic combinations derived from co-expression patterns

For each sample, overall marker and phenotype levels were aggregated across all ROIs. These values were compared to whole-tissue measurements from the same slide. Matched-pair analyses were conducted in JMP statistical analysis software, enabling direct comparison between ROI-based and whole-slide quantification.

Results: Image analysis results - examples of visualizations

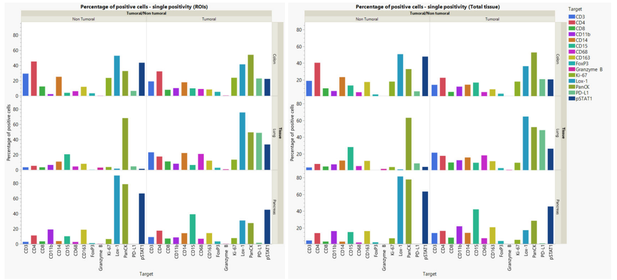

Fig 3. Quantification of single-positive cell percentage and cell density.

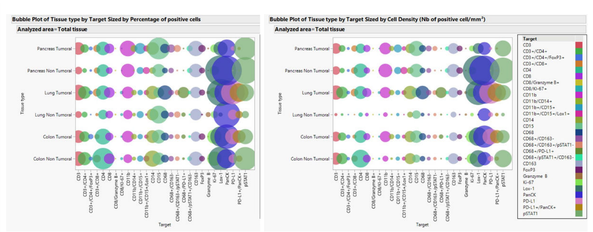

Fig 4. Visualization of the proportion of positive cells and cell density for single-marker positivity and phenotypes for each target/phenotype in total tissue.

Results: Total tissue vs ROIs comparison

Reportable parameter	Target vs Phenotype	Correlation
All	All	0.93
Cell Density (Nb of positive cell/mm²)	All	0.93
	Phenotype	0.95
	Single positivity	0.91
Percentage of positive cells	All	0.96
	Phenotype	0.95
	Single positivity	0.95

Table 1. Total tissue vs ROIs comparison results.

Matched-pair analysis demonstrated a strong correlation between ROI-based data and whole-tissue marker expression, confirming that the selected ROIs accurately represent the overall tissue context. This applied to:

- Single-marker quantification
- Phenotypic combinations
- All reportable parameters

Multiplex panels exhibited high versatility across multiple tumor indications, offering clear discrimination between immune profiles in healthy versus tumor tissue.

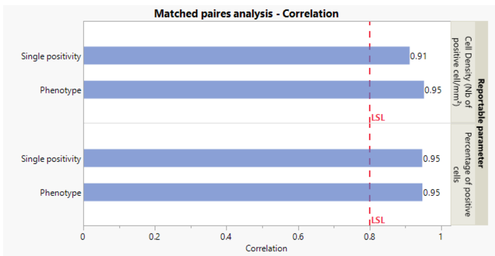

Fig 5. Matched pairs analysis using JMP software for percentage of positive cells and cell density for single positive cells and phenotypes.

Quantification of single-marker positivity (e.g., CD8, CD68, PD-L1) and derived phenotypes (e.g., macrophage subsets, activated cytotoxic T cells, regulatory T cells) provided detailed insight into the spatial distribution and functional organization of immune subsets within the TME.

The study further highlighted:

- Strengths of ROI-based analysis for focused, pathologist-driven evaluation
- Complementary value of whole-slide analysis for global tissue characterization

Conclusion

mIHC proved to be a robust, flexible, and informative tool for immune profiling across solid tumors. The combination of automated staining platforms, high-resolution imaging, and quantitative image analysis allowed comprehensive characterization of immune markers and phenotypes, capturing both cellular abundance and spatial context. By integrating ROI-based and whole-slide quantification, this work supports a deeper understanding of tumor–immune interactions, enabling cross-tissue and cross-tumor comparisons, assessment of immune infiltration patterns and evaluation of functional immune states within the TME. **Overall, these multiplex IHC workflows provide a powerful analytical framework for immuno-oncology biomarker development and contribute valuable insights for the advancement of precision immunotherapy.**

Disclosures: EB, SI, AF, MM, RB, M G-D are Cerba Research employees or past employees. RG is a consultant. Study is sponsored by Cerba Research.