Spatial and multiomics analysis reveals immune interactions as key drivers of immunotherapy outcomes in melanoma patients with in-transit metastases

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Background

- In-transit metastasis (ITM) of melanoma occurs between the primary tumor and draining lymph nodes [1].
- ITM can be managed by adjuvant immune checkpoint inhibitors (ICIs) after surgical resection, or ICI alone when unresectable [2].
- The outcomes for patients with ITM disease following ICI therapies are under-reported in prospective trials [3].
- A small retrospective analysis (n=54) identified that 46% of ITM patients are primarily resistant to ICIs [4].
- Multiomic spatial profiling offers insights into features influencing immunotherapy response, but the tumor microenvironment (TME) and biological determinants of ITM remain poorly defined.

Aims

1. To characterise the molecular profiles and spatial TME landscape in ITM patients.
2. To determine the features associated with immunotherapy outcomes in ITM patients.

Methods

Cohort: Fifty-four samples from ITM patients treated with immunotherapy were collected at pre-treatment (PRE) and progression (PROG).

Patients with a complete or partial response in the advanced setting, or recurrence-free survival over 12 months in the adjuvant setting, were classified as "responsive," and others as "resistant".

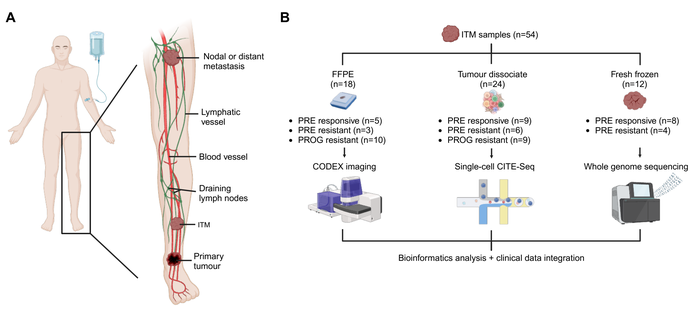

Figure 1. Overview of ITM and study workflow. (A) An illustration showing the sites of primary tumour, ITM and distant metastasis in a melanoma patient treated with ICI. **(B)** Study overview showing the collected ITM samples, breakdown of patients in each response group, and the experimental workflow. Abbreviations: CITE-Seq, cellular indexing of transcriptomes and epitopes; CODEX, co-detection by indexing; FFPE, formalin-fixed paraffin-embedded; ITM, in-transit metastasis; PRE, pre-treatment; PROG, disease progression.

Results

1. Clinical characteristics and response to ICI

Table 1. Clinicopathological characteristics and response outcomes of patients with ITM stratified by responsive and resistance status.

Characteristic	Responsive (n=9)	Resistant (n=17)	P-value*
Age (median, IQR)	67 (57-73)	68 (56-76)	0.852
Sex			
Female	4 (44%)	10 (59%)	0.683
Male	5 (56%)	7 (41%)	
BRAF			
Mutant	5 (63%)	6 (35%)	0.389
Wildtype	3 (38%)	11 (65%)	
Not reported	1	0	
Baseline LDH			
Elevated	1 (13%)	9 (69%)	0.0237
Normal	7 (88%)	4 (31%)	
Not reported	1	4	
Breslow thickness			
<1 mm	0 (0%)	2 (13%)	0.808
1-2 mm	3 (38%)	3 (20%)	
2-4 mm	2 (25%)	5 (33%)	
>4 mm	3 (38%)	5 (33%)	
Not reported	1	2	
Ulcerated primary tumour			
Absent	6 (86%)	6 (43%)	0.159
Present	1 (14%)	8 (57%)	
Not reported	2	3	
Vascular invasion			
Absent	3 (60%)	10 (71%)	>0.999
Present	2 (40%)	4 (29%)	
Not reported	4	3	
Lymphatic invasion			
Absent	3 (75%)	9 (69%)	>0.999
Present	1 (25%)	4 (31%)	
Not reported	5	4	
ITM site			
Upper limb	2 (22%)	3 (18%)	0.936
Lower limb	3 (33%)	6 (35%)	
Trunk	4 (44%)	6 (35%)	
Head & neck	0 (0%)	2 (12%)	
Treatment			
Anti-PD-1	4 (44%)	10 (59%)	0.683
Anti-PD-1 + anti-CTLA-4	5 (56%)	7 (41%)	
RECIST response^			
Complete response	5 (83%)	0 (0%)	<0.0001
Partial response	1 (17%)	1 (7%)	
Progressive disease	0 (0%)	14 (93%)	
Lesion # at treatment^			
1	1 (17%)	2 (13%)	0.802
2-5	3 (50%)	10 (67%)	
>5	2 (33%)	3 (20%)	
Concurrent nodal met^			
Yes	3 (50%)	6 (40%)	>0.999
No	3 (50%)	9 (60%)	
Progression^			
Yes	1 (17%)	15 (100%)	0.0003
No	5 (83%)	0 (0%)	

IQR: interquartile range; LDH: lactate dehydrogenase.
*Mann-Whitney U test for age; Fisher's exact test for contingency tables.
^For patients treated in advanced metastatic setting only (n=21).

2. Spatial and single-cell profiling of the ITM tumour microenvironment and immune cell interactions

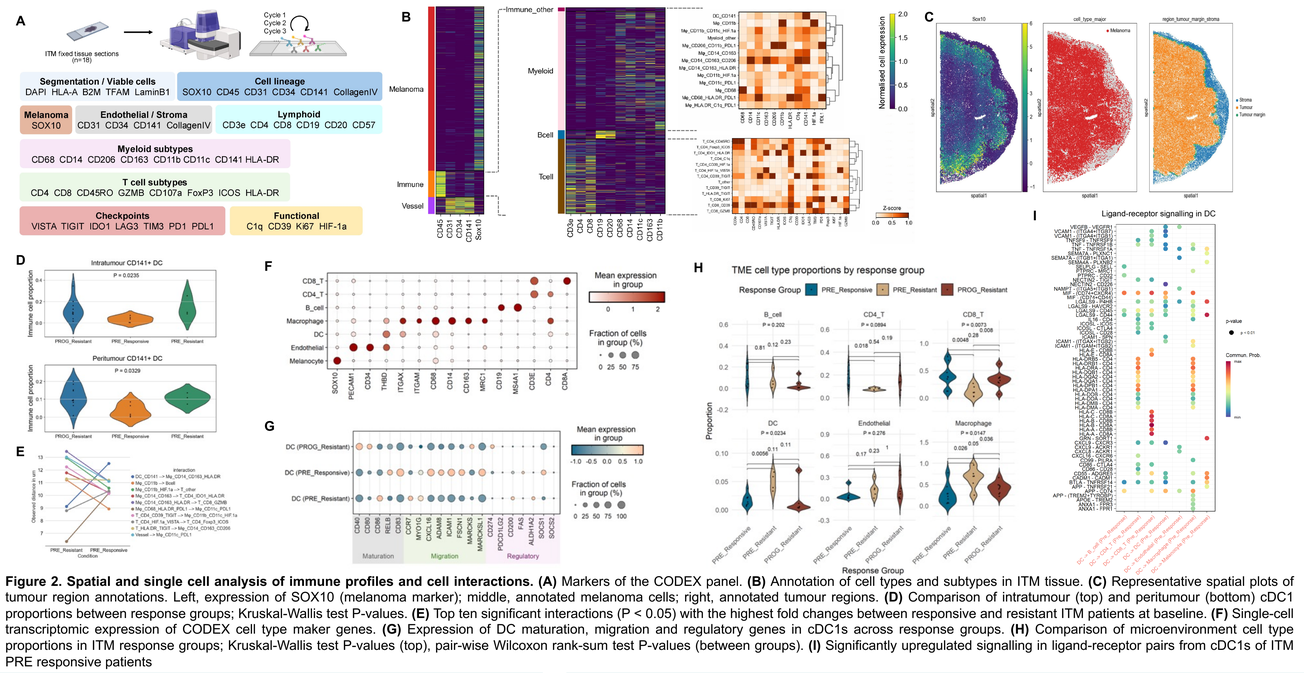

Figure 2. Spatial and single cell analysis of immune profiles and cell interactions. (A) Markers of the CODEX panel. **(B)** Annotation of cell types and subtypes in ITM tissue. **(C)** Representative spatial plots of tumour region annotations. Left, expression of SOX10 (melanoma marker); middle, annotated melanoma cells; right, annotated tumour regions. **(D)** Comparison of intratumour (top) and peritumour (bottom) cDC1 proportions between response groups; Kruskal-Wallis test P-values. **(E)** Top ten significant interactions ($P < 0.05$) with the highest fold changes between responsive and resistant ITM patients at baseline. **(F)** Single-cell transcriptomic expression of CODEX cell type maker genes. **(G)** Expression of DC maturation, migration and regulatory genes in cDC1s across response groups. **(H)** Comparison of microenvironment cell type proportions in ITM response groups; Kruskal-Wallis test P-values (top), pair-wise Wilcoxon rank-sum test P-values (between groups). **(I)** Significantly upregulated signalling in ligand-receptor pairs from cDC1s of ITM PRE responsive patients

3. Impaired T cell activation in resistant ITM

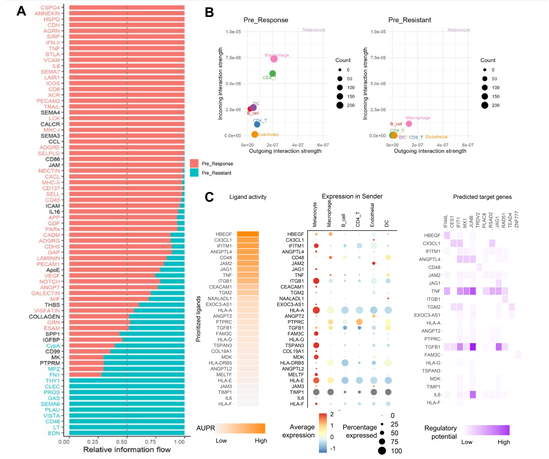

Figure 3. Supressed immune signalling in resistant patients. (A) Overall information flow for conserved and group-specific signalling pathways between response groups. **(B)** Cell populations with changes in sending and receiving signals of ligand-receptor interactions. **(C)** Ligand induced transcriptional response in CD8 T cells of resistant ITM; AUPR, area under the precision-recall curve.

4. Angiogenic remodelling by melanoma cells in resistant ITM

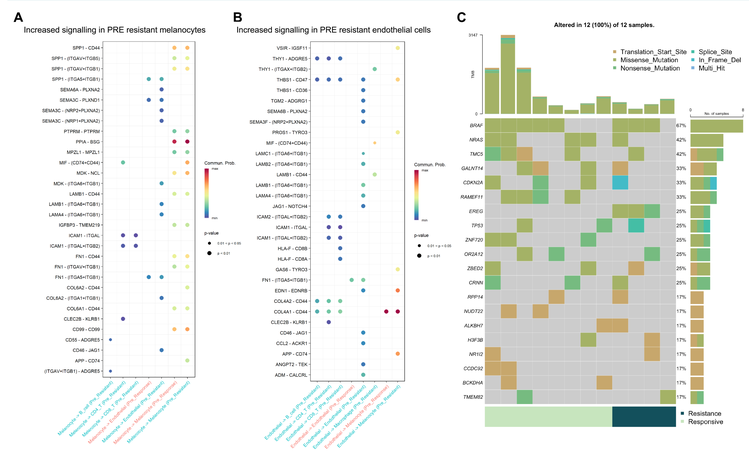

Figure 4. Tumour-driven signalling and somatic variants in baseline ITM. (A) Significantly upregulated signalling ligand-receptor pairs from melanoma cells (melanocytes) of ICI-resistant ITM patients compared to responsive patients at baseline. **(B)** Significantly upregulated signalling ligand-receptor pairs from endothelial cells of ICI-resistant ITM patients compared to responsive patients at baseline. **(C)** Key somatic variants identified in ICI-resistant (n=4) and responsive (n=8) ITM patients at baseline. Abbreviations: PRE, pre-treatment.

Conclusions

- ICI-responsive ITM patients have lower cDC1 density, but increased antigen presentation via MHC-I and MHC-II pathways from cDC1s, macrophages and B cells to CD8+ and CD4+ T cells in ICI responders, suggestive of enhanced cDC1 functions and alternative antigen presenting cells for local T cell stimulation.
- Mutations in the *EREG* gene were exclusively found in resistant ITMs, which may be linked to immunoregulation and tumour invasion through the EGFR pathway; in line with this, ICI-resistant patients displayed enhanced tumour proliferation and angiogenesis interactions, and downregulated HLA-I interactions with CD8+ T cells.
- The present study deepen our understanding of tumour-immune interactions in ITM patients and offers a foundation for refining personalised immunotherapy strategies tailored to their unique tumour-immune landscape.

References

1. Testori et al. *European Journal of Surgical Oncology* (2017)
2. Russano et al. *Cancers* (2024)
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Acknowledgements

- All patients and their families
- Project funded by Melanoma Research Alliance
- Travel funded by Sydney Cancer Partners with funding from Cancer Institute NSW (2021/CBG0002)

Proudly supported by Cancer Institute NSW