

Single cell spatial features of in-transit melanoma associated with patient outcome to immunotherapy

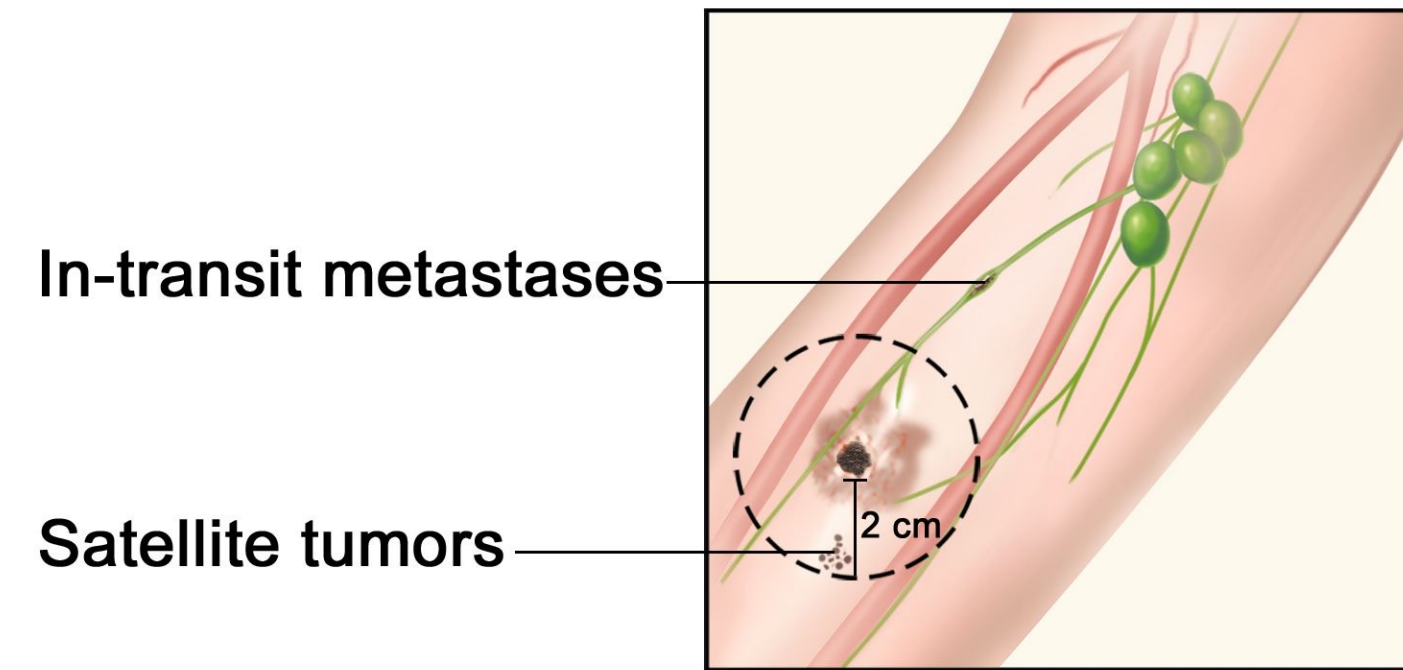
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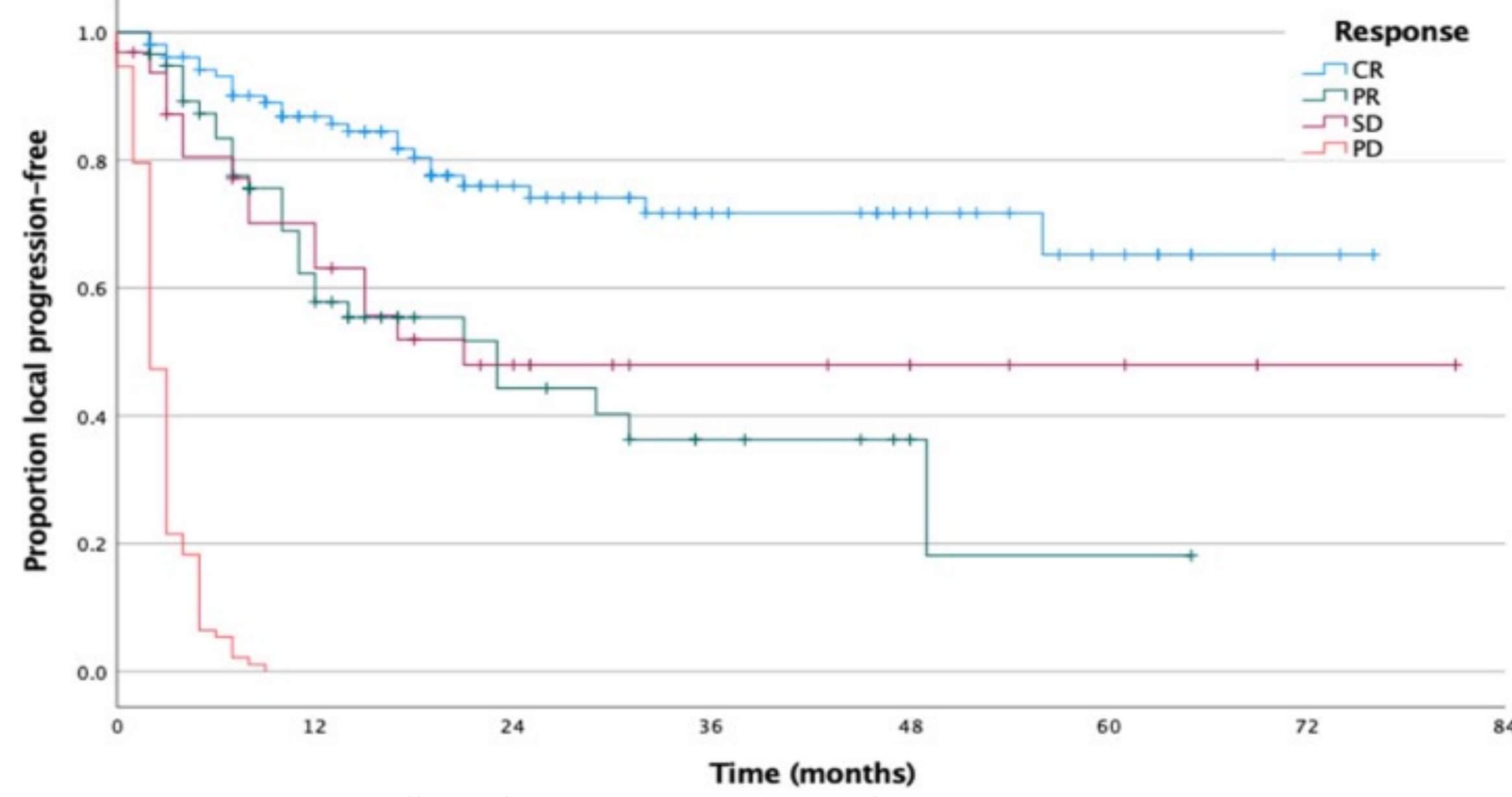


Background

- Melanoma in-transit metastasis (ITM) refers to lesions between the primary melanoma and its nearest regional lymph node basin.
- ITM occurs in up to 20% of patients with cutaneous melanoma.



- Anti-PD-1-based immunotherapy is used when patients develop unresectable stage III disease or distant metastasis (stage IV).
- The tumour microenvironment (TME) of ITM melanoma remains poorly understood, where distinct cellular and spatial features can influence patients' outcome to therapy. This study aims to characterise the spatial TME in ITM melanoma patients.



Holmberg CJ, Ny L, Hieken TJ, et al. The efficacy of immune checkpoint blockade for melanoma in-transit with or without nodal metastases - A multicenter cohort study. *Eur J Cancer*. 2022;169:210-222. doi:10.1016/j.ejca.2022.03.041

Objectives

- To deeply characterise the TME spatial features of ITM melanoma.
- To understand the features of primary and acquired resistance to anti-PD-based immunotherapies.
- To identify novel drug targets to circumvent these resistance mechanisms.

Conclusions

- ITMs that completely regressed following systemic checkpoint therapies demonstrated co-localisation of T cells, B cells and HLA-A⁺ melanoma cells.
- Baseline samples from ITM that progressed post treatment demonstrated tumour heterogeneity and immune exclusive features.
- Expression of alternate immune checkpoint receptors (LAG3, TIM3, ICOS, VISTA) in resistant patients suggest novel combinatory targets.

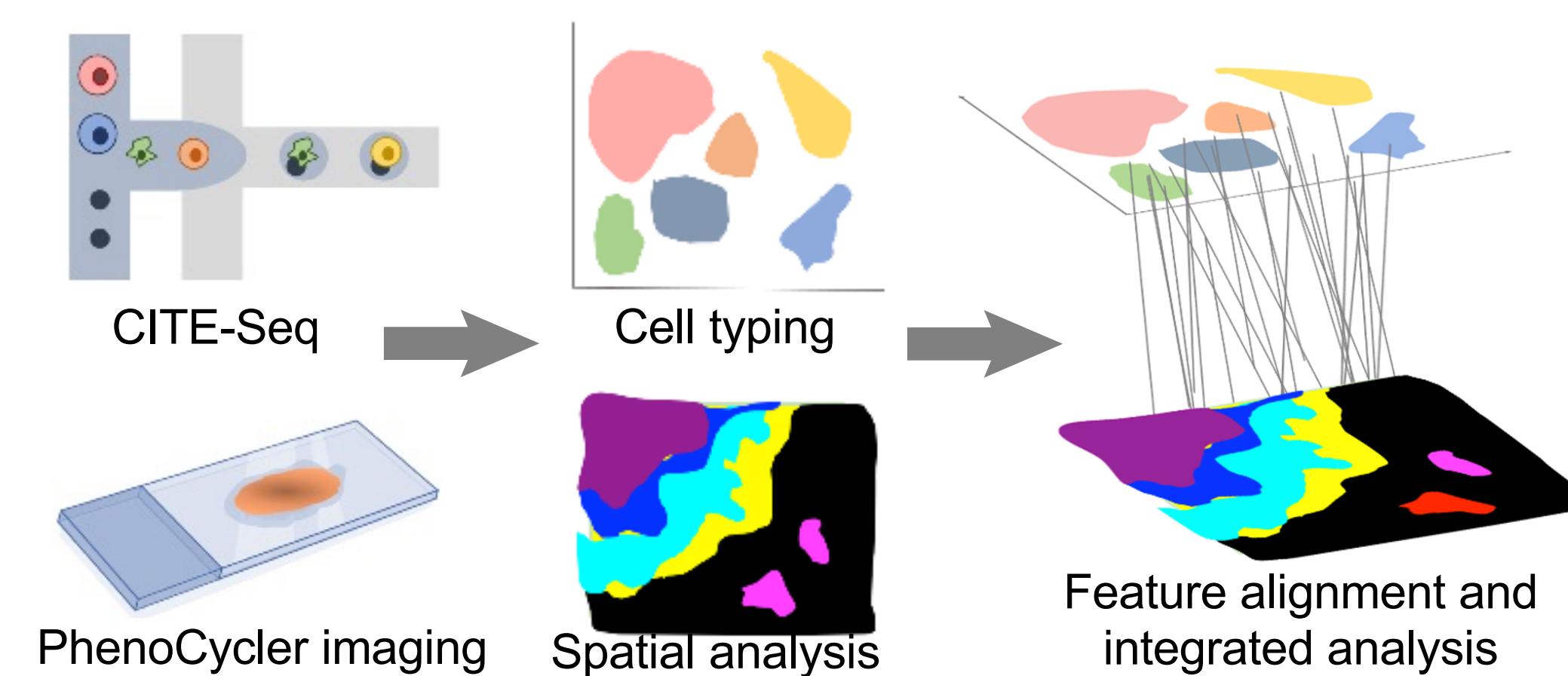
Our results demonstrate patterns of immune cell recruitment, functional phenotypes and cellular neighbourhoods associated with immunotherapy response and tumour progression/therapy resistance in ITM melanoma patients treated with immunotherapy.

Methods

- We performed 40-plex PhenoCycler imaging on whole-tissue slides from 20 ITM melanoma patients treated with ICI, 10 biopsied at baseline and 10 biopsied at the time of disease progression.

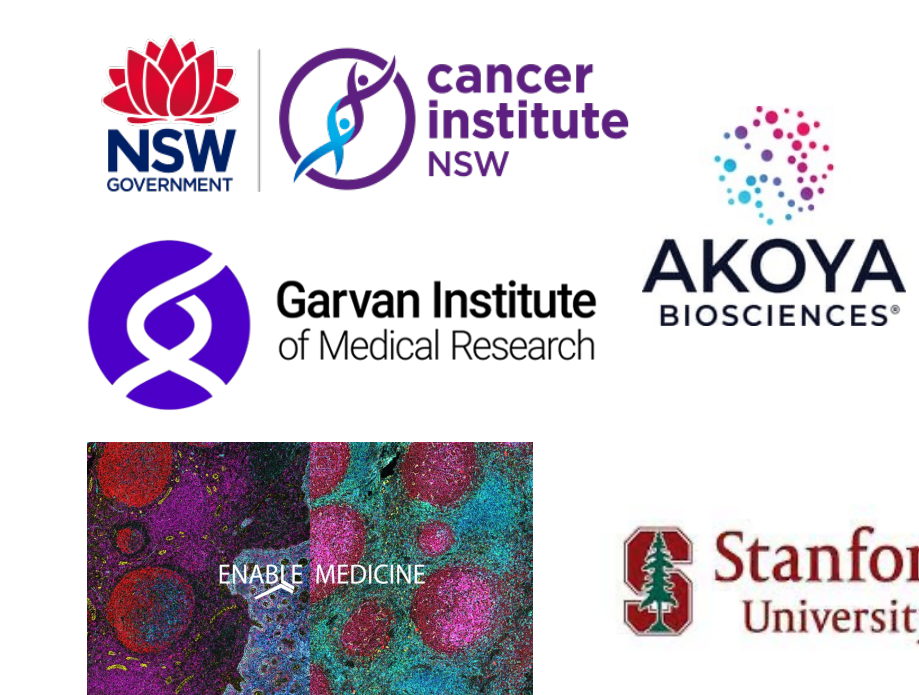
Patient characteristics	PRE (n=10)	PROG (n=10)
Stage		
IIIc	2	1
IV (M1a)	0	2
IV (M1b)	2	3
IV (M1c)	3	3
IV (M1d)	3	1
Treatment		
Adj. anti-PD-1	1	1
Adj. anti-PD-1+anti-CTLA-4	0	1
Met. anti-PD-1	4	4
Met. anti-PD-1+anti-CTLA-4	5	4
RECIST response (met. only)		
CR	4	0
PR	2	1
PD	3	7
Recurred/Progressed?		
Yes	5	10
No	5	0

- Using integrated bioinformatic analysis, we characterised the cellular and spatial profiles of ITM TME and investigated the features associated with immunotherapy response and resistance.



Acknowledgement

- XB has no conflicts of interest to declare.



Result 1. Spatially defined immune cell interactions are enriched in responders to immunotherapy

- With spatial and cell interaction analysis, we showed that the pre-treatment TME in responders were enriched with spatial neighbourhoods harbouring T cell and B cell interactions, as evident by cell colocalisation, interaction scores and neighbourhood enrichment scores.

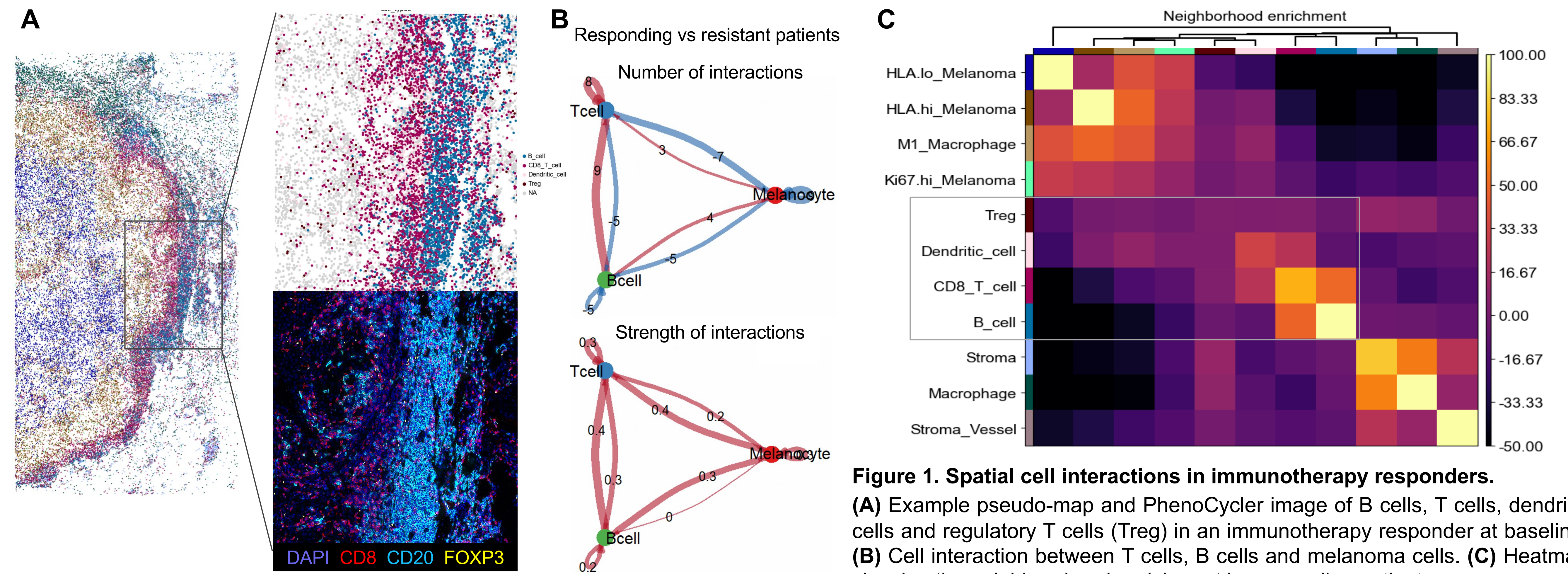


Figure 1. Spatial cell interactions in immunotherapy responders. (A) Example pseudo-map and PhenoCycler image of B cells, T cells, dendritic cells and regulatory T cells (Treg) in an immunotherapy responder at baseline. (B) Cell interaction between T cells, B cells and melanoma cells. (C) Heatmap showing the neighbourhood enrichment in responding patient.

Result 2. Melanoma heterogeneity and immune exclusion are associated with immunotherapy resistance

- Tumours in ITM patients resistant to immunotherapy have heterogeneous phenotype and transcriptional profiles, with intrinsic and extrinsic features associated with dampened immune recruitment and T cell infiltration.

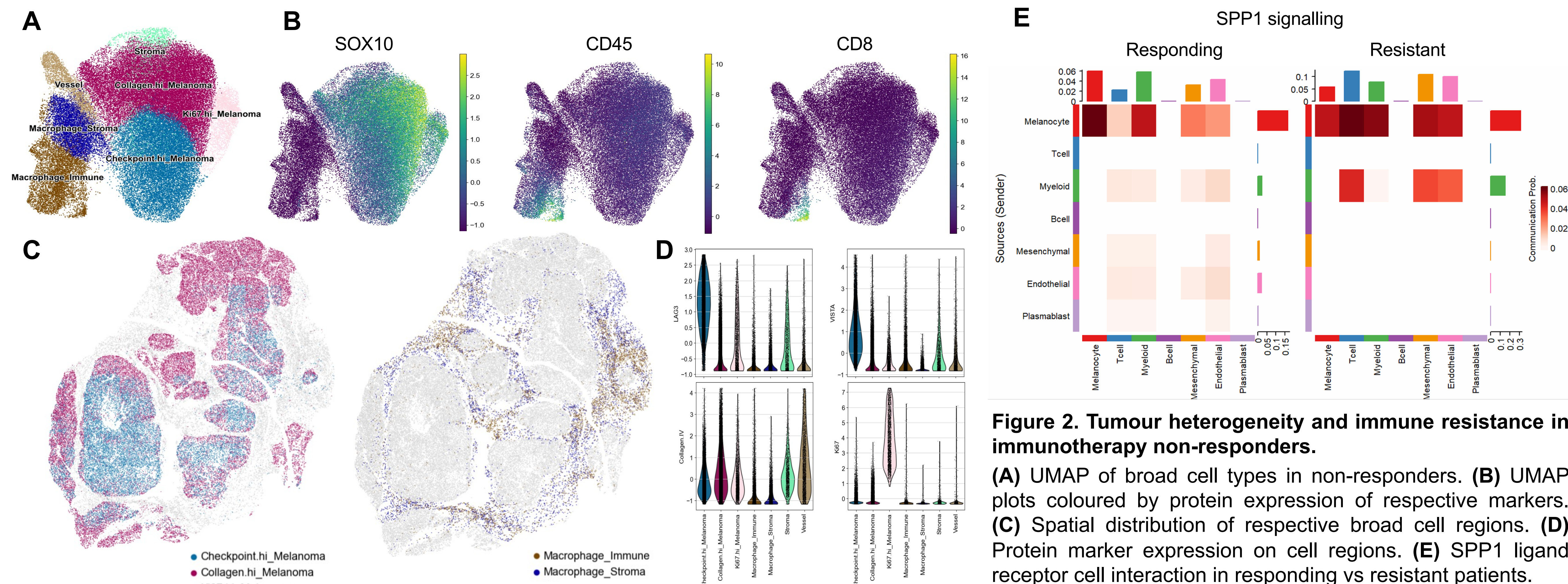


Figure 2. Tumour heterogeneity and immune resistance in immunotherapy non-responders. (A) UMAP of broad cell types in non-responders. (B) UMAP plots coloured by protein expression of respective markers. (C) Spatial distribution of respective broad cell regions. (D) Protein marker expression on cell regions. (E) SPP1 ligand receptor cell interaction in responding vs resistant patients.

