

Therapeutic Vulnerability of Prostate Cancer to VPAC Antagonism

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Background: Tumor-associated macrophages (TAM) represent a dominant immunosuppressive force in the prostate cancer (PC) microenvironment, driving therapeutic resistance and progression. Their abundance correlates with poor clinical outcomes and with acquired resistance to androgen-deprivation therapy (ADT). Our preclinical work has established that macrophage phagocytosis can partially restrain PC growth at both castration-sensitive and -resistant stages; however, this response remains incomplete. We therefore hypothesized that activating phagocytosis by targeting androgen-regulated checkpoints on macrophages empowers ADT and provides a combinatorial therapeutic strategy to control PC via anti-tumor immunity.

Methods: We profiled bone marrow-derived macrophages (BMDM) using RNA sequencing and Western blot to identify androgen-regulated immune checkpoints, followed by functional validation in polarization and phagocytosis assays. *In vivo* anti-tumor efficacy of these molecules was tested using syngeneic TrampC1, Myc-CAP, cMyc-Rb-knockout models in both wildtype and immune checkpoint-knockout mice. These studies included treatment with androgen receptor (AR) and immune checkpoint blockade alone and the combination arms with relevant untreated controls. Translational relevance was assessed using ADT-treated patient specimens, plasma vasoactive intestinal peptide (VIP) levels, and cBioPortal datasets.

Results: Transcriptomic and proteomic profiling revealed selective upregulation of VIP receptor 1 (VPAC1) on immunosuppressive M2-like BMDM vs anti-tumor M1-like. Enzalutamide further upregulated VPAC1 on both M1/M2-like BMDM. Whereas there were no alterations in other immune checkpoints. Functional assays revealed an increased IL-12 expression in BMDM following AR and VPAC co-blockade. Also, enzalutamide increased phagocytosis of PC cells in VIP-knockout BMDM relative to untreated control, but not in wildtype, suggesting that VPAC1 serves as an androgen-regulated macrophage-suppressive checkpoint. Strikingly, bioinformatic analysis showed positive correlations between 1) VPAC1 and AR, and 2) VIP and synaptophysin (SYP) in prostate adenocarcinoma samples. In neuroendocrine PC, there was no detectable VIP or VPAC1 expression, outlining their role in initiating lineage plasticity toward neuroendocrine fate. Corroborating our hypothesis, plasma VIP levels were increased following ADT in subsets of PC patients.

Furthermore, degarelix improved the survival of syngeneic TrampC1 tumor (harboring both adenocarcinoma and neuroendocrine phenotypes)-bearing mice, particularly in VPAC1-knockout mice, compared to VIP or VPAC2-knockout and wildtype controls, with corresponding benefits in prostate tumor growth control. In the androgen-sensitive Myc-CAP syngeneic model, a potent VIP receptor antagonist, ANT308, increased degarelix-mediated tumor control. Also, ANT308 treatment yielded similar effects in androgen-independent Myc-Rb-knockout PC (Rb loss-of-function mutation is a known neuroendocrine driver) model, highlighting the synergy of co-blocking VIP/VPAC and AR pathways for controlling PC.

Conclusions: VPAC1 is an androgen-regulated macrophage checkpoint. VPAC inhibition with ANT308 leads to anti-tumor phagocytosis and enhances ADT-mediated control of prostate tumors in both adenocarcinoma and mixed adeno-neuroendocrine diseases. Further mechanistic investigation is needed to elucidate the intrinsic *versus* extrinsic role of VIP/VPAC at those stages, informing the design of clinical trials for ANT308 in PC patients.

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