Lung cancers are the leading cause of cancer-related deaths with a 5-year survival of only ~20%. Whilst immunotherapies have led to durable and prolonged survival, only a subset of patients remain responsive. Additional biomarkers are thus needed to better predict if patients will respond or develop resistance against immune checkpoint inhibitor (ICI) therapies. Spatial phenotyping of the tumor microenvironment (TME) is now recognized as a predictive proxy for ICI therapy outcomes. We phenotyped pretreatment biopsies from non-small-cell lung cancer (NSCLC) patients enrolled in a single-agent Nivolumab clinical trial. We first performed 57-plex whole-slide Single Cell Spatial Phenotyping on the Phenocycler®-Fusion platform. Our analyses revealed high phenotypic diversity in the TME of responding and non-responding patients. We then deployed customisable PhenoCode™ Signature Panels (PS) for high-throughput immune profile (IP) characterization (CD3/CD16/17/19; CD3/CD2/CD68/PanCK + CD4 add-in) and immunity-contexture (IC: CD68/CD3/CD2/CD4/PD-1/ FoxP3/PanCK + PD1 add-in) analyses on the Phenocycler® HT. The PS panels were deployed on 37 biopsies and afforded comprehensive evaluation of patient cohorts. Our PS data revealed no difference in the immune cell makeup in responders vs. non-responding TMEs. However, we discovered multiple quantifiable and statistically significant Spatial Signatures that appear to be predictive of a positive treatment outcome.

**3. Ultrahigh-plex & High-throughput Single-Cell Spatial Phenotyping Analyses of NSCLC Cohorts**

57-plex Spatial Phenotyping identified 11 Immune Cell Populations in Responder vs. Non-Responder


NSCLC Biopsies Contain 7 Distinct Cellular Neighborhoods

Cellular Neighborhood (CN) Analyses of NSCLC tissues analyzed with the PS-PIC panel. Images on top show a representative pretreatment biopsy and its CN representation (right panel). The heatmap contemplates 7 CNs. Circos plots show dynamic nearest-neighbor interactions between CNE across cohorts. Notably, less immune-infiltrating monocytes (TN) and tumor cells show how less direct interactions in non-responding patients.

**5. A Predictive Spatial Signature for NSCLC Treatment Outcomes**

The SpatialScore is the ratio of the physical distance between CD8+ T-cells and the nearest tumor cell, validated in a multi-center (M2) clinical study. Both, Treg infiltration and immune suppressive effects on CD8+ T-cells, which then results in a higher SpatialScore. Indeed, the postedsuppressives from pretreatment biopsies were significantly higher in the Non-Responder (NR) condition when compared to the responders (R). A high spatial score can thus be interpreted as: higher CD8+ T-cell suppression – lower tumor activity – lower survival rate. See Kaiser-Kerzen’s line in illustrations.

**6. Conclusions and Outlook**

- The study authors report a unique comprehensive Single Cell Spatial Phenotyping analysis of pretreatment NSCLC biopsies from a single-agent Nivolumab clinical trial.
- Our data illustrate the diverse immune microenvironment of NSCLC and indicate that immune cell quantification is insufficient to stratify patient cohorts. The identification of unique spatial signature such as a spatial score may be developed to predict patient response.
- Single-cell Spatial Phenotyping with Akoya’s unified Phenocycler Fusion and Phenocycler solution along with PhenoCode Discovery and Signature panels enables the discovery of multiple quantifiable and statistically significant Spatial Signatures in Responders vs. Non-Responders.
- This study shows how Akoya’s solutions are uniquely positioned to enable discovery to translational workflows thereby accelerating the development of clinical candidates and highly predictive spatial signatures.

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**References:**