NOVEL, HIGH-PLEX, AND FLEXIBLE BIOMARKER PANELS FOR RAPID DEVELOPMENT OF SPATIAL SIGNATURES TO IMPROVE STRATIFICATION OF RESPONSE TO COMBINATION THERAPIES

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Background Pre-clinical and clinical studies into new checkpoints as therapeutic targets have raised the possibility that combining immune checkpoint inhibitors or other immunotherapies may be an effective strategy in improving both the response and survival rates. The development of clinically useful biomarkers to select responders for combination therapies will be critical for the advancement of such treatments. Profiling the tumor microenvironment (TME) by assessing spatial relationships and protein co-expression within specific cellular subsets could lead to improved patient stratification of response to these combination therapies. To provide researchers with an end-to-end, automated workflow for the functional evaluation of tumors in the context of patient immunity, we have developed a new single-cell multiplexed staining method.

Methods PhenoCode Signature panels utilize barcode-based antibody labeling chemistry, allowing primary antibodies to be applied as a cocktail in a single incubation step followed by amplified detection using Opal fluorescent dye technology. These panels feature a flexible design component allowing for the easy integration of a novel checkpoint or immune cell marker into a 5-plex panel, resulting in the detection of up to six biomarkers simultaneously on a single tissue section. Human formalin-fixed, paraffin-embedded (FFPE) lung cancer tissues were stained using the PhenoCode Signature panels and DAB. Staining was performed on the Leica BOND RX™ automated stainer. Multispectral imaging was performed on the PhenoImager™ platform, and image analysis was performed with a phenotyping algorithm in inForm™ software. Intensity analysis was performed in R using Phenoptr and PhenoptrReports.

Results Three PhenoCode Signature 6-plex panels were developed and applied to lung cancer samples to help characterize distinct immune landscapes. We demonstrate a fully automated, yet robust and flexible workflow for assessing spatial relationships and profiling the tumor microenvironment. The staining of each marker within the panels qualitatively matches chromogenic DAB staining. High assay reproducibility was demonstrated by both qualitative and quantitative analysis of staining quality. Single markers were substituted from each panel to show the flexibility of the panel design.

Conclusions In this study, we demonstrate the utility of three 6-plex PhenoCode Signature panels for easy and reproducible profiling of the TME. The flexible design component of the panels allows for the substitution of a single biomarker enabling the rapid assessment of additional cell phenotypes. The flexible configuration combined with shortened assay development time are designed to enable researchers to rapidly develop more predictive spatial phenotypic signatures, aiding in the development of more targeted combination immunotherapies.