

5647: Multiomic Spatial Phenotyping of a Partial Immunotherapy Response in Head and Neck Cancer

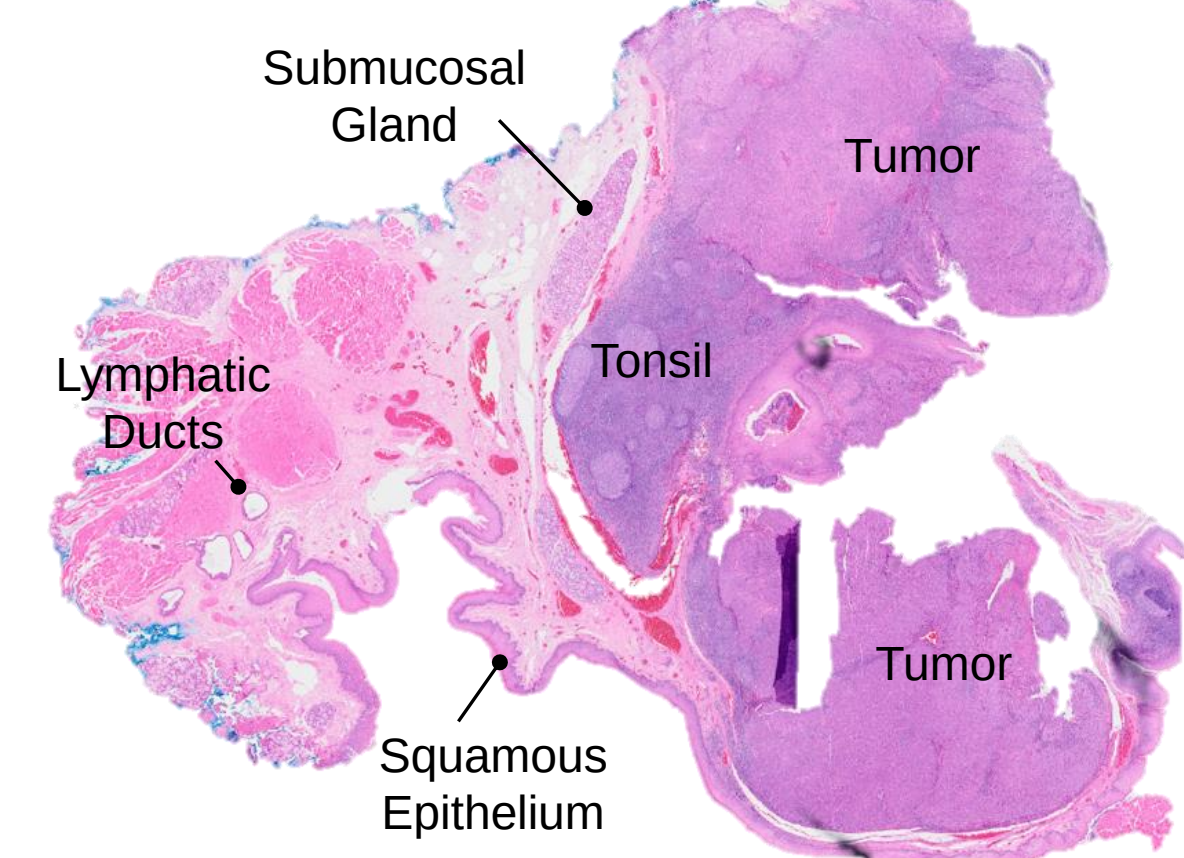
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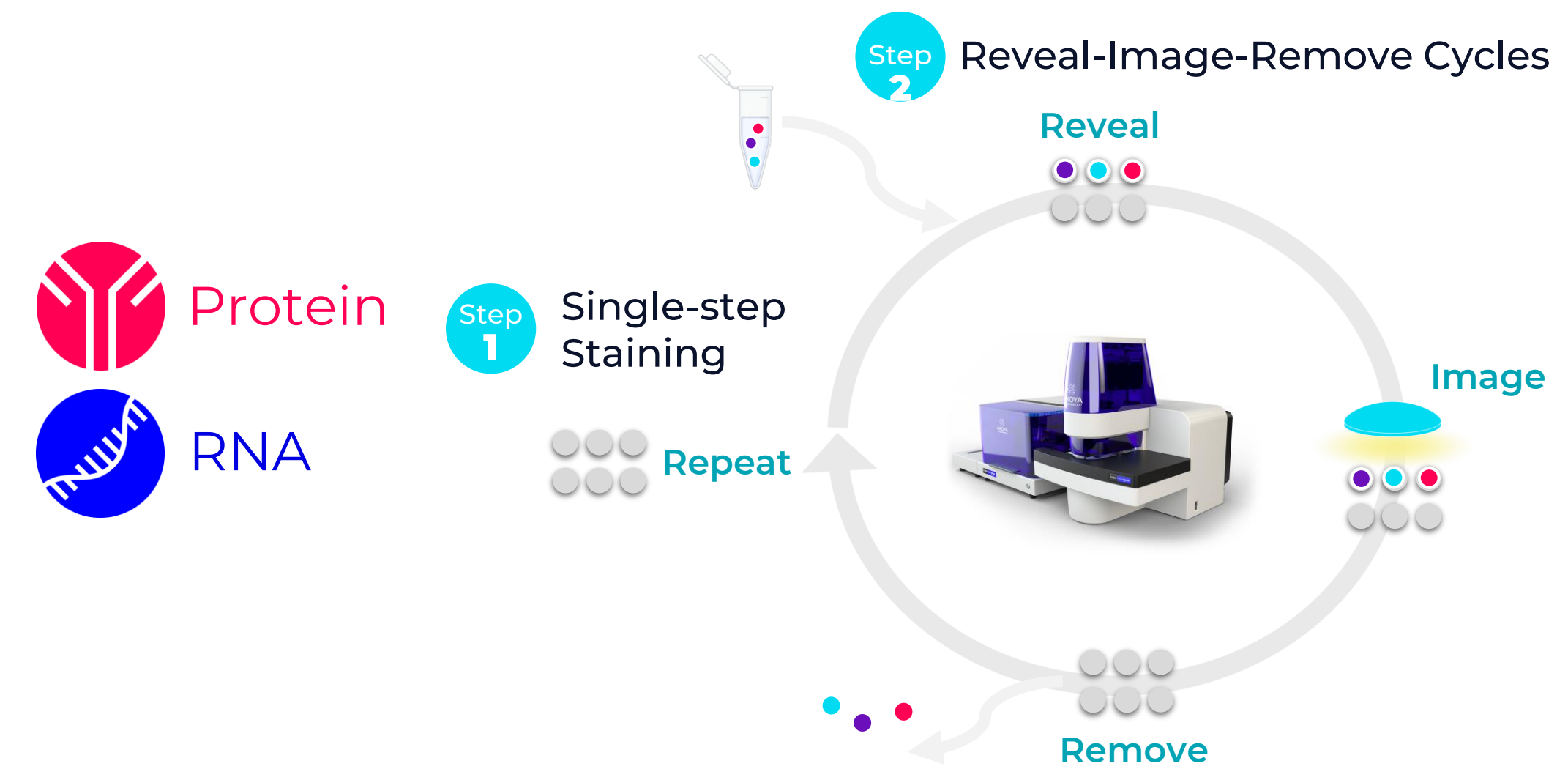
1. Introduction

Immune checkpoint inhibitors (ICI) have proven to be game-changing treatments for mucosal head and neck squamous cell cancer (HNSCC). The tumor microenvironment (TME) composition, contexture, and cellular architecture are key to understanding immune responsive and resistant HNSCC phenotypes. In this study, we performed multiomic spatial phenotyping to characterize a HNSCC tissue from a patient with a partial response to Pembrolizumab/Nivolumab treatment (H&E-stained section shown below). Pathology annotations indicate a large tumor mass, a regionally intact tonsil, as well as an Esophageal Submucosal Gland, Lymphatic Ducts and normal Squamous Epithelium.

Here, we demonstrate how **Multiomic Spatial Phenotyping** can produce a uniquely comprehensive analysis of the patient's TME and how it may explain the patient's partial response to ICI therapy.

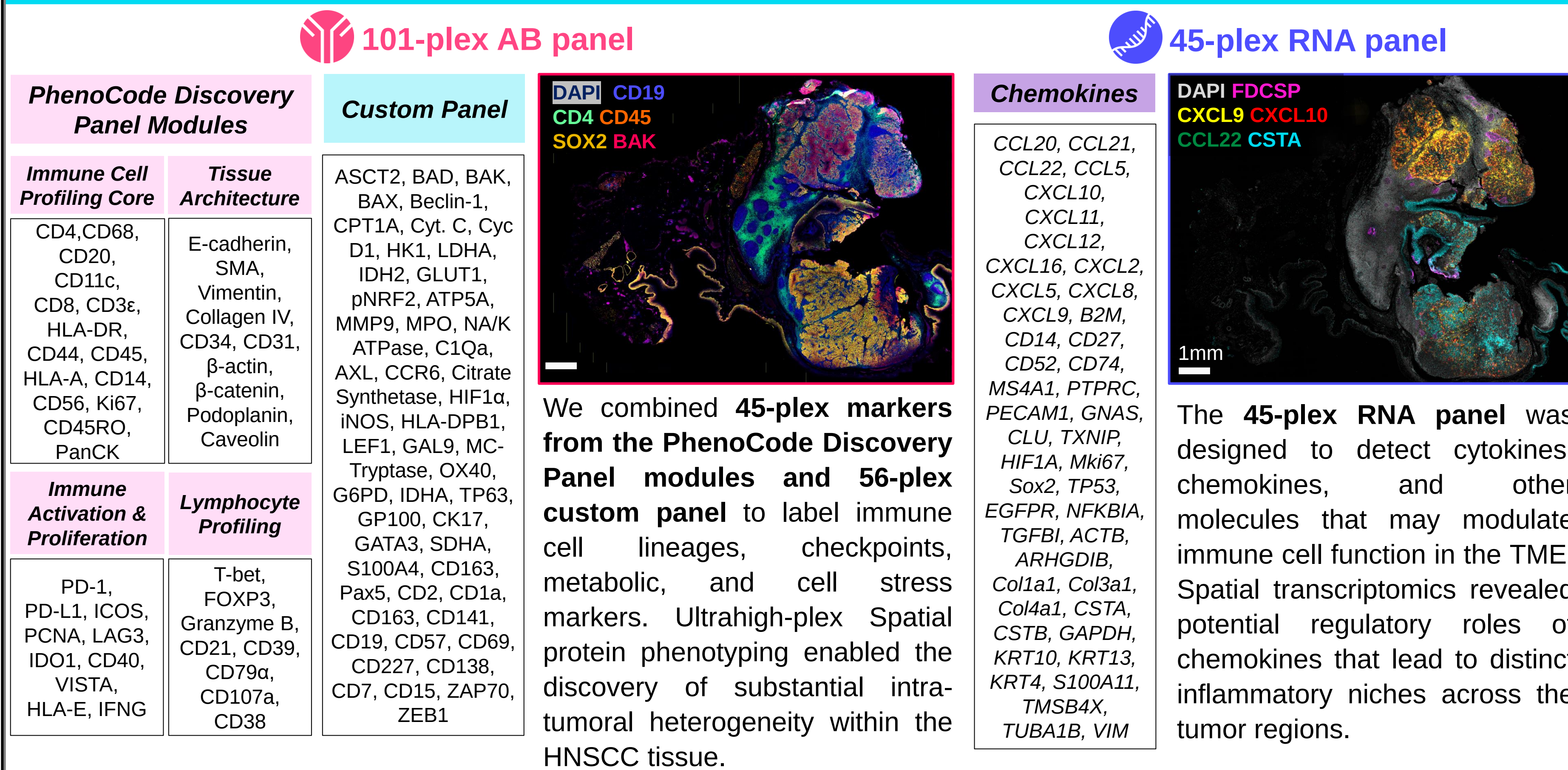


2. Multiomic (Protein & RNA) Spatial Phenotyping on PhenoCycler-Fusion



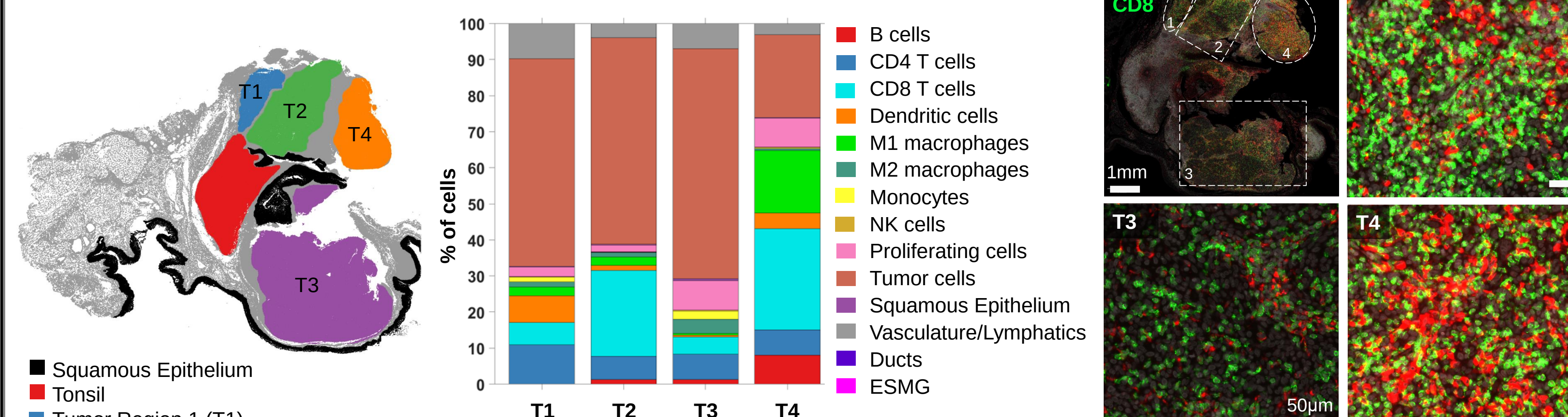
The **PhenoCycler®-Fusion** system is a spatial biology solution that enables multiomic ultrahigh-plex imaging of whole tissues at single-cell resolution. The high-throughput coupled with in-flight data processing and file compression algorithms enables routine analyses of whole-slide tissue sections, which often contain millions of cells. The unified PhenoCycler chemistry can be used to detect both RNA and protein targets, thereby allowing thorough interrogation of complex tissues. We first analyzed HNSCC tissue with **101 antibodies** (a combination of 45-plex selected from the PhenoCode™ Discovery Panel modules and a 56-plex custom panel) that comprehensively label both immune and metabolic markers. We then imaged **45 RNA probes** that label TME cyto- and chemokines on a serial section from the same tissue. Multiomic phenotyping readouts were obtained by aligning the protein & RNA datasets with a rigid affine transform using QuPath's alignment extension.

3. Multiomic Panel Design

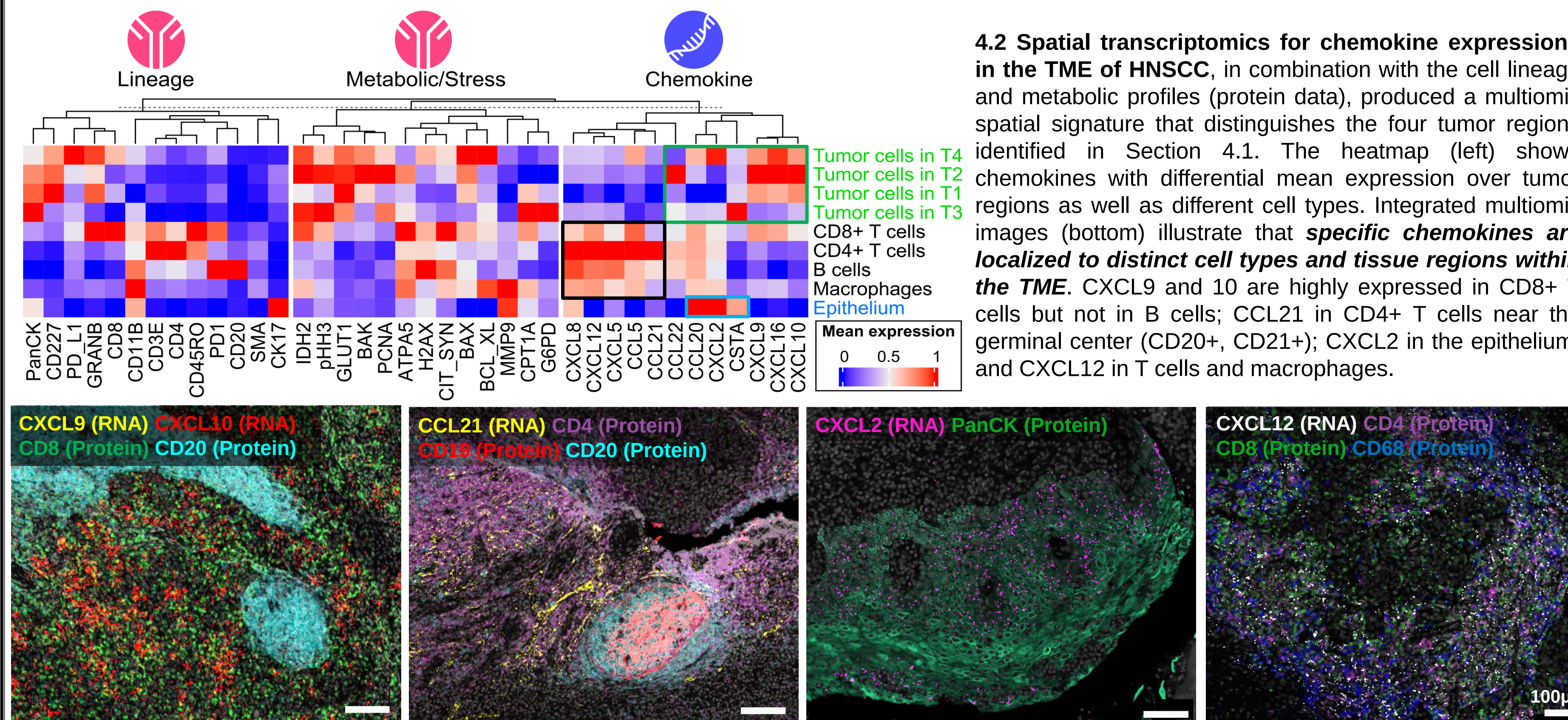


4. Integrated Multiomic Spatial Phenotyping Reveals Distinct Immune and Chemokine Signatures in Different Tumor Regions

4.1. Immune Spatial Phenotyping Illustrates Tumor Heterogeneity

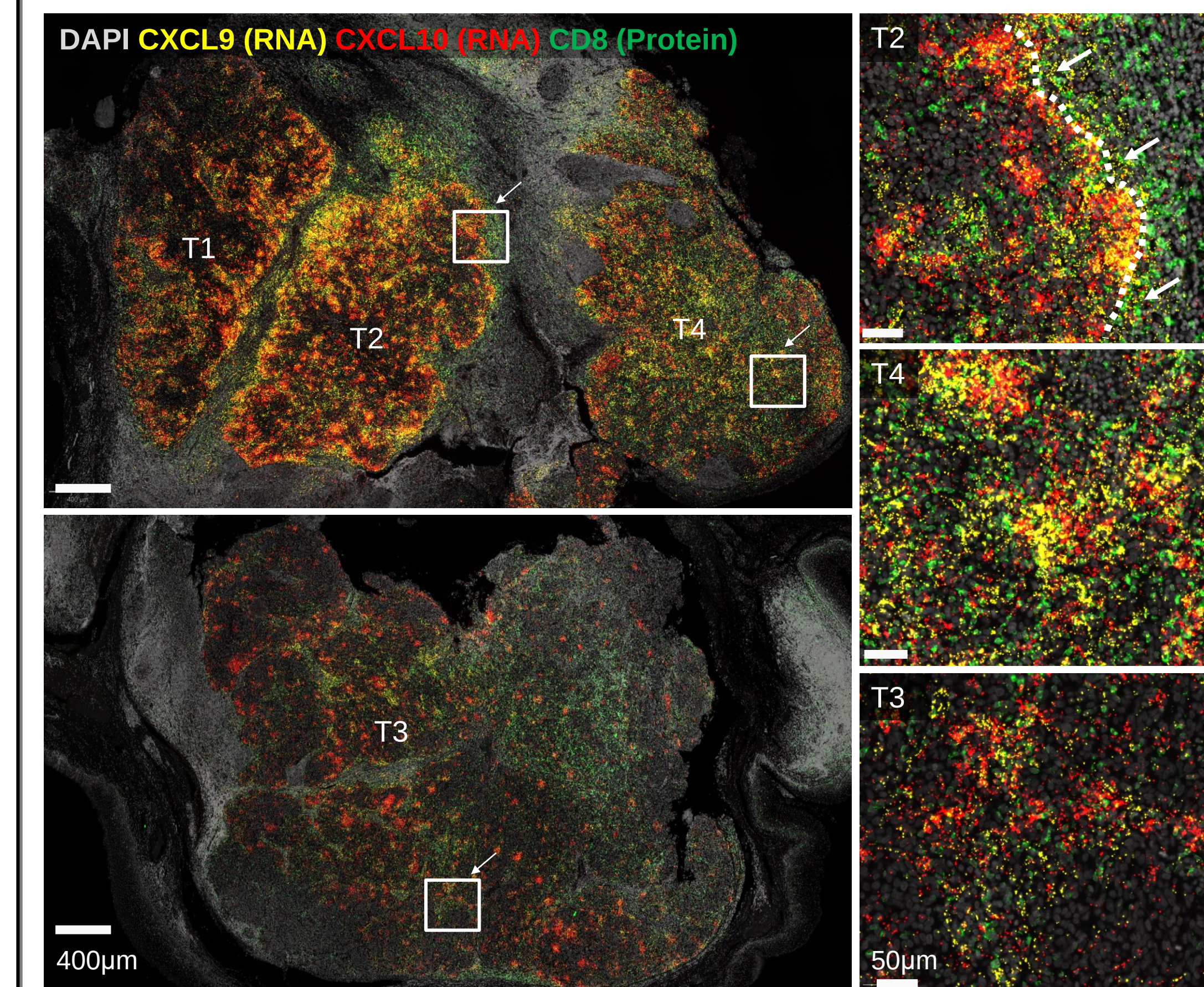


4.2. Multiomic Spatial Profiling Reveal Unique Chemokine Expression Landscape within the TME



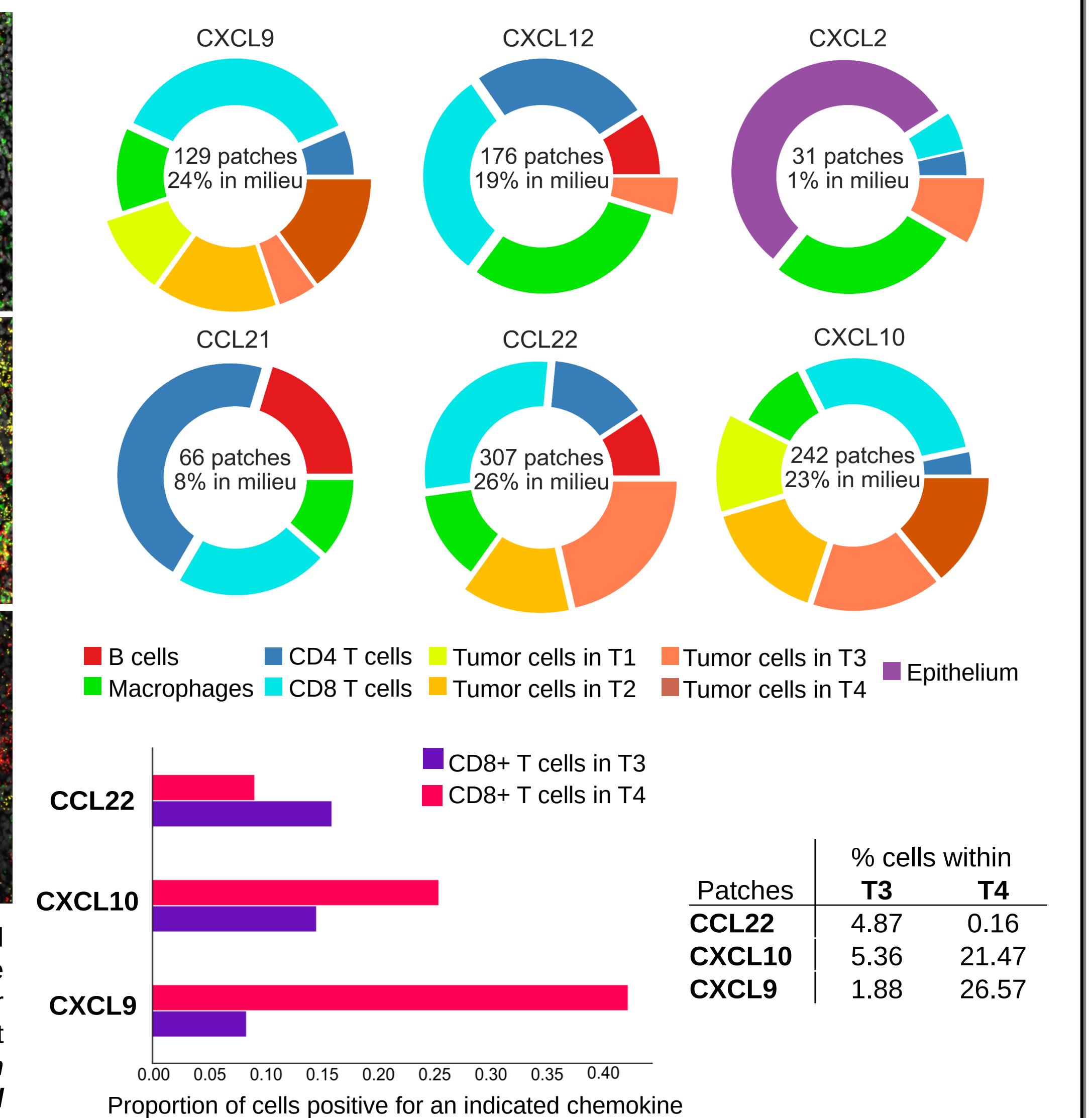
5. Localized Patterns of Chemokine Expression Indicates Distinct Chemokine-driven CD8+ T Cell Recruitment in Different Tumor Regions

5.1. Intra-Tumoral Chemokine-driven CD8 T Cell Infiltration



5.1. Chemokine-driven immune cell recruitment to tumor regions. Integrated multiomic images (right) show **CXCL9 & CXCL10** overlaid on **CD8 T-cell** positions in the four tumor regions. Boxed regions, magnified on the right, illustrate distinct marker expression per tumor region. White dotted lines and arrows in the magnified T2 depict putative CD8 T-cell recruitment at the T2 margin. **T4 & T3 show clear differences in the amount of CD8 T-cell infiltration, which appears to be recruited by CXCL9 and CXCL10.**

5.2. Chemokine-expressing cells form specialized milieus



6. Multiomic Spatial Phenotyping Provides Mechanistic Insights into Partial Immune Response of a HNSCC Patient

