Dissecting myeloid-driven mechanisms of immunotherapy resistance in prostate cancer bone metastases

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Background: Patients with metastatic castration-resistant prostate cancer (mCRPC) are generally refractory to immune checkpoint inhibitors (ICIs), particularly those with bone metastases, the most frequent site of disease progression (70-90%). The bone marrow (BM) harbors diverse immune populations, with myeloid cells comprising >70% of its cellular compartment. Among these, neutrophils, known mediators of ICI resistance across multiple malignancies, predominate, whereas macrophages, including *SPP1*-high tumor-associated macrophages (*SPP1*^{hi}-TAMs), are relatively sparse, implicating neutrophils as potential key drivers of ICI resistance. However, their role in human mCRPC bone metastases remains poorly characterized. We hypothesize that single-cell characterization of immunosuppressive neutrophil subsets can find new therapeutic opportunities to enhance immunotherapeutic efficacy.

Methods: We performed single-cell profiling of prostate cancer biopsies across disease stages and tumor sites, including bone metastases, as well as bone samples from tumor-free patients who underwent hip replacement surgery. To validate these findings, we also employed a murine intraosseous CRPC model, combining multi-omic single-cell analyses with functional assays and mechanistic studies.

Results: Single-cell profiling of patients revealed abundant neutrophil populations in the bone compared to other soft tissues, with distinct subsets corresponding to developmental trajectories. Among mature neutrophils, an inflammatory subset expressing elevated *IL1B* expression (*IL1B*^{hi}-mNeu) was significantly enriched in mCRPC bone metastases versus tumor-free bone tissues and expressed elevated immunosuppressive transcriptional programs. Multi-omic analyses of the intraosseous CRPC murine model identified an analogous *Il1b*^{hi}-mNeu population that suppressed CD8^{+} T cell function in co-culture. *In vivo* studies showed that targeting *Il1b*^{hi}-mNeu with a CXCR1/2 antagonist (SX-682) or adoptive transfer of these neutrophils into CRPC-bearing mice demonstrated that *Il1b*^{hi}-mNeu are key drivers of ICI resistance in intraosseous CRPC. Pathway analyses implicated pro-tumor inflammatory signaling as a major mechanism, in particular through IL-1β-mediated pathways. Pharmacologic inhibition of IL-1R diminished *Il1b*^{hi}-mNeu -mediated CD8^{+} T cell suppression *in vitro* and improved ICI responsiveness *in vivo*.

Conclusions: Our studies identify a distinct mediator of immunotherapy resistance in prostate cancer bone metastases. We demonstrate that mature inflammatory neutrophils (*IL1B*^{hi}-mNeu) are enriched in human mCRPC bone metastases and drive ICI resistance through IL-1R signaling. These findings suggest that targeting these cells and their signaling pathways could represent promising therapeutic strategies for overcoming ICI resistance in advanced prostate cancer.

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