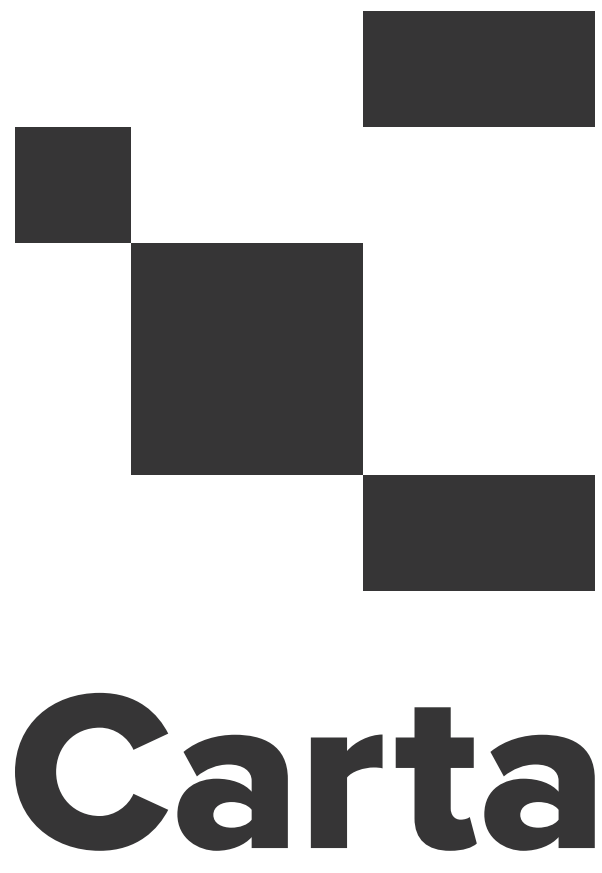


Tumor-specific MHC class II upregulation associated with response to anti-PD-L1 therapy in patients with urothelial cancer

İlayda Hasakioğulları¹, Mark Kockx¹, Bernadett Szabados², Matthew Young² and Thomas Powles²
¹CellCarta, Antwerp, Belgium and ²Barts Cancer Institute, Queen Mary University of London, London, United Kingdom

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INTRODUCTION

- Muscle invasive bladder cancer (MIBC) poses significant therapeutic challenges due to the aggressive nature of the tumor and its potential for metastasis.
- The abacus study (NCT02662309) investigated the atezolizumab (anti-PD-L1) efficacy in a neoadjuvant setting. Pathological complete response (pCR) rate was 31% and 8% of the patients showed major pathological response (MPR, >90% necrosis and immune cell presence).
- The presence of activated CD8+ T cells in the baseline samples was correlated with outcome, but no treatment-related biomarker was discovered (1).
- Signatures of response in tumor during/ after therapy in pCR cannot be studied because the tumor is completely eradicated. Understanding MPR tumors is crucial because their responsiveness to therapy remains uncertain. Identifying specific response biomarkers in these tumors could potentially serve as valuable surrogate indicators for overall treatment response, thereby enhancing the evaluation of therapeutic outcomes.
- In this study, we compared different response groups using spatial transcriptomics to study the molecular mechanisms of resistance and response and looked for biomarkers of response after the treatment.

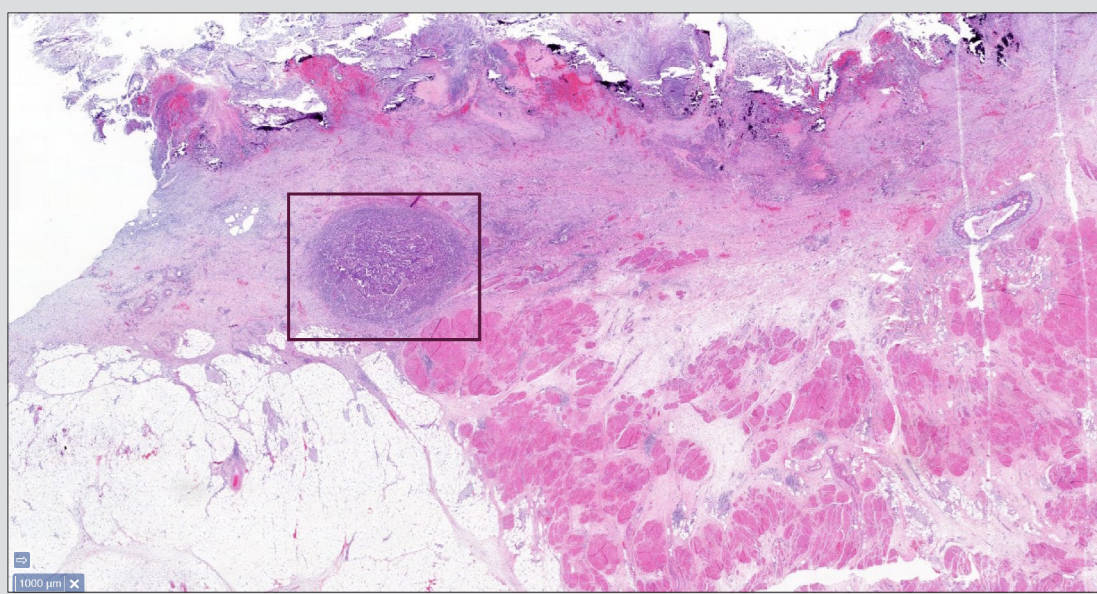


Figure 1: Example of a residual tumor lesion in an MPR patient.

METHODS

Spatial transcriptomics – GeoMx Digital Spatial Profiler (DSP)

Twelve paired biopsies, consisting of 3 pCR, 3 MPR, and 6 relapse samples, were collected. Morphology markers SYTO 13, PanCK (tumor), CD45, and CD3 were used for region of interest (ROI) selection and segmentation of transcripts. A total of 200 ROIs were collected across 23 samples. GeoMx DSP Data Analysis Suite and BioTuring Lens were used for data analysis and visualization.

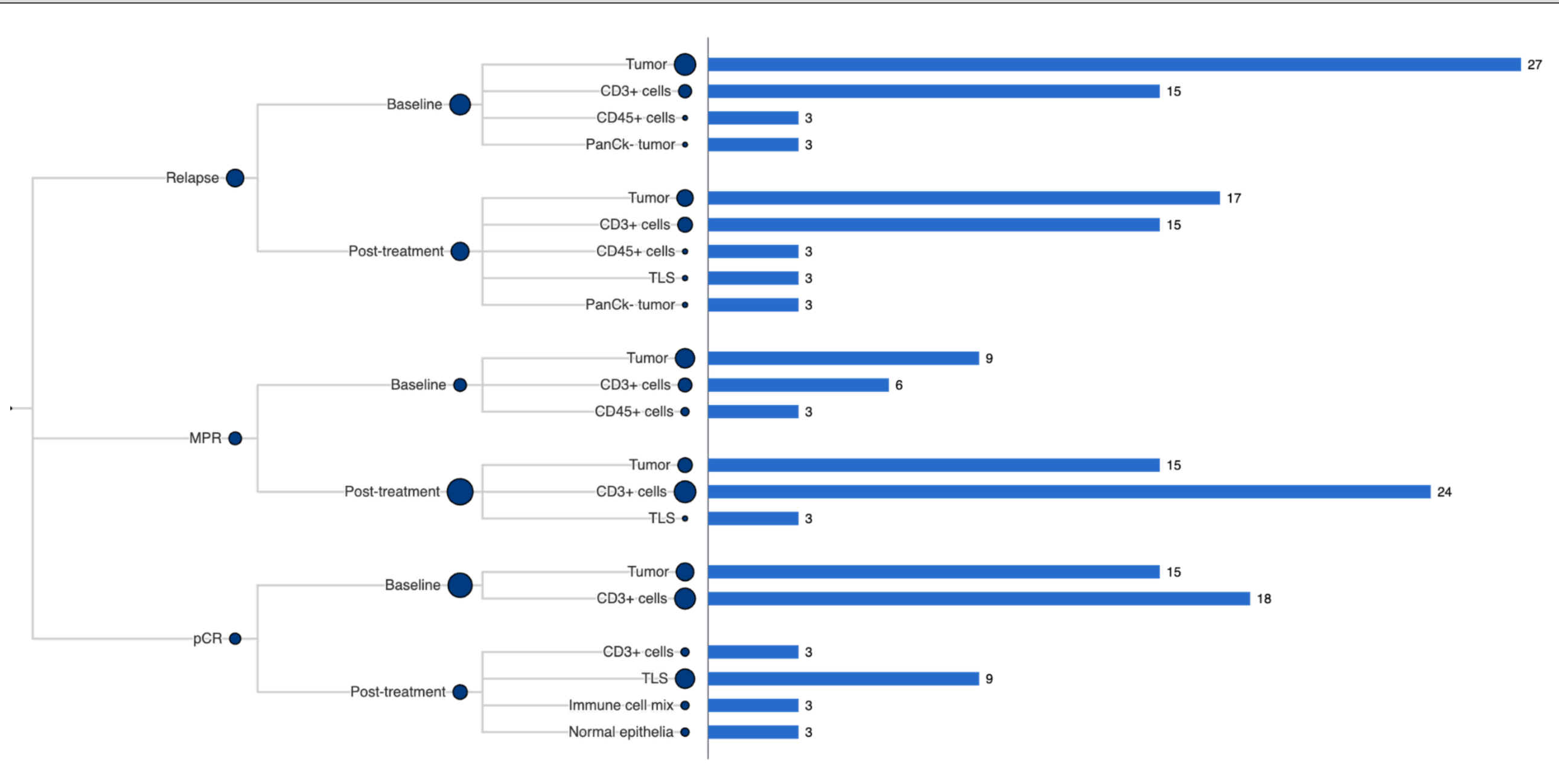


Figure 2: Composition chart illustrating the number of ROIs and their collection strategy based on the morphology markers.

Immunohistochemistry (IHC)

HLA-DR (LN3, eBioscience) used to stain all available baseline and post-treatment samples (n= 91) on LabVision autostainer. Percentage of HLA-DR positive tumor cells were scored by a pathologist. Data is visualized and statistics is performed using R Studio.

RESULTS

GeoMx Results

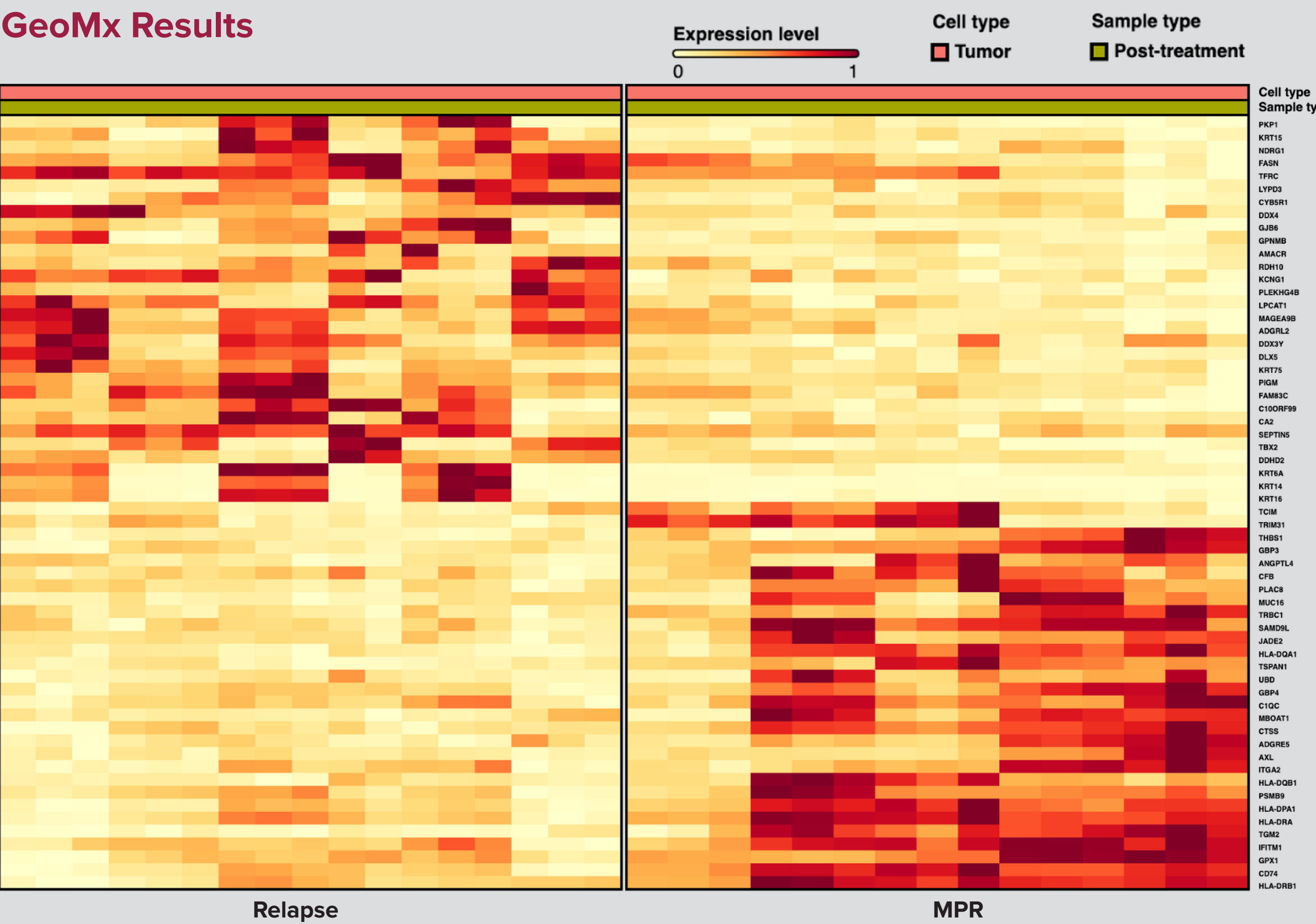


Figure 3: Heatmap displaying the differential gene expression analysis between MPR and relapse post-treatment tumor segments, based on t-test analysis.

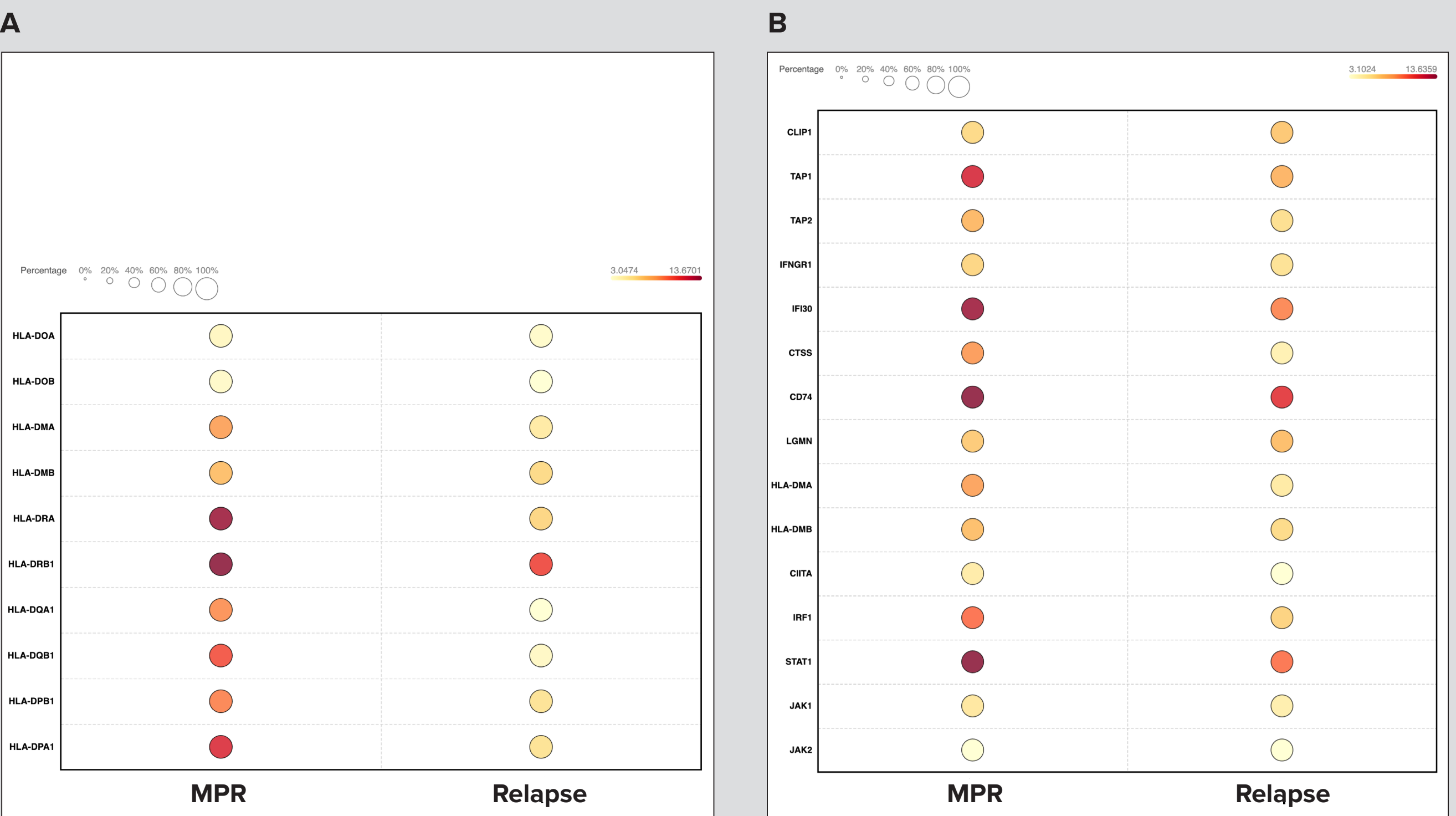


Figure 4: Bubble heatmap comparing all MHC II (A) and MHC II machinery (B) genes in MPR and relapse post-treatment tumor groups. Bubble size represents the percentage of ROIs showing gene expression, while color indicates log-normalized expression levels.

IHC Results

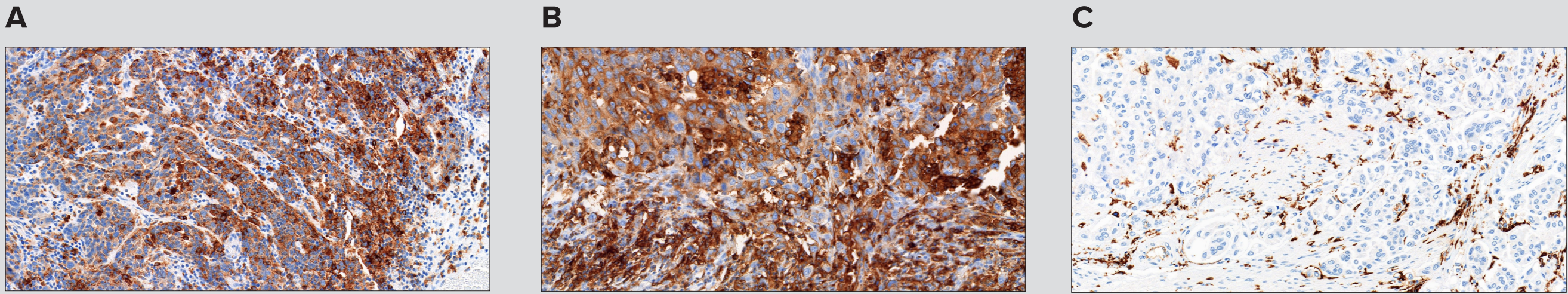


Figure 5: HLA-DR staining on resection samples post-treatment. In panels (A) and (B), the MPR sample exhibits variable HLA-DR expression among tumor cells. Panel (C) shows a sample from a relapsed patient, where HLA-DR expression is observed on macrophages and/or antigen-presenting cells (APCs) but not on tumor cells.

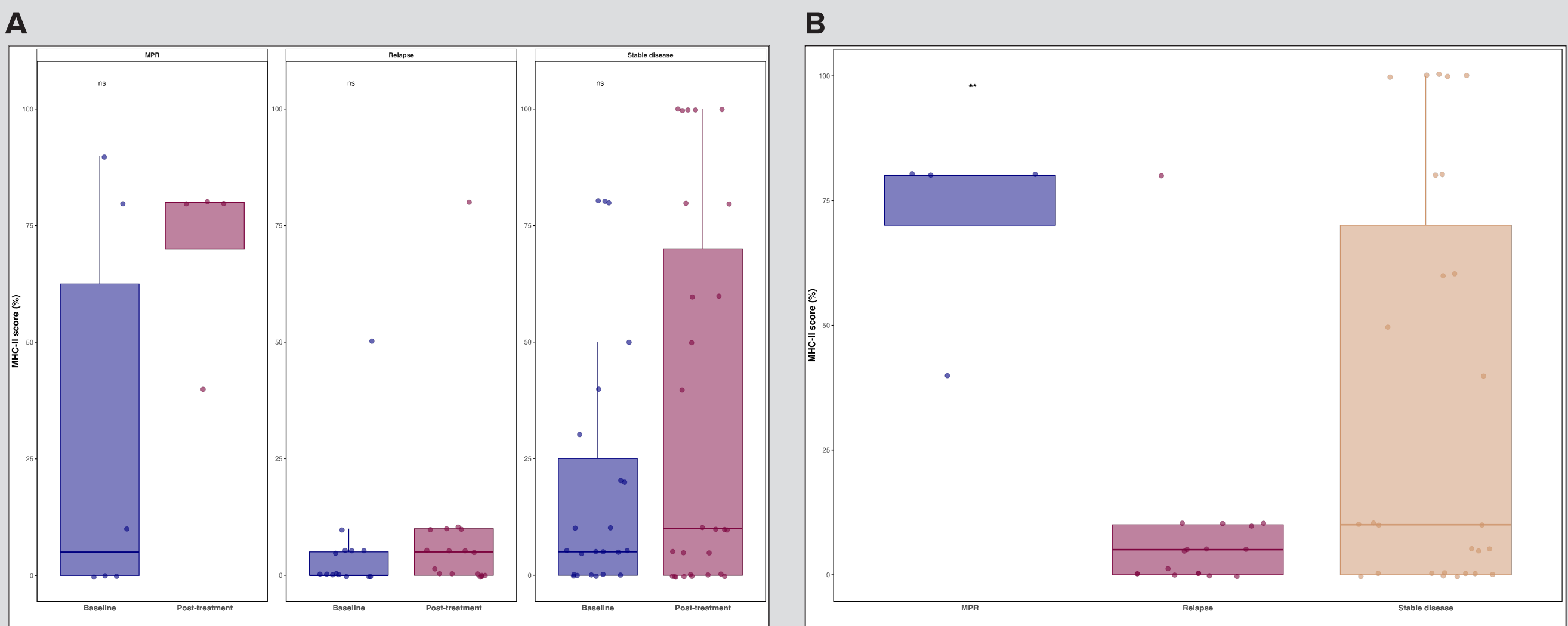


Figure 6: (A) Dynamic MHC II (%) Changes in Different Response Groups (B) Post-Treatment MHC II (%) Scores Compared Across Response Groups (p < 0.005).

CONCLUSIONS

While tumor cells expressing MHC II are relatively rare, they are not entirely unprecedented (2, 3). Prior exploratory studies utilizing bulk RNA sequencing (RNAseq) data have demonstrated that an MHC II signature is associated with anti-tumor immunity and can predict responses to anti-PD-L1 therapy in bladder cancer (4). However, these studies assumed that the upregulation of MHC II genes was predominantly from immune cells. Our study advances beyond bulk analysis by employing an unbiased whole transcriptome spatial approach to investigate dynamic changes following treatment. GeoMx technology enabled the examination of MHC II expression in both tumor cells and immune cells separately, offering a more detailed understanding of the tumor microenvironment and immune response post-treatment. Our findings were corroborated by IHC on a larger cohort.

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Send inquiries to ilayda.hasakioğullari@cellcarta.com with ASCO-MHC II on bladder tumor in email subject.