Unraveling functionally distinct metabolic programs to predict response and resistance to immunotherapies in patients with non-small cell lung cancer (NSCLC)

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Background

- NSCLC accounts for most lung cancers and has a poor 5-year survival.
- With an increasing number of systemic and targeted therapies, including immune checkpoint inhibitors (ICIs), it is becoming more important to develop predictive biomarkers to identify patient response to ICIs.
- Developing predictive spatial biomarkers and targeting cancer and stromal cell metabolism could be key to bypassing immune checkpoint blockade (ICB) resistance.

Methods

- Study performed on a retrospective cohort of 28 second-line nivolumab-treated NSCLC tissue cores – N = 28 (responders = 10/non-responders = 18) [1].
- Cohort was profiled using a custom 44-plex immunofluorescence panel (See Table 1) (incl. functional/metabolic markers) with the Phenocycler Fusion platform (Akoya Biosciences).
- We applied an unbiased spatial analytics and explainable AI pipeline, **SpaceIQ™**, to capture emergent metabolic programs in spatial arrangements of unbiased cell types (microdomains, μD1 and μD2) predictive of ICI response.
- Predictive spatial networks implicated in known metabolic pathways are currently being verified by spatial transcriptomics.

	Lineage	Description
1	CD3e	T cell surface glycoprotein
2	CD4	Surface marker on Helper T cells, monocytes, macrophages, dendritic cells
3	CD8	T cell surface glycoprotein
4	CD11b	Macrophage marker
5	CD11c	Dendritic cell marker
6	CD14	Macrophage membrane co-receptor with TLR-4 and MD-2
7	CD19	Immature B cells surface glycoprotein
8	CD20	B cell membrane protein
9	CD21	Complement receptor type 2 on B cells for activation and maturation
10	CD31	Surface protein on endothelial cells, platelets, macrophages, granulocytes, lymphocytes
11	CD34	Endothelial progenitor cell marker
12	CD44	Cancer stem cell marker
13	CD45	Surface protein on leucocytes and hematopoietic cells (except erythocytes and plasma cells)
14	CD45RO	T memory cell marker
15	CD57	Senescent T-cells and NK cells
16	CD68	Macrophage marker
17	CD163	M2 macrophage marker
	Structural	**Description**
18	Collagen IV	Structural protein in extracellular basement membrane
19	E-cadherin	Epithelial marker (tumor suppressor)
20	NAKATPASE	Sodium-potassium pump (electrogenic transmembrane ATPase)
21	Pan-Cytokeratin	Epithelial marker
22	SMA	Smooth muscle actin expressed in myofibroblasts
23	Vimentin	Cytoskeletal protein expressed in mesenchymal cells

	Metabolic	Description	Known Pathways
24	SDHA	Succinate-ubiquinone oxidoreductase in mitochondrial respiratory chain, tumor suppressor activity	OXPHOS
25	ASCT2	Neutral amino acid transporter from SLC1 family	PPP
26	ATPA5	ATP synthase subunit	OXPHOS
27	Citrate Synthase	Mitochondrial enzyme involved in TCA cycle (oxidative phosphorylation)	TCA cycle
28	CPT1A	Carnitine palmitoyltransferase 1A involved in fatty acid oxidation	Fatty Acid Synthesis
29	G6PD	Glucose-6-phosphate dehydrogenase involved in glycolysis and Pentose Phosphate Pathway	PPP
30	GLUT1	Glucose transporter membrane protein	Glycolysis
31	Hexokinase1	Enzyme producing glucose-6-phosphate for glucose metabolism	Glycolysis
32	IDH2	Isocitrate dehydrogenase, mitochondrial enzyme involved in energy production	TCA cycle
33	LDHA	Lactate dehydrogenase A converting lactate to pyruvate for energy production	Glycolysis & TCA cycle
34	pNRF2	Txn factor regulating anti-oxidative stress	PPP
	Status	**Description**	
35	FOXP3	Regulatory CD4+ T cells	
36	Granzyme B	Proteolytic activity of NK cells and cytotoxic T cells	
37	HLA-A	Surface receptor on antigen presenting cells	
38	HLA-DR	Surface receptor on antigen presenting cells (dendritic cells, B cells, macrophages, monocytes)	
39	ICOS (CD278)	Immune checkpoint surface protein on activated T cells	
40	Ki67	Cell proliferative marker	
41	LAG3	Immune checkpoint protein on activated T cells, B cells, NK cells, and plasmacytoid dendritic cells	
42	PAX5	Promotes lymphoid progenitor to B lymphocyte lineage, tumor suppressor transcription factor	
43	PD-1	Immune checkpoint surface protein on T and B cells	
44	PD-L1	Immune checkpoint surface protein on tumor cells, macrophages, and monocytes	

Table 1: Biomarker panel used for multiplexed immunofluorescence imaging includes lineage (=17), structural (6), metabolic (11) and status (10) markers.

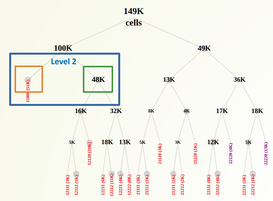
Results

- Non-responders had higher proportions of CD4 T cells with upregulated TCA cycle/downregulated glycolysis and pentose phosphate pathway (PPP).
- Unbiased and recursive cell typing allowed for functional characterization of tumor/stromal cells and reveals differential metabolic activity in tumor cell types in non-responders and stroma cell types in responders.
- μD1 and μD2 were spatially anchored around tumor cells with upregulated TCA cycle and oxidative phosphorylation (OXPHOS) with additional NK cells and dendritic cells along with upregulated PPP in μD2.
- Each microdomain had distinct metabolic programs relating to catabolic (energy utilization) and anabolic (cellular biogenesis) pathways.
- μD1/μD2 were prognostic for overall survival (mean AUC = 0.92/0.90 +/- 0.06/0.07), with high median sensitivity and specificity for nivolumab-treated response.

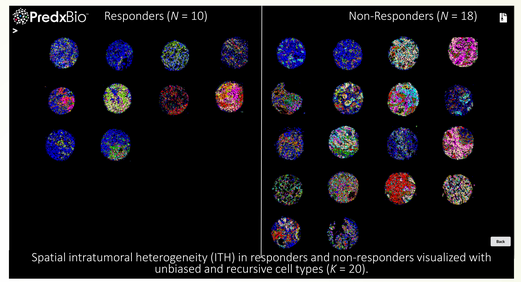
References

[1]Monkman et al Immunology, 2023; [2]Spagnolo D et al, JPI, 2016; [3]Uttam, S, et al Nat. Comm., 2020; [4]Furman SA, Cell. Rep. Met., 2021

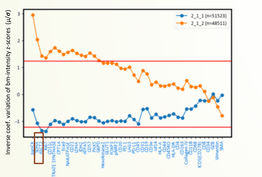
Unbiased Cell Typing Extracts Numerically Stable, Spatially Distinct and Biologically Interpretable Recursive Cell Types



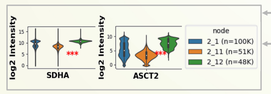
Unbiased and recursive cell typing algorithm extracts numerically stable, spatially distinct and biologically interpretable cell types and yields 20 different sub-populations (red and purple nodes) in the NSCLC data.

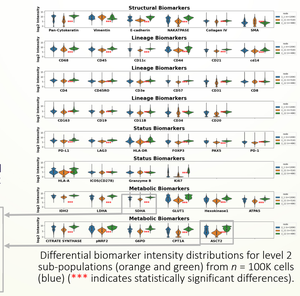


Spatial intratumoral heterogeneity (ITH) in responders and non-responders visualized with unbiased and recursive cell types (*K* = 20).

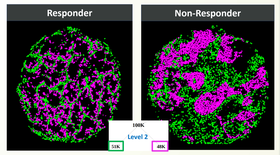


For illustration, the discriminating biomarkers for level 2 split (shown above) incl. metabolic SDHA and ASCT2 markers (highlighted), among others.



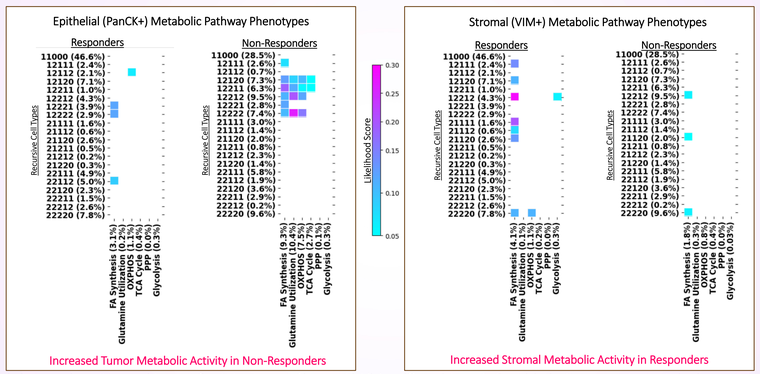


Differential biomarker intensity distributions for level 2 sub-populations (orange and green) from *n* = 100K cells (blue) (*** indicates statistically significant differences).



Spatial distribution of the discriminating biomarkers for level 2 – SDHA (green) and ASCT2 (pink) on a responder and a non-responder core.

Unbiased Cell Typing Reveals Differential Metabolic Activity in Tumor (Stroma) Cell Types in Non-Responders (Responders)



Increased Tumor Metabolic Activity in Non-Responders

Increased Stromal Metabolic Activity in Responders

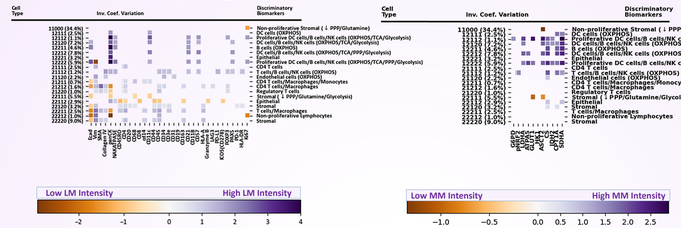
Associating recursive cell types with pre-defined phenotypes of interest highlights differential metabolic activity in the tumor sub-population in non-responders, but stromal sub-population in responders. In terms of the metabolic pathways triggered, the heterogeneity in the tumor metabolic activity is higher than the stromal metabolic activity.

Conclusions

- The SpaceIQ platform infers distinct metabolic programs revealing spatially mediated roles for anabolic/catabolic pathways to predict immunotherapy response in NSCLC.
- Unbiased and recursive cell typing allowed for functional characterization of tumor/stromal cells and reveals differential metabolic activity in tumor (stroma) cell types in non-responders (responders).
- Distinct spatial organization of metabolic activity encompassing glycolysis, TCA cycle, PPP, and OXPHOS may play a significant role in affecting clinical outcomes induced by ICI therapy.

Microdomains Emerge as Spatial Networks of Immuno-Metabolic Cell Types

Discriminatory Lineage Markers (LM) and Metabolic Markers (MM) in Recursive Cell Types

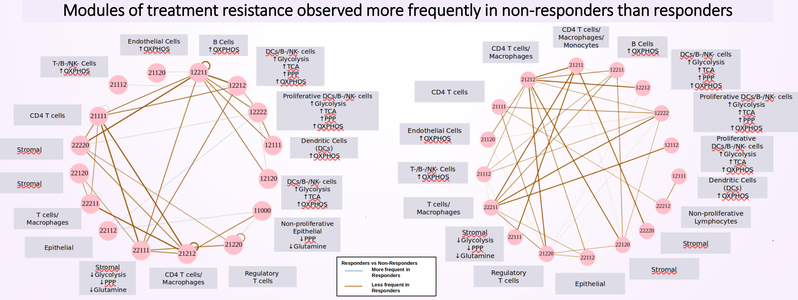


Discriminate lineage markers differentiated immune cells, epithelial, and other stromal cells. Metabolic pathway annotation based on probable pre-defined phenotypes delineates metabolic activity in recursive cell types.

Microdomain-1 (μD1 - 36% of cells)

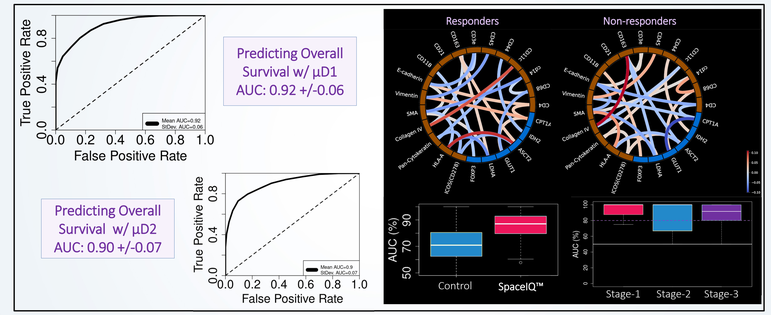
Microdomain-2 (μD2-14% of cells)

Modules of treatment resistance observed more frequently in non-responders than responders



Microdomains μD1 and μD2 emerge as spatial networks of immuno-metabolic cell types based on pointwise mutual information [2-4]. The spatial interactions suggest modules of treatment resistance, where predominantly significant interactions between recursive cell types are frequently observed in non-responders (brown) compared to the responders. There are only a limited number of interactions (blue) that are favorable in the responders.

Microdomains are Spatially Distinct Catabolic and Anabolic Programs that Predict ICI Response



Microdomains μD1 and μD2 are highly predictive of overall survival. Microdomains μD1 and μD2 are spatially anchored around tumor cells with upregulated TCA cycle and oxidative phosphorylation (OXPHOS) with additional NK cells and dendritic cells along with upregulated PPP in μD2. Each microdomain captures spatially distinct metabolic programs relating to catabolic (energy utilization) and anabolic (cellular biogenesis) pathways.

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Acknowledgments

- Akif Burak Tosun, PhD of PredxBio, Inc.

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