Flexible high-plex biomarker panels enable rapid development of spatial signatures to improve stratification of response to combination therapies

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

SITC 2022: #160

1. Background

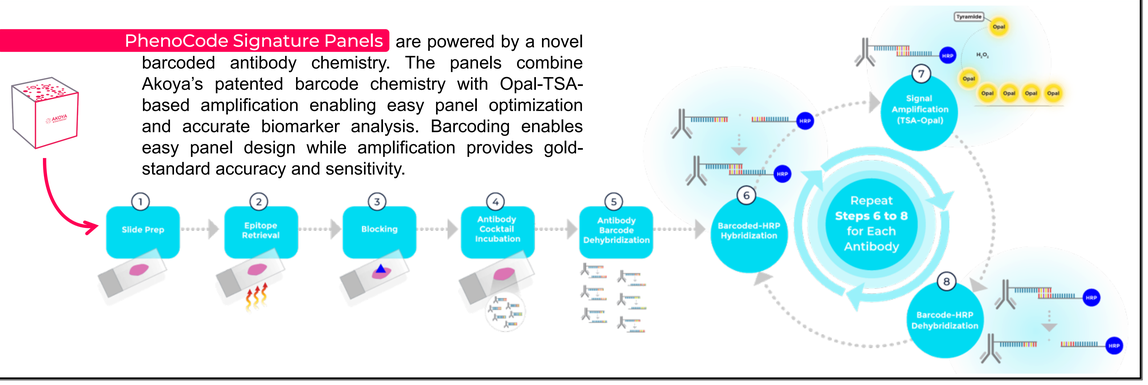
Immune checkpoints are established therapeutic targets for cancer treatment, and it is increasingly thought that combination therapies, which target multiple checkpoints at once, lead to improved patient outcomes. The development of clinically relevant prognostic tools to stratify patients will be critical for the advancement of such treatments. Spatial Phenotyping of the tumor microenvironment (TME) by assessing both, protein co-expression and spatial relationships between cells, appears to improve patient stratification. To provide researchers with an automated end-to-end workflow for the functional evaluation of tumors & stratification of patients, we have thus developed a new single-cell multiplexed staining solution.

2. Design and Development of PhenoCode Signature Panels

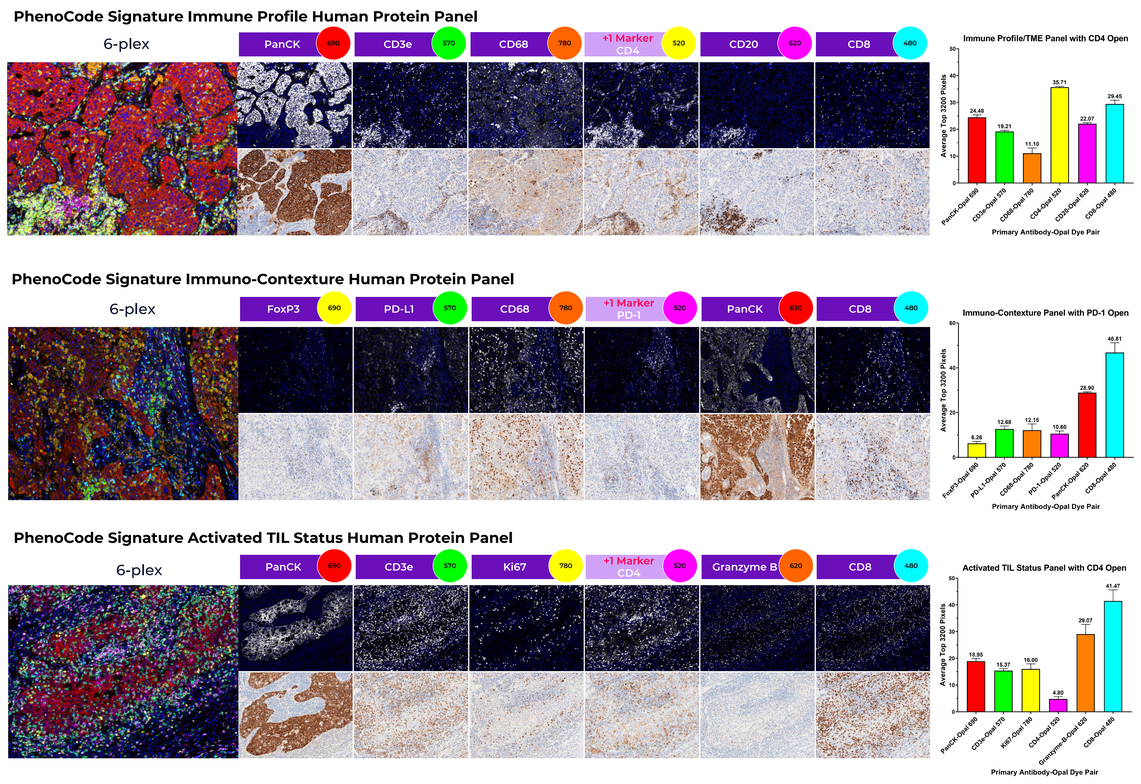
PhenoCode™ Signature Panels (PSP) use Akoya's barcode-based antibody labeling chemistry; this allows cocktailed primary antibodies to be applied in a single incubation step followed by amplified detection using Opal™ fluorescent dye technology. Furthermore, PSPs feature a flexible design component and allow easy integration of a novel checkpoint or immune cell marker into a 5-plex base panel. Ultimately, this results in the detection of up to six biomarkers simultaneously on a single tissue section. Here, we demonstrate the performance of 3 PSPs using human formalin-fixed, paraffin-embedded (FFPE) lung cancer (LuCa) tissues. Staining was performed on the Leica BOND RX™; multiplexed imaging was performed on the PhenoImager® HT platform, and image analysis was performed with inForm® software. Intensity analyses were performed in R using Phenoptr and PhenoptrReports.

3. PhenoCode Signature Chemistry

PhenoCode Signature Panels are powered by a novel barcoded antibody chemistry. The panels combine Akoya's patented barcode chemistry with Opal-TSA-based amplification enabling easy panel optimization and accurate biomarker analysis. Barcoding enables easy panel design while amplification provides gold-standard accuracy and sensitivity.

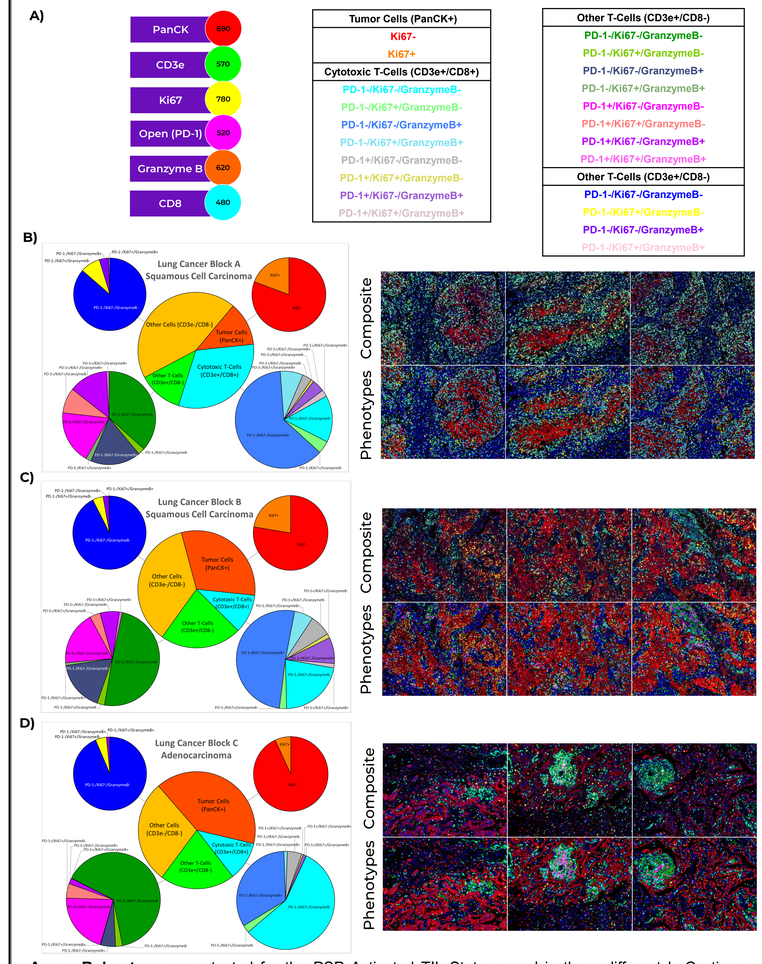


4. Validation of PhenoCode Signature Chemistry vs DAB IHC



Comparison between PhenoCode Signature Panels and DAB IHC in LuCa Tissue
Imaging data from three PhenoCode Signature Panels are shown with their antibody-Opal pairing and order of Opal deposition. An a la carte antibody was run as an open marker with Opal 520 for each panel. The open channel provides the flexibility to add any biomarker of choice using an antibody conjugation kit for PhenoCode Signature. The six multiplexed biomarkers for each panel are to their DAB counterparts (pathologist reviewed). For average pixel intensity, the average top 3200 Pixels of each marker was determined from 5 ROIs for each LuCa tissue.

5. Robustness of PhenoCode Signature Assay Performance



Assay Robustness was tested for the PSP Activated TIL Status panel in three different LuCa tissue blocks. A. Phenotyped PSP assay data yielded 22 cell types/activation states (same colors used in B-D). B-D. Each row shows, on its left, the InForm phenotype quantification for each experiment; to the right, we show a representative imaging result with phenotypes overlaid in the bottom image. In each row, the center piechart summarizes identified cell phenotypes, while subpopulations/activation states are shown in the 4 smaller piecharts. **Phenotyping was representative and robust across all three tissue blocks**. This result confirms assay robustness of our PSP Activated TIL Status Panel.

6. Conclusions and Outlook

In this study, we demonstrate the performance of three 6-plex **PhenoCode Signature Panels** for easy and reproducible profiling of the TME. Powered by Akoya's novel barcoded antibody chemistry combined with Opal-TSA-based signal amplification, these panels enable shortened assay development time and gold-standard sensitivity and accuracy. Furthermore, the complementary design aspect of these panels enable systematic interrogation of the TME. Additionally, the inherent flexibility provides easy integration of novel checkpoint markers enabling researchers to adapt with the dynamic combination therapy landscape.

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