Single-cell, high-throughput spatial profiling of in-transit and lymph node metastases reveals distinct immune populations and spatial regions linked to recurrence and immunotherapy response

Eva R. Shteinman[1,2,3], Xinyu Bai[1,2,3], Yizhe Mao[1,2,3], Paola Cornejo-Paramo[1,2,3], Nurudeen A. Adegoke[1,2,3], Nigel G. Maher[1,2,3,4], Camelia Quek[1,2,3], Nicola Waddell[5], Nicholas K. Hayward[5], John V. Pearson[5], John F. Thompson[1,3,7], Robyn P. M. Saw[1,3,7], Andrew J. Spillane [1,3,6], Mainthan Palendira[2,3], Inês Pires da Silva[1,3,6], Richard A. Scolyer[1,2,3,4], Georgina V. Long[1,2,3,6], James S. Wilmott[1,2,3,*], Ismael A. Vergara[1,2,3,*]

[1]Melanoma Institute Australia, The University of Sydney, Sydney, Australia; [2]Charles Perkins Centre, The University of Sydney, Sydney, Australia; [3]Faculty of Medicine and Health, The University of Sydney, Sydney, Australia; [4]Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital & NSW Health Pathology, Sydney, Australia; [5]Cancer Program, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia ; [6]Department of Medical Oncology, Royal North Shore and Mater Hospitals, Sydney, Australia; [7]Department of Melanoma and Surgical Oncology, Royal Prince Alfred Hospital, Sydney, Australia

Background

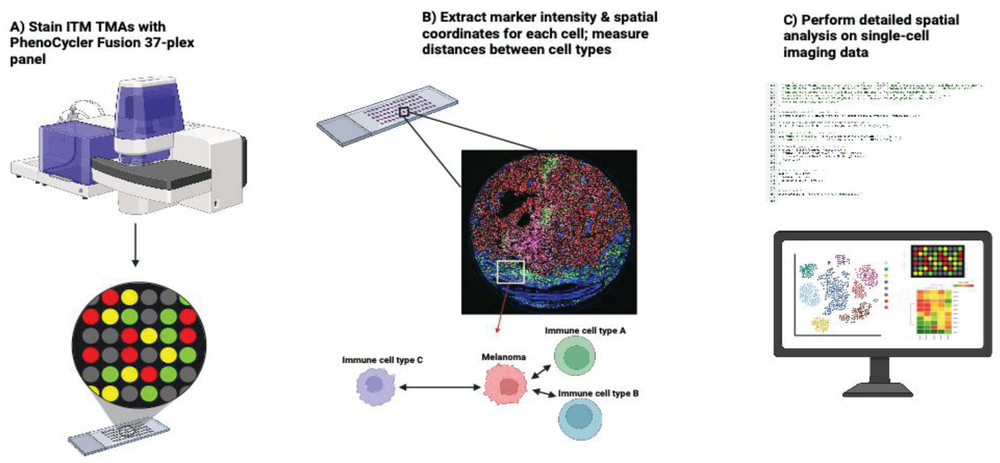
The differential biology of in-transit metastases (ITMs) compared to lymph node metastases (LNMs) is not well understood, impacting the ability to predict recurrence and response to immune checkpoint inhibitors (ICIs).

Objectives

Here, we aim to identify features in ITM and LNM that are (i) predictive of ICI response, (ii) prognostic of disease aggressiveness, and (iii) indicative of differential biology between ITM and lymph node metastases (LNMs).

Methods

- A cohort of 271 samples were collected from ITM and LNM patients subsequently treated with ICIs (ITM n=41, LNM N=20), as well as surgery-alone ITM (N=90) and LNM (N=120) patients.
- Snap-frozen ITMs (n=124) and LNMs (n=112) underwent whole-genome sequencing for genomic profiling.
- For immune profiling, FFPE tissue from representative intratumour sections of ITMs (n=69) and LNMs (n=65) were used to create tumour micro-arrays for high-plex image analysis with PhenoCycler Fusion (*Akoya Biosciences*) using a 37-plex panel targeting melanoma, immune and stromal cell populations.



Results

1. ITMs have higher intratumour myeloid cell proportions, including M2 macrophages, while LNMs have higher B cell infiltration

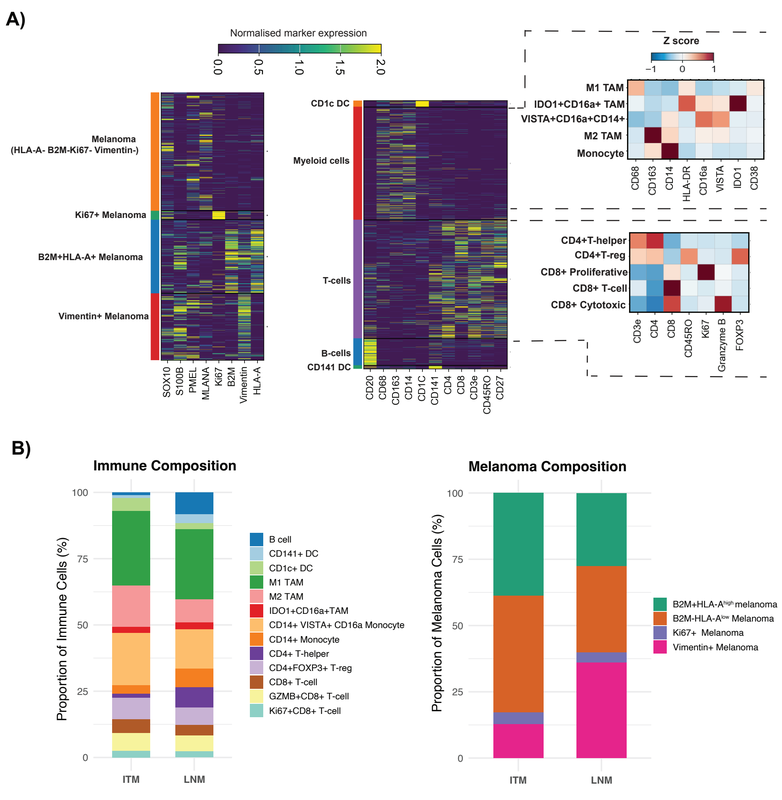


Figure 1A. PhenoCycler Fusion imaging was used for single-cell characterisation of the melanoma & immune landscape across ITM and LNM tumour micro-arrays

Figure 1B. LNMs (n=65) were enriched for Vimentin+ melanoma (p<0.00001) and B-cells (p=0.003)

ITMs (n=69) had greater proportions of overall myeloid cells (0.012) and M2 macrophages (p=0.001)

2. In ITM, ICI responders have closer proximities between melanoma cells and distinct T-cell and macrophage populations

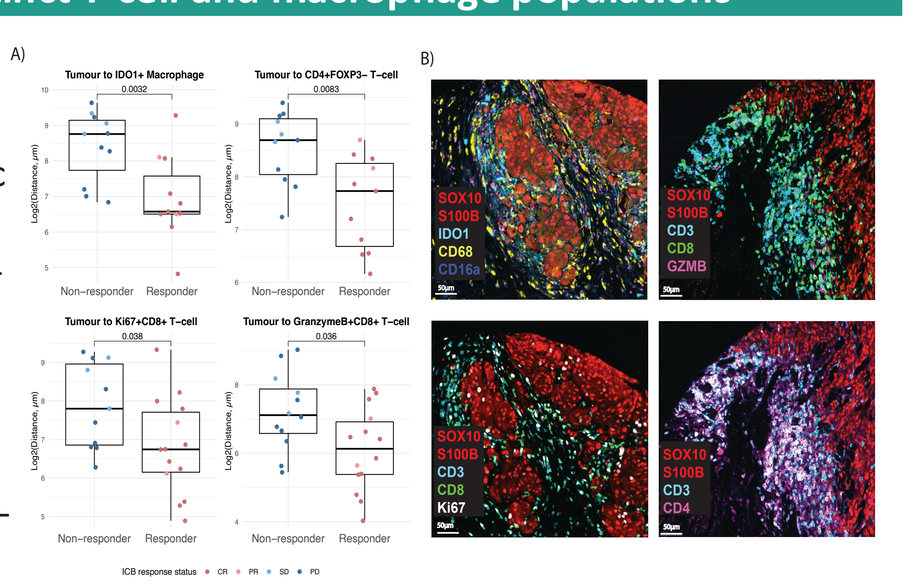


Figure 2. In pre-ICI -treatment ITMs (n=28 treated in the metastatic setting), melanoma cells were closer to distinct T-cell & macrophage populations, incl. IDO1+CD16a+HLA-DR+ TAMs in ICI responders (n=14) compared to non-responders (n=14)

3. In LNMs, distinct T-cell and macrophage populations are prognostic for distant metastasis and survival

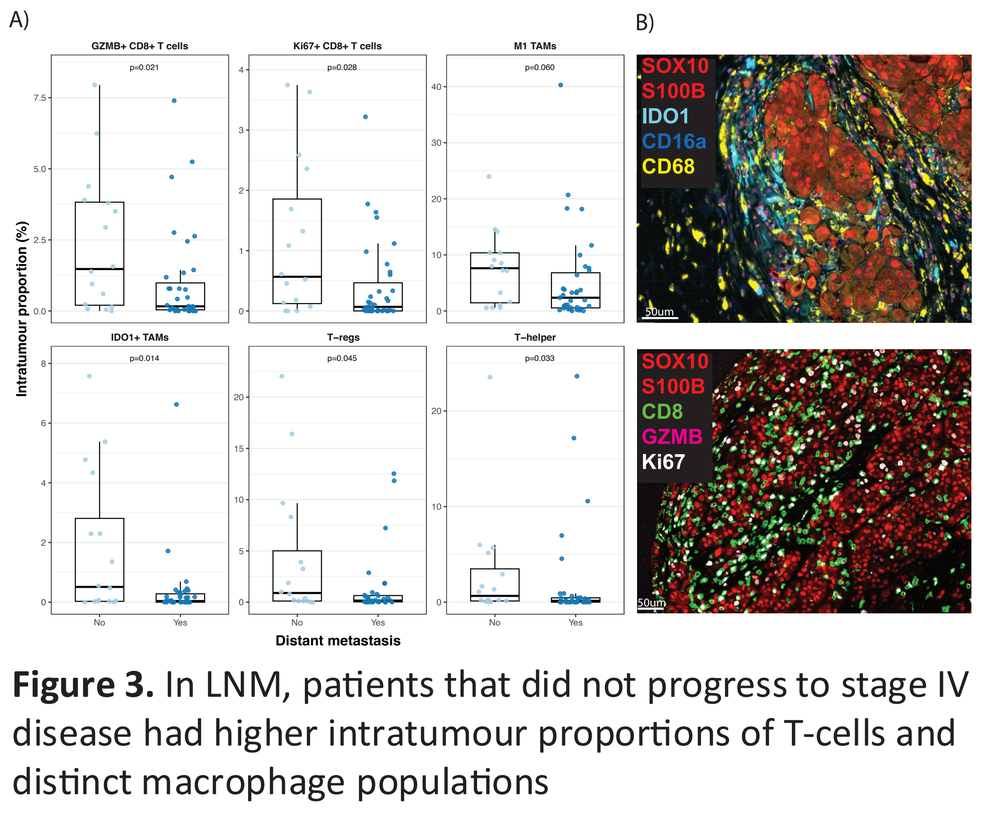


Figure 3. In LNM, patients that did not progress to stage IV disease had higher intratumour proportions of T-cells and distinct macrophage populations

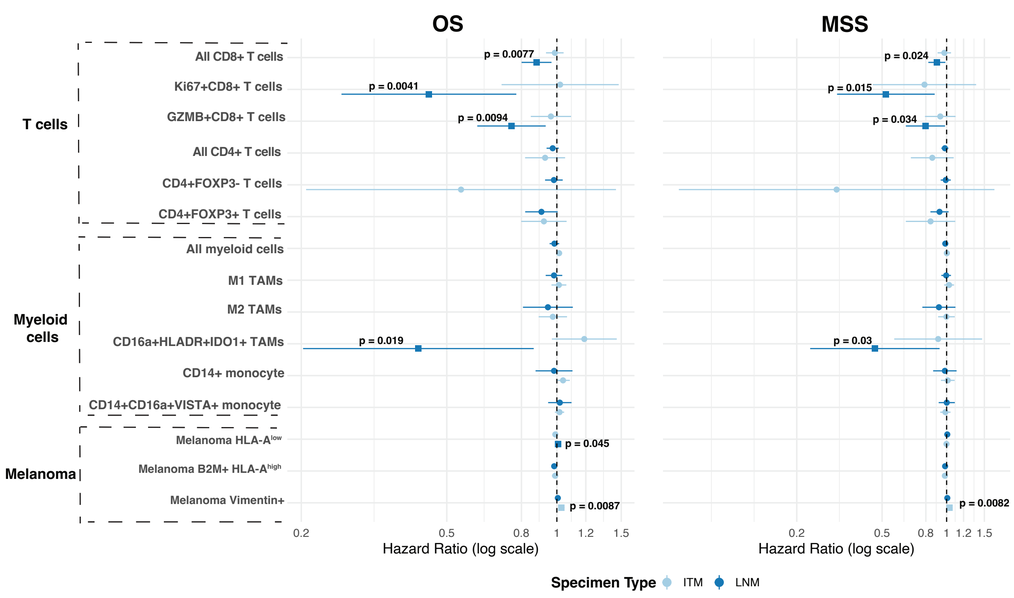


Figure 4. In LNM, CD8+ T-cells and IDO1+CD16a+HLA-DR+ TAMs were associated with improved survival, while Vimentin+ melanoma was associated with worse survival in ITM

Conclusions

There are **distinct predictive & prognostic roles** of immune and melanoma cell populations in ITMs and LNMs, and a differential biology between tumour types. Analysis of the genomic landscape is ongoing

Acknowledgements

- Eva Shteinman received conference support funding from Sydney Cancer Partners via Cancer Institute NSW (2021/CBG0002)
- Melanoma Institute Australia & The University of Sydney

Proudly supported by Cancer Institute NSW

Sydney Cancer Partners
Collaboration, Translation, Impact