Androgen receptor blockade primes NLRP3 in tumor-associated macrophages to enhance NLRP3 agonist-mediated phagocytosis and tumor control

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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. One hypothesis for immunotherapy failure in mCRPC is a sparse immune infiltrate, with predominance of immunosuppressive tumor-associated macrophages (TAM) in the tumor microenvironment. Therefore, therapeutic strategies to overcome TAM-mediated immunosuppression are critically needed in advanced PC. NLR family pyrin domain containing 3 (NLRP3) is an intracellular innate immune sensing protein, which when activated, facilitates IL-1β, IL-18 cytokine release and pyroptosis, a form of inflammatory cell death. 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 and bulk-RNAseq was utilized to assess NLRP3 expression/inflammasome activity within TME of androgen deprivation therapy (ADT)-treated metastatic PC patients. Mechanistic *ex vivo* polarization/western blotting/phagocytosis assays were performed on immortalized bone marrow derived macrophages (iBMDM) and human moncytic THP-1 cell line treated with androgen receptor blockade (ARB) +/- NLRP3 agonist (N). For *in vivo* studies, mice with established c-myc-driven syngeneic 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 is highly expressed in TAM of metastatic PC patients treated with ADT, relative to benign bone marrow controls, other tumor types or untreated primary PC. 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-like TAM. Mechanistically, CHIPseq analysis revealed androgen (R1881)-induced binding of AR at H3K27ac marked sites across the NLRP3 gene locus in macrophage THP-1 line. In contrast, anti-tumor M1-like TAM had high *de novo* NLRP3 expression, regardless of AR activity. The combination of ARB and N significantly enhanced phagocytosis of cancer cells by M2-like TAM independent of pyroptosis, whereas N treatment alone was sufficient to induce phagocytosis in M1-like TAM. Following ARB/N treatment, all TAMs acquired a distinct phenotype with high

PD-L1 and CD86 expression, indicative of phagocytic TAM. Critically, ADT in combination with N resulted in significant TAM-dependent tumor control in an aggressive c-myc driven advanced PC model, with 55% of mice achieving complete tumor clearance.

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.

Funding Acknowledgements

Michael & Lori Milken Family Foundation-Prostate Cancer Foundation Challenge Award

Conflicts of Interest

Dr. Patnaik has consulting for Exelixis, Tolmar Therapeutics, Janssen, Novartis and BostonGene and received research support from BMS, Clovis Oncology, Progenics, Janssen, Laekna, Astrazeneca, Xencor, Zenith, and Exelixis. None of these relationships have influenced the content of this submission, and the other authors declare no competing interests.