

Identification of neutrophil-driven mechanisms underlying immunotherapy resistance in prostate cancer bone metastasis

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Background: Patients with metastatic castration-resistant prostate cancer (mCRPC) show limited responses to immune checkpoint inhibitors (ICIs),^[1,2] particularly those with bone metastases, a common site of spread (70-90%).^[3,4] As a primary hematopoietic organ, the bone marrow (BM) serves contains abundant immune cells,^[5] with myeloid cells comprising >70% of the hematopoietic compartment.^[6,7] Our previous work demonstrated that an immunosuppressive macrophage population contributes to ICI resistance in human mCRPC.^[8] Although neutrophils are known drivers of ICI resistance across multiple cancer types and represent the most abundant myeloid cell population in the BM, their immunosuppressive functions in human mCRPC bone metastases remain poorly understood. We hypothesize that single-cell characterization of neutrophil subsets will identify therapeutic targets to improve immunotherapeutic efficacy.

Methods: We generated single-cell profiles from prostate cancer biopsies representing multiple disease stages and metastatic sites, including the prostate, bone, lymph nodes, and liver, as well as tumor-free bone controls. Findings were validated in a murine intratibial CRPC model using comprehensive multi-omic single-cell approaches, functional and mechanistic studies, and *in vivo* ICI response analyses. We analyzed a published SU2C/PCF East Coast Dream Team consortium dataset to extend the clinical relevance of our findings.

Results: Single-cell profiling of patient biopsies identified significantly neutrophil abundance in bone metastases versus other soft tissues, with heterogeneous developmental states. A mature *IL1B*^{hi}-mNeu neutrophil subset (*IL1B*^{hi}-mNeu) was expanded in mCRPC bone metastases compared to tumor-free bone samples and exhibited elevated immunosuppressive transcriptional programs.^[9–12] Multi-omic analyses in an intraosseous CRPC mouse model identified an analogous *IL1B*^{hi}-mNeu subset that suppressed CD8+ T cell activity in culture. Pharmacologic CXCR1/2 inhibition and adoptive transfer studies demonstrated that *IL1B*^{hi}-mNeu promotes ICI resistance *in vivo*. Pathway and computational analyses implicated *IL1B*-driven inflammatory signaling,^[13] and IL-1R blockade reduced *IL1B*^{hi}-mNeu-mediated CD8+ T cell suppression while enhancing responses to combined PD-1 and CTLA-4 inhibition *in vivo*. In SU2C/PCF East Coast Dream Team data, higher *IL1B*^{hi}-mNeu enrichment correlated with stronger immunosuppressive signatures and worse outcomes,^[14] supporting *IL1B*^{hi}-mNeu as a potential immunotherapeutic target in patients.

Conclusions: Our study identifies a distinct mediator of immunotherapeutic resistance in bone metastases. We demonstrate not only an increased prevalence of *IL1B*^{hi}-mNeu in human bone mCRPC metastases, but also that these neutrophils drive ICI resistance through IL-1-driven inflammatory signaling pathways. These findings suggest that targeting *IL1B*^{hi}-mNeu and/or their associated signaling axes may represent a promising therapeutic strategy for overcoming immunotherapy resistance in mCRPC.

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Conflicts of Interest: L.F. has received research support from Roche/Genentech, Abbvie, Bavarian Nordic, Bristol Myers Squibb, Dendreon, Janssen, Merck and Partner Therapeutics; has served on the scientific advisory boards of Actym, Astra Zeneca, Bioatla, Bristol Myers Squibb, Daiichi Sankyo, Immunogenesis, Innovent, Merck, Sutro and Roche/Genentech; and reports ownership interest in Actym, BioAtla, ImmunoGenesis, Nutcracker, and Therapaint. E.M.V. has received research support from Novartis, BMS, and Sanofi, and served on the scientific advisory boards of Tango Therapeutics, Genome Medical, Genomic Life, Enara Bio, Manifold Bio, Monte Rosa, Novartis Institute for Biomedical Research, Riva Therapeutics, and Serinus Bio. The remaining authors declare no competing interests.

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