Markers of interferon signaling and glutamine utilization within the stromal microenvironment in Head & Neck Squamous Cell Carcinoma (HNSCC) predict cancer recurrence

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Background

- Intra- and inter-tumor heterogeneity in head and neck cancers can lead to phenotypic diversity that makes cancer prognosis difficult to assess accurately.
- The heterogeneity that lies within the tumor-stroma interactions are poorly understood.
- There is an unmet clinical need to develop phenotypic signatures that can be predictive of clinical progression and therapeutic outcomes.

Methods

- In this study, we applied our SpaceIQ™ platform to identify a subset of key markers characteristic of the complex tumur microenvironment features that can be used to derive a predictive test for a patient's likelihood of cancer recurrence.
- We applied cell segmentation and unbiased cell typing on spatial proteomics data (**43 cores of 67-plex mIF panel across 15 pre-treated patients with 5 non-recurring status**) yielding **23 cell types**.
- **Recurrence-specific microdomains**, which are spatial arrangements of cells that are differentially expressed between the two response groups, were derived from **pointwise mutual information between the unbiased cell types**.
- Further, **a de-plexing algorithm** was implemented to obtain a **low-dimensional proxy representation** for each of the unbiased cell types involved in the microdomains based on their biomarker profiles.
- Finally, a prognostic modeling strategy, based on de-plexed representation, employing a **spatial score** between cell types and an estimated mean biomarker expression as **predictive features** to the recurrence outcome.

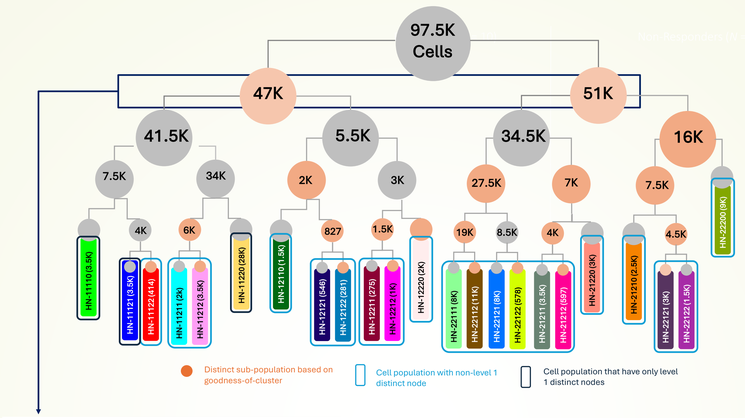
Results

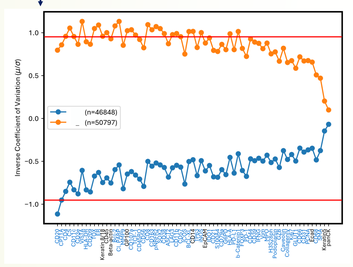
- We identified several spatial interactions associated with non-recurrence:
  1. spatial interaction between M2 macrophages (unbiased cell type 21111) with anti-inflammatory Tregs (unbiased cell type 22200) was predictive of recurrence outcome AUC=0.73 (0.71,0.74)
  2. spatial interaction between regulatory T cells (unbiased cell type 12121) with IFNG-activated, glutamine-dependent (ASCT1) mature NK cells (unbiased cell type 12212) was predictive of recurrence outcome with AUC=0.80 (0.79,0.81)
  3. spatial interaction between iNOS-mediated regulatory T cells (unbiased cell type 12220) with epithelial cells of Microdomain 3 was predictive of recurrence outcome with AUC=0.75 (0.74,0.76)

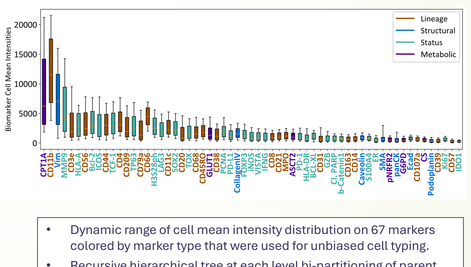
Conclusions

- We uncovered key **spatial signatures of tumor non-recurrence in HNSCC** patients involving interactions between **M2 macrophage and Tregs**, **stromal immune cells**, and **mature NK cells**.
- These spatial interactions demonstrate the complexity of the TME affected by **Wnt/b-Catenin signaling** regulation on ECM remodeling, fibrotic processes, and iNOS-mediated immune regulation, in addition to potential **glutamine-dependent facilitation of epithelial tumor invasion by IFNG mature NK cells**.
- Through the SpaceIQ platform, we were able to **de-plex a 67-plex IF panel to a few key predictive biomarkers involving macrophages, Tregs, and glutamine-dependent NK cells** yielding good predictive performance for recurrence outcome in HNSCC patients.

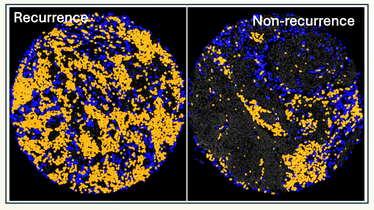
Unbiased Cell Typing Extracts Numerically Stable, Spatially Distinct and Biologically Interpretable Recursive Cell Types



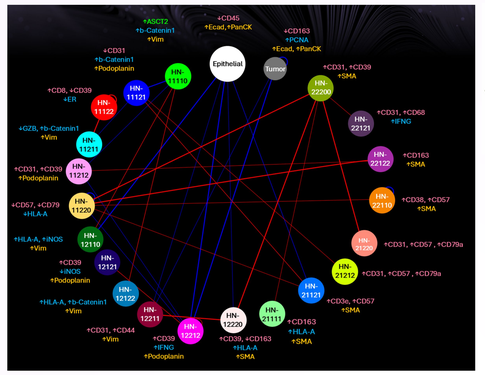


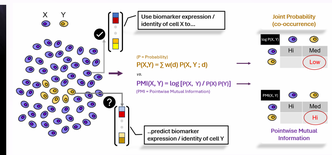


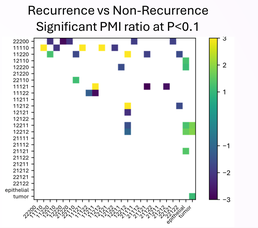
- Dynamic range of cell mean intensity distribution on 67 markers colored by marker type that were used for unbiased cell typing.
- Recursive hierarchical tree at each level bi-partitioning of parent node cell population into children nodes. The root node represents all nonepithelial cells undergoing unbiased cell typing. Brown nodes denote distinct clusters at a given level based on a positive estimated Silhouette score. Red leaf nodes denote descendants that have a distinct ancestral node beyond the first level split. Purple leaf nodes denote descendants that do not have a distinct ancestral node beyond the first level split.
- Estimated inverse coefficient of variation of the z-scores for each marker for each of the cell population clusters from the first level split of unbiased cell typing.
- Recurrence and non-recurrence cores showing the cells from the first level split. Cells in gray are the epithelial cells.



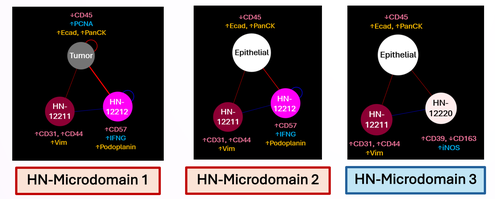
µDs Emerge as Spatial Networks of Immuno-Metabolic Cell Types



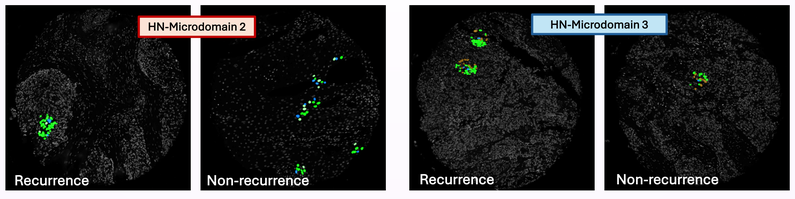




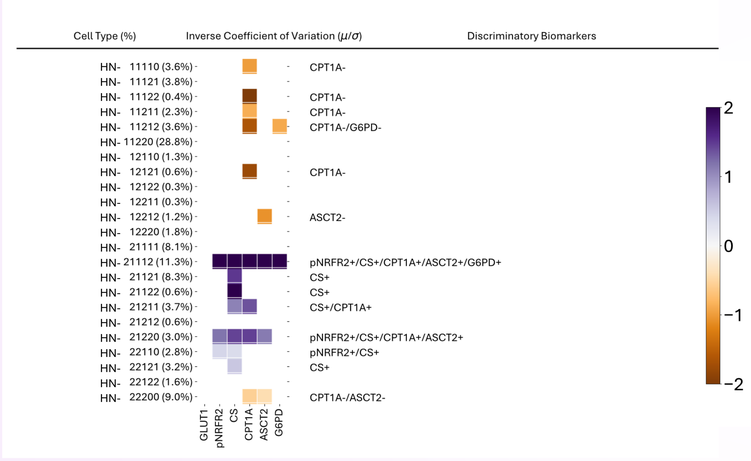
More frequent in non-recurrence — More frequent in recurrence



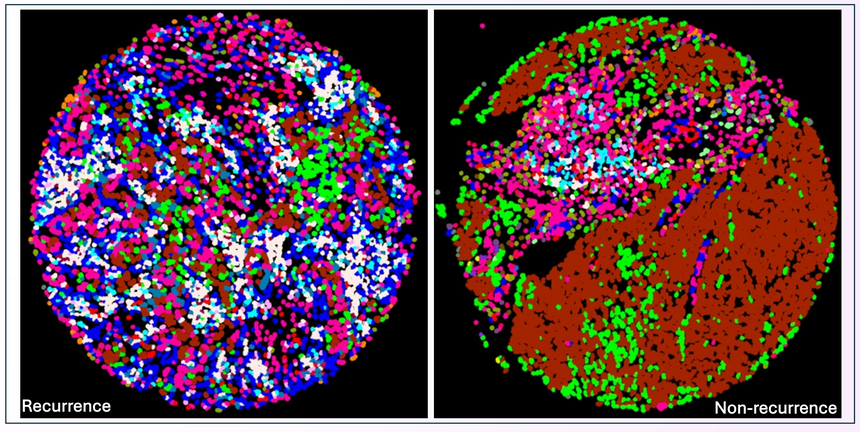
- Spatial domain network representing microdomain families of co-localization among unbiased cell types and epithelial cells
- Red (blue) edges denote more frequent co-localization in recurrence (non-recurrence) group
- Each cell type node shows de-plexed markers that are used to approximate the cell type (up/down arrows denote the above/below cell mean intensities in the approximated cell type relative to mean intensities of overall cell population)
- Microdomains present in the spatial domain network



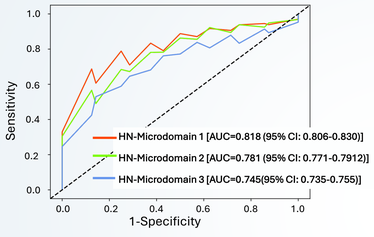
Discriminatory Markers for Biological Interpretation of Unbiased Recursive Cell Types

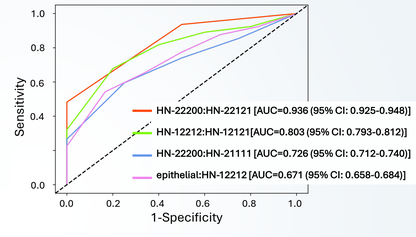


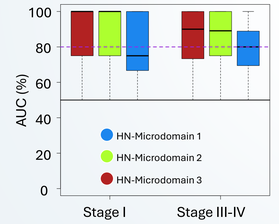
Cellular Heterogeneity Visualized

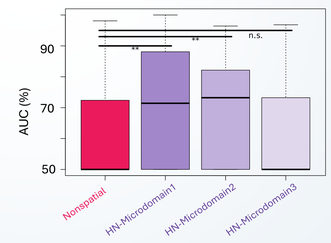


Microdomains are Spatially Distinct IFNG and iNOS Modulated Immuno-Regulatory Programs that Predict ICI Response









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