

A large-scale patient-derived xenograft trial uncovers a CHK1 vulnerability in advanced prostate cancer and a strategy to overcome resistance

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Background: Advanced prostate cancer (PCa), particularly castration-resistant disease, remains a major cause of PCa mortality. PARP inhibitors primarily benefit patients with homologous recombination-deficient tumors, whereas radiation is often unsuitable for disseminated metastases. Genomic alterations common in advanced PCa increase replication stress, creating a potential vulnerability to checkpoint kinase 1 (CHK1) inhibition. We evaluated the CHK1 inhibitor prexasertib in castration-resistant PCa (CRPC) patient-derived xenograft (PDX) models and investigated biomarkers of response and strategies to overcome resistance.

Methods: Prexasertib efficacy was evaluated in 27 metastatic PCa PDX models with diverse genomic backgrounds and benchmarked against radiation and the PARP inhibitor talazoparib. Response was determined by comparing tumor-growth slopes in treated and vehicle groups using prespecified cutoffs. Pretreatment RNA sequencing was used to identify features associated with response. CRISPR/Cas9 knockout screens targeting ~1,800 DNA damage-response genes were performed in PC-3, 22Rv1, and LNCaP cells. Druggable candidates were prioritized and evaluated in proliferation and clonogenic assays, followed by *in vivo* validation in PDX models.

Results Prexasertib produced >80% tumor-growth inhibition in 26% (7/27) of PDX models. Integration of genomic and transcriptomic analyses with CRISPR screening linked CDK2 and E2F-target expression to prexasertib response. The screens also identified RRM2 as a synthetic-lethal vulnerability. Pharmacologic RRM2 targeting with gemcitabine restored prexasertib sensitivity in resistant models *in vitro* and *in vivo*.

Conclusions: Prexasertib demonstrated activity across CRPC PDX models, including models unresponsive to radiation or talazoparib, and achieved higher response rate than talazoparib. CDK2 and E2F-target expression emerged as candidate response biomarkers, while RRM2 inhibition with gemcitabine provided a strategy to overcome resistance. Together, these findings provide a strong preclinical rationale for clinical evaluation of prexasertib, alone and in combination with gemcitabine, in advanced PCa.

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