Characterization of patient (polymorphonuclear neutrophils) PMNs reveals a novel role for androgen receptor regulation of neutrophil immune response

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Metastatic disease of prostate cancer (PCa) is currently incurable. Bone is the most common tissue site for prostate cancer metastasis and progression in bone is largely dictated by tumor-stromal interactions in the bone microenvironment. We showed previously that bone marrow neutrophils inhibit bone metastatic PCa growth, however metastatic PCa becomes resistant to neutrophil response and progresses within the bone compartment. It is unclear how these findings translate to a clinical setting. Further, the mechanisms associated with tumor resistance to neutrophil immune response remain unknown and presents a new avenue for therapeutic intervention. Thus, the goals of this study were: 1) to define the impact of metastatic PCa on neutrophil function throughout prostate cancer progression and 2) to determine the potential of neutrophils as predictive biomarkers of PCa disease progression.

To do this, in a prospective clinical study, peripheral blood polymorphonuclear neutrophils (PMNs) were collected from 4 patient cohorts (localized PCa, metastatic hormone-sensitive PCa (mHSPC), metastatic castration-resistant (mCRPC) and healthy men) and PMN molecular and functional properties assessed, including: cancer cell cytotoxicity, cell surface markers, and transcriptome alterations (bulk RNA sequencing). We observed a significant decrease in PMN expression of CD10, a marker for differentiation, with disease progression while in tandem, there was an increase in pro-inflammatory markers, including CD88 (complement receptor) in mCRPC men compared to mHSPC. Similarly, there a was pro-inflammatory gene signature in mCRPC PMNs compared to healthy and mHSPC men, including elevated granule enzymes (*lactoferrin* and *MMP8*) and genes associated with neutrophil activation (*CYBB, C3AR1, SERPINB1*).

PMNs from all disease stages and healthy men were equally cytotoxic to human PCa cell lines (LNCaP, C42B, and PC3). Surprisingly, second generation androgen deprivation therapy (ADT), compared to first-line therapy, suppressed patient PMN cytotoxicity against mCRPC cells (C42B, PC3). This suppression was due to androgen receptor (AR)-mediated regulation of the pleotropic and immune suppressant cytokine, transforming growth factor beta receptor I (TβRI). PMNs from mCRPC patients who received 2nd generation ADT, expressed significantly more TβRI than patients who only received 1st line ADT. Further, we found that PMNs in CRPC patient bone metastases express more TβRI than PMNs in matched liver metastases, which express little to no TβRI. Treatment of mouse bone marrow neutrophils with enzalutamide, increased neutrophil TβRI expression in a dose dependent manner and blocked cytotoxicity against PCa cells. Exogenous testosterone treatment *in vivo*, pharmacologic inhibition (using Repsox, a small molecule kinase inhibitor of TβRI) or genetic deletion (using conditional TβRI knockout mice) rescued enzalutamide-mediated neutrophil suppression and restored neutrophil cytotoxicity. These data collectively suggest that AR inhibition suppresses anti-tumor neutrophil responses via TβRI (Figure 1) however, this study highlights the ability to leverage standard-care ADT to generate neutrophil anti-tumor responses against bone metastatic PCa.

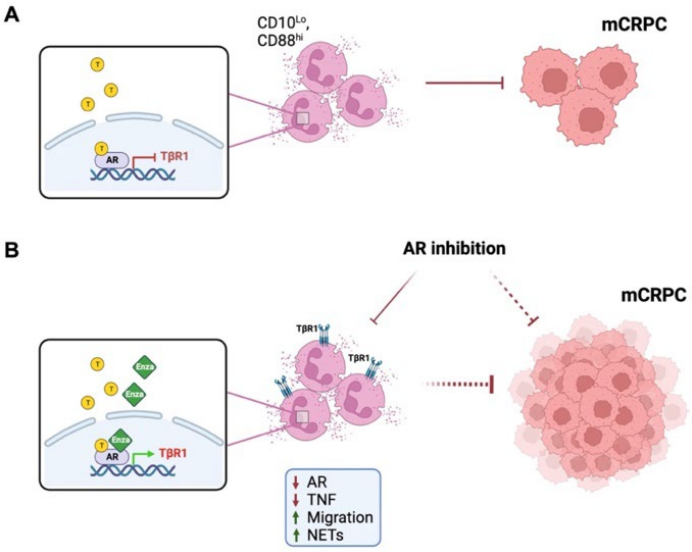

Figure 1. AR inhibition and 2nd generation ADT regulates neutrophil immune function via TBRI.

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