

Title:

Type I Interferon priming in macrophages is associated with poor outcomes in patients with advanced prostate cancer

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Background: Metastatic castration-resistant prostate cancer (mCRPC) remains a significant clinical challenge with limited therapeutic options. Through our studies of Bipolar Androgen Therapy (BAT)—an innovative treatment that cycles serum testosterone from supraphysiological to near-castrate levels—we sought to understand immune dynamics in the tumor microenvironment and their association with therapeutic response.

Methods: We performed spatial transcriptomics on 28 matched tumor biopsies from 14 mCRPC patients enrolled in the COMBAT clinical trial, collected before and after BAT treatment. To overcome resolution limitations inherent to spatial profiling, we generated a comprehensive prostate cancer single-cell atlas comprising approximately 450,000 cells from 96 patients with hormone-sensitive and castration-resistant metastatic disease. This atlas enabled robust deconvolution of our spatial transcriptomic data, allowing cell type-specific gene expression analysis while preserving spatial context. We complemented tumor profiling with single-cell RNA sequencing of peripheral blood mononuclear cells (PBMCs) from 10 patients (5 responders, 5 non-responders) and validated findings using flow cytometry in an expanded cohort of 17 patients. Integration across platforms was performed using established computational pipelines to construct differentiation trajectories linking circulating monocytes to tumor-associated macrophages.

Results: Spatial transcriptomic profiling revealed that mCRPC tumors are dominated by cancer cells, fibroblasts, and macrophages, with notably poor lymphocyte infiltration. Macrophage populations were spatially resolved, localizing both at the tumor periphery and near tumor cores. Critically, differential gene expression analysis of macrophages identified Interferon-alpha associated transcripts as specifically enriched in macrophages from non-responding patients, with this signature increasing post-treatment. Integration with PBMC single-cell data revealed that circulating classical monocytes in non-responders displayed an analogous Type I IFN transcriptional program. We identified a discrete population of *MX1*-expressing monocytes, characterized by surface markers CD169 (*SIGLEC1*) and LY6E, that was expanded in non-responding patients. Notably, the frequency of CD169⁺LY6E⁺ monocytes strongly correlated with tumor volume in non-responders. Analysis of an independent publicly available dataset of 43 tumor biopsies demonstrated that *SIGLEC1* expressing macrophages were most abundant in mCRPC compared to hormone-sensitive or treatment-naïve cases, suggesting Type I IFN priming is a general feature of advanced disease rather than BAT-specific. Furthermore, these macrophages exhibited transcriptional signatures of altered lipid processing, a hallmark of tumor-promoting macrophages. In vitro differentiation of isolated CD169⁺LY6E⁺ monocytes confirmed their capacity to generate macrophages with enhanced SREBF1 activity and dysregulated lipid metabolism transcriptional programs.

Conclusions and outlook: Through integration of spatial transcriptomics with single-cell profiling across a large patient cohort, we identified Type I IFN primed macrophages as a population associated with poor outcomes in advanced prostate cancer. Our multi-modal approach—linking spatially-resolved tumor microenvironment analysis with circulating immune cell profiling—reveals that CD169⁺LY6E⁺ monocytes may serve as accessible biomarkers of disease progression. These findings provide mechanistic insight into immune evasion in mCRPC and identify Type I IFN signaling in macrophages as a potential therapeutic target.

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