Inhibitors of Ataxia-Telangiectasia Related (ATR) protein lead to innate immune pathway activation and enhanced response to immune therapy

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Background: In prostate cancer (PCa), androgen receptor (AR) signaling regulates the cell cycle and mitigates replication stress via upregulation of CDC6 and the TopB1-ATR-Chk1 pathway, the key axis in replication stress response (RSR). Preclinical studies and early phase clinical trials of DDR inhibitors, including PARP inhibitors, have shown synergy with immune checkpoint therapy, whereby DDR inhibitors lead to S-phase specific damage, accumulation of cytosolic DNA and activation of innate immune pathways. DDR inhibitors have rapidly expanded, now including inhibitors of ATR. However, the impact of these novel, potent, highly-specific inhibitors of ATR on immunocompetent PCa preclinical models has not been fully investigated. Furthermore, dynamic changes in DDR and innate immune gene expression profiles that result from various targeted therapies have not been reported. In this study we use preclinical, immunocompetent models of advanced PCa to investigate the efficacy, transcriptional targets, induction of immunostimulatory cytosolic DNA, and synergy with anti-PD-L1 therapy associated with ATR inhibition.

Methods: We used PCa cell lines derived from ras+myc—induced mouse PCa tumors (RM-1, RM-9 and RM-1-BM) to analyze ATRi (BAY1895344 or VX-970) effects on ATR-driven survival/proliferation, DNA damage, cGAS-STING signaling, and chemokine expression in comparison to olaparib. MTS assay was used to analyze cell growth. PicoGreen staining was performed to detect cytosolic DNA. Western blot analysis was used to detect DNA damage, and activation of cGAS-STING signaling, and qRT-PCR was used to examine the mRNA expression of cGAS-STING—regulated chemokines. RM-1-BM model was used to assess the efficacy and safety of single agent ATR inhibitor, single-agent anti-PD-L1, and the combination in vivo.

Results: Treatment of mouse PCa cell lines with ATRis (BAY1895344 or VX-970) or a PARP inhibitor (olaparib) demonstrated dose-dependent (0.125-8μM) growth suppression and cytosolic DNA accumulation, while ATRis showed greater growth suppression than olaparib. ATRi and olaparib treatment generated similar levels of DNA damage (γH2AX); however, ATRi treatments resulted in significantly increased CCL5 and CXCL10 levels compared to olaparib (2-10 fold, $P < 0.05$), with BAY1895344 demonstrating superior induction of chemokine expression ($P < 0.05$). cGAS, STING, TBK1 or IRF3 siRNA knockdown significantly suppressed all of these responses ($P < 0.05$). The combination of BAY1895344 with anti-PD-L1 was safe and resulted in significantly greater tumor response than either single agent in RM-1-BM model.

Conclusions: ATR inhibition induces S-phase specific DNA damage, accumulation of cytosolic DNA, activation of cGAS-STING signaling, CCL5 and CXCL10 expression, and suppression of cancer growth in immunocompetent, advanced PCa models. ATRi had greater impact on innate immune pathway activation than olaparib, and the combination of ATRi with anti-PD-L1 was safe and resulted in significant efficacy in our model system. These results will directly inform biomarker-directed clinical trials of ATR inhibitors in combination with immune checkpoint blockade for patients with advanced solid cancers.

COI & Funding: There are no conflicts of interest in direct relation to this work. Bayer contributed scientific insight through the Bayer-MDACC alliance.