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## Immune Cell Types and Biology

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## ANDROGEN BLOCKADE PRIMES NLRP3 INFLAMMASOME IN MACROPHAGES TO INDUCE TUMOR CELL PHAGOCYTOSIS

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Background While immune-based therapies induce durable remissions in subsets of patients across a wide range of malignancies, efficacy in metastatic castrate-resistant prostate cancer (mCRPC) is limited. <sup>1-3</sup> One hypothesis for immunotherapy failure in mCRPC is a sparse immune infiltrate in the tumor microenvironment (TME), with predominance of immunosuppressive tumor-associated macrophages (TAM). Therapeutic strategies to overcome TAM-mediated immunosuppression are critically needed in advanced PC. Here we investigated the impact of NLR family pyrin domain containing 3 (NLRP3) inflammasome activation as a strategy to overcome TAM immunosuppression in advanced PC.

Methods Single cell RNAseq was utilized to assess NLRP3 expression/inflammasome activity within TME of ADT-treated metastatic PC patients. Mechanistic *ex vivo* polarization/west-ern blotting/phagocytosis assays were performed on immortalized bone marrow derived macrophages (iBMDM) treated with androgen receptor blockade (ARB) ± NLRP3 agonist (N). For *in vivo* studies, syngeneic mice with established c-myc-driven prostate tumors (Myc-CAP) were treated with N singly or in combination with ADT, with or without clodronate (Cl, systemically depletes phagocytic macrophages). Immune profiling/NLRP3 expression and tumor growth were assessed by flow cytometry and Vernier Caliper, respectively.

Results Using unbiased single cell RNA sequencing studies in metastatic PC patients, we discovered that NLRP3, an innate immune sensing protein, is highly expressed in TAM from metastatic PC patients treated with ADT, relative to benign bone marrow controls, other tumor types or untreated primary PC (figures 1–2). Based on these findings, we hypothesized that ARB will enhance NLRP3 expression and innate immune tumor control in advanced PC. We discovered that blockade of TAM-intrinsic androgen receptor (AR) activity enhances NLRP3 expression, but not inflammasome activity within tumor-promoting M2-TAM. In contrast, anti-tumor M1-TAM had high *de novo* NLRP3 expression, regardless of AR activity (figure 3). The combination of ARB and N

significantly enhanced inflammasome activity and phagocytosis of cancer cells by M2-TAM, whereas N treatment alone was sufficient to induce inflammasome activity/phagocytosis in M1-TAM (figures 3, 4A). Following N treatment, all TAMs acquired a distinct phenotype with high PD-L1 and CD86 expression, indicative of phagocytic TAM (figure 4B). Critically, N in combination with ADT resulted in significant TAM-dependent tumor control in an aggressive c-myc driven advanced PC model, with 55% of mice achieving complete tumor clearance (figure 5).

Conclusions Collectively, our results credential the NLRP3 inflammasome as an AR-regulated 'macrophage phagocytic checkpoint' that can be inducibly expressed and activated in TAM following ADT/NLRP3 agonist treatment, respectively, resulting in TAM-mediated phagocytosis and tumor control (figure 6).

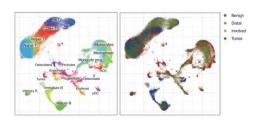
Acknowledgements We thank the Prostate Cancer Foundation and Bristol-Myers Squibb (BMS) for funding this work, and to BMS for providing BMS-392959 (NLRP3 agonist).

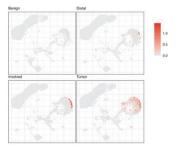
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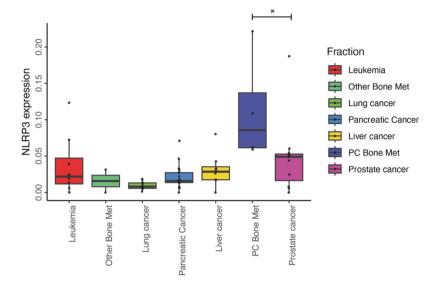
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Ethics Approval All human samples were obtained following written informed consent and the studies were conducted in accordance with Declaration of Helsinki ethical guidelines. Bone metastatic (BMET) PC samples (n = 7), BMET non-PC samples (n = 3) and bone marrow from patients with benign inflammation (n = 3) were collected and handled in accordance to protocol approved by the Institutional Review Board (Dana Farber/Harvard Cancer Center protocol 13–416 and Partners protocol 2017P000635/PHS). All murine studies were performed in accordance with NIH guidelines and protocol approved by the Institutional Animal Care and Use Committee (IACUC) at University of Chicago (protocol #72483).

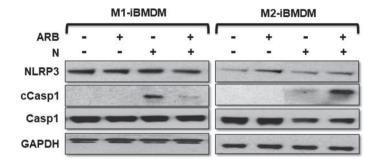
Consent Written informed consent was obtained from patients for all human samples in accordance with Declaration of Helsinki ethical guidelines.



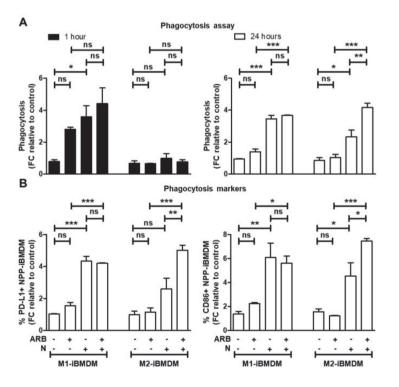




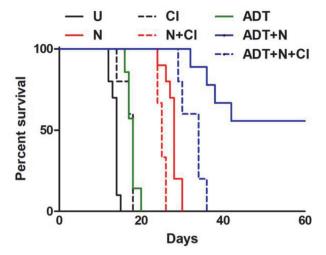
Abstract 1537 Figure 2



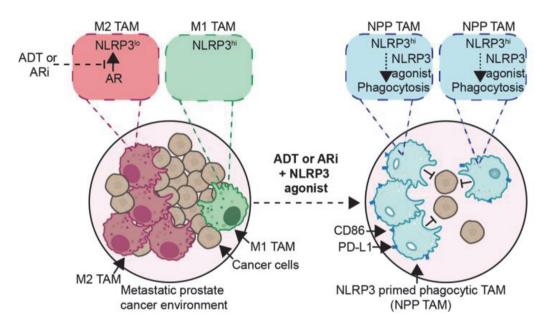
Abstract 1537 Figure 3



Abstract 1537 Figure 4



Abstract 1537 Figure 5



Abstract 1537 Figure 6

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