5626: Multomic Spatial Profiling of the Tumor Immune Microenvironment

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1. Introduction
Effective strategies for cancer immunotherapies will require a deep understanding of the factors that shape both the tumor microenvironment (TME) and the immunological components within these TMEs, known as the tumor immune microenvironment (TIME). Here, we describe a spatial multomics approach that utilizes RNAscope™ ISH technology paired with high-plex whole-slide spatial phenotyping on the PhenoCycler® Fusion platform. This two-step approach is compatible with human FFPE tissues and enables researchers to characterize the spatial biology of the TME more accurately by detecting RNA and protein markers on serial sections. Cell phenotypes and spatial associations are further analyzed through the Visiopharm Phenocode™ software. The resulting multomic spatial data more accurately reveals the interplay between TIME and TME by giving insight into cell lineages, surrounding structures, as well as secreted chemokines and cytokines that exist within the TIME ecosystem.

2. Multomic Spatial Phenotyping Workflow
2.1. Spatial Protein Phenotyping on the PhenoCycler-Fusion
PhenoCycler-Fusion seamlessly integrates automated fluidics and imaging, creating an end-to-end solution for high-resolution spatial imaging with rapid turnaround times. Here we analyzed human FFPE tissues using the PhenoCode™ Discovery Panels that comprehensively labels both immune and metabolic markers.

2.2. RNAscope HiPlex v2 on the PhenoCycler-Fusion
RNAscope HiPlex v2 assay with PhenoCycler-Fusion to perform the iterative fluorescent imaging to label a custom RNAscope chemo panel.

2.3. Data Analysis with Visiopharm Phenocode software
Phenocode software offers end-to-end workflow including importing multiplex images, Paint-to-Train AI-based tissue segmentation, deep-learning-based nuclear and cell segmentation, as well as a guided workflow for cellular phenotyping.

3. Multomic Panel Design
PhenoCode Discovery Panels are designed for immune cell phenotyping, evaluation of immune contexture and proliferation across the TIME. 12-plex Immune-encodacy panel of RNA target probes was selected to detect macrophages, chemokines, and cytokines within tumors.

4. RNAscope ISH paired with PhenoCycler-Fusion on Human FFPE Tissues
4.1. Detection of RNAscope 12-plex probes in Human FFPE Tonsil with PhenoCycler-Fusion
Detection of 12 target genes in a Human FFPE Tonsil tissue. To demonstrate the feasibility of automating RNAscope HiPlex v2 on PhenoCycler-Fusion, we utilized probes targeting a housekeeping human gene in each of 12 separate channels, in addition to DAPI staining in a human FFPE tonsil tissue (top images). Additionally, the custom RNAscope panel (listed in Section 1) was used to identify immune cells, tumor cells, chemokines and cytokines in a Human FFPE tonsil tissue (bottom images).

4.2. Multomic Detection of Protein & RNA Panel on Human Squamous Cell Carcinoma tissue
Multomic detection of protein markers and RNA target genes in FFPE tumor serial sections. Phenocode Discovery Panels were utilized to design protein panels that can phenotypically detect cells, immune cell subtypes, and immune activation states that contribute to the TIME. In a serial section, probes were used to detect target genes for various chemokines and cytokines within the TIME.

5. Multomic Spatial Profiling of Human FFPE Head and Neck Cancer Tissue Reveals Distinct Chemokine and Immune Signatures within the TIME
5.1. Whole-Slide Multomic Spatial Profiling Reveals Intra-tumoral Heterogeneity
Tumor Region 1 (T1) Tumor Region 2 (T2) Tumor Region 3 (T3) Tumor Region 4 (T4)

5.2. Chemokine-driven Immune Cell Landscape Leads to Distinct TIME in Different Tumor Regions
5.2.1. The prevalence of specific chemokine combinations in each tumor region: it shows a clear distinction in the immune signatures driven in T1 and T4. CCL19 and CXCL1 are prominent chemokines in T1 and 4, while CCL2 and CXCL12 are expressed in T3 and 4. CCL19 and CXCL1 are ant-inflammatory chemokines for cancer progression, whereas CCL2 and CXCL12 are pro-inflammatory for cancer progression.

5.3. Multomic Spatial Analysis Uncover Interplay of Chemokines and Immune Cells within the TIME
In this proof-of-concept study, we demonstrate the utility of multomic spatial profiling on the PhenoCycler-Fusion platform with the RNAscope assay. Analysis of the resulting multiplex imaging data not only revealed the structural organization of cells within the TIME, but also the regulatory role of chemokines within the TIME. Together, this information provides a more complete functional map of immune cells within the TIME and thereby enriches our understanding of tumor biology that may be deterministic of immunotherapeutic responsiveness.