149 COMPLEMENTARY PHENOCODE SIGNATURE PANELS COMPREHENSIVELY MAP CELL INTERACTIONS AND IDENTIFY SPATIAL PHENOTYPIC SIGNATURES IN THE TUMOR MICROENVIRONMENT

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Background 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 tumor microenvironment (TME). An emerging new biomarker class in the TME are Spatial Phenotypic Signatures (SPS), defined by the measurement of the interactions between, and cell densities of, tumor and immune cells. Akoya's PhenoCode™ Signature Panels have been designed to enable comprehensive mapping of the TME and to help identify SPS. Three PhenoCode Signature Panels were used to investigate SPS for immune checkpoint inhibitor (ICI) therapy in non-small cell lung cancer (NSCLC) patients where ICI have shown durable benefit.

Methods PhenoCode Signature Panels provide off-the-shelf multiplexed imaging customization (6-plex Panel via 5-plex core plus 1 open channel configuration) with minimal user development requirements. PhenoCode uses barcode-based antibody chemistry from Akoya's PhenoCycler® platform integrated with the signal amplification capabilities of Opal chemistry from Akoya's PhenoImager® platform. A tissue microarray (TMA) comprising 41 formalin-fixed paraffin-embedded (FFPE) pre-treatment samples from second-line PD-L1/PD-1 ICI-treated NSCLC cohorts (Durvalumab, Nivolumab, or Pembrolizumab, 16 responders and 25 non-responders) was screened with three PhenoCode Signature Panels. The PhenoCode Signature Panels were: Immuno-contexture Human Protein Panel (CD8/CD68/PDL1/FoxP3/CK core + CD20 or PD1 in the open channel) and Immune Profile Human Protein Panel (CD3/CD8/CD20/CD68/CK core + CD4 in the open channel). FFPE TMAs were stained on a Leica Bond RX™. Slides were imaged on a Phenoimager HT multispectral imaging system and analysis algorithms were developed using inForm®, with cell counts, densities, and spatial parameters calculated using PhenoptrReports.

Results Meta-analysis[1] on anti-PD-1/PD-L1 therapy data pooled from 50+ studies (10+ tumor types and 8,000+ patients) examined the predictive value of single-marker immunohistochemistry (PDL1), tumor mutation burden (TMB), gene expression profiling (GEP) and multiplexed imaging; findings revealed that spatial approaches performed significantly better compared to the other assay types. Multispectral spatial analysis of FFPE TMA samples using PhenoCode Signature Panels uncovered putative SPS within ICI-sensitive NSCLC, indicating enrichment and significance of cell frequencies and cell interactions in responder/non-responder cohorts.

Conclusions 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. The ability to deploy Signature Panels supported by PhenoCode chemistry to investigate the immune landscape of the TME will accelerate the finding of SPS that may reliably predict response for ICI therapy.

REFERENCE

1. Lu S, Stein JE, Rimm DL, *et al*. Comparison of Biomarker Modalities for Predicting Response to PD-1/PD-L1 Checkpoint Blockade: A Systematic Review and Meta-analysis. *JAMA Oncol*. 2019; **5**(8):1195–1204. https://jamanetwork.com/journals/jamaoncology/fullarticle/2738418

http://dx.doi.org/10.1136/jitc-2022-SITC2022.0149