STING Activation to Overcome Resistance to Immune Checkpoint Blockade in PTEN-deficient Prostate Cancer

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Background: There has been a resurgent interest in prostate cancer (PC) immunotherapy, partly based on the profound and durable clinical responses to immune checkpoint blockade (ICB) antibodies targeting CTLA-4 and PD-1/PD-L1 in multiple other cancers, and partly due to emergence of resistance to various androgen receptor-based therapies. However, only approximately 10% of metastatic castrate-resistant prostate cancer (mCRPC) patients respond to CTLA4 and/or PD1 pathway directed therapy. While there is ongoing work to identify predictive biomarkers to ICB responsiveness, early preclinical data from our group suggests that targeting fundamental DNA repair and oncogenic signaling pathways could markedly increase the fraction of patients responsive to immunotherapeutic interventions.

Methods: For *in vitro* work, myc-CAP and B6-myc cell lines were grown in DMEM medium containing 10% FBS, 1% P/S, and 2% L-glutamine. Bone marrow derived macrophages from wild type and STING$^{-/-}$ mice used for co-culture experiments were differentiated in 10% RPMI containing 1% P/S and 30% L-conditioned media for a total of 5 days. For immune profiling by flow cytometry, cells were stained using the following antibodies: anti-mouse CD45, CD11b, F480, MHC-II, CD86, PD-L1, PD-1, CD4, CD8. The supernatants from the co-culture experiments were utilized for quantification of IFNB1 (Biolegend) and related cytokines by ELISA. To look for biomarkers of DNA damage and signaling proteins downstream of STING activation, confocal staining for cytosolic dsDNA and western blot for polyADP-ribosylation, p-gamma-H2AX, cGAS/ STING, pIRF3/IRF3, pTBK1/TBK1, and GAPDH was completed on cell lines. Microvesicles (MVs) were isolated by ultracentrifugation and utilized for quantitative and qualitative analysis. For *in vivo* experiments, Myc-CAP cells and B6-MYC tumor were engrafted subcutaneously into FVB and C57Bl/6J mice, respectively. Statistical analysis was done by one-way ANOVA.

Results: Our recent preclinical data demonstrate that PARP inhibitors (PARPi) in combination with PI3K inhibitors (PI3Ki) activate the innate immune DNA-sensing cGAS/STING pathway within the tumor microenvironment (TME), which was accompanied by enhanced T cell infiltration and tumor clearance of PTEN$^{+/+}$ myc^{hi} murine PC models *in vivo*. This anti-tumor response elicited by PARPi/PI3Ki was abrogated in immunodeficient mice, demonstrating an immune-based mechanism. Critically, PARPi/PI3Ki-mediated tumor regression in myc-driven murine models was abolished by concomitant clodronate treatment, suggesting that macrophages within the TME were driving the anti-cancer innate immune response. *Ex vivo* co-culture assays showed that DNA damage within myc-driven cancer cells induced by PARPi/PI3Ki, resulted in STING-dependent activation of tumor-associated M2 macrophages (TAMs), and a functional shift to anti-cancer M1 phenotype, with high MHC-II and enhanced antigen presentation capacity. Interestingly, STING activation within myeloid suppressive cells induced by the PARPi/PI3Ki combination was mediated by dsDNA-containing MVs released by PARPi-treated tumor cells, coupled with PI3Ki-mediated de-repression of c-GAS enzymatic activity within TAMs. Interestingly, *ex vivo* reconstitution studies revealed that DNAse treatment of the MVs completely abolished the STING activation response elicited within macrophages, thus demonstrating that the DNA fragments on the surface of exosomes drives STING pathway activation within macrophages. Strikingly, CRISPR/CAS9-mediated PTEN deletion in the myc^{hi} model abrogated STING pathway activation and T cell infiltration within the TME, resulting in resistance to PARPi/PI3Ki treatment in the presence or absence of ICB. Critically, MVs from PARPi-treated PTEN$^{-/-}$ tumor cells (which undergo DNA damage) were unable to activate STING within macrophages, due to absence of dsDNA fragments on their surface. This block to DNA damage-induced STING pathway activation within PTEN-deficient cancers can be overcome by administration of direct STING agonists/PI3K combinations *in vivo*. Preliminary correlative genomic and immune profiling of paired metastatic biopsies from a combination co-clinical trial of PARPi/ICB in castrate-resistant prostate cancer patients, supports the preclinical data described above, and will be presented at the meeting.

Conclusions: These results demonstrate that DNA damage elicited by PARPi/PI3Ki can reprogram the myeloid tumor microenvironment to enhance anti-tumor immunity within PTEN$^{+/+}$ myc-driven cancers. The block to DNA damage-induced STING pathway activation in PTEN$^{-/-}$ cancers can be overcome by administration of direct STING agonist/PI3K combinations *in vivo*.

Conflicts of Interest: Dr. Patnaik has received research funding from Bristol Myers Squibb (preclinical studies and clinical trial) and Clovis (clinical trial).

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