

## SINGLE-CELL RNA SEQUENCING AND CITE-SEQ ANALYSIS OF BLADDER CANCER PATIENT URINE WITH MATCHED TUMOR AND PERIPHERAL BLOOD SUGGESTS URINE AS A WINDOW INTO THE TUMOR IMMUNE MICROENVIRONMENT

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**Background** FDA-approved immunotherapies for early and advanced stage bladder cancer have response rates of 15–65% in bladder cancer, suggesting that tumor-associated resistance mechanisms undermine their efficacy. Accordingly, there is an unmet need to identify accessible biomarkers that predict response. Urine, which is in direct contact with urothelial tumors, represents an easily accessible patient material that may reflect cellular and/or genetic signatures related to immune resistance. It has been demonstrated that urine from bladder cancer patients contains not only tumor cells, which are routinely assessed by clinical urinalyses, but also immune cells that previous studies suggest may reflect the tumor microenvironment (TME).<sup>1</sup> However, the concordance between cells in the urine and those in bladder tumors is unknown. Here, we characterized patient urine in an unbiased fashion by performing the first single-cell RNA sequencing (scRNAseq) and Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) on matched bladder cancer patient urine, tumor, and peripheral blood.

**Methods** Matched tumor tissue, urine, and peripheral blood were collected from bladder cancer patients (n=7) during surgery; either trans-urethral resection of bladder tumor or cystectomy. All three tissues were processed to single-cell suspensions and sequenced using the 10X Genomics platform (scRNAseq: 17 samples, CITE-seq: 3 samples). These sequencing approaches permitted quantification of both transcriptomic and surface protein expression of 54,469 cells total.<sup>2–3</sup> Analysis was performed using Seurat, Enrichr, and Monocle packages and platforms.<sup>4–6</sup>

Results scRNAseq of urine from bladder cancer patients revealed several immune populations including CD4+ and CD8+ T cells, Treg cells, NK cells, B cells, neutrophils, dendritic cells, monocytes, and macrophages in addition to non-hematopoietic lineages including bladder epithelial cells, neuronal cells, prostate epithelial cells, fibroblasts, myofibroblasts, and endothelial cells. The composition and transcriptional profiles of urine immune cells were more similar to TME immune cells than to peripheral blood immune cells. Urine immune cells expressed gene signatures associated with hypoxia, anergy, pro-inflammation, and glucose deprivation that were more similar to tumor immune cells than those in the peripheral blood.

**Conclusions** Our work represents the first scRNAseq and CITE-seq profiling of cancer patient urine. Our study suggests several viable immune cells shed in bladder cancer patient urine that look more transcriptionally and phenotypically similar to the TME than peripheral blood cells. This important finding has several implications for future research and clinical applications as urine can be sampled non-invasively in scenarios when tumor resection may not be feasible.

### REFERENCES

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**Ethics Approval** The study was approved by Mount Sinai Institution's Ethics Board, approval number 10–1180. Participants gave informed consent before taking part in the study.

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