Metabolic reprogramming of glycolysis, fatty acid oxidation, and anti-oxidative stress in macrophages is predictive of immunotherapy response in patients with non-small cell lung cancer (NSCLC)

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Background

- Lung cancer remains the tumor type with the highest mortality rate globally.
- Whilst therapeutic options, both systemic and targeted are increasing, selecting patients for these therapies become a critical factor.
- The tumor microenvironment (TME) has been recognized as an increasingly important component to profile, both **phenotypically and functionally**, to understand the cellular composition and how cells communicate in the TME.
- Moreover, these profiles can be used in the development of **predictive signatures** and companion diagnostic assays for patient triage.

Methods

- Using our SpaceIQ™ platform, we developed a simple prognostic test on key biomarkers predictive of the likelihood of patients responding to adjuvant immunotherapies.
- Briefly, we applied cell segmentation and unbiased cell typing on the spatial proteomics data (**28 patient cores in 44-plex mIF panel with 10 responders and 18 non-responders**) yielding **23 different cell types**.
- Differential spatial arrangements of cells based on pointwise mutual information between response groups resulted in **response-specific microdomains**.
- Biomarker profiles from each of the cell types involved in the microdomains served as the basis for a de-plexing algorithm in obtaining a **low-dimensional proxy representation** of the cell types.
- Finally, a **spatial score** between the de-plexed representation of cell types and its estimated mean biomarker expression were derived as **predictive features for response outcome** in a simplified prognostic model.

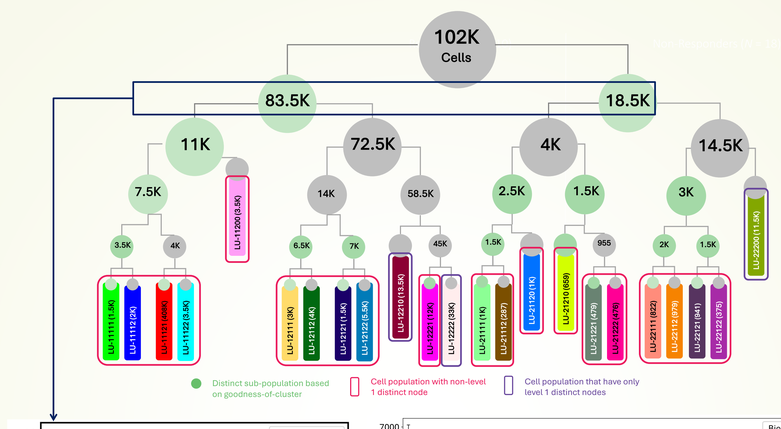
Results

- Predictive spatial interactions for response outcome emerged from our analysis involving metabolic activity in immune cells, in particular macrophages.
- **Resistant signature** defined **by spatial interactions between immune cell subpopulations of Microdomain 1 and 3 show increased LDHA and decreased CPT1A activity that were predictive for IO response with AUC=0.87 (0.86,0.88) and AUC=0.79 (0.79,0.80),** respectively.
- **Response signature** promoting **spatial interaction** between **epithelial/tumor cell population with infiltrating macrophages of Microdomain 2** was predictive for IO response with AUC=0.83 (0.82,0.84).

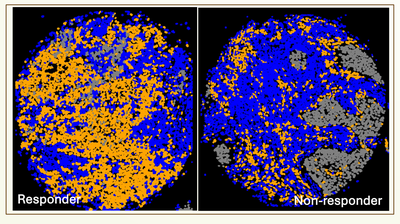
Conclusions

- Through the SpaceIQ™ platform, we were able to **de-plex a 44-plex IF panel** to a **few key predictive biomarkers involving metabolic and macrophage signatures** that yielded good predictive performance on immunotherapy response in NSCLC patients.
- We hypothesize that **metabolic reprogramming of glycolysis in macrophages through lowered CPT1A stabilization of LDHA may lead to less effective pro-inflammatory response of M1 macrophages contributing to the resistance of IO therapy.**
- We also observed evidence that interaction between **epithelial/tumor cells with mitigated proliferation of GLUT1-active tumor-associated macrophages led to improved response to IO therapy.**

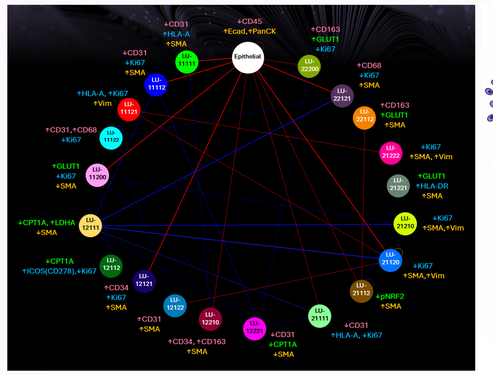
Unbiased Cell Typing Extracts Numerically Stable, Spatially Distinct and Biologically Interpretable Recursive Cell Types

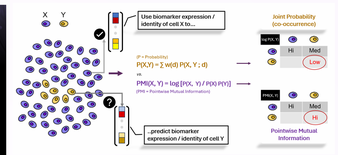


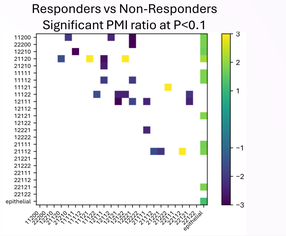
- Dynamic range of cell mean intensity distribution on 40 biomarkers colored by marker type that were used for unbiased cell typing.
- Recursive hierarchical tree at each level bi-partitioning of parent node cell population into children nodes. The root node represents all nonepithelial cells undergoing unbiased cell typing. Green 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 for each marker for each of the cell population clusters from the first level split of unbiased cell typing.
- Responder and non-responder cores showing the cells from the first level split. Cells in gray are the epithelial cells.



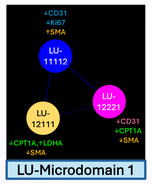
μDs Emerge as Spatial Networks of Immuno-Metabolic Cell Types

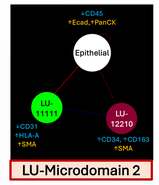


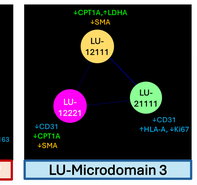




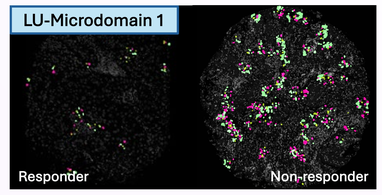
More frequent in non-responders — More frequent in responders

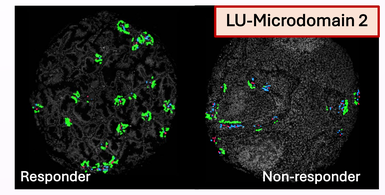




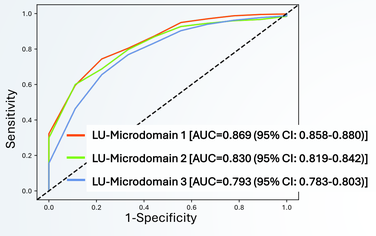


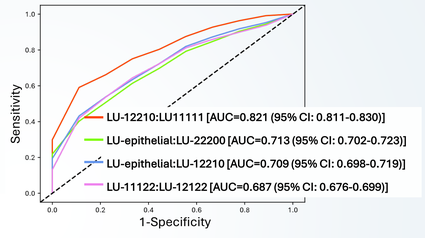
- Spatial domain network representing microdomain families of co-localization among unbiased cell types and epithelial cells.
- Red (blue) edges denote more frequent co-localization in responder (non-responder) group.
- Each cell type node shows de-plexed markers that are used to approximate the cell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

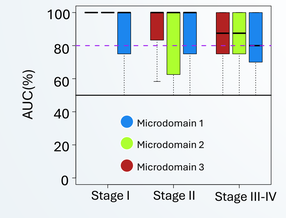


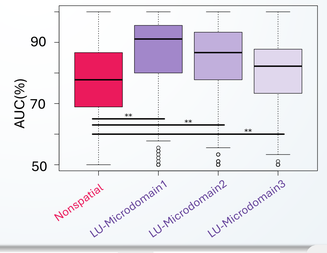


De-plexed markers involving metabolic and macrophage signatures yield good predictive performance in IO response in NSCLC patients

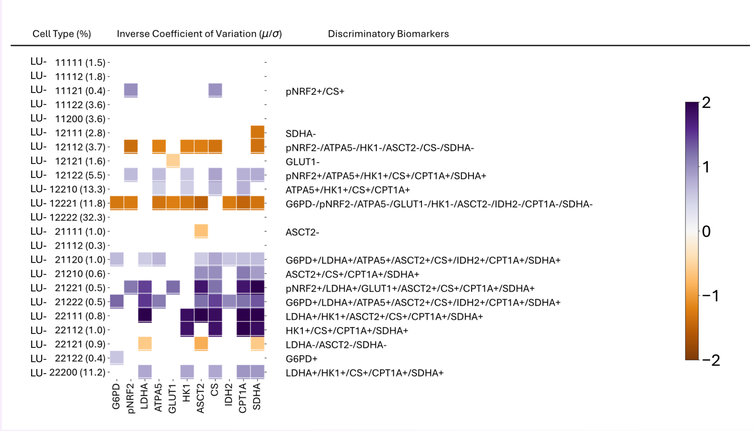




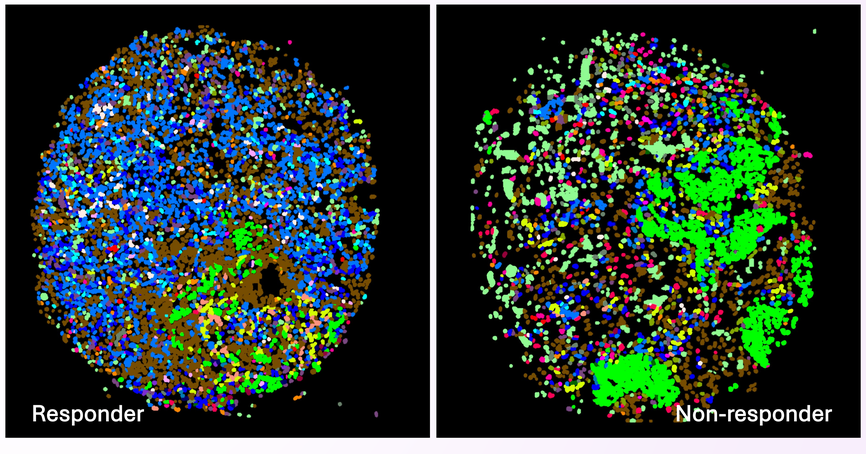




Discriminatory Markers for Biological Interpretation of Unbiased Recursive Cell Types



Cellular Heterogeneity Visualized



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