

# Single-cell trajectories of metastatic urothelial cancer and individual patterns of resistance to immune checkpoint inhibitors

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## BACKGROUND

Immune checkpoint inhibitors (ICI) improved survival in patients with metastatic urothelial cancers (mUC) but only ~20% derive long-term responses, and most ultimately experience disease progression. Primary and acquired mechanisms of resistance are unknown.

## OBJECTIVE

Identify primary and acquired mechanisms of resistance to single-agent immune checkpoint inhibitors in patients with mUC, using single sequential single-nuclei RNAseq of metastatic biopsy samples.

## PATIENTS AND METHODS

The MATCH-R trial (NCT02517892) included patients with mUC treated with single-agent PD-(L)1 inhibitors.

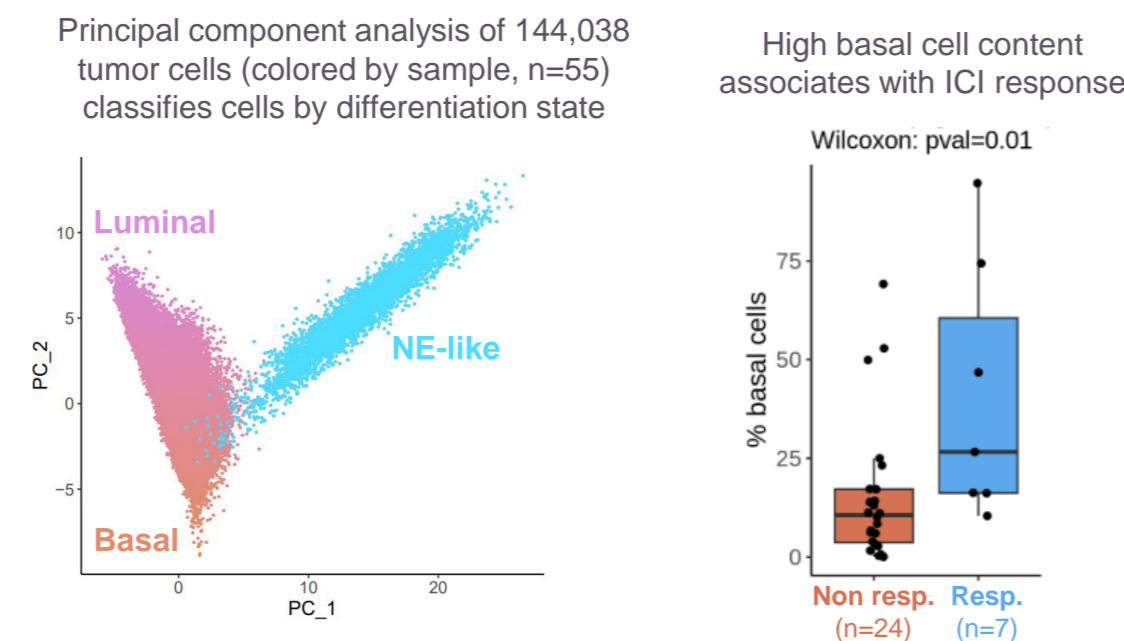
We performed longitudinal single-nuclei RNA-seq analyses of metastatic samples to explore tumor and immune features predicting ICI response at baseline or associated with acquired resistance at relapse.

After stringent quality control and cell type annotation, we compared the proportions and transcriptomic signatures of tumor and immune cell subsets between responders and non-responders and explored their evolution during treatment.

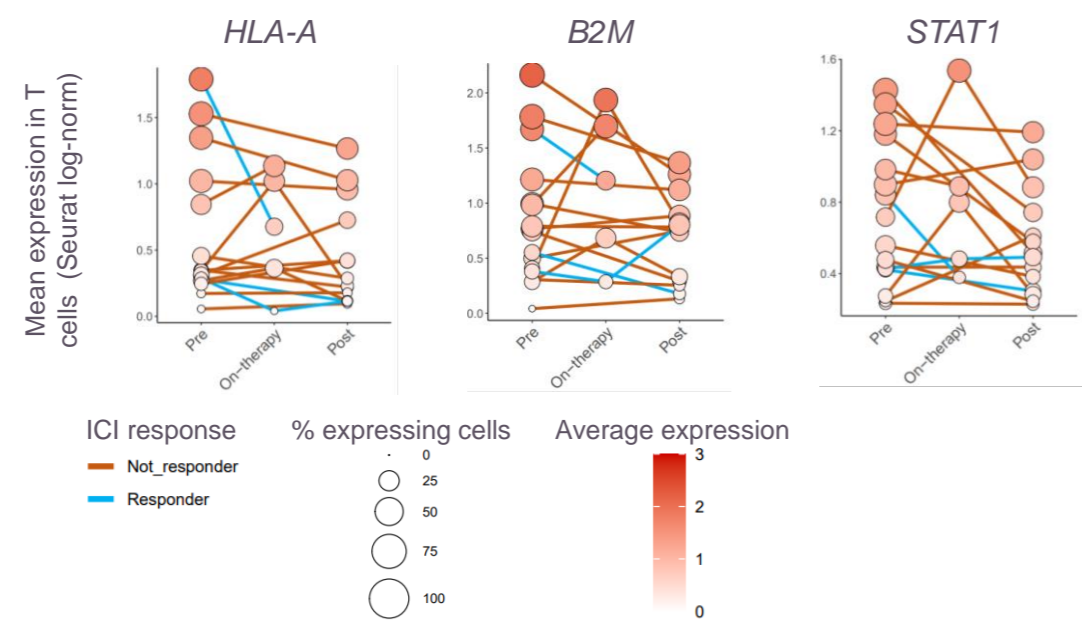
A total of 32 mUC patients were included.  
- 7/32 (22%) achieved objective response.  
- 55 biopsies were performed: all underwent biopsies at baseline, 6 (19%) on therapy and 17 (53%) at progression.

## RESULTS

### Differentiation states drive tumor heterogeneity and ICI response

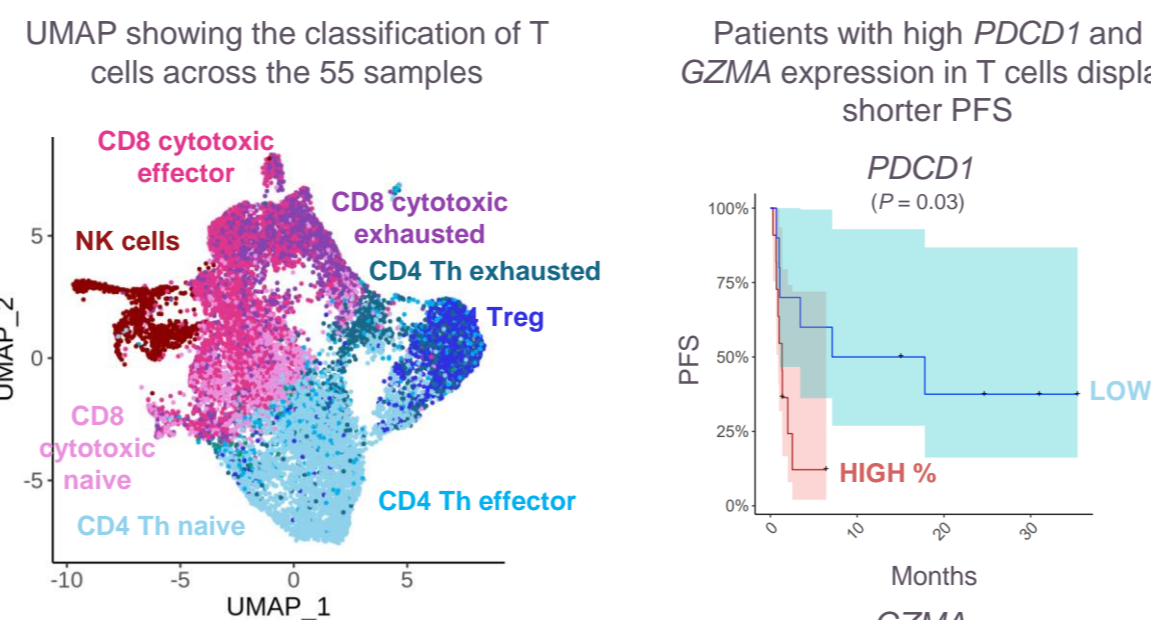


Longitudinal analysis reveals down-regulation of antigen presentation and IFN/JAK/STAT signaling at relapse in some patients

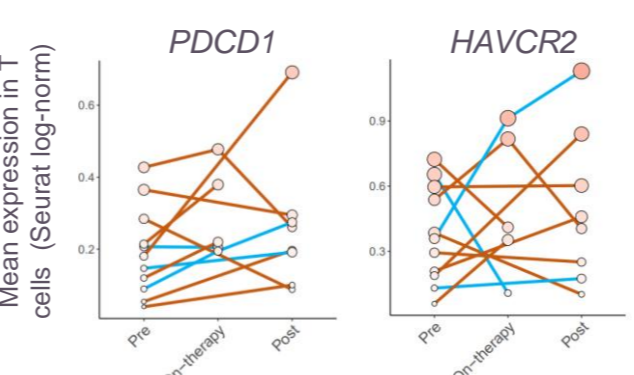


**Fig. 1.** Tumor cell heterogeneity and evolution upon ICI treatment

### CD8 T cell exhaustion is associated with primary and secondary resistance to ICI

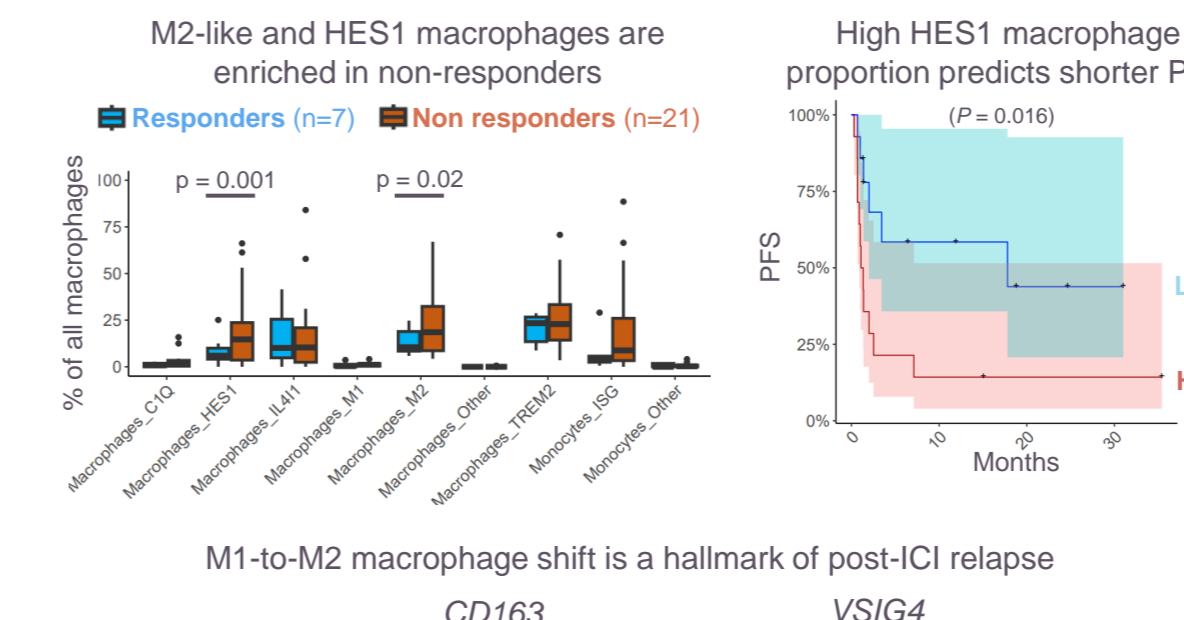


Immune checkpoint genes are overexpressed at relapse in some patients

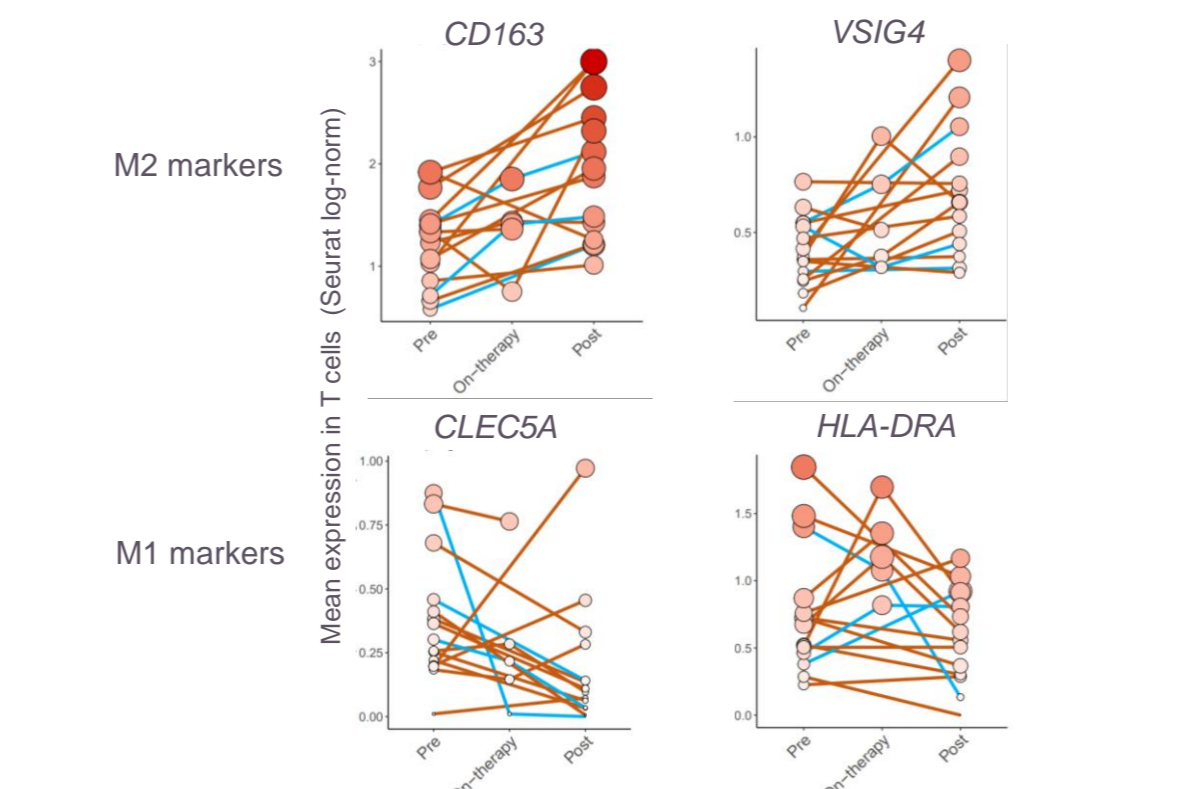


**Fig. 2.** Exhausted CD8 T cells predicts poor ICI response and increase post-treatment

### Pro-tumoral macrophage shift is a hallmark of ICI resistance in mUC



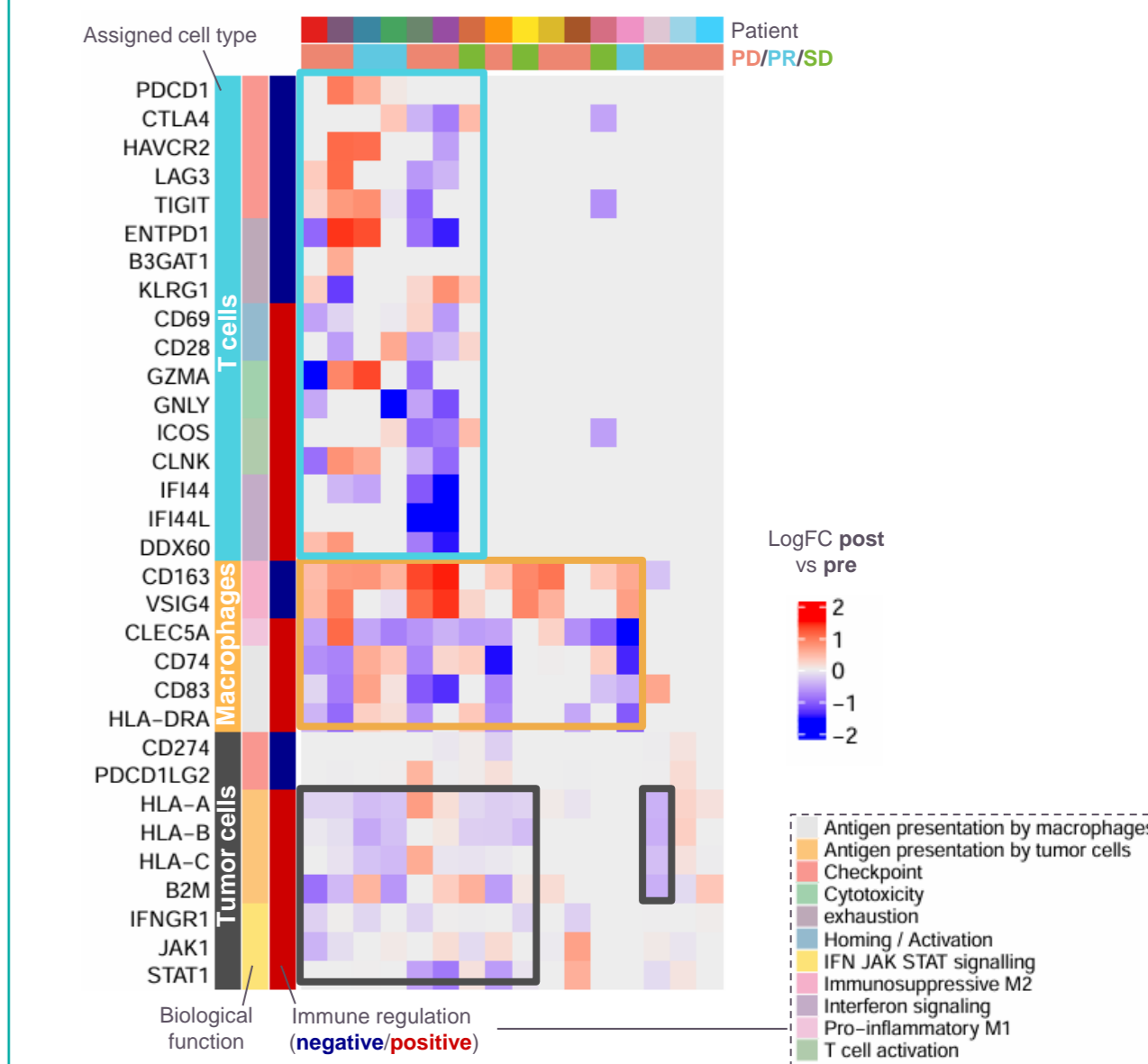
M1-to-M2 macrophage shift is a hallmark of post-ICI relapse



**Fig. 3.** Pro-tumoral macrophages predict poor ICI response and increase upon treatment.

### Single-nucleus RNA sequencing pinpoints individual trajectories towards ICI resistance

Overview of genes and pathways deregulated at relapse in each cell type. Each column represents a patient, with red/blue squares indicating genes that are up/down-regulated between its pre- and post-treatment samples



**Fig. 4.** Overview of tumor and immune cell reprogramming after ICI.

## CONCLUSION

Single cell states may inform outcomes on ICI for patients with mUC and identify individual patterns of resistance

Basal tumor cell proportion at baseline predict mUC response to ICI. Conversely, adverse immune features include abundant pro-tumoral HES1 macrophages (poor response) and a higher proportion of exhausted CD8+ lymphocytes expressing immune checkpoint genes (poor response).

Longitudinal analyses uncovered molecular shifts linked to ICI progression, involving both tumor and immune compartments: downregulation of HLA genes and IFN signaling in tumor cells; a shift from M1 to M2 macrophage polarization; increased expression of immune checkpoints and downregulation of type-I interferon induced genes in T cells.

Implementation of single-cell transcriptomics in a clinical setting may help predict ICI response and to enable dynamic, personalized therapeutic strategies.

