Second-generation EZH2 inhibitor CPI-0209 has therapeutic potential in androgen receptor-positive prostate cancer

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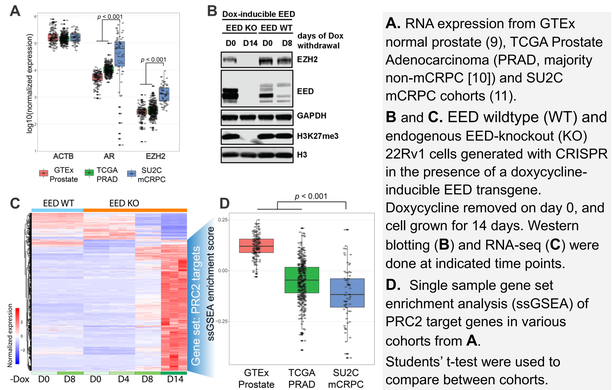
Introduction

- Enhancer of Zeste Homolog 2 (EZH2), a subunit of Polycomb Repressive Complex 2 (PRC2), catalyzes trimethylation of histone H3 on lysine 27 (H3K27me3) and functions in suppression of gene expression.
- Amplification and overexpression of EZH2 along with down-regulation of its target genes correlate with treatment resistance and poor prognosis in various tumor types including metastatic castration resistant prostate cancer (mCRPC) (1-4).
- While most mCRPC patients are initially responsive to agents targeting the androgen receptor (AR) signaling pathway (ARS inhibitors), tumors eventually develop resistance and progress.
- Other than EZH2 overexpression (5), mechanisms of resistance involve AR alterations, including AR amplification (6), expression of AR splice variants (e.g. AR V7) which constitutively activate ARS (7), and AR mutations that affect native ligand-binding specificity (8).
- CPI-0209 is an orally bioavailable and selective EZH2 and EZH1 inhibitor with highly enhanced potency and extended residence time compared to first-generation EZH2 inhibitors. CPI-0209 is currently being developed [NCT04104776].

High EZH2 and low PRC2 target genes expression in mCRPC



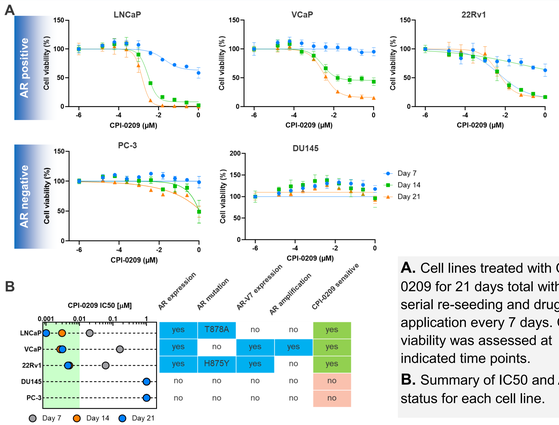
A. RNA expression from GTEx normal prostate (9), TCGA Prostate Adenocarcinoma (PRAD, majority non-mCRPC [10]) and SU2C mCRPC cohorts (11).

B and **C.** EED wildtype (WT) and endogenous EED-knockout (KO) 22Rv1 cells generated with CRISPR in the presence of a doxycycline-inducible EED transgene. Doxycycline removed on day 0, and cell grown for 14 days. Western blotting (**B**) and RNA-seq (**C**) were done at indicated time points.

D. Single sample gene set enrichment analysis (ssGSEA) of PRC2 target genes in various cohorts from **A**.

Students' t-test were used to compare between cohorts.

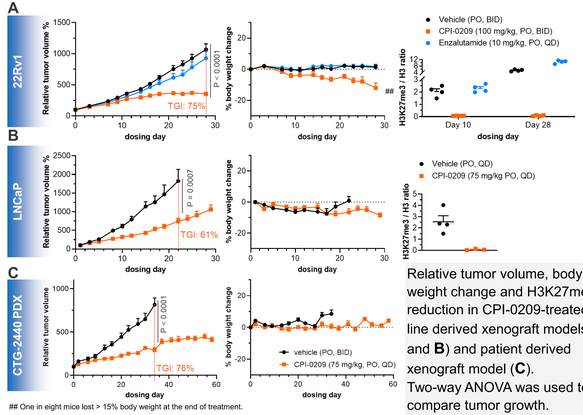
CPI-0209 preferentially inhibits growth of *AR* positive prostate cancer cell models



A. Cell lines treated with CPI-0209 for 21 days total with serial re-seeding and drug re-application every 7 days. Cell viability was assessed at indicated time points.

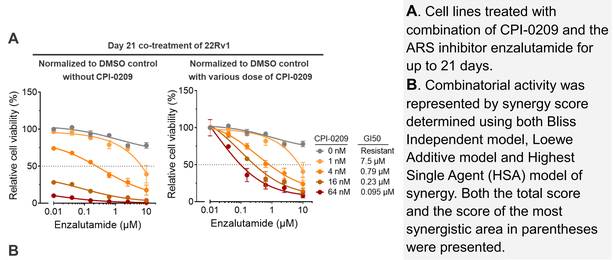
B. Summary of IC50 and AR status for each cell line.

CPI-0209 potently inhibits tumor growth of AR positive prostate cancer xenograft models



Relative tumor volume, body weight change and H3K27me3 reduction in CPI-0209-treated cell line derived xenograft models (**A** and **B**) and patient derived xenograft model (**C**). Two-way ANOVA was used to compare tumor growth.

Combination of CPI-0209 and Enzalutamide synergistically inhibit growth of AR positive PC cells



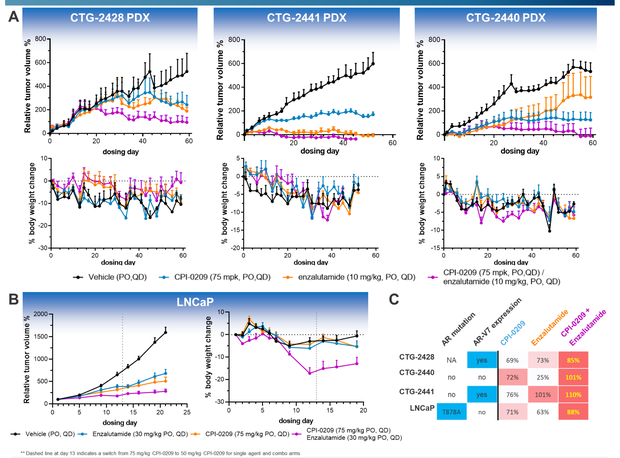
Cell Line	Treatment	GI50 day 7	GI50 day 14	GI50 day 21	Bliss synergy score day 7	Bliss synergy score day 14	Bliss synergy score day 21	Loewe synergy score day 7	Loewe synergy score day 14	Loewe synergy score day 21	HSA synergy score day 7	HSA synergy score day 14	HSA synergy score day 21
VCaP	CPI-0209	> 1	0.21	0.093									
	Enzalutamide	0.7	0.27	0.057	-0.7 (2.2)	-2.4 (3.9)	-3.5 (0.9)	0.4 (5.16)	7.9 (14.8)	6.0 (13.1)	2.9 (5.67)	8.0 (13.5)	5.9 (13.9)
22Rv1	CPI-0209	> 1	0.015	0.007									
	Enzalutamide	> 10	> 10	> 10	-5.6 (21.4)	5.3 (21.5)	5.5 (17.3)	10.3 (29.2)	7.0 (20.3)	-2.5 (6.5)	10.4 (29.6)	11.3 (29.1)	9.3 (26.9)

> 10 Strong synergy
5 ~ 10 Synergy
-5 ~ 5 Additive
-10 ~ -5 Antagonism
< -10 Strong antagonism

A. Cell lines treated with combination of CPI-0209 and the ARS inhibitor enzalutamide for up to 21 days.

B. Combinatorial activity was represented by synergy score determined using both Bliss Independent model, Loewe Additive model and Highest Single Agent (HSA) model of synergy. Both the total score and the score of the most synergistic area in parentheses were presented.

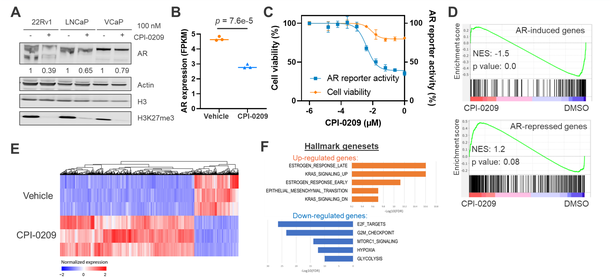
Combination of CPI-0209 and enzalutamide shows enhanced efficacy in prostate cancer xenograft models



A, B. Relative tumor volume and body weight change in CPI-0209-treated patient derived xenograft models (**A**) and cell line derived xenograft models (**B**).

C. Summary of AR status and tumor growth inhibition of each model.

CPI-0209 treatment modulates both AR-dependent and AR-independent pathways



A, B. AR expression in CPI-0209 treated cell lines for 7 days (**A**) or 22Rv1 derived xenograft model end of treatment samples (**B**).

C. Cell viability and AR response element driven luciferase reporter activity of 22Rv1 cells treated with CPI-0209 for 7 days.

D. Gene set enrichment analysis of AR-induced genes and AR-repressed genes in 22Rv1 derived xenograft models treated with CPI-0209 for 28 days.

E. Heatmap of differentially expressed genes in 22Rv1 derived xenograft models treated with CPI -0209 for 28 days.

F. Overrepresented Hallmark genesets in CPI-0209 up-regulated and down-regulated genes.

Conclusions

- Here we demonstrate that AR-positive prostate cancer cell lines are sensitive, while AR-negative cell lines are completely insensitive to CPI-0209. Sensitivity to CPI-0209 are also observed in AR-positive cell line-derived xenografts (CDX) and patient-derived xenografts (PDX) models.
- In AR-positive cells, CPI-0209 synergizes with enzalutamide and overcomes the anti-androgens resistance induced by AR alterations *in vitro*. In prostate cancer CDX and PDX models, the combination treatment of CPI-0209 and enzalutamide shows greater tumor growth inhibition compared to enzalutamide monotherapy.
- As we demonstrated previously with our first generation inhibitor (12), transcriptomic analysis reveals that CPI-0209 treatment modulates both AR-related and AR-independent pathways, which reveals a potential mechanism to explain the synergy of CPI-0209 and AR inhibitors.
- Thus, our results indicate that CPI-0209 may have the potential to be effective for AR-positive mCRPC sensitive or resistant to current ARS inhibitors, supporting both monotherapy and ARS inhibitor combination clinical development paths for CPI-0209 in mCRPC.

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Glossary

GTEx: Genotype-Tissue Expression project; TCGA: The Cancer Genome Atlas; SU2C: Stand Up To Cancer program; CDX: Cell line-derived xenograft; PDX: patient-derived xenograft