Multiplex-immunofluorescence-based spatial characterization of the tumor-microenvironment of a large biologic clinical non-small cell lung cancer cohort

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Background
Non-small-cell lung cancer (NSCLC) is the leading cause for cancer death with an estimated 1.4 million deaths worldwide. While modern targeted and immunotherapies trigger response in a fraction of cases, causes for non-response and tumor relapse as well as the complexity of the immune reaction are not sufficiently well understood. It is hypothesized that the tumor microenvironment (TME) and its immune cell spatial composition are a key to alleviate that. Arguably, a sufficiently large study cohort, a high-plex (immune) cell characterization and an accordingly scalable analysis is required to advance the discovery of complex patterns. In this study, we tackle this by means of a large NSCLC cohort, a multiplex panel consisting of hematoxylin and eosiin (H&E) and 11 immunofluorescence markers (mIF), and a scalable AI-based image analysis approach, which combines histopathological and multiplex immunofluorescence (mIF) data to characterize the TME for detection of prognostic and predictive biomarkers.

Materials and methods
We gathered formalin-fixed paraffin-embedded tissue and clinicopathological data from 1288 patients with resected stage I-IV NSCLC from two large German hospitals (Charité Berlin and University Hospital Cologne). Four 1.5 mm tissue cores were punched in formalin-fixed tissue and were correlated with clinical data. All stains were scanned and co-registered at single tissue microarray. Sections were stained with a 12-plex mIF panel from high-purity tumor regions of each case for constructing a multiplex-immunofluorescence-based spatial analysis

Table 1: Pixel-wise evaluation of the tissue segmentation model performance.

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<th>Model</th>
<th>Carcinoma</th>
<th>Stromal</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
<th>F1</th>
<th>Segmentation</th>
<th>Precision</th>
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Results
The tissue segmentation model achieved a macro averaged F1-score of 92% and the cell classification models predicted positivity for 12 markers with an F1-score of at least 95%, both on hold-out data.

Figure 2: Tissue segmentation model

Figure 3: Identification of spatially resolved immune cell signatures within the tumor stroma. (A) Clustering analysis of signatures showed six separate population subgroups (B), which revealed significantly different overall survival times in Kaplan-Meier curve (C).

Conclusion
In our study we demonstrate that the combination of a large cohort, high-plex panel, and automated AI-based analysis have the potential to discover complex, predictive TME signatures. Initial results show that data-driven and spatially resolved immune cell signatures can be found that significantly separate population subgroups with respect to overall survival.

* Both authors contributed equally to this work.