6764: Multi-Parametric Comparison of Ultrahigh-Plex Spatial Phenotyping of Proteins in FFPE Head and Neck Tumor Biopsies

Aditya Pratapa[1], Shawn M. Jensen[2], Niyati Jhaveri[1], Christopher Paustian[2], Quyen C. Vu[2], Tarsem Moudgil[2], Yoshinobu Koguchi[2], Venkatesh Rajamanickam[2], Brady Bernard[2], Tanisha Christie[2], Brian Piening[2], Rom S. Leidner[2], Oliver Braubach[1], Bernard A. Fox[2]

[1]Akoya Biosciences, Marlborough, MA; [2]Providence Cancer Institute of Oregon, Earle A. Chiles Research Institute, Portland, OR

EARLE A. CHILES RESEARCH INSTITUTE
A division of Providence Cancer Institute

AKOYA BIOSCIENCES®
THE SPATIAL BIOLOGY COMPANY®

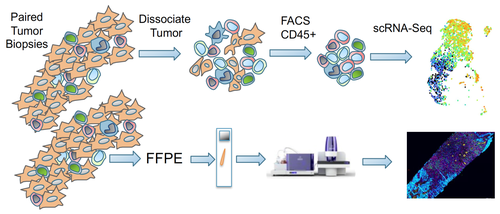
1. Study Design

Characterizing pre-treatment and on treatment changes to the tumor microenvironment (TME) holds great promise for developing strategies to improve patient outcomes. Single cell RNA-seq (scRNA-seq) performed on cell suspensions isolated from tumor biopsies provides substantial insights into the transcriptional state of the cells in the TME.

Complementing this work with multi-omic Cellular Indexing of Transcriptomes and Epitopes (CITE-seq), which adds barcoded antibodies to label the surface of isolated cells, allows us to additionally characterize surface proteins.

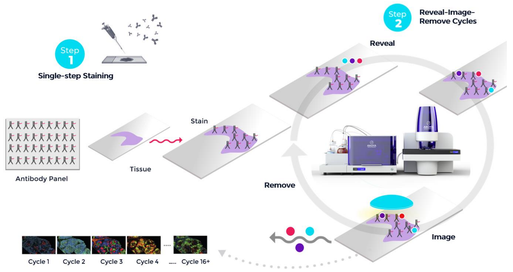
While powerful, these studies fail to provide insights into cell-cell relationships in the TME.

Here we sought to compare scRNA-seq on single-cell suspensions isolated from tumor biopsies and sorted for CD45+ cells against paired biopsies that were formalin-fixed/paraffin embedded (FFPE) and analyzed using ultrahigh-plex spatial phenotyping. While preliminary, these data encourage the performance of additional studies.



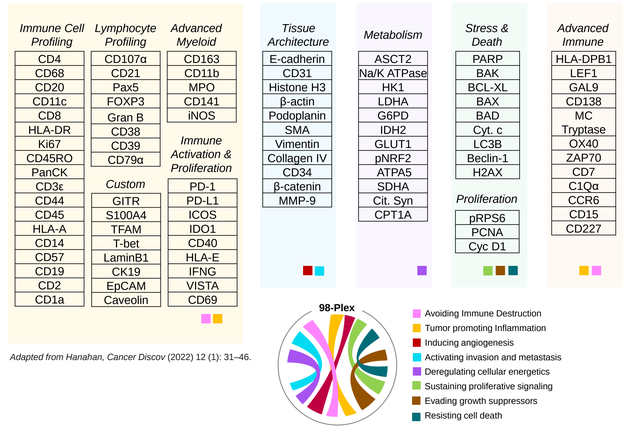
2. Rapid and Deep Spatial Phenotyping

Single-cell spatial phenotyping has transformed cancer research and is poised to play formative roles in the development of effective therapeutic strategies. Here, we present ultrahigh-plex single-cell spatial phenotyping of whole-slide FFPE head and neck cancer tissues with nearly 100 protein biomarkers encompassing *immune cell lineage*, *activation states*, *immune checkpoints*, *tissue structure*, *apoptosis*, *DNA damage & metabolism*.



Deployment of 98-plex deep Spatial Phenotyping panel occurs within 48hrs on the PhenoCycler®-Fusion. The PhenoCycler (formerly CODEX®) employs cocktails of DNA-tagged antibodies to stain tissue, which is then followed by iterative steps of hybridization with complementary, fluorescently labeled probes for imaging.

3. Design and Development of Ultrahigh-Plex Protein Panel

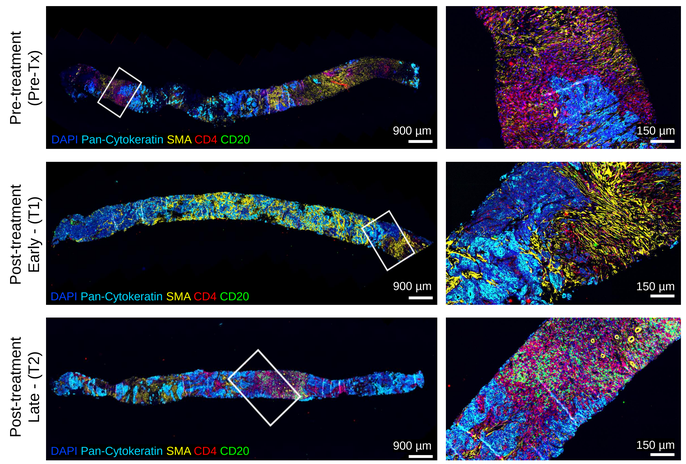


Adapted from Hanahan, Cancer Discov (2022) 12 (1): 31–46.

- Avoiding Immune Destruction
- Tumor promoting Inflammation
- Inducing angiogenesis
- Activating invasion and metastasis
- Deregulating cellular energetics
- Sustaining proliferative signaling
- Evading growth suppressors
- Resisting cell death

3.1 The 98-plex antibody panel is based on the **hallmarks of cancer**. The panel includes markers for cell lineage, immune activation and checkpoints, cellular energetics, mediators of proliferation, metastasis and stress responses, and more. Each marker and each module has been carefully selected to reveal unique information on different pathways and, when multiplexed together, provides an integrated overview of the landscape of cancer progression

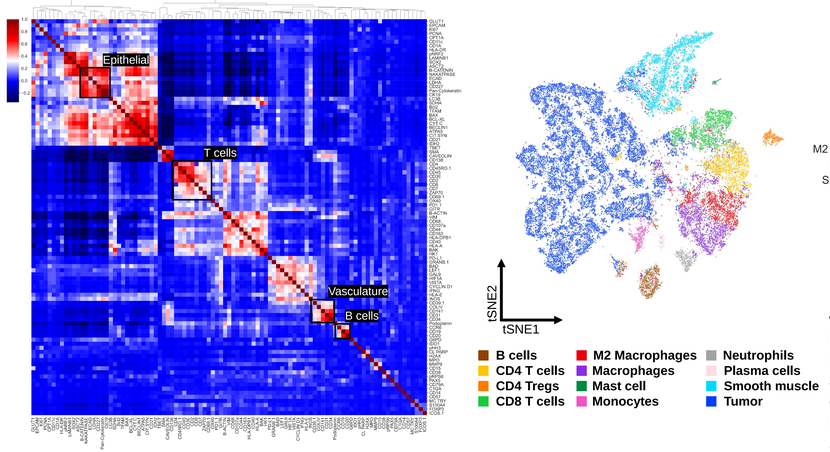
3.2 Whole-Slide Imaging of HNC Tumor Biopsies



3.2 Whole-Slide multiplex imaging at single-cell resolution of three different HNC tumor biopsies (left) demonstrating the heterogeneity of the spatial landscapes observed across biopsies and timepoints (biomarkers as indicated). We observed an increased immune infiltration in the late timepoint (T2, bottom row) relative to pre-treatment (Pre-Tx, top row) and early timepoint post-treatment (T1, middle row). A closer view (right) of the tissue in the areas highlighted in white box.

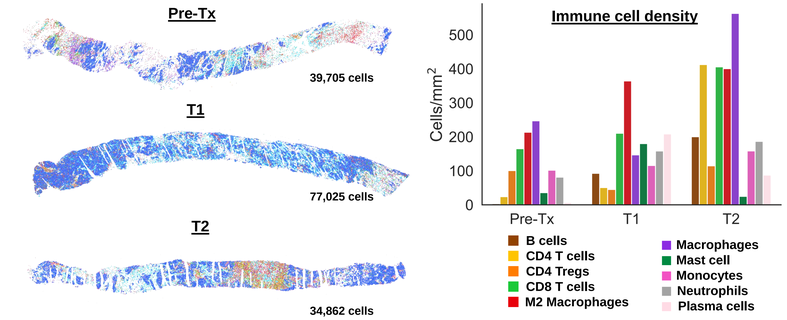
4. Ultrahigh-Plex Single-Cell Spatial Phenotyping of Proteins in Head and Neck Tumor Biopsies

4.1 Single-Cell Spatial Phenotyping Using PhenoCycler-Fusion Reveals 12 Major Cell Phenotypes



4.1 Cross-correlation analysis (left) of 98 markers reveals cell lineages and functional niches with varying metabolic, immune, and stress signatures. **Cellular phenotypes** depicted using a t-SNE plot (middle) and the heatmap (right) corresponding to the average expression of cell-type defining markers and the major cell-types identified.

4.2 Comparison of Cell-Type Distribution Across Different Timepoints

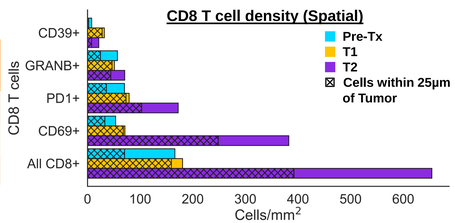


4.2 Immune cell phenotypes identified at different treatment timepoints. Spatial phenotyping diagrams (left) of 151,592 cells across three samples are shown on the left. The bar plot (right) shows the cell type densities for each time point. We observed a general increase in immune cell populations (in cells/mm^2) in both early (T1) and late (T2) timepoints post-treatment.

4.3 Comparison of CD8 T Cell Subtypes From CITE-Seq

CD8 T cells in T2 (CITE-seq)

	CD39+ (ADT)	*GRNB+* (gene)	*PD1+* (gene)	CD69+ (ADT)
CD4 T cells	62.1%	6.8%	7.9%	76.6%
CD8 T cells	**70.8%**	**44.5%**	**25.4%**	**74.1%**
CD4 Tregs	98.9%	2.3%	4.2%	52.5%



4.3 Comparison to CITE-seq reveals CD8 T cell subtypes that are involved in response to the treatment. The table (left) shows the percentage of cells in CITE-seq data for three immune cell subtypes that are positive for ICOS, GRNB, PD1, and CD69. The bar plot (right) shows the abundance of various CD8 T cell subtypes in PhenoCycler-Fusion data. The shaded region in each bar shows the proportion of cells that are within 25µm of a tumor cell. Preliminary evaluation of the data shows T cells with CD69 and PD1 expression have a high correlation with CITE-seq data.

5. Correlating Multiple Analytical Approaches for a Uniquely Comprehensive TME Analysis

- Cell phenotyping data from the PhenoCycler-Fusion compares favorably to CITE-seq data obtained from paired biopsies, thus opening the door for comprehensive analysis of deep spatial and multi-omic phenotypic analyses of human tumor biopsies.
- This study represents a novel approach to integrate high dimensional data from bulk tissue and single-cell spatial technologies to provide uniquely comprehensive insights into tumor biology and, in turn, to develop more informed strategies for immunotherapy development efforts.

Akoya Biosciences, Inc., 100 Campus Drive, 6th Floor, Marlborough 01752, MA USA (855) 896-8401 www.akoyabio.com