

## DISSECTING MYELOID CELL-MEDIATED MECHANISMS OF RESISTANCE TO IMMUNE CHECKPOINT BLOCKADE IN BLADDER CANCER

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**Background** PD-(L)1 immune checkpoint blockade (ICB) therapy yields objective responses in 15-25% of patients with bladder cancer (BC), suggesting that tumor-associated resistance mechanisms undermine their efficacy.<sup>1-6</sup> We previously identified two gene signatures derived from pre-treatment tumor tissue independently associated with ICB outcomes in BC patients: 1) a signature enriched in adaptive immune response genes and associated with better ICB outcomes named the “adaptive immune response signature” and 2) a signature enriched in innate immune and inflammation genes and associated with worse ICB outcomes labelled the “pro-tumorigenic inflammation signature”.<sup>7</sup> The adaptive immune response: pro-tumorigenic inflammation score ratio, named the 2IR score, was highly correlated with ICB response and validated in an independent cohort.

**Methods** Single-cell RNA sequencing (scRNAseq) was performed on BC tumors (n=26) and normal-adjacent tissue (n=3) to resolve the cellular composition underlying the 2IR score. Ingenuity Pathway Analysis was applied to predict upstream ligands skewing MΦ phenotypes and such ligands were tested for their effects on healthy donor peripheral blood monocytes differentiated with (G)M-CSF. Patient plasma was assessed for pro-inflammatory cytokines across disease stage using O-Link Proteomics and Enzyme-Linked Immunosorbent Assay.

**Results** From our scRNAseq, we discovered the adaptive immune response and pro-tumorigenic inflammation signatures were enriched in distinct MΦ subsets we labelled immunostimulatory (is)MΦs and pro-tumorigenic (pt)MΦs, respectively. ptMΦs upregulated inflammatory markers: SPP1, TREM1, and CLEC5A; expressed pro-angiogenic and hypoxic programs; and downregulated antigen presentation machinery. ptMΦs were enriched in tumor versus normal-adjacent tissue, which was confirmed by flow cytometry on additional patients. From our screen of predicted upstream ligands, lipopolysaccharide (LPS) induced the highest amount of ptMΦs (p<.05), as defined by Clec5a and Trem1 surface expression, in GM-CSF-induced MΦs, while IL-1β induced the most for M-CSF-induced MΦs (p<.005). ptMΦs also upregulated SPP1 on a transcriptional level. Inflammatory cytokines (e.g. IL-1β, IL-6, IL-8) as well as macrophage-colony stimulating factor (CSF-1) were elevated in the plasma of advanced stage BC patients as compared to early stage and healthy donors.

**Conclusions** From our BC scRNAseq cohort, we discovered a pro-tumorigenic inflammatory MΦ subset underlying a gene signature previously linked to ICB resistance in bladder cancer. We recapitulated these MΦs using blood monocytes differentiated with G(M)-CSF and skewed with LPS/IL-1β. We also found inflammatory cytokines associated with these MΦs were elevated in the peripheral blood of advanced patients. Together, these findings identify a distinct MΦ transcriptional state that may underlie tumor-promoting inflammation and ICB resistance and be therapeutically modulated to overcome ICB resistance.

## REFERENCES

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**Ethics Approval** The study was approved by Mount Sinai Institution's Ethics Board, approval number 10-1180.

**Consent** Participants gave informed consent before taking part in the study.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0946>