# 4658: A Comprehensive Approach to Immunotherapy Response Prediction: Unlocking Spatial Signatures with Complementary PhenoCode Signature Panels

Jacob Circelli[1], Rachel Schaefer[1], Oscar Perez[1], Linying Liu[1], Michael McLane[1], Yi Zheng[1]
[1]Akoya Biosciences, Marlborough, Massachusetts, USA

## 1. Background

Characterization of the tumor microenvironment (TME) is a fundamental step in identifying distinct immunologic phenotypes in various types of cancer, with the spatial arrangement of cells and

co-expression patterns serving as an increasingly important tool for the identification of highly predictive markers called spatial signatures for immunotherapy response. To study the complex biological processes within the TME and develop clinically useful predictive biomarkers, it is imperative to take an approach that combines relevant content with flexibility, speed, and throughput. We recently introduced PhenoCode™ Signature Panels that offer researchers the ability to stain for multiple biomarkers at single cell resolution on a single tissue in a scalable end-to-end automated workflow. The rapid nature of PhenoCode Signature Panels allows for multiple panels to be used in succession to stratify response and accurately evaluate the TME.

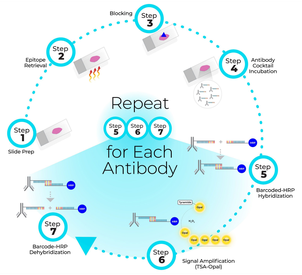
## 2. Methods

A new TSA-based Opal® method (see section 4) was used in this study for multiplexed immunofluorescence (mIF) staining of human formalin-fixed, paraffin embedded (FFPE) lung cancer tissue using 5 PhenoCode Signature panels. All staining was performed on the Leica® BOND® RX autostainer, imaging was performed on the Akoya Biosciences PhenoImager® HT and image analysis was performed using inForm software. H-score and phenotype quantitation were obtained in R via Phenoptr and PhenoptrReports. H-score was used to quantitatively assess signal intensity and percent of stained cells at each intensity level. Optimized mIF protocols were validated against chromogenic (DAB) singleplex protocols on consecutive tissue sections.

## 3. PhenoCode Signature Panels

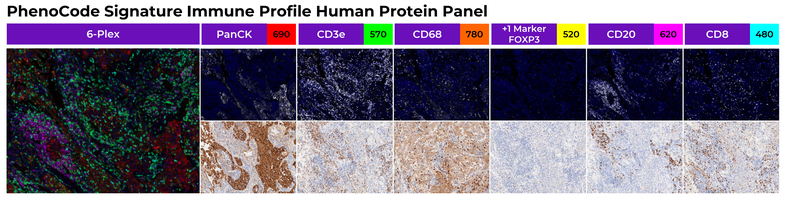

## 4. PhenoCode Signature Chemistry

PhenoCode Signature Panels are powered by a novel barcoded antibody chemistry. Following steps of baking, dewaxing, and epitope retrieval, slides are stained with a primary antibody cocktail, in which antibodies have been conjugated to unique oligo-based barcodes. A single antibody is revealed at a time, beginning with the hybridization of a complementary barcode conjugated to HRP. Signal amplification is then performed using Opal-TSA chemistry. Once complete, the HRP conjugate is dehybridized. The process (steps 5-7) is repeated for each antibody, labeling the markers with different Opal dyes.

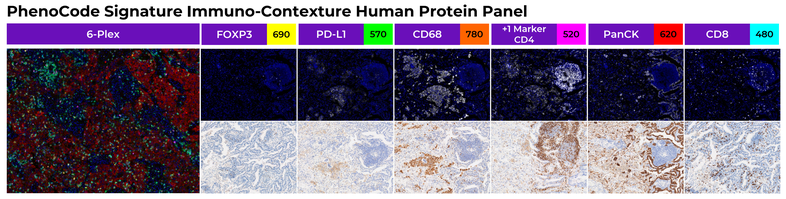



## 5. Validation of PhenoCode Signature Staining vs DAB IHC

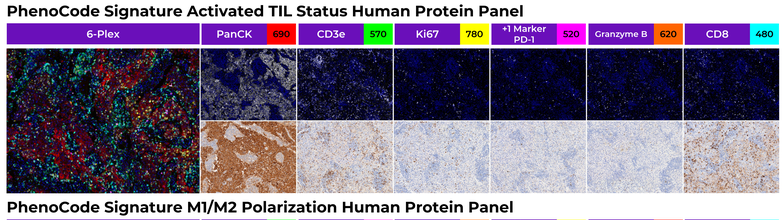
**PhenoCode Signature Immune Profile Human Protein Panel**



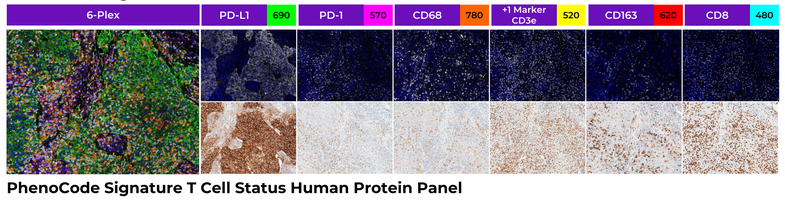

**PhenoCode Signature Immuno-Contexture Human Protein Panel**



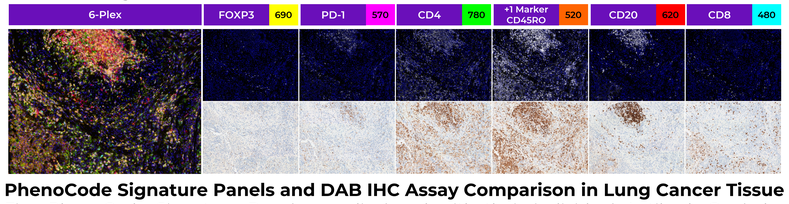

**PhenoCode Signature Activated TIL Status Human Protein Panel**




**PhenoCode Signature M1/M2 Polarization Human Protein Panel**



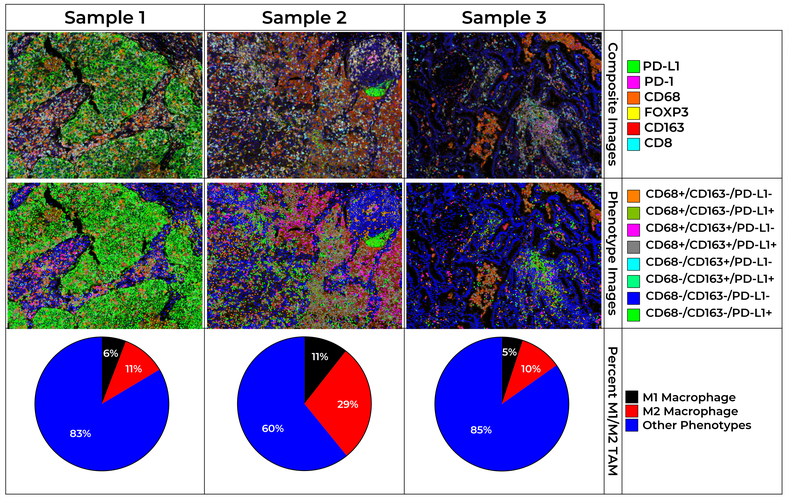

**PhenoCode Signature T Cell Status Human Protein Panel**



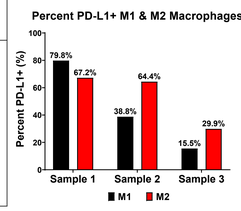

**PhenoCode Signature Panels and DAB IHC Assay Comparison in Lung Cancer Tissue**
Five PhenoCode Signature Panels are displayed with their individual Antibody-Opal dye pairings shown as stained on human lung cancer FFPE samples. An *a la carte* "+1" *antibody is* paired with Opal 520 and added to the flexible base panel to create a 6-plex panel. Each marker includes a DAB comparison.

## Assessment of TAM Polarization and T Cell Status using PhenoCode Signature Panels

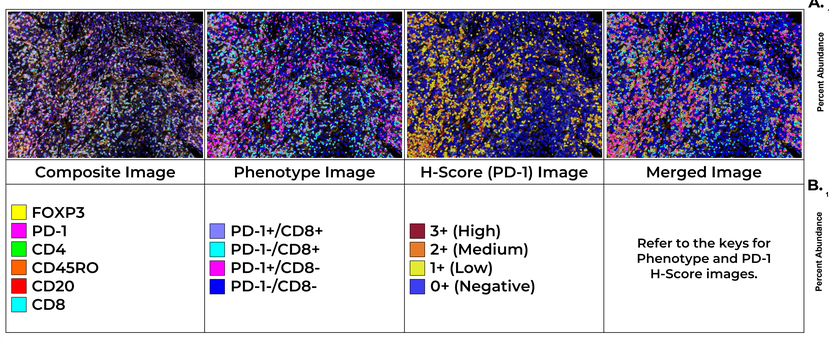
### 6.1 Analysis of TAM Polarization and PD-1/PD-L1 Immune Checkpoint Axis in Non-Small Cell Lung Cancer (NSCLC)

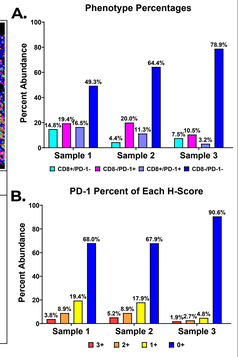



**6.1 PD-L1 Co-Expression Levels in Tumor Associated M1/M2 Macrophages**
The M1/M2 Polarization Panel was run on three human NSCLC FFPE samples. Representative 6-plex images (composite) are displayed along with PD-L1, CD68, and CD163 phenotypes. Pie charts summarize percent M1, M2, and other cell phenotypes. Bar chart (below) represents percent of M1 and M2 macrophages that are PD-L1+.

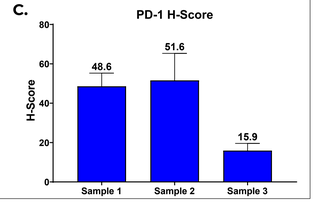



### 6.2 PD-1 Immunostaining of NSCLC Reveals Differences in Cytotoxic T Cell Status




**6.2 Cytotoxic T Cell Effector Function Analysis and H-Scoring**
The T Cell Status Panel was run on three human lung cancer FFPE samples. A representative image of 6-plex staining (composite) is displayed along with the phenotype map (PD-1 and CD8), PD-1 H-Score map, and the merged phenotype and H-Score maps. **A.** Bar chart contains a summary of percent CD8, PD-1, CD8 & PD-1, and other phenotypes for the three lung cancer tissue samples. **B.** Bar chart represents percent of PD-1 high (3+), medium (2+), low (1+), and negative (0+) expressors for the three lung cancer tissue samples. **C.** Bar chart displays the PD-1 H-Score for the three lung cancer tissue samples. H-Score was calculated using five regions of interest on each sample across three technical replicates.




## 7. Conclusion and Outlook

PhenoCode Signature panels, powered by Akoya's novel barcode chemistry, provide an off-the-shelf, flexible 6-plex immunofluorescence staining option that requires minimal optimization. Here, we have shown how these panels can be leveraged to investigate PD-L1 expression in M1 or M2 polarized TAMs and PD-1 H-Scoring and co-expression with CD8 T Cells. The five PhenoCode Signature Panels featured here are designed to be complementary and allow for thorough and rapid interrogation of the TME to gain key biological insights and accelerate the development of spatial signature that can more reliably predict immunotherapy response.



Akoya Biosciences, Inc., 100 Campus Drive, 6th Floor, Marlborough 01752, MA USA (855) 896-8401 www.akoyabio.com