

Title: Pharmacological activation of the GCN2-mediated integrated stress response enhances the efficacy of BH3 mimetics in castration-resistant prostate cancer

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Background: Advanced prostate cancer (PCa) remains lethal, largely due to the inevitable emergence of treatment resistance. Therefore, therapeutic strategies that directly induce tumour cell death are urgently needed. BH3 mimetics promote apoptosis in haematological malignancies by inhibiting anti-apoptotic BCL2 family proteins but have shown limited efficacy in CRPC. This study aims to identify combination strategies that enhance the therapeutic activity of BH3 mimetics in CRPC.

Methods: A CRISPR interference (CRISPRi) loss-of-function screen targeting kinases, phosphatases and druggable genes (n=2,318) was performed in the presence or absence of navitoclax (BCLXL/BCL2 inhibitor) or S63845 (MCL1 inhibitor). Drug responses were assessed by viability and apoptosis assays. Mechanistic studies included siRNA-mediated gene silencing and pharmacological inhibition of the integrated stress response (ISR). Findings were validated in patient-derived xenograft organoids (PDX-Os), and BIM expression was analysed in two independent CRPC clinical cohorts

Results: Pathway enrichment analysis of CRISPRi sensitizers identified aminoacyl-tRNA biosynthesis as a top enriched pathway for both navitoclax and S63845, implicating activation of the GCN2-mediated ISR. Accordingly, NXP800, a GCN2 activator developed at the ICR, synergised with MCL1 and BCLXL inhibitors in CRPC models, inducing robust apoptosis. Cell death was dependent on BAX/BAK and was abolished by ISR inhibition, confirming an ISR-mediated mechanism. Genetic studies further identified GCN2, ATF4 and the BH3-only protein BIM as essential mediators of the apoptotic response. NXP800 rapidly induced BIM expression, and sensitivity to the combinations was associated with high basal BIM expression in PCa cell lines and PDX-Os. Furthermore, high tumour BIM expression was associated with shorter overall survival in patients with CRPC, indicating that this biomarker-driven strategy may target a clinically highly-aggressive subgroup.

Conclusions: Pharmacological activation of the GCN2-mediated ISR sensitizes CRPC cells to BH3 mimetics through BIM-dependent apoptosis. These findings support combining GCN2 activators with BH3 mimetics as a promising therapeutic strategy for advanced prostate cancer.

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