Complementary PhenoCode Signature Panels Comprehensively Map Cell Interactions and Identify Spatial Signatures in the Tumor Microenvironment

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BACKGROUND

In cancer research, advancing our understanding of the underlying mechanisms driving disease progression is key to developing new therapeutic regimens and improving patient outcomes. Over the past several years, multiplexed imaging has played a vital role in elucidating novel immune-tumor interactions and identifying targets of interest for drug discovery and development.

Emerging studies utilizing multiplexed imaging have revealed complex cell-to-cell interactions within the tumor microenvironment (TME). Spatial biology using multiplexed imaging provides advantages over other biomarker modalities by enabling deeper interrogation of cellular- and protein-level co-expression, localization, and arrangements within the TME.

A developing new biomarker class in the TME are Spatial Signatures, defined by the measurement of the interactions between, and cell densities of, tumor and immune cells.

Akoya's PhenoCode™ Signature Panels (PSP) have been designed to enable comprehensive mapping of the TME and to help identify spatial signatures. PhenoCode Signature Panels incorporate the barcode-based antibody chemistry from Akoya's PhenoCycler® platform integrated with the signal amplification capabilities of Opal chemistry from Akoya's PhenoImager® platform. These 6-plex kits are designed with 5 locked-down markers and 1 open marker configuration that provide an off-the-shelf, multiplexed capability with minimal user development requirements.

In this study, we utilized three PSPs to investigate and identify potential spatial signatures in a tissue microarray comprised of responders and non-responders non-small cell lung cancer (NSCLC) patient biopsy samples who received immune checkpoint inhibitor (ICI) therapy.

METHODS

Sample Staining: Three tissue microarray (TMA) slides comprising 41 formalin-fixed paraffin-embedded (FFPE) NSCLC patient samples from second-line PD-L1/PD-1 ICI-treated cohorts (Durvalumab, Nivolumab, or Pembrolizumab, 16 responders and 25 non-responders) were stained using three PhenoCode Signature Panels on a Leica Bond RX™.

Tissue Image Acquisition and QC: Multispectral images were acquired of each of the TMA slides using the PhenoImager HT. TMA cores were identified and selected in Phenochart. All TMA cores were visually inspected. Those that contained tissue folds, high levels of adipose tissue, or significant tissue loss were excluded from analysis.

Data Analysis: Scans were unmixed using the tissue specific library using Akoya's inForm® image analysis software. An analysis algorithm was developed with images undergoing tissue and cell segmentation, cell phenotyping, and thresholding. Samples were then batch processed and data exported. Cell densities and spatial measures were computed using the R-script package phenoptrReports. Graphs were generated in GraphPad Prism.

Figure 1. PhenoCode Signature Panel Workflow Overview. The Akoya PhenoCode Signature Panels were stained on TMA slides using the Leica Bond RX autostainer. Whole slide multispectral images were acquired on the PhenoImager HT and TMA cores identified. Images were multispectrally unmixed then underwent tissue segmentation, cell segmentation, phenotyping, and thresholding in inForm. Data exported from inForm was processed in phenoptrReports.

PHENOCODE SIGNATURE PANEL TECHNOLOGY

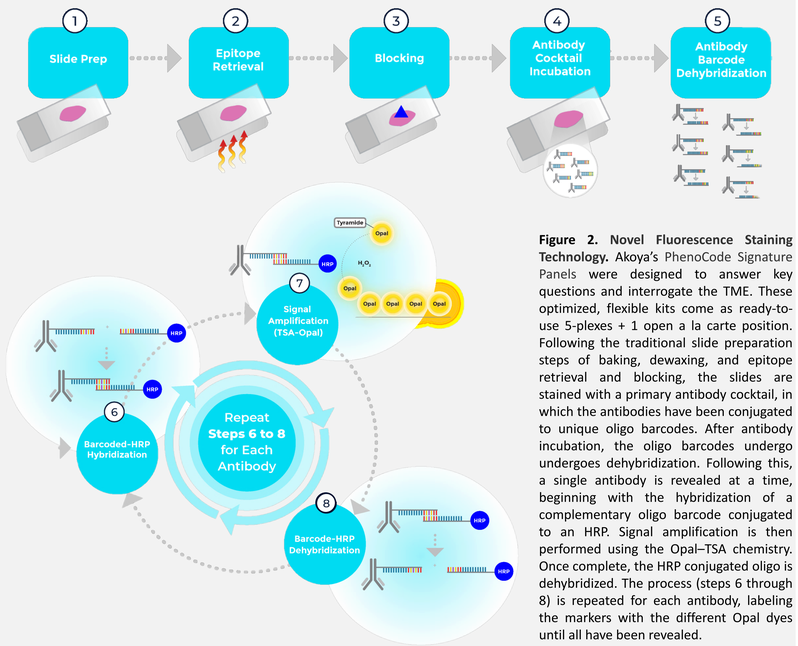

Figure 2. Novel Fluorescence Staining Technology. Akoya's PhenoCode Signature Panels were designed to answer key questions and interrogate the TME. These optimized, flexible kits come as ready-to-use 5-plexes + 1 open a la carte position. Following the traditional slide preparation steps of baking, dewaxing, and epitope retrieval and blocking, the slides are stained with a primary antibody cocktail, in which the antibodies have been conjugated to unique oligo barcodes. After antibody incubation, the oligo barcodes undergo undergoes dehybridization. Following this, a single antibody is revealed at a time, beginning with the hybridization of a complementary oligo barcode conjugated to an HRP. Signal amplification is then performed using the Opal–TSA chemistry. Once complete, the HRP conjugated oligo is dehybridized. The process (steps 6 through 8) is repeated for each antibody, labeling the markers with the different Opal dyes until all have been revealed.

ICI-SENSITIVE NSCLC TMA AND PANEL OVERVIEW

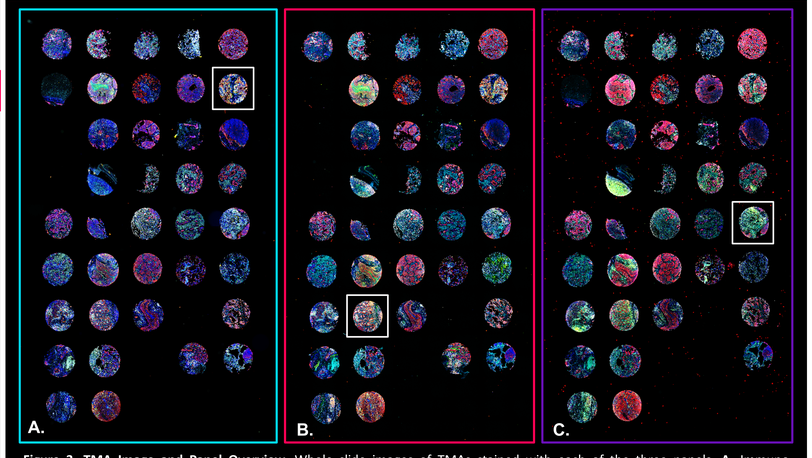

Figure 3. TMA Image and Panel Overview. Whole slide images of TMAs stained with each of the three panels. **A.** Immuno-contexture + PD-1 (teal); **B.** Immuno-contexture + CD20 (pink); and **C.** Immune Profile + CD4 (purple). White outlines highlight representative cores shown in greater detail in Figure 4.

Panel	Position 1 Opal 690	Position 2 Opal 570	Position 3 Opal 780	Position 4** Opal 520	Position 5 Opal 620	Position 6 Opal 480
Immuno-contexture + PD-1	FoxP3	PD-L1	CD68	PD-1	PanCK	CD8
Immuno-contexture + CD20	FoxP3	PD-L1	CD68	CD20	PanCK	CD8
Immune Profile + CD4	PanCK	CD3e	CD68	CD4	CD20	CD8

Table 1. PhenoCode Signature Panels. The 5-plex Spatial Signature Panels were designed to address key questions to interrogate the TME landscape. An open position** provides the flexibility to incorporate an additional marker of choice. Users can select from a choice of markers with pre-conjugated barcodes that can be easily integrated into any of the panels. Conversely, users can utilize Akoya's labeling kit to conjugate their own antibody of choice to add into any panel.

ANALYSIS OF TMA SAMPLES USING PHENOCODE SIGNATURE PANELS

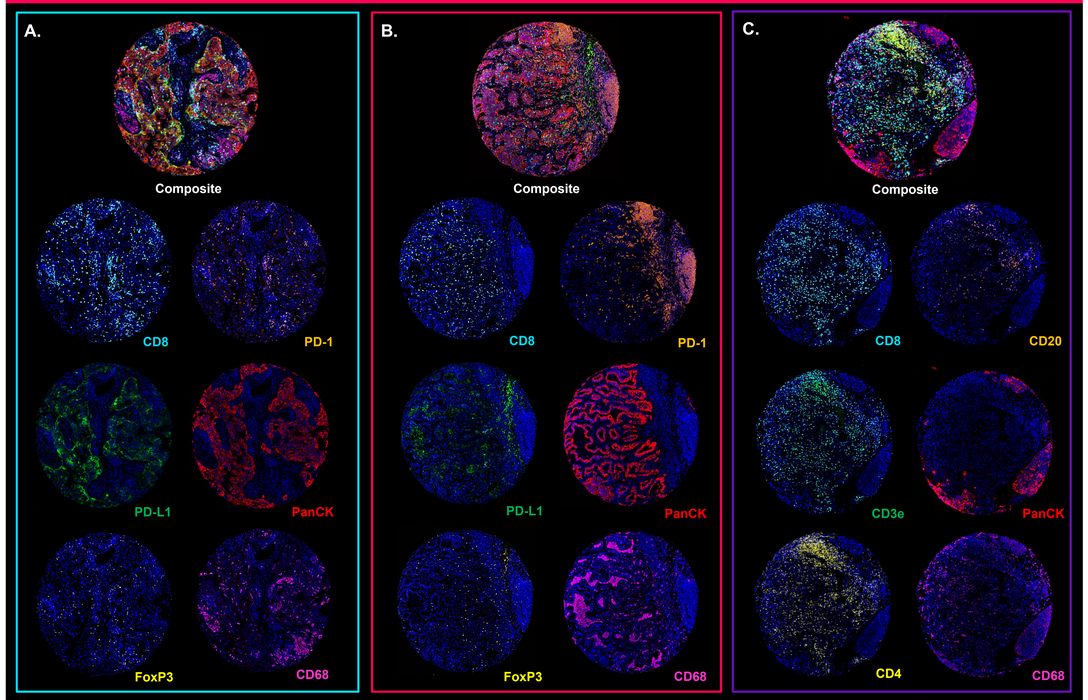

Figure 4. Representative Panel TMA Cores. One core from each slide stained with **A.** Immuno-contexture + PD-1 (teal); **B.** Immuno-contexture + CD20 (pink); and **C.** Immune Profile + CD4 (purple). Images show spectrally unmixed composite image along with each marker in their individual channels. Marker and Opal pairings can be found in Table 1.

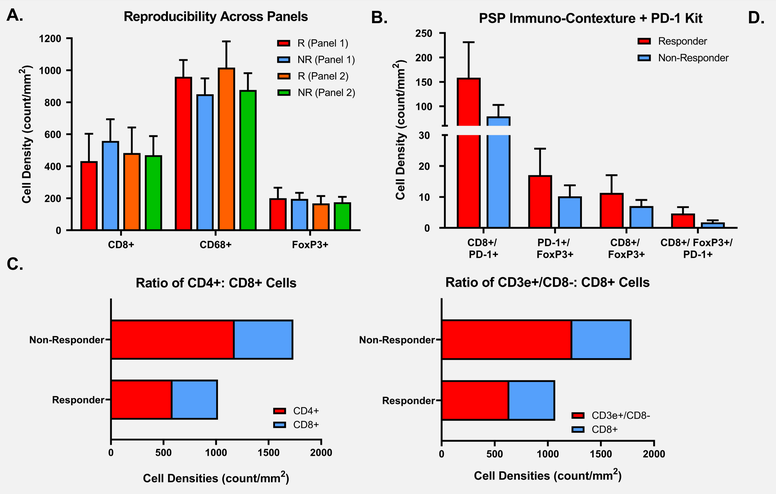

Figure 5. Analysis of TMA samples using PhenoCode Signature Panels. A. Same markers in different PSP kits run on serial sections demonstrate reproducibility. Data also shows there are no significant differences from single marker analysis in responder/non-responder (R/NR) cohorts. **B.** Putative signatures indicating enrichment of cell phenotypes and interactions in R/NR cohorts were observed. C. Assessment of cell density ratios confirms staining accuracy of multiple colocalized markers. **D.** Spatial relationships within ICI-sensitive NSCLC responder cores. Visualization of the nearest CD3e+/CD4+ cells (top) to each CD3e+/ CD8+ cells, and CD8+/PD-1+ cells (bottom) to each membrane PD-L1+ cells.

CONCLUSION

A new frontier of biomarker discovery based on spatial biology presents a path toward the use of multiplexed imaging in the clinic, as multiplexing technologies and workflows become more practical, high-throughput, and analytically robust. PhenoCode Signature Panels provide an off-the-shelf, flexible 6-plex option that targets key questions to thoroughly interrogate the TME with minimal user development requirements. The ability to deploy signature panels supported by PhenoCode chemistry to investigate the immune landscape of the TME can be used to accelerate the finding of spatial signatures that may reliably predict response for ICI therapy in clinical trials.