Characterizing the Spatial Transcriptomic Landscape of Metastatic Melanoma Patients Treated with Combination Anti-LAG-3 + Anti-PD-1 Immunotherapies

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Background

- Lymphocyte-activation gene-3 (LAG-3), an immune checkpoint receptor, negatively regulates T-cell function and facilitates immune escape of tumors[1].
- Dual blockade of LAG-3 and PD-1 has demonstrated improved survival in patients with metastatic melanoma compared to anti-PD-1 therapy alone[2].
- Despite these advances, a significant subset of patients still develops resistance or fails to respond[3].
- Spatial transcriptomics allows us to capture both gene expression and spatial context at a single-cell level, offering insight to potential biomarkers of response/resistance.

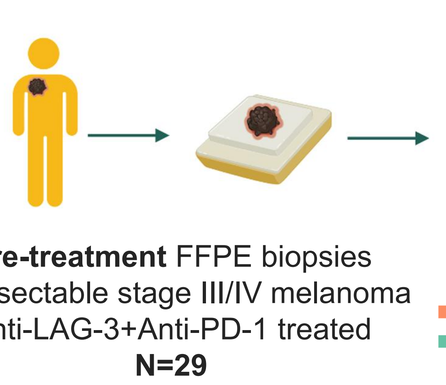
Objectives

To characterize the spatial transcriptomic profiles associated with response and resistance to combined anti-LAG-3+anti-PD-1 immunotherapy in metastatic melanoma by:

1) Defining cell type, gene expression, and pathway differences between response groups
2) Identifying cell–cell communication networks and spatial niches linked to response or resistance

Methods

Patient Cohort



Pre-treatment FFPE biopsies
Unresectable stage III/IV melanoma
Anti-LAG-3+Anti-PD-1 treated
N=29

Spatial Transcriptomic Sequencing

480 gene panel on the **Xenium** In Situ (*10X Genomics*) platform

Analysis

Cell type deconvolution (linear mixed-effect models)
Differential expression (diffxpy)
Gene set enrichment (GSEApy)

CellPhoneDB Cell-cell communication

squidpy Spatial neighbourhood enrichment

Results

Tumour and Peritumour regions included in TMA

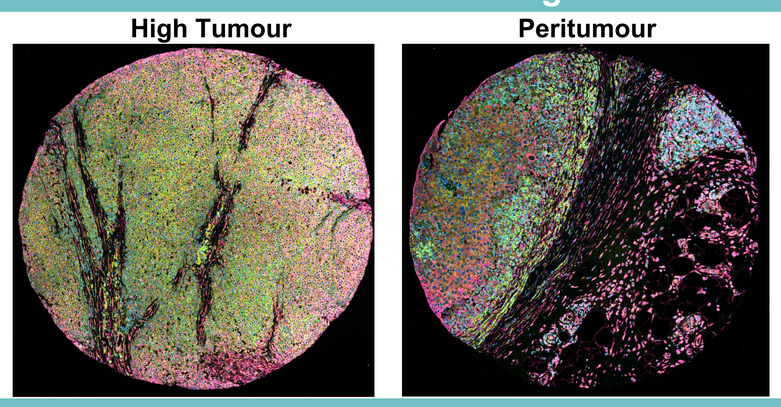
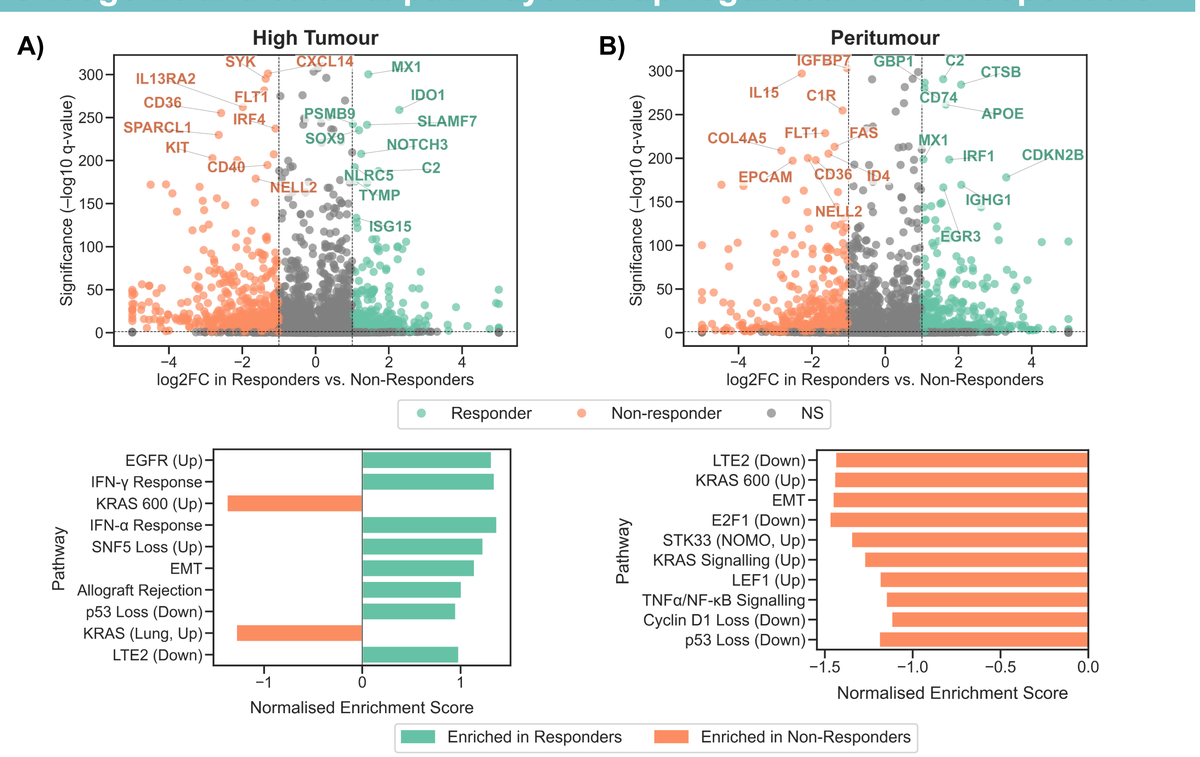


Figure 1: Representative images of a high tumour and peritumour melanoma tissue core following spatial transcriptomic sequencing.

Oncogenic and stromal pathways are up-regulated in non-responders



Immune and stromal cell proportions differ between R and NR

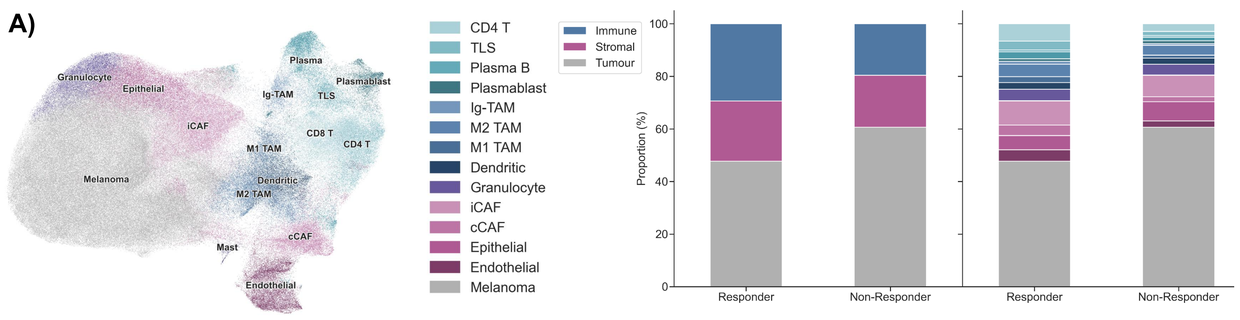


Figure 2: A) UMAP of cell clusters pre-deconvolution and comparison of cell type proportions between responders (R) and non-responders (NR). **B)** Comparison of specific cell types between responders and non-responders in high tumour and **C)** peritumour regions.

VEGF interactions are associated with resistance

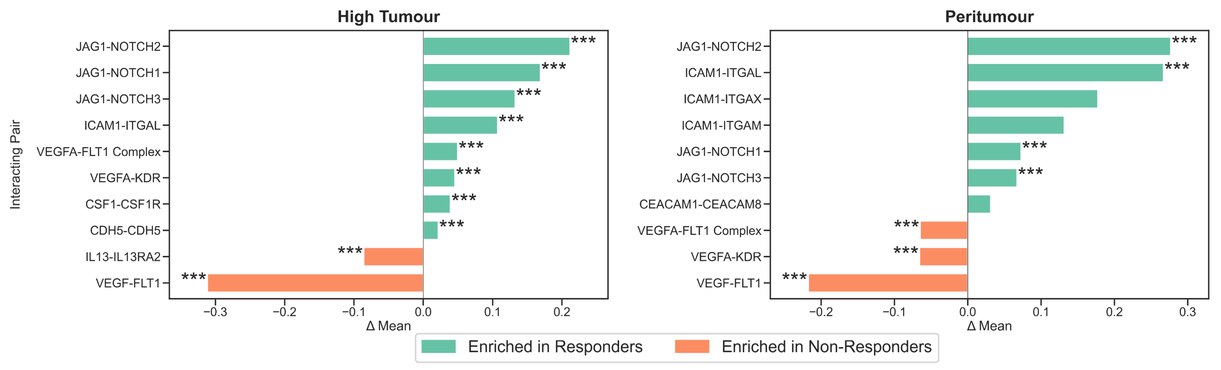


Figure 4: Top 10 ligand-receptor pair interactions enriched in responders and non-responders in high tumour and peritumour regions.

Non-responders exhibit diffuse cell distribution

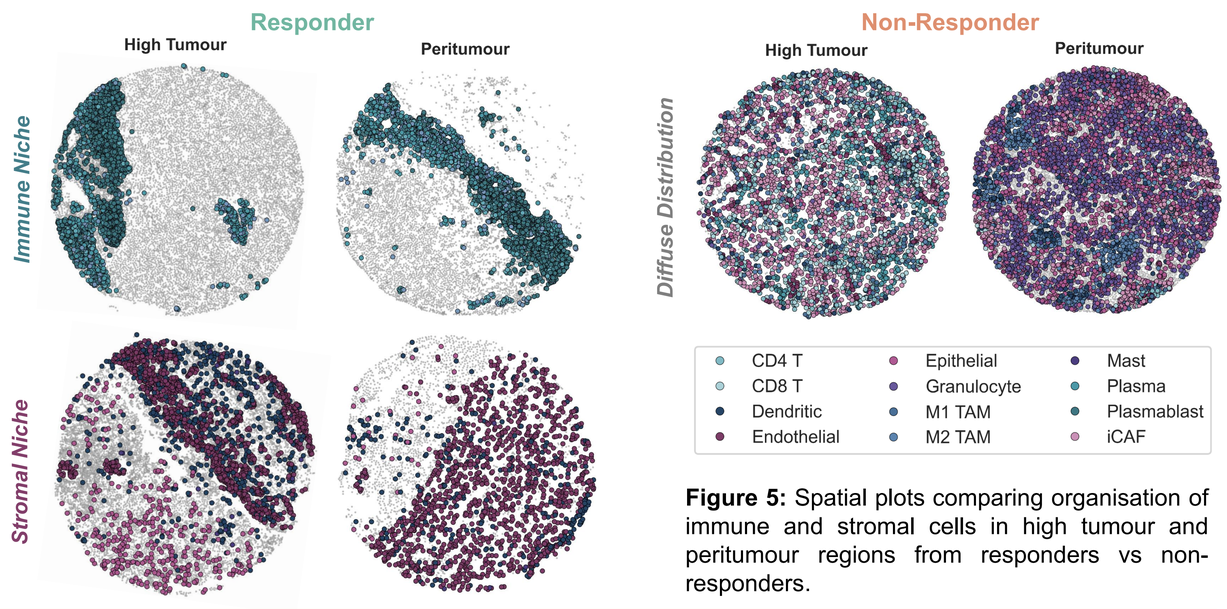


Figure 5: Spatial plots comparing organisation of immune and stromal cells in high tumour and peritumour regions from responders vs non-responders.

Conclusions

Responders and non-responders showed distinct spatial transcriptomic profiles:

- Responders: ↑ CD8+/CD4+ T and plasma cells; ↑ Immune activation and NOTCH signalling; Distinct spatial architecture
- Non-responders: ↑ Epithelial and melanoma cells; ↑ VEGF signalling and oncogenic pathways; Disorganised, diffuse cell distribution

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References

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