Spatial transcriptomics reveals distinct niches of resistance to immune checkpoint blockade in melanoma patients

Xiaohang Fu[1,2,3], Paola Cornejo-Páramo[1,2,3], Tuba N. Gide[1,2,3], Yizhe Mao[1,2,3], Nurudeen A. Adegoke[1,2,3], Eva R. Shteinman[1,2,3], Grace H. Attrill[1,2,3], Andrew J. Hoy[3,4], Jeff Holst[5], Umaimainthan Palendira[1,2,3,6], Matteo S. Carlino[1,2,7], Inês Pires da Silva[1,2,3], Danielle Vignati[1,2,3], Nigel G. Maher[1,2,3], Serigne N. Lo[1,2,3], Richard A. Scolyer[1,2,3,8], Alexander M. Menzies[1,2,9,10], Georgina V. Long[1,2,3,9,10^], Ismael A. Vergara[1,2,3^], James S. Wilmott[1,2,3^]

[1] Melanoma Institute Australia, The University of Sydney, NSW 2065, Australia; [2] Faculty of Medicine and Health, The University of Sydney, NSW 2006, Australia; [3] Charles Perkins Centre, The University of Sydney, NSW 2006, Australia; [4] School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, NSW 2006, Australia; [5] School of Biomedical Sciences, University of New South Wales, NSW 2052, Australia; [6] Centenary Institute, The University of Sydney, Sydney, NSW 2050, Australia; [7] Westmead and Blacktown Hospitals, Sydney, NSW, Australia; [8] Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital and NSW Health Pathology, Sydney, Australia; [9] Mater Hospital, North Sydney, NSW 2060, Australia; [10] Royal North Shore Hospital, St Leonards, NSW 2065, Australia; ^ These authors contributed equally

Background

- A large proportion of melanoma patients fail to achieve durable benefit from immune checkpoint blockade (ICB) targeting PD-1 and CTLA-4, and the underlying biological determinants of resistance to ICB remain poorly understood.
- Recent studies have highlighted the importance of the spatial organisation of cells in the tumour microenvironment (TME), which influences key cellular interactions and functional states that in turn, affect tumour progression and therapeutic response.
- However, prior studies were limited by small sample sizes, focused on response rather than resistance, and used low spatial resolution to profile gene expression.

Objectives

- To develop an interpretable deep learning-based framework to accurately predict resistance to ICB from single-cell spatial transcriptomics.
- Capture cellular spatial neighbourhoods, phenotypes, and interactions contributing towards response and resistance across large cohorts of melanoma patients.
- Examine the role of lymphoid aggregates in response versus resistance to ICB.
- Discover potential therapeutic targets by identifying biomarkers and signalling pathways associated with resistance.

Methods

Patient cohorts

Advanced cohort (n = 91)
Unresectable stage III/IV melanoma
Metastatic treatment setting

Responders n = 53
Non-responders n = 38
Total samples: 192

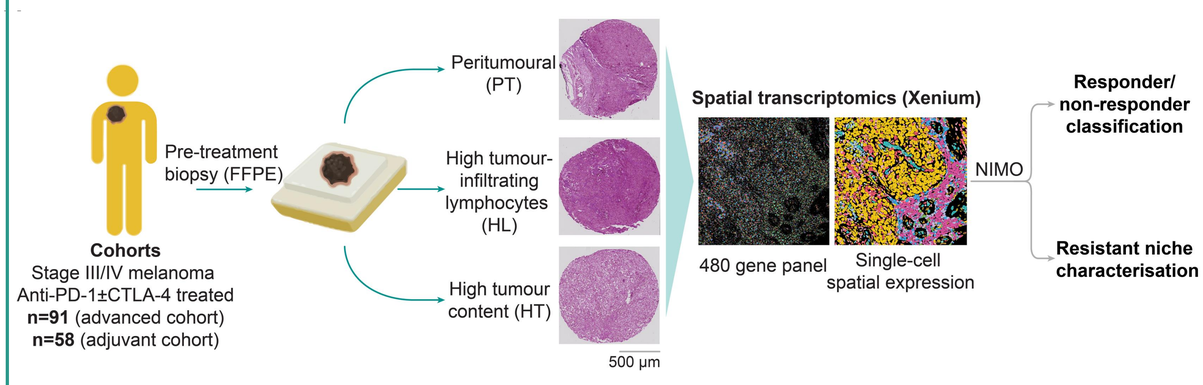
Responders: Progression-free survival (PFS) > 6 months.
Non-responders: PFS < 6 months.
anti-PD-1: n = 42
anti-PD-1 + anti-CTLA-4: n = 49

Adjuvant cohort (n = 58)
Stage III resectable melanoma
Adjuvant treatment setting

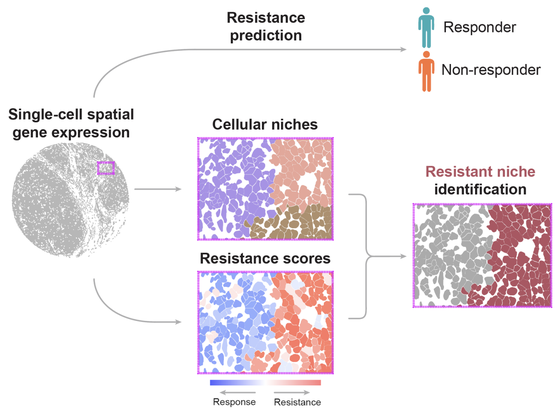
Responders n = 34
Non-responders n = 24
Total samples: 95

Responders: Recurrence-free survival (RFS) > 12 months.
Non-responders: RFS < 12 months.
anti-PD-1: n = 46
anti-PD-1 + anti-CTLA-4: n = 12

Capturing the single-cell spatial landscape of melanoma



NIMO (Niches and biomarkers of IMmunOtherapy resistance)



Results

Distinct spatial niches are associated with response and resistance

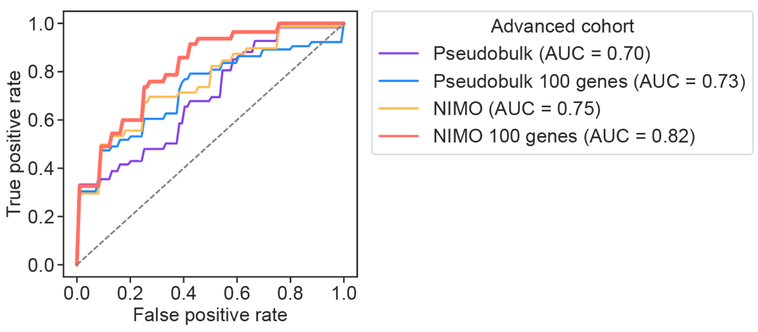


Figure 1. ROC AUC curves of different approaches for resistance prediction in the advanced cohort. NIMO achieved AUC of 0.79 on the adjuvant cohort without further tuning.

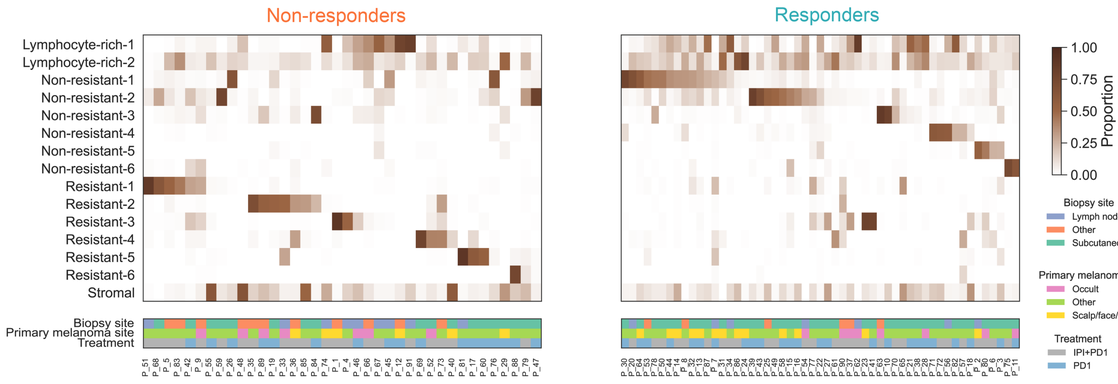


Figure 2. Proportions of niches within each patient in the advanced cohort. Six Resistant niches were identified, which were enriched in non-responders across both cohorts.

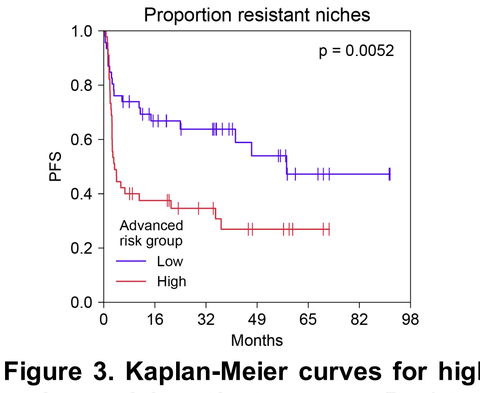


Figure 3. Kaplan-Meier curves for high and low risk patient groups. Resistant niche proportion stratified patients by PFS risk.

Signatures and signalling pathways in niches associated with ICB resistance

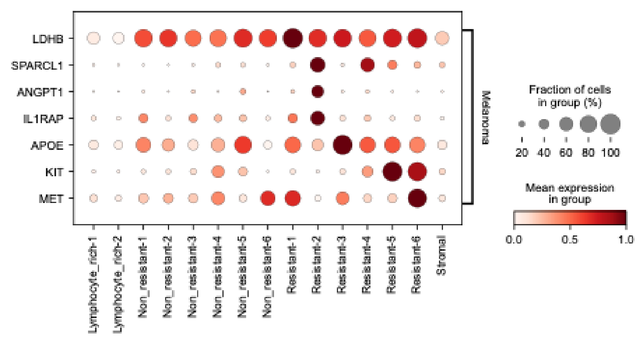


Figure 4. Top differentially expressed genes (DEGs) in melanoma cells of resistant niches, showing distinct expression patterns.

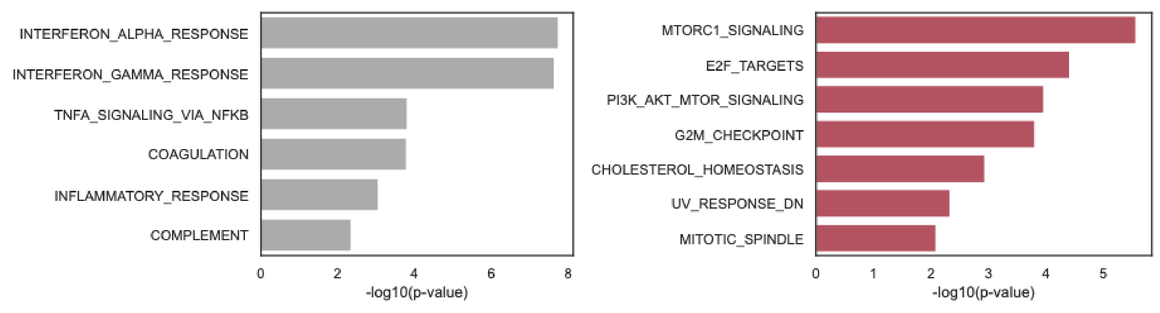


Figure 5. Pathway enrichment analysis based on hallmark gene set enrichment of DEGs in melanoma cells in non-resistant and resistant niches (p-value < 0.01).

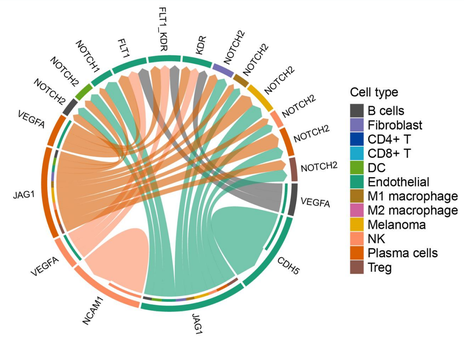


Figure 6. Elevated LR signalling within Resistant niches, highlighting the JAG1-NOTCH axis.

Non-responders show altered cellular composition and immune status within lymphocyte-rich niches

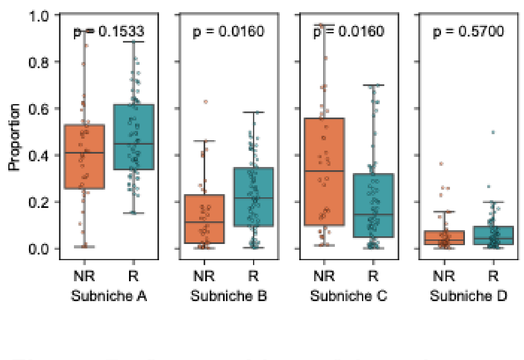


Figure 7. Composition of Lymphocyte-rich-1 subniches. Subniche B was enriched in Responders (R) and subniche C was enriched in Non-responders (NR).

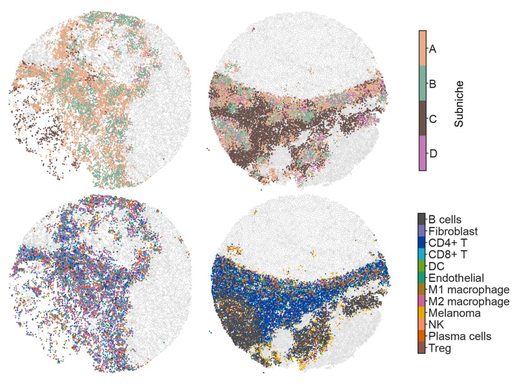


Figure 8. Organisation of subniches B and C. NR had lower infiltration of B and higher infiltration of C in intratumour regions.

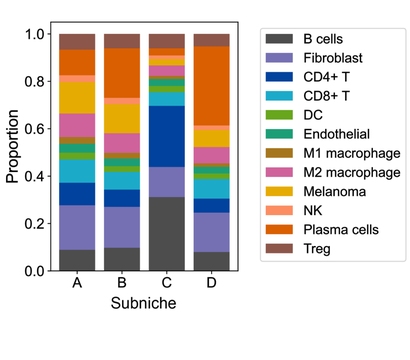


Figure 9. Cell type proportions in subniches. C had higher proportions of B and CD4+ T cells.

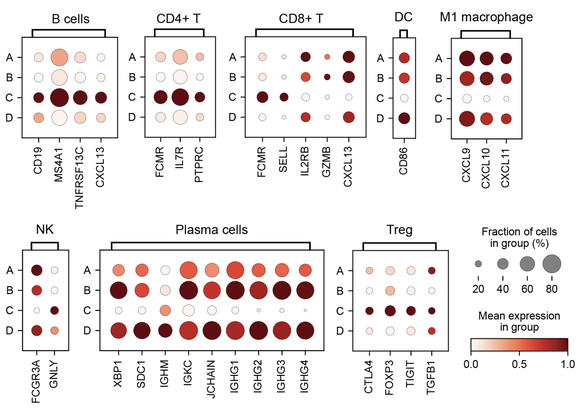


Figure 10. Expression of markers across cell types in lymphoid aggregate subniches. Subniche C (enriched in NR) showed distinct molecular profiles.

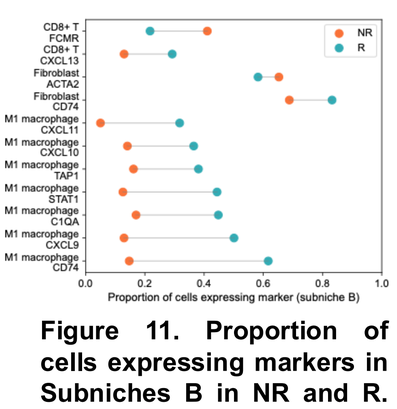


Figure 11. Proportion of cells expressing markers in Subniches B in NR and R. Subniche B, which had a favourable molecular profile towards response, is further compromised in NR.

Conclusions

- Single-cell spatial transcriptomics revealed heterogeneity of resistance to ICB across large melanoma cohorts.
- We identified distinct resistance-associated cellular niches with distinct melanoma phenotypes, elevated endothelial cell signalling, and expression of potential drug targets.
- We further reveal a lymphoid aggregate profile in non-responders exhibiting a molecular signature that fails to support effective antitumour immunity, including elevated immunoregulation and diminished chemokine expression.
- Our findings underscore the spatial-molecular complexity of ICB resistance and suggest therapeutic opportunities targeting multiple TME components.

Acknowledgements

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xiaohang.fu@melanoma.org.au