Spatial organization of the tumor immune microenvironment (TIME) in primary and metastatic melanoma is associated with patient outcome

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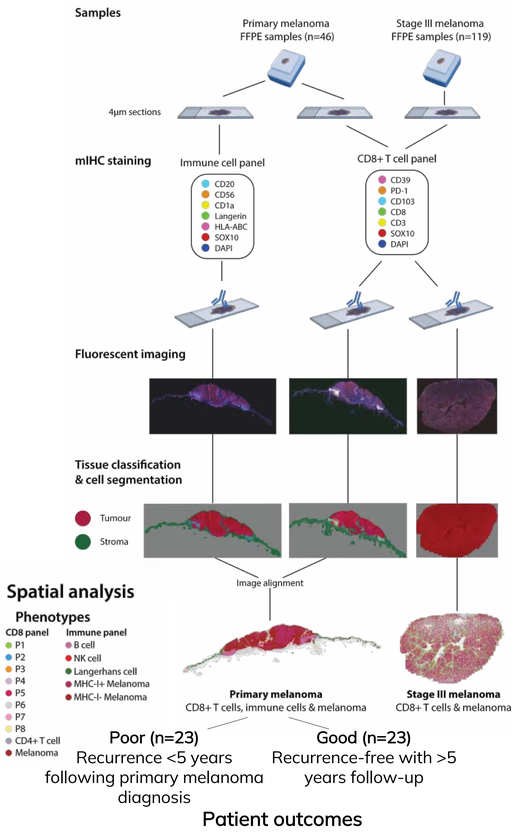
Introduction

- Immunospatial interactions in the melanoma TIME provide insights into immune cell functions[1-3]
- TIME immune cells often form aggregates – for example tertiary lymphoid structures, which are sites of a localized anti-tumour immune response
- However, immune aggregates are difficult to computationally identify and characterize, and immune interaction networks are intricate and complex
- Novel multiplex immunofluorescence (mIHC) and spatial analytic techniques enable comprehensive investigation of the immunospatial TIME (isTIME) in melanoma[4-6]
- Immunospatial structures & interactions revealed by this analysis could indicate immune mechanisms of melanoma clearance and recurrence

This project aims to:

1. Determine immunospatial patterns associated with patient outcome in primary melanoma
2. Develop a high-throughput cohort-wide workflow for tumor immunospatial analysis

Methods



Identification of immune neighborhoods from mIHC-stained primary melanoma tissue using SPIAT[7,8]

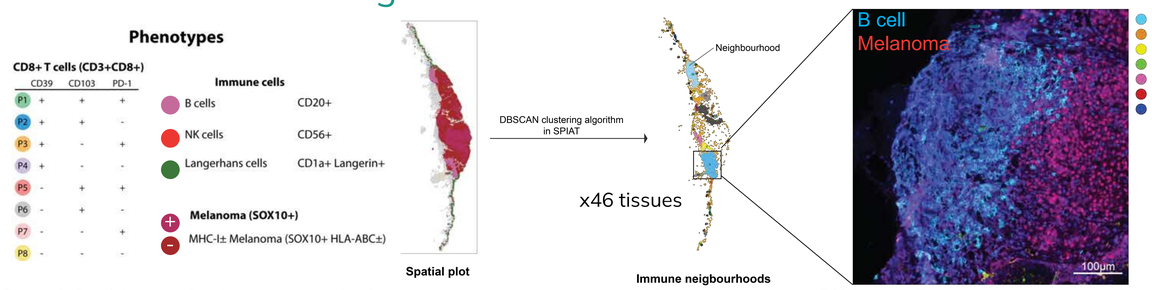


Figure 1. Spatial maps of melanoma comparing immune phenotypes were generated from whole-tumour mIHC images. These maps were exported to the SPIAT package in R[3,4], which used the DBSCAN algorithm to identify distinct cell clusters, or 'neighborhoods'. Neighborhoods were visually compared with mIHC images to assess algorithm accuracy.

Neighborhood metacluster (NMC) analysis finds patterns of neighborhood composition associated with patient outcomes

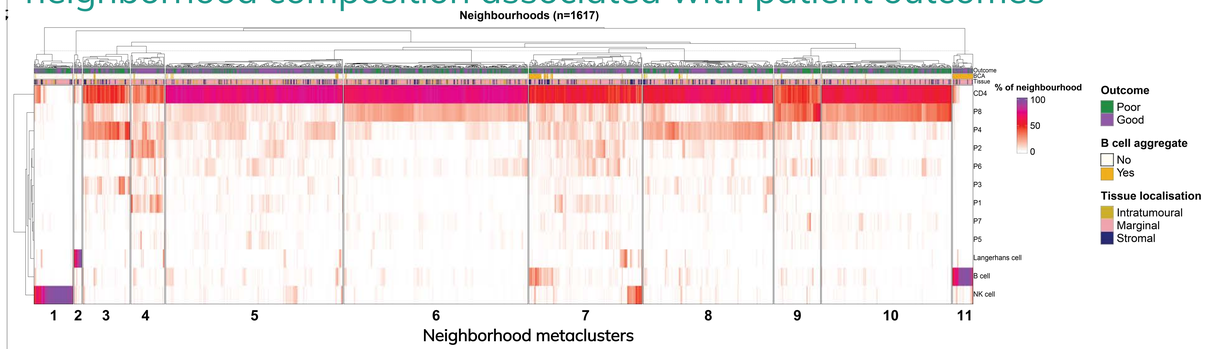


Figure 2. Heatmap showing immune composition of 1617 neighborhoods identified from 46 primary melanoma and annotated with patient outcome, B cell composition (>10% B cells = B cell aggregate), and tissue localization. Neighborhoods were then divided into 11 'neighborhood metaclusters' (NMCs) by k-means clustering based on their cellular composition.

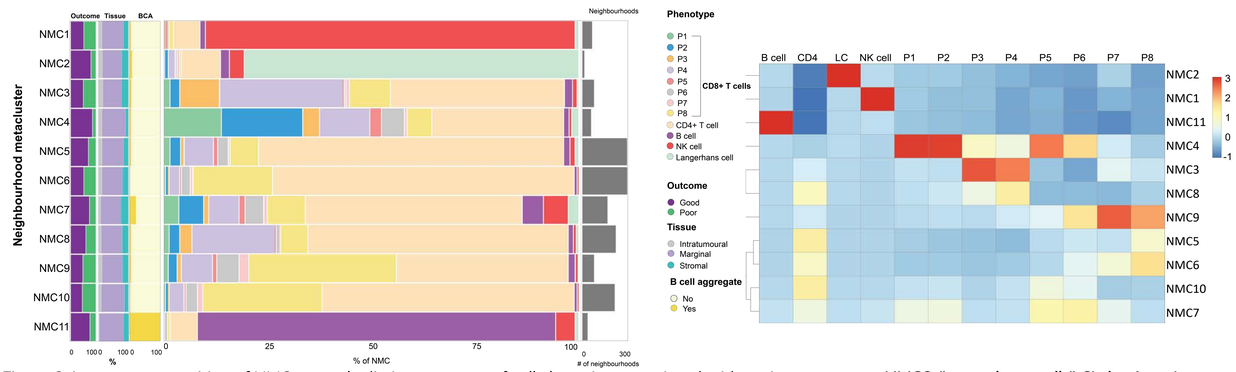
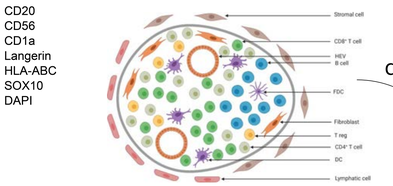


Figure 3. Immune composition of NMCs reveals distinct patterns of cell clustering associated with patient outcomes. **NMC2 (Langerhans cell (LC) dominant), NMC4 (CD39+CD103+ CD8+ T cell^{hi}), and NMC11 (B cell dominant)** were the most likely to be observed in patients with good outcomes, while **NMC3, NMC9, NMC10** all featured high levels of CD103-PD-1- CD8+ T cell populations and were the mostly likely to be observed in patients with poor outcomes

B cell aggregate (BCA) profiles resemble tertiary lymphoid structures



B cell aggregates (BCAs) are precursors of tertiary lymphoid aggregates[9,10] and were analyzed separately

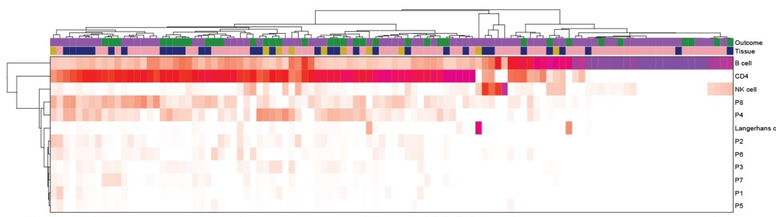


Figure 4. Heatmap showing immune composition of 106 BCA neighborhoods, annotated with patient outcome and tissue localization.

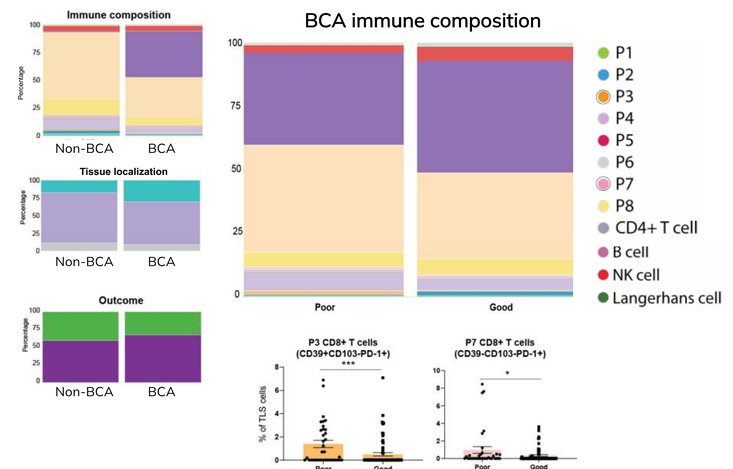


Figure 5. BCA and non-BCA profiles were compared based on immune composition, tissue localization and patient outcomes, with BCAs more likely to be stromal and dominated by B cells. BCA profiles were compared between patients based on their outcome group, with two CD103-PD-1+ CD8+ T cell populations – P3 and P7 –significantly increased in patients with poor outcomes.

Conclusions

- NMC composition is closely associated with patient outcome, with the presence of B cell, CD39+CD103+ CD8+ T cell, and LCs linked to recurrence-free survival
- BCAs can be reliably identified using neighborhood analysis in SPIAT
- CD103-PD-1+ CD8+ T cells are increased in the BCAs of melanoma patients with poor outcomes
- BCAs may represent trafficking sites for non-tumour-reactive CD8+ T cells in patients who recur

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Image sources

Methods figure: created in Biorender
TLS image: Munoz-Erazo 2020, *Nature*

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