Background  Non-small cell lung cancer (NSCLC) patients can have impaired immune responses within the tumor microenvironment (TME), leading to a progression of tumor growth and poorer prognosis. Accurate cell phenotyping combined with spatial profiling of the immune contexture and checkpoint expression, can provide a deeper understanding of complex cellular interactions underpinning the tumor-immune response. The aim of this study was to utilize spatial multiplexed imaging technology and associated data analysis methods to identify populations of immune cells, their functional status, as well as their interactions with the tumor in a set of NSCLC tissue cores from patients treated with first line standard-of-care chemotherapy, and second line immuno-oncology treatment.

Methods  Formalin-fixed paraffin-embedded (FFPE) NSCLC tissue microarrays (TMA), comprised of n=41 cores containing a range of carcinomas and pathological Tumor-Node-Metastasis (pTNM) stages, were stained on a Leica Bond RX™ using the Akoya PhenoCode™ Signature Immuno-contexture Human Protein Panel, which includes markers for CD8, CD68, PD-1, PD-L1, FoxP3, and PanCK as a tumor indicator. Stained TMAs were scanned at 20x magnification on a PhenolImager HT multispectral imaging system. Image analysis was performed using Visiopharm software deep learning algorithms for multiplexed images to segment specific tissue regions of interest (ROI) and to perform accurate detection and classification of different cell phenotypes. Cell object data files per core were exported for spatial and heterogeneity analysis using proprietary python scripts.

Results  Immune cell counts, phenotypes and spatial interactions were identified within the tumor and stroma ROI per core. Data included total and negative cell phenotype counts, cell density in tumor and stroma, as well as cell distance and clustering spatial interactions in each of the 41 cores in the TMA set. Specific immune cell subsets of interest quantified included CD8+/PD-1+, CD8+/FoxP3+/PD-1+ and CD68+/PD-L1+, FoxP3+, and FoxP3+/PD-1+. Tumor cells of interest included PanCK+ and PanCK+/PD-L1 +.

Conclusions  The combination of high quality, spatial multiplex imaging data provided by the PhenoCode Panel, coupled with deep learning quantitative image analysis techniques, enables detailed characterization of the complex cellular interactions, at both the functional and spatial level, within the TME of IO-treated NSCLC tissue. This study further highlights the importance of a robust technical workflow to deliver optimal staining, imaging, cell detection and phenotyping in generating high quality data to facilitate spatial profiling and interpretation in pathologically complex tissue samples.

Ethics Approval  Commercially sourced TMA from Tr-Star Technology Group