Spatial analysis characterises cellular immunotypes of in-transit melanoma associated with response to anti-PD1-based checkpoint inhibitor immunotherapy

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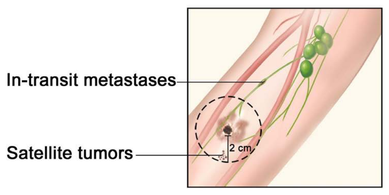
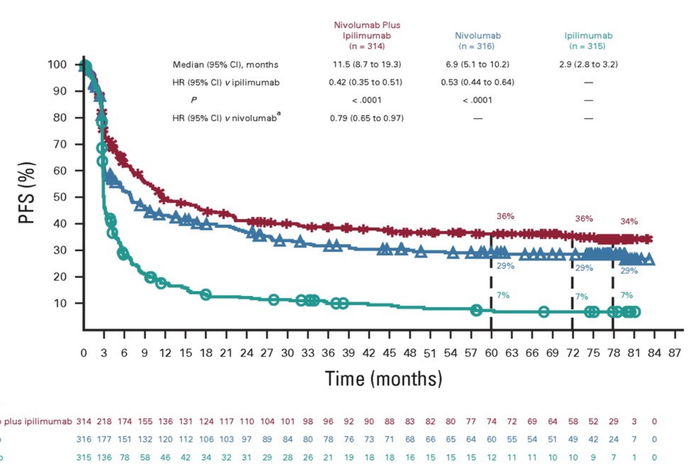
Background

- In-transit metastasis (ITM) refers to metastatic lesions between the primary melanoma and nearest regional lymph node basin.
- Patients with ITM are classified as Stage III or above and have a 5-year survival rate of 25-30%.
- The role of anti-PD-1-based immunotherapies in the management of patients with ITMs is evolving. However, the tumour microenvironment (TME) of ITM remains poorly defined, where distinct cellular constitution, intercellular interactions and molecular signals may influence tumour progression and therapy outcomes.





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.

Methods

- We performed 41-plex PhenoCycler imaging on whole-tissue slides from 20 ITM melanoma patients treated with ICI, 10 biopsied at baseline and a further 10 biopsies on treatment at the time of progressive disease.
- High resolution imaging was used to characterise the phenotypes of cancer, stroma and immune cells, and architecture of the TME using advanced bioinformatics analyses including deep learning classifier, spatial deconvolution, and expression profiling.

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

Samples retrieved at pre-treatment, PRE; at progression, PROG. Treated in adjuvant setting, adj.; in metastatic setting, met.. Complete response, CR; partial response, PR; progressive disease, PD.

Result 1. Immune cell enrichment at the tumour invasive margin in patient with complete response to immunotherapy

- Pre-treatment ITM sample showed co-location of CD4 T cells (cluster 0), CD8 T cells (cluster 7), B cells (cluster 8) and HLA-A^{high} melanoma cells (cluster 5).

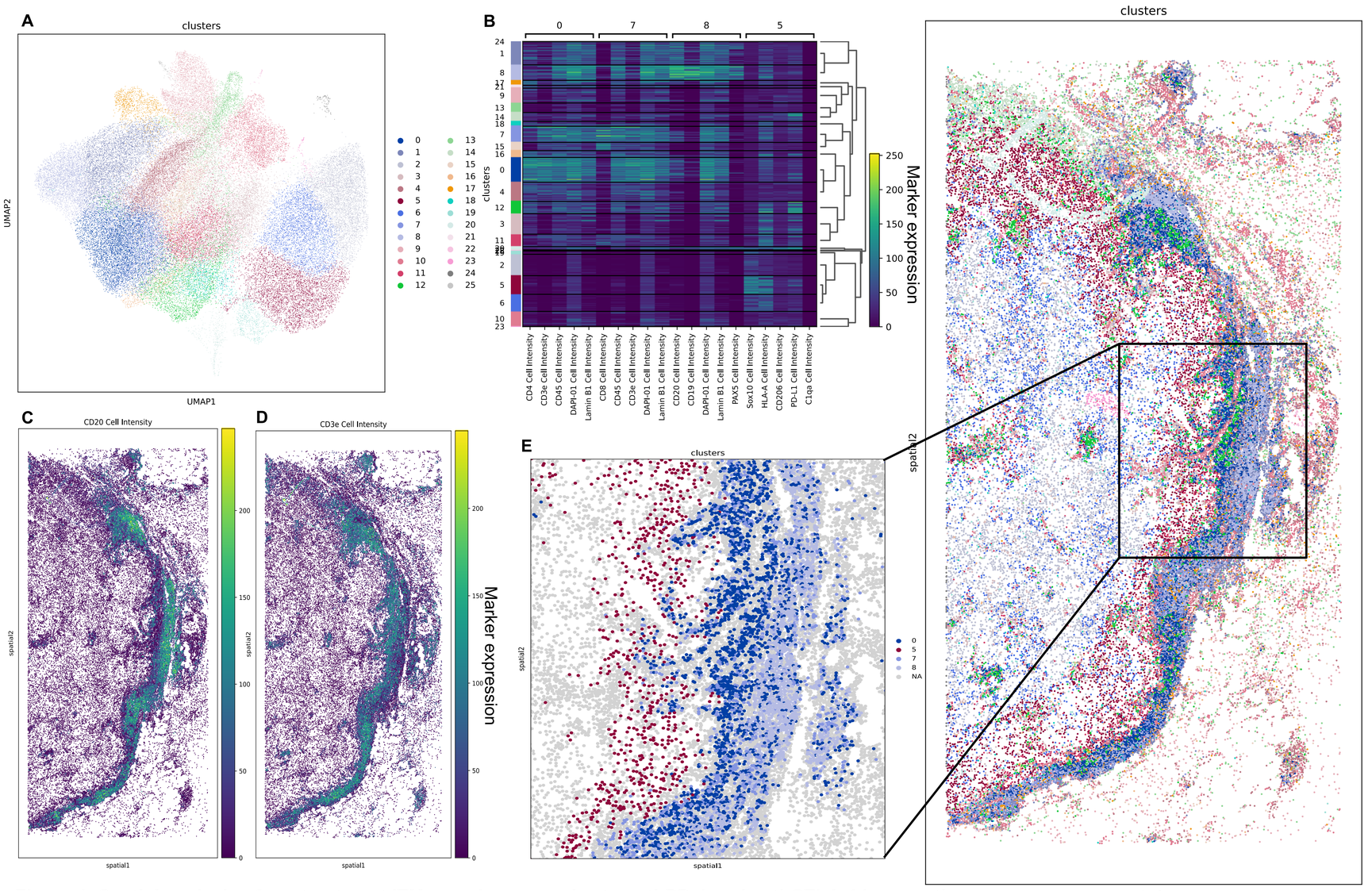


Figure 1. Spatial analysis of pre-treatment ITM sample responsive to anti-PD-1 and anti-CTLA-4 immunotherapy.
(A) Clustering (UMAP) of all cells using average cell marker expression. (B) Average cell expression of top 5 markers in clusters of tumour and immune cells. Spatial expression of CD20 (B cell marker) (C) and CD3 (T cell marker) (D). (E) Spatial location of tumour cells and lymphocytes at the invasive margin.

Result 2. Intratumoural heterogeneity in immunotherapy resistant patient

- At pre-treatment, non-responder tumour harboured:
  - Inter-lesional heterogeneity in tumour phenotype, as demonstrated by the expression of proliferative or MHC markers (e.g. Ki67^{high} and Ki67^{low}).
  - Heterogenous Immune cell phenotypes and recruitment within the tumour (high vs low accumulation at the invasive margin).
  - Evasive features (such as collagen deposition forming physical barrier for lymphocyte infiltration) associated with distinct tumour subtype.
- Proliferative tumour subtypes (Ki67^{high}) showed reduced recruitment of lymphocytes and less proliferative tumour subtypes (Ki67^{low}) demonstrate increased collagen deposition.
- This may suggest tumour evolutionary pathways associated with host immunological selection, we are conducting single cell RNA sequencing to understand these mechanisms.

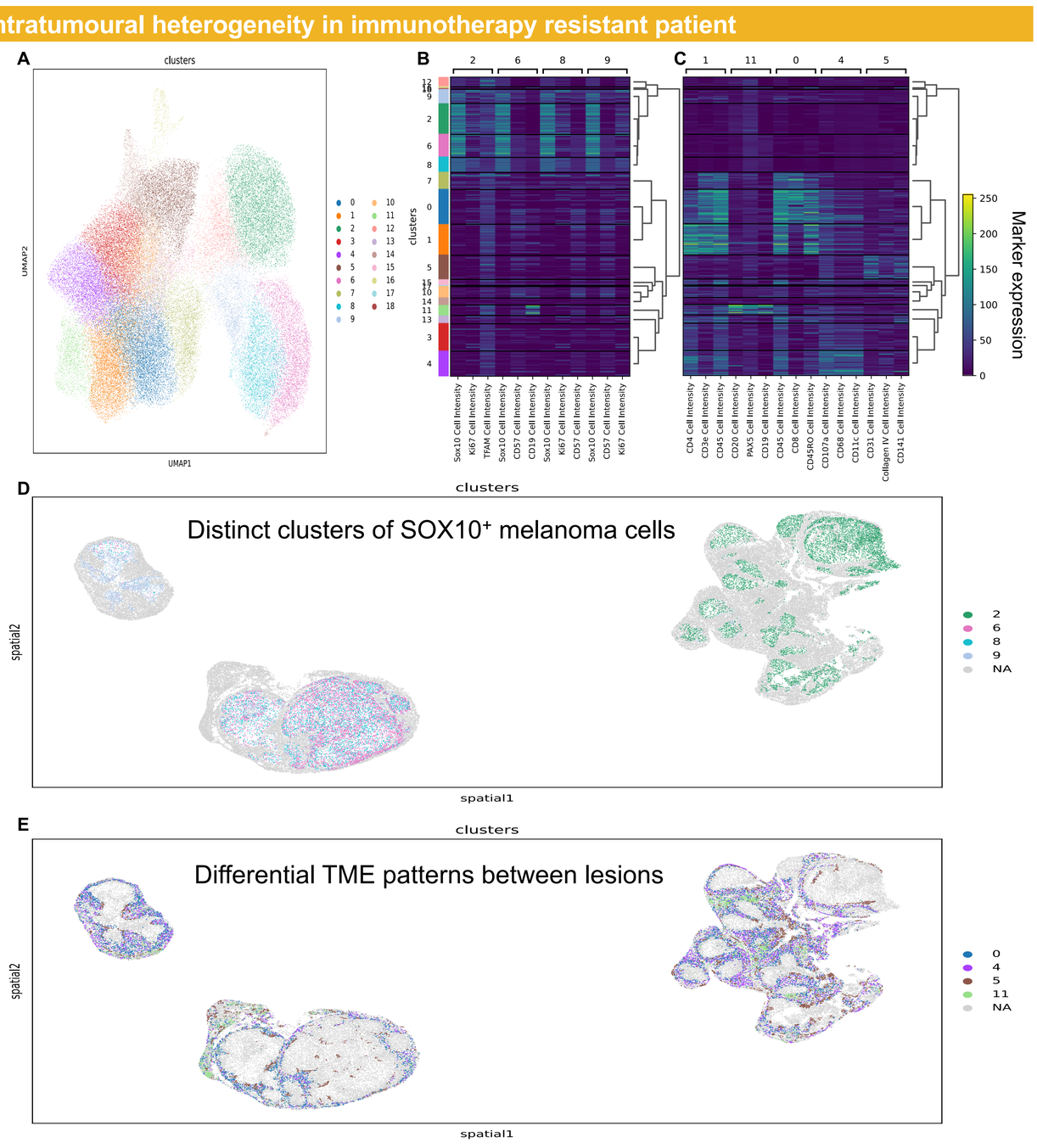
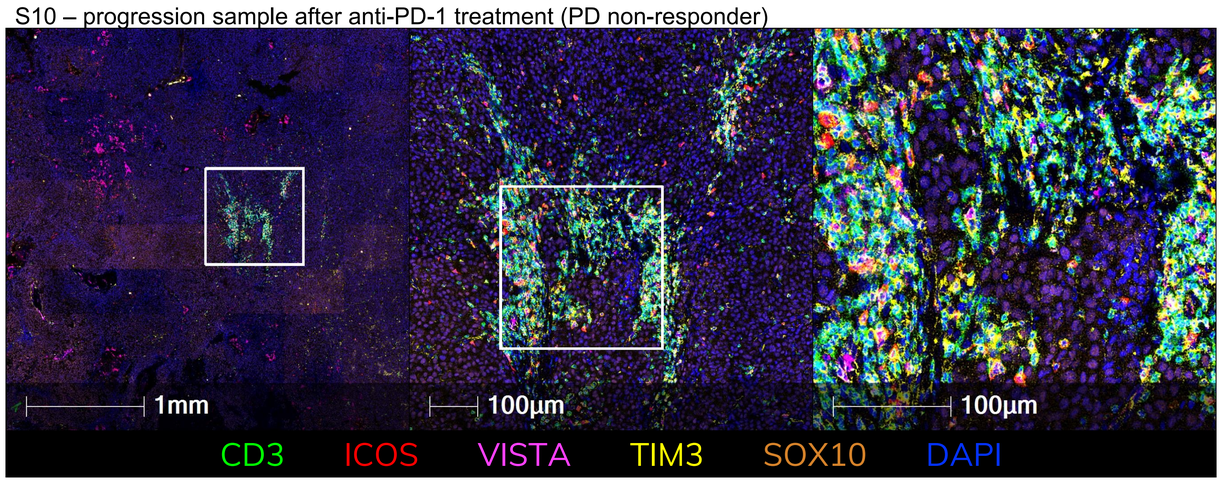


Figure 2. Spatial analysis of pre-treatment ITM sample non-responsive to anti-PD1 immunotherapy.
(A) Clustering (UMAP) of all cells using average cell marker expression. (B) Average cell expression of top 3 markers in clusters of tumour cells. (C) Average cell expression of top 3 markers in clusters of immune and stromal cells. (D) Spatial location of tumour cell clusters (subtypes). (E) Spatial location of immune and stromal cell clusters.

Result 3. Alternative checkpoint receptor expression in non-responder at progression

S10 – progression sample after anti-PD-1 treatment (PD non-responder)



CD3 T cell, ICOS inducible T cell costimulator, VISTA V-domain immunoglobulin suppressor of T cell activation, TIM3 checkpoint receptor, SOX10 melanoma, DAPI nucleus stain

Conclusions

1. ITMs that completely regressed following systemic checkpoint therapies demonstrated co-localisation of T cells, B cells and HLA-A^{+} melanoma cells.
2. Baseline samples from ITM that progressed post treatment demonstrated tumour heterogeneity and immune exclusive features.
3. 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.

References

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