

P3.112: Spatial Multi-Omics in Mesothelioma Identifies Marked Intra-Tumour Heterogeneity in the Tumour Immune Microenvironment

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INTRODUCTION

Malignant pleural mesothelioma (MPM) demonstrates variable responses to immune checkpoint inhibitors (ICI)¹, highlighting the need for reliable predictive biomarkers. Intra-tumoural heterogeneity is a major cause of treatment failure in cancer patients treated with ICI^{2,3}. We used spatial multi-omics to characterise tumour and immune microenvironment heterogeneity in prospectively collected multi-region, pre- and post-treatment samples. Our preliminary findings demonstrate substantial spatial variation in tumour phenotype and immune contexture, while spatially resolved ligand–receptor interactions show potential to predict response to second-line immunotherapy, independent of histological subtype. These findings support the development of spatially informed biomarkers to guide immunotherapy selection in MPM.

METHODS

Design:

Prospective, multicentre observational cohort study

Enrolled adults with MPM undergoing clinically indicated video-assisted thoracoscopic surgery (VATS) for diagnosis

Sample collection: Fresh and formalin-fixed samples were obtained from 12 patients at the time of diagnosis (28 samples in total). Multi-region biopsies were taken from 5 patients (13 samples).

Pathology and imaging correlation: Fresh samples underwent frozen section and histologic analysis to confirm tumour content; CT-PET imaging findings were correlated with the pleural biopsy site.

Spatial transcriptomics (ST): 28 samples were analysed using the Visium CytAssist platform, capturing whole-transcriptome data at ~5,000 spots (55 µm spacing; ~5–10 cell resolution) over a 6.5 mm² area⁴.

Pathologist review: Each ST analysis region was independently reviewed by a pathologist for cellular content.

Sequencing depth and bioinformatics for ST: Visium libraries were sequenced to >25,000 reads per spot; data were processed with Space Ranger and Loupe, then further analysed (filtering, normalisation, dimensionality reduction, visualisation) using Scanpy, STUtility, Seurat, and CellChat.

Whole-genome sequencing (WGS): In one patient, fresh samples from three distinct pleural sites were sequenced at 60× WGS and compared against a peripheral blood mononuclear cell control.

Primary ICI-resistant = Progression ≤ 4 months of treatment

Acquired ICI-resistant = Progression after 4 months

DISCUSSION AND CONCLUSION

Actionable Take Away Message

- MPM shows limited genomic but significant transcriptomic heterogeneity
- Diverse immune response displayed within the same tumour mass
- Spatial analysis of the tumour microenvironment**, combined with **machine learning**, identified spatial heterogeneity in signals associated with ICI efficacy.
- Spatially resolved ligand–receptor interactions show potential to predict response to second-line ICI independent of histological subtype.
- The multiregional spatial transcriptomics may overcome heterogeneity and inform single-region transcriptional expression signature that may accurately predict response to ICI.

Future Direction for Research

- Prospective validation:** The preliminary findings will inform a prospective study incorporating multi-region biopsy collection.
- Clinical utility:** This study will support the development of spatially informed ICI- biomarkers in MPM
- Translational pathway:** Linking spatial molecular features with biomarker-guided clinical trial referral will establish a pathway from discovery to clinical implementation.
- Precision oncology impact:** This approach may accelerate the development of targeted therapies and clinically actionable precision oncology tools.

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RESULTS

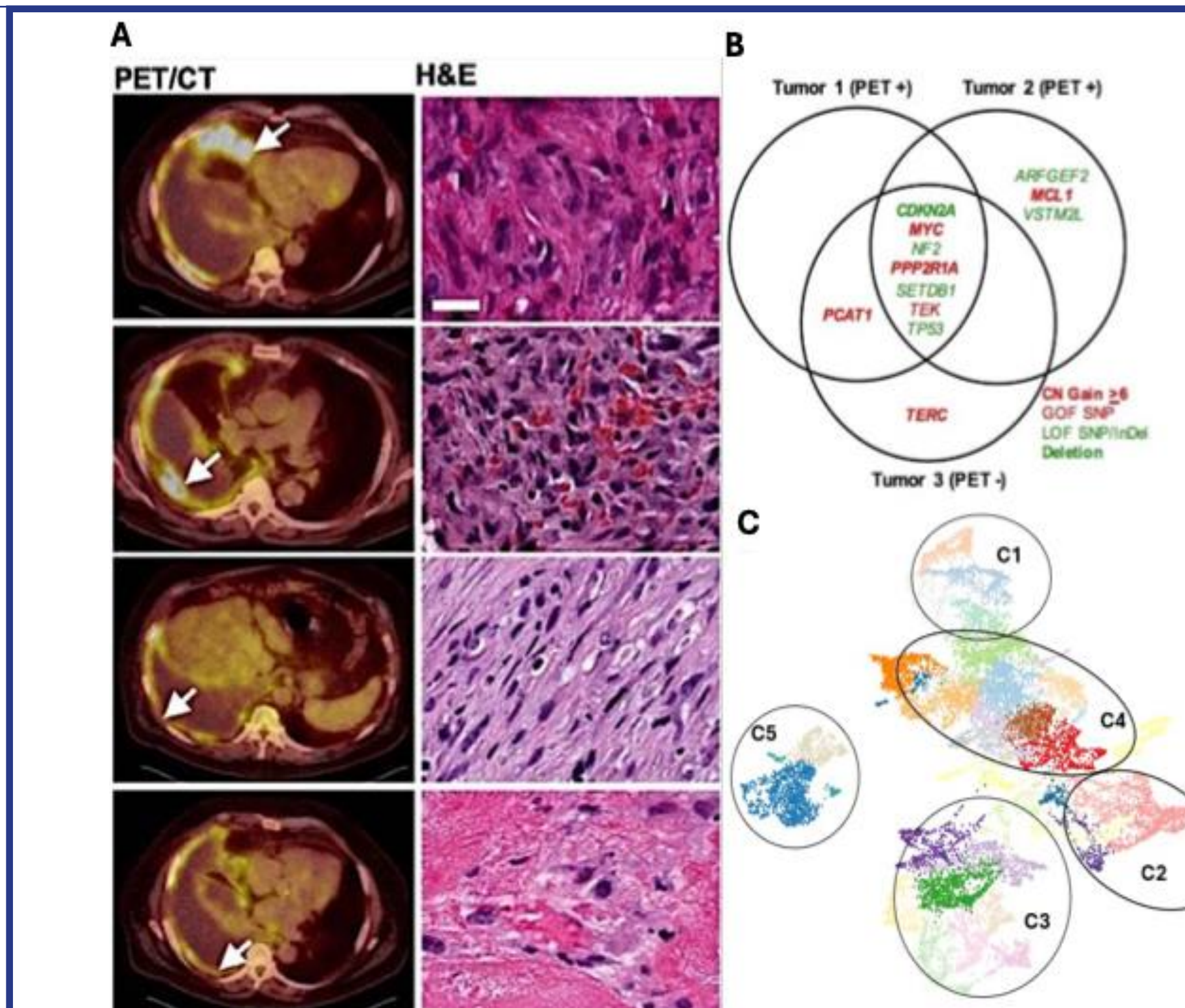


Fig 1. Histological, genomic and transcriptomic heterogeneity in multi-region biopsies. (A) PET/CT and histology of multi-region biopsies (B) WGS identified driver mutations that were unique to or shared between multiple biopsy sites. (C) UMAP projection of ST data from 5 biopsy regions revealed significant transcriptional heterogeneity.

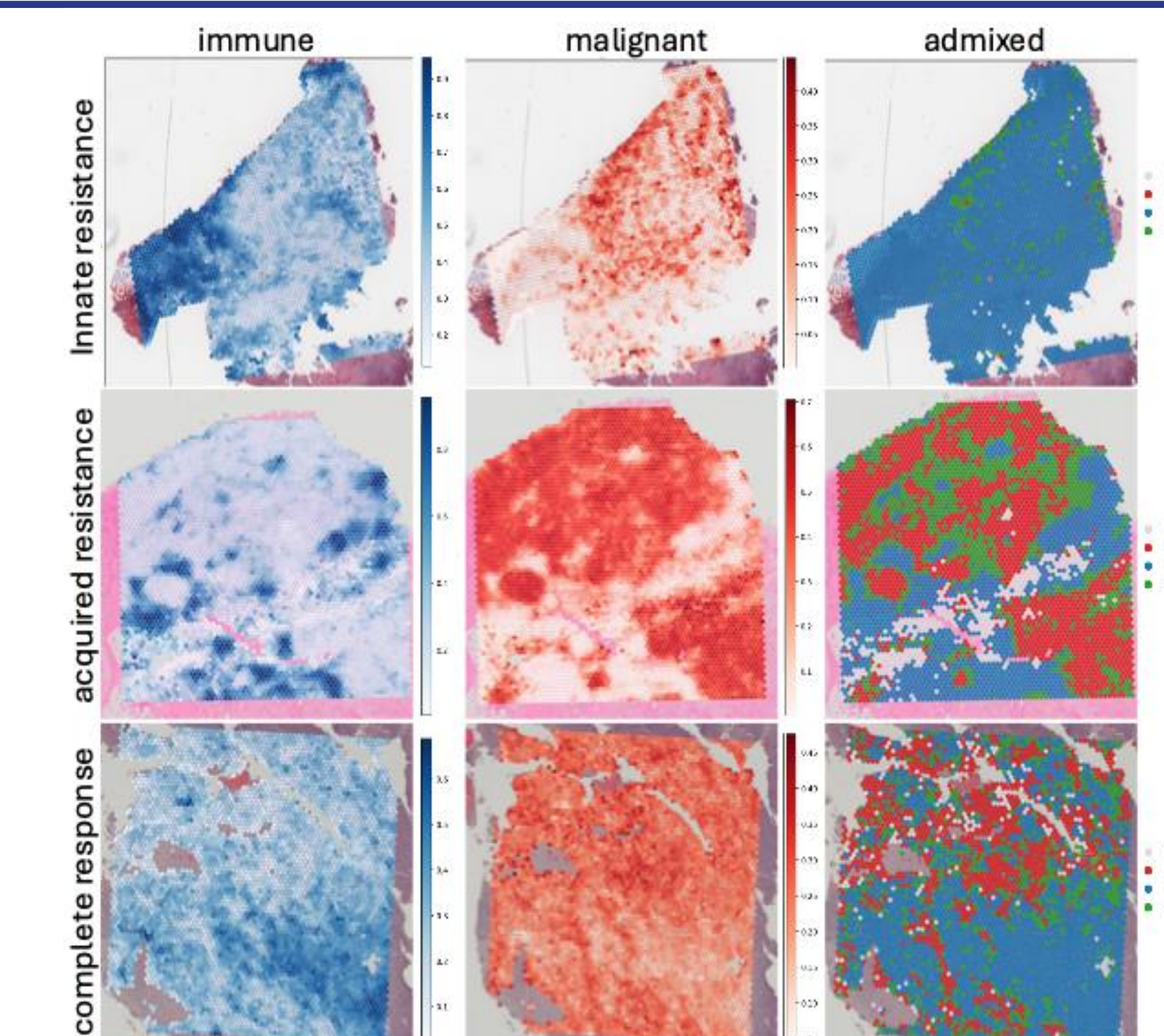


Fig 2. Immune composition does not correlate to IO response. Patients representing innate resistance, acquired resistance and complete response were assessed for spatial distribution of immune signatures (immune), tumour signatures (malignant) and whether signatures were dominant or balanced in each spot (admixed).

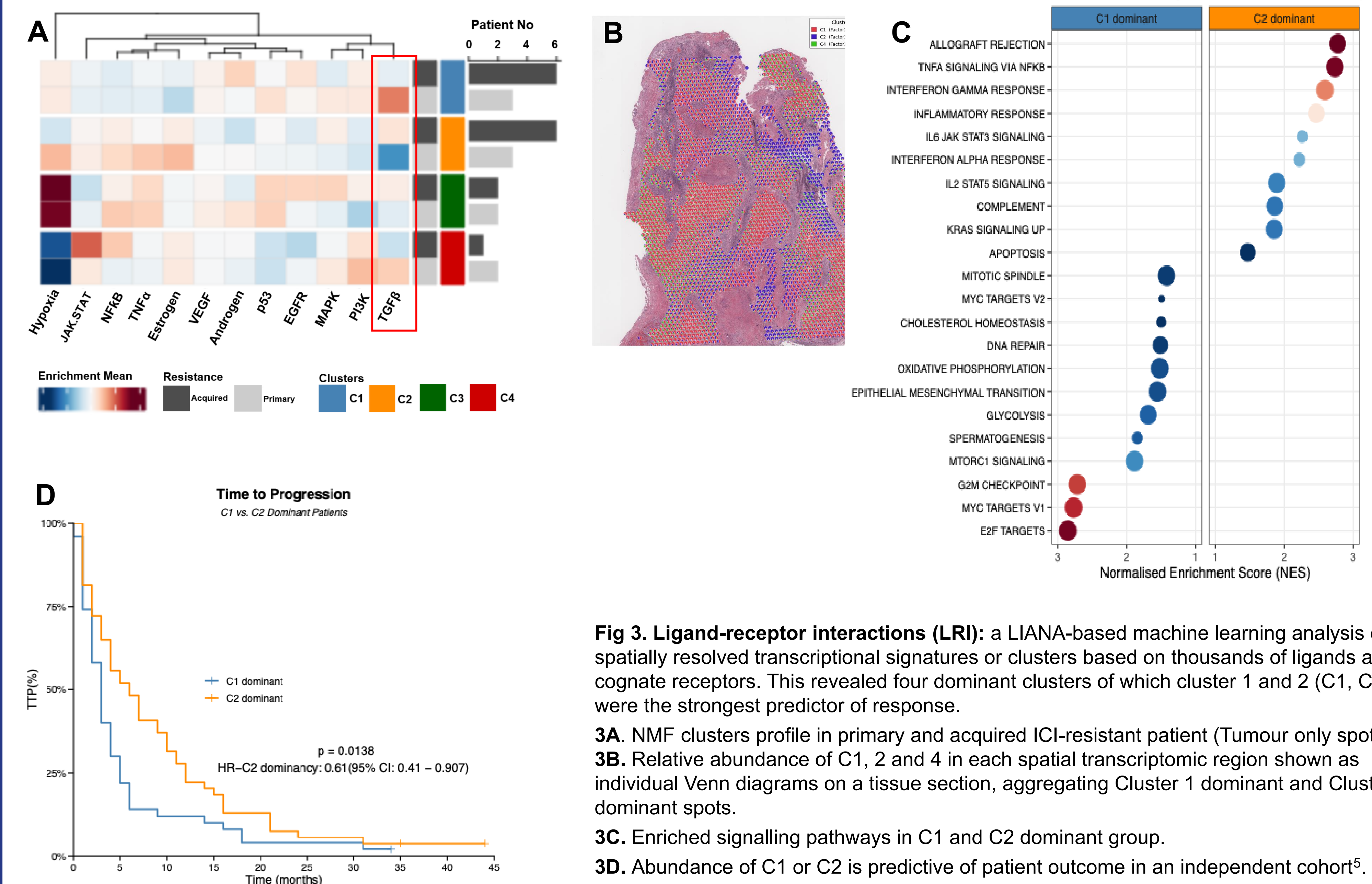


Fig 3. Ligand-receptor interactions (LRI): a LIANA-based machine learning analysis of spatially resolved transcriptional signatures or clusters based on thousands of ligands and cognate receptors. This revealed four dominant clusters of which cluster 1 and 2 (C1, C2) were the strongest predictor of response.

3A. NMF clusters profile in primary and acquired ICI-resistant patient (Tumour only spots)
3B. Relative abundance of C1, 2 and 4 in each spatial transcriptomic region shown as individual Venn diagrams on a tissue section, aggregating Cluster 1 dominant and Cluster 2 dominant spots.

3C. Enriched signalling pathways in C1 and C2 dominant group.

3D. Abundance of C1 or C2 is predictive of patient outcome in an independent cohort⁵.