Background
Prostate cancer is the most diagnosed cancer in men worldwide and the leading cause of cancer death in men worldwide. Checkpoint immunotherapy has yielded meaningful responses across many cancers but has shown modest efficacy in advanced prostate cancer. B7 homolog 3 protein (B7-H3/CD276) is an immune checkpoint molecule and has emerged as a promising therapeutic target. However, much remains to be understood regarding B7-H3’s role in cancer progression, predictive biomarkers for B7-H3 targeted therapy, and combinatorial strategies.

Methods
Genetic inactivation of PTEN and TP53 is common in advanced prostate cancers. To identify PTEN- and p53-associated immune checkpoints, we performed multi-omics analyses of expression patterns of 51 checkpoint molecules in human prostate cancer samples. Then, we generated a novel genetically engineered mouse model to elucidate the role of B7-H3 in tumor development and progression of PTEN/p53-deficient prostate cancer. We also performed unbiased immunoprofiling using Mass Cytometry (CyTOF) and Immunofluorescence to visualize B7-H3’s impact on immune components in prostate tumors. In addition, we tested the B7-H3 inhibitor alone or in combination with other checkpoint blockades in preclinical models of castration-resistant prostate cancer (CRPC).

Results
Our multi-omics analyses identified B7-H3 as one of the most abundant immune checkpoints in prostate tumors containing PTEN and TP53 genetic inactivation. Here, we sought in vivo genetic evidence for, and the mechanistic understanding of, the role of B7-H3 in PTEN/TP53 deficient prostate cancer. We found that loss of PTEN and TP53 induced B7-H3 expression by activating transcriptional factor Sp1. Prostate-specific deletion of Cd276 resulted in delayed tumor progression and reversed the suppression of tumor-infiltrating T cells and NK cells in Pten/Tp53 genetically engineered mouse models. Furthermore, we tested the efficacy of the B7-H3 inhibitor in preclinical models of CRPC. We demonstrated that enriched regulatory T cells and elevated Programmed Cell Death Ligand 1 (PD-L1) in myeloid cells hinder the therapeutic efficacy of B7-H3 inhibition in prostate tumors. Finally, we showed that B7-H3 inhibition combined with blockade of PD-L1 or Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) achieved durable anti-tumor effects and had curative potential in PTEN/TP53-deficient CRPC model.

Conclusions
Given that B7-H3 targeted therapies have been evaluated in early clinical trials, our studies provide new insights into the potential of biomarker-driven combinatorial immunotherapy targeting B7-H3 in prostate cancer, among other malignancies.

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