6768: The Potential Predictive Role of Spatial Phenotyping in Non-small Cell Lung Cancer (NSCLC)

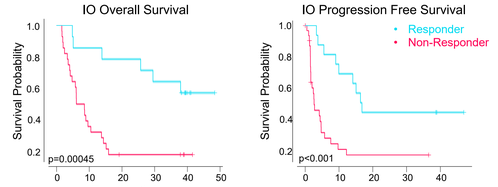
Ning Ma[1], Aditya Pratapa[1], James Monkman[2], Ken O'Byrne[3], Oliver Braubach[1], Arutha Kulasinghe[2]

[1]Akoya Biosciences, Inc, Marlborough, Massachusetts, USA; [2]University of Queensland, Brisbane, QLD, AUS; [3]Princess Alexandra Hospital, Brisbane, QLD, AUS

1. Introduction

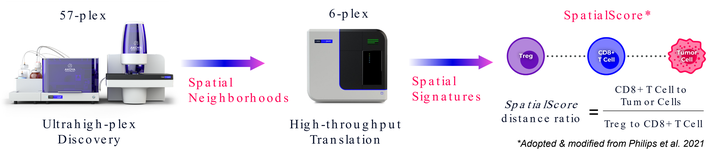
Lung cancers are the leading cause of cancer-related deaths with a 5-year survival of only ~20%. Whilst immunotherapies have led to durable and prolonged survival, only a subset of patients remains responsive. Additional biomarkers are thus needed to better predict if patients will respond or develop 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 customizable **PhenoCode™ Signature Panels** (**PSP**) for high-throughput immune profile (IP: CD3ε/CD8/CD20/CD68/PanCK + CD4 add-in) and immuno-contexture (IC: CD8/CD68/PD-L1/FoxP3/PanCK + PD-1 add-in) analyses on the PhenoImager® HT. The PSP panels were deployed on 37 biopsies and afforded comprehensive evaluation of patient cohorts. Our PSP data revealed no difference in the immune cell makeup in responder vs. non-responder TMEs. However, we discovered multiple quantifiable and statistically significant **Spatial Signatures** that appear to be predictive of a positive treatment outcome.

Patient cohort	(n=41)
CR	2
PR	12
PD	13
SD	14
Sub-cohort (current study)	**(n=21)**
CR	2
PR	7
PD	7
SD	5



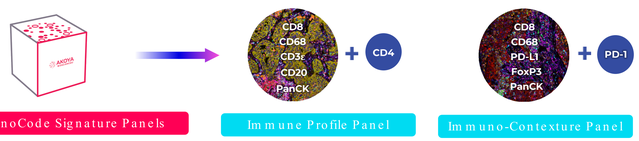
Kaplan-Meier curves of patient cohorts: CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease. **Non-responders (NR) are pooled PD & SD; Responders (R) are pooled CR & PR.**

2. Enabling IO Discovery to Translation with Akoya's Unified Solutions



*Adopted & modified from Philips et al. 2021

Discovery to Translational Workflow: NSCLC pretreatment core biopsies (FFPE) were phenotyped using a 57-plex antibody panel on **PhenoCycler®-Fusion** using commercially available **PhenoCode™ Discovery Panel** encompassing markers for cell lineage, activation states, immune checkpoints and tissue structure along with a custom module comprising key markers of cellular metabolism. Serial sections were then stained with **PhenoCode Signature Panels**: Immune Profile Panel + CD4 in the open channel (PSP-IP) and Immuno-Contexture Panel + PD-1 in the open channel (PSP-IC); these samples were imaged on the **PhenoImager HT** with preoptimized acquisition parameters and analyzed with inForm®. Spatial analyses, including the **SpatialScore** *vs.* ICI responses, were adopted & modified from *Philips et al. 2021*[1] and implemented in a custom analysis pipeline.

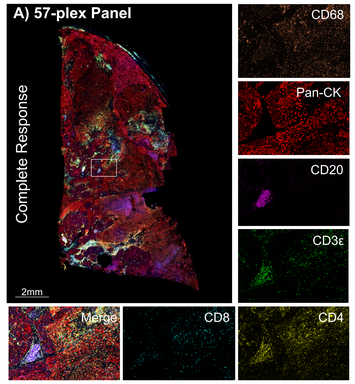


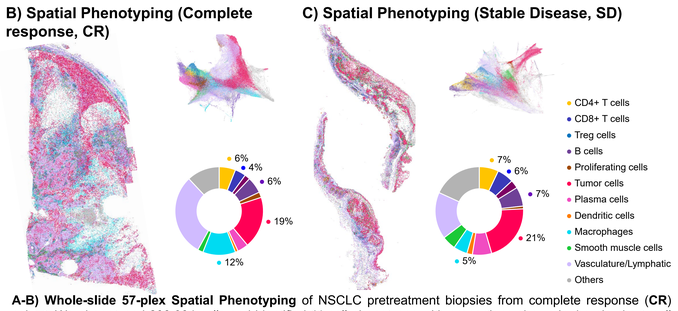
PhenoCode Signature Panels | Immune Profile Panel | Immuno-Contexture Panel

PhenoCode Signature Panels use Akoya's barcode-based antibody labeling chemistry and are validated for the PhenoImager® HT workflow. Featuring a flexible design component that allows for the easy integration of a novel checkpoint or immune cell marker, these panels offer 3-fold faster assay development and optimization times when compared to other custom 6-plex panels.

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

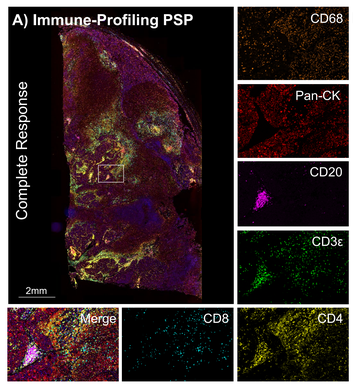
57-plex Spatial Phenotyping Identified 11 Immune Cell Populations in Responder vs. Non-responder

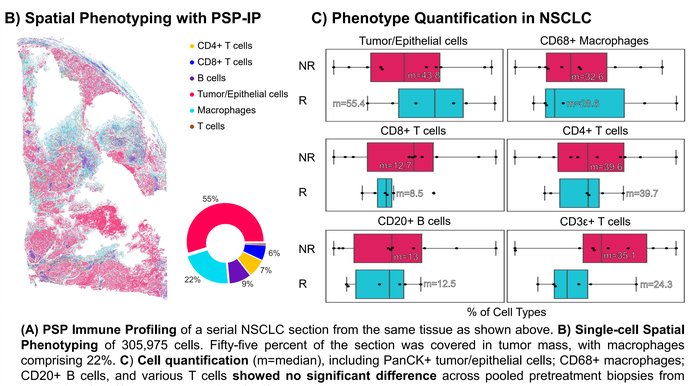




A-B) Whole-slide 57-plex Spatial Phenotyping of NSCLC pretreatment biopsies from complete response (**CR**) cohort. We phenotyped 298,084 cells and identified 11 cell phenotypes with proportions shown in the pie chart; cell type percentages are listed only for the phenotypes that we also identified with PSP panels below. **C)** Pretreatment biopsy from the stable disease (**SD**) group; 254,272 cells & 11 phenotypes. Differences in the immune cell makeup are evident but hard to establish statistically, due to low throughput. We thus deployed high-throughput PSP panels to analyze more samples from responders vs. non-responders.

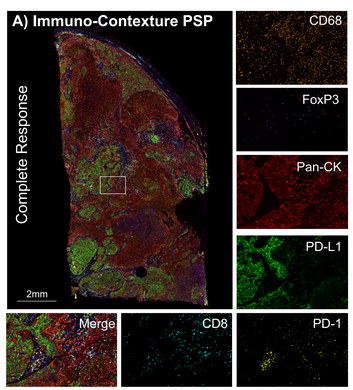
6-plex Spatial Phenotyping Validates Diverse NSCLC Immune Landscape but Rules out Differences in Immune Composition



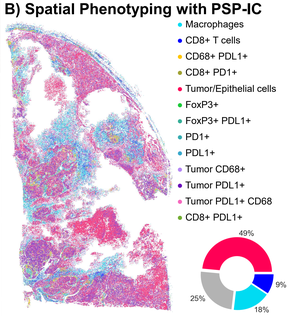


(A) PSP Immune Profiling of a serial NSCLC section from the same tissue as shown above. **B) Single-cell Spatial Phenotyping** of 305,975 cells. Fifty-five percent of the section was covered in tumor mass, with macrophages comprising 22%. **C) Cell quantification** (m=median), including PanCK+ tumor/epithelial cells; CD68+ macrophages; CD20+ B cells, and various T cells **showed no significant difference** across pooled pretreatment biopsies from responders (n=10) vs. non-responders (n=6).

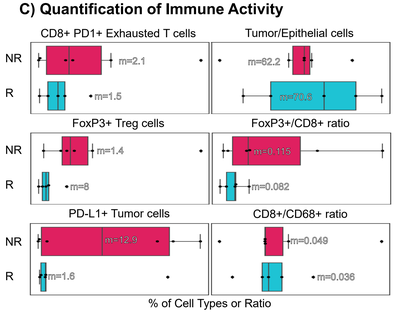
A) Immuno-Contexture PSP



B) Spatial Phenotyping with PSP-IC



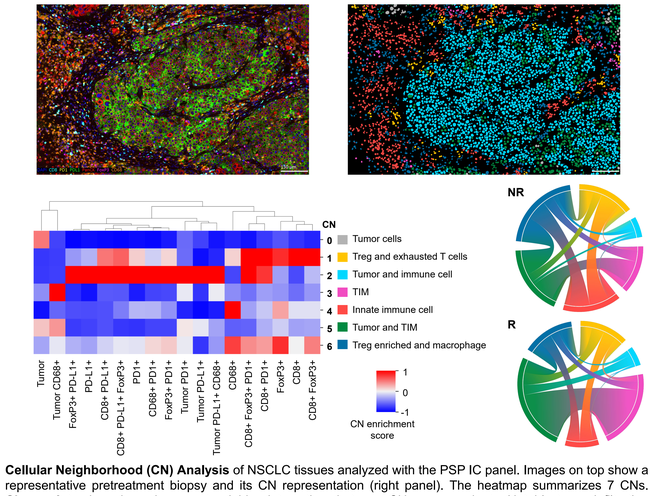
C) Quantification of Immune Activity



(A) PSP Immuno-contexture analysis of serial NSCLC section as above. **B) Single-cell Spatial Phenotyping** of 320,656 cells. Cell quantities were similar as in PSP Immune Profiling above. **C) Quantification** of CD8+PD1+ exhausted T cells; FoxP3 regulatory Treg cells; PanCK+PD-L1+ tumor cells (PD-L1 test), FoxP3+/CD8+ ratio and CD8/CD68+ ratio **showed no significant difference** between responders (n=6) vs. non-responders (n=5).

4. Cell Neighborhood Analyses Indicate Distinct Spatial Biology of Responder vs. Non-Responder TME

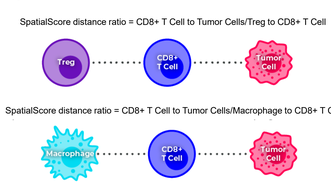
NSCLC Biopsies Contain 7 Distinct Cellular Neighborhoods



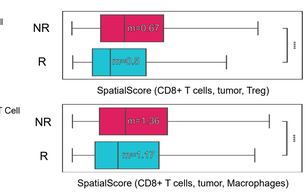
Cellular Neighborhood (CN) Analysis of NSCLC tissues analyzed with the PSP IC panel. Images on top show a representative pretreatment biopsy and its CN representation (right panel). The heatmap summarizes 7 CNs. **Circos plots** show dynamic nearest-neighbor interactions between CNs across cohorts. Notably, tumor-infiltrating macrophages (TIM) and tumor cells show less direct interactions in non-response groups.

5. A Predictive Spatial Signature for NSCLC Treatment Outcomes

SpatialScore



SpatialScore predicts ICI response



The **SpatialScore** is the ratio of the physical distance between CD8+ T-cells and the nearest tumor cell, relative to its nearest Treg (top) or Macrophage (bottom). Both, Treg and Macrophages can exert immuno-suppressive effects on CD8+ T-cells, which then results in a higher SpatialScore. Indeed, the pooled SpatialScore from pretreatment biopsies were significantly higher in the Non-responder (NR) condition when compared to the responder (R) condition. A high spatial score can thus be interpreted as: higher CD8+ T-cell suppression - lower anti-tumor activity - lower survival rate (see Kaplan Meier curve in Introduction).

6. Conclusions and Outlook

- This study amounts to a uniquely 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 PhenoImager solution along with PhenoCode Discovery and Signature panels enables the discovery of multiple quantifiable and statistically significant Spatial Signatures in Responder vs. Non-responder cohorts.
- **This study shows how Akoya's solutions are uniquely positioned to enable discovery to translational workflows thereby accelerating the development of clinically relevant and highly predictive spatial signatures.**

[1]Philips et al. (2021): Immune cell topography predicts response to PD-1 blockade in cutaneous T cell lymphoma. *Nature Comm.*