

ATM-deficient prostate cancer cells exhibit therapeutic sensitivity to cell cycle checkpoint inhibition

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Background: One frequently altered pathway in advanced prostate cancer (PC) includes genes involved in DNA Damage Response (DDR). Tumors harboring DDR mutations are associated with more aggressive disease and poor prognosis, due in part to the accumulation of DNA damage and increase in genomic instability. Among these DDR mutated genes, those involved in homologous recombination repair (HRR) are the most common (23-28%), with the ataxia-telangiectasia mutated (*ATM*) gene being the second most frequently altered. Although poly (ADP-ribose) polymerase (PARP) inhibitors are approved for patients with HRR-deficient prostate cancer, *ATM*-mutant tumors often exhibit lower sensitivity to these agents compared to *BRC42*-mutated tumors, particularly in the castration-resistant setting. This highlights an unmet clinical need for more effective targeted therapies. We, therefore, sought to identify synthetic lethal vulnerabilities that could be exploited therapeutically in *ATM*-deficient prostate cancer.

Methods: We performed a genome-scale CRISPR interference (CRISPRi) screen in *ATM*-deficient prostate cancer cells to identify genetic dependencies associated with *ATM* loss. Candidate druggable targets nominated by the screen were validated using genetic and pharmacologic approaches across hormone-sensitive and castration-resistant *ATM*-deficient prostate cancer cell line models. Therapeutic efficacy was further evaluated *in vitro* using single-agent and combination treatments as well as *in vivo* using xenograft models.

Results: We showed *ATM*-deficient prostate cancer cells exhibit selective dependencies on DDR and cell cycle checkpoint pathways. Inhibition of genes involved in these pathways significantly repressed the growth of *ATM*-deficient PC cells, compared to *ATM*-wild-type PC cells. In particular, we evaluated WEE1, a kinase which regulates the cell cycle transition from G2 to mitosis and prevents mitotic entry in the presence of DNA damage, as a therapeutic vulnerability in *ATM*-deficient PC. WEE1 inhibition significantly impaired the viability of *ATM*-deficient PC cells *in vitro* across hormone-sensitive and castration-resistant models and reduced tumor burden *in vivo*, compared to *ATM*-wild-type prostate cancer cells.

Furthermore, combining WEE1 inhibition with DDR-targeting agents, such as PARP and ATR inhibitors, synergistically decreased the viability of ATM-deficient PC cells *in vitro*.

Conclusions: Our results demonstrate cell cycle checkpoint dependence as a therapeutically actionable vulnerability in *ATM*-mutated prostate cancer and highlight WEE1 as a promising therapeutic target. Taken together, these findings provide a rationale for evaluating WEE1 inhibition, alone and in combination with PARP or ATR inhibitors, as a precision treatment strategy for patients with *ATM*-mutated prostate cancer.

Funding Acknowledgements: LNC acknowledges funding from the Benioff Initiative for Prostate Cancer Research and the UCSF Prostate Cancer Program Pilot Research Award. AKS acknowledges funding from the Benioff Initiative for Prostate Cancer Research. MS acknowledges funding from the Swedish Cancer Society Junior Clinical Investigator Award and Prostate Cancer Foundation Young Investigator Award. AF acknowledges support from the Benioff Initiative for Prostate Cancer Research, UCSF Prostate Cancer Program Pilot Research Award, and UCSF Sandler Program for Breakthrough Biomedical Research. RB acknowledges National Institutes of Health grants DP2-CA290244 and K08CA226348, American Cancer Society grant RSG-22-021-01-CDP, US Department of Defense grants PC220482 and PC141542, Prostate Cancer Foundation grant 16YOUN01, the Benioff Initiative for Prostate Cancer Research, and the Bakar Aging Research Institute. MK acknowledges funding from the NIH R35GM156189; Welch Foundation AQ-2287-20260402. DAQ acknowledges funding from the Benioff Initiative for Prostate Cancer Research, the Prostate Cancer Foundation, NCI SPORE P50CA275741, and Department of Defense awards W81XWH-22-1-0833 and HT94252410252. JML acknowledges funding from P01CA298991, P50CA269011, DOD PC240511, and 1R37CA264518-01A1. FYF acknowledges funding from the Benioff Initiative for Prostate Cancer Research, Prostate Cancer Foundation Challenge and Tactical Awards, and R01CA230516.

Conflicts of Interest Disclosure Statement: MS receives speaker fees from Astellas and consultant fees and advisory board from Veracyte/Adelphi Targis unrelated to this work. RB has served on advisory boards for Pfizer and Johnson & Johnson. JML is a paid consultant with Astellas, Pfizer, Gilead, Janssen, Sanofi, Foundation Medicine, Cytogen, AstraZeneca, Arvinas, MacroGenics, and Daiichi Sankyo and founder of EOLAS, Inc. FYF consulted for Astellas, Bayer, Blue Earth Diagnostics, BMS, EMD Serono, Exact Sciences, Foundation Medicine, Janssen Oncology, Myovant, Roivant and Varian, and served on the Scientific Advisory Board for BlueStar Genomics and SerImmune. FYF has patent applications with Decipher Biosciences and PFS Genomics/Exact Sciences in breast cancer unrelated to this work. A.A. is a co-founder of Azkarra Therapeutics, Ovibio Corporation, Tango Therapeutics and Tiller Tx; a member of the board of Cambridge Science Corporation, Cytomx and Ovibio; a member of the scientific advisory board of Ambagon, BlueStar/Clearnote Health, Circle, Deciphera, Genvivo, GLadiator, Interdict Bio Inc., Earli, Leapfrog Bio, ORIC, Phoenix Molecular Designs, Trial Library, Yingli/280Bio and Z prime; a consultant for Catalio, Longitude, Novartis, Overwater, ProLynx and YK bioventures; and holds patents on the use of PARP inhibitors held jointly with AstraZeneca from which he has benefited financially (and may do so in the future).