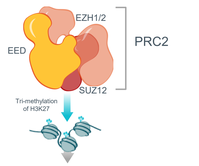
Rinzimetostat, an Allosteric EED Inhibitor With Best-In-Class Properties for the Treatment of Prostate Cancer, Is Effective in PRC2 Methyltransferase-resistant Settings in Preclinical Studies

Xi Chen, Livia Ulicna, Eunice Lopez-Fuentes, Anjana Ramnath, Ashley Pereira, Archana Kumar, Robert Warne, Krista Bowman, Kyle Edgar, Aleksandr Pankov, Jason E. Long, Ajit Narang, Lori S. Friedman, Anneleen Daemen, Melissa R. Junttila

ORIC Pharmaceuticals, Inc., South San Francisco, CA 94080

Poster #7120

BACKGROUND



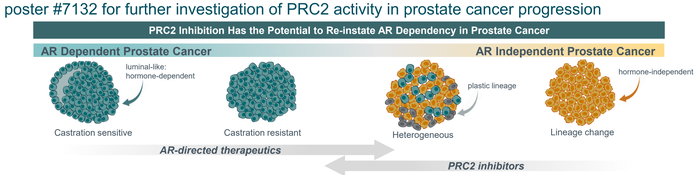
Polycomb Repressive Complex 2 (PRC2):

- Comprised of three core subunits EZH2, EED and SUZ12
- Methylates histone H3 at lysine 27 (H3K27), leading to long-term transcriptional modulation with implications for cell growth and differentiation
- Dysregulated in multiple solid tumors, and increased activity is associated with poor prognosis in prostate cancer patients

PRC2 Inhibition in Prostate Cancer:

- PRC2 activity increases in early prostate cancer and associates with prostate cancer lineage and therapeutic resistance to androgen receptor (AR) pathway inhibitor (ARPI) therapies
- Preclinical data indicates that PRC2 inhibitors (PRC2i) constrain this process by restricting lineage-escape programs and enforcing a luminal, AR-dependent cell state
- Emerging clinical data suggest that combining AR and PRC2 inhibitors may improve outcomes for patients

See poster #7132 for further investigation of PRC2 activity in prostate cancer progression



1. PRC2 Inhibition Induces Luminal Transition in Prostate Models When Targeting Either EED or EZH2

Equivalent Transcriptional Effects of Combining ARPI with Rinzimetostat or Mevrometostat In Vivo

PRC2 Inhibitors Enhance Expression of Luminal Markers In Vivo

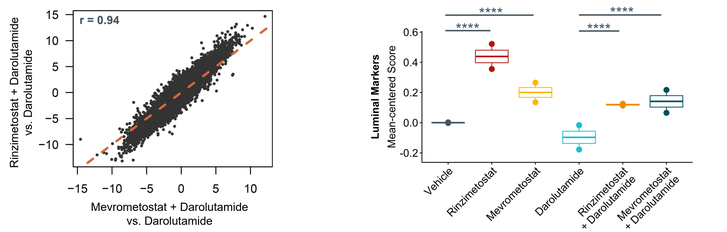


Figure 1. Left. RNA sequencing correlation analysis of DESeq2-derived test statistics for rinzimetostat + darolutamide and mevrometostat + darolutamide vs. darolutamide in castration sensitive (CSPC) LNCaP xenografts, N = 2/treatment. *r* = Pearson correlation. **Right.** Luminal marker signature [Liang et al., Prostate Cancer and Prostatic Disease (2022)] expression in LNCaP xenografts. Significance based on weighted Stouffer test applied to DESeq2 results using inversed log2 fold change standard errors as weights: ****, p<0.0001.

2. PRC2 Inhibition Via EED or EZH2 Similarly Restrains Chromatin Accessibility in Prostate Cancer

Rinzimetostat and Mevrometostat Combined With ARPI Alter Chromatin Similarly In Vivo

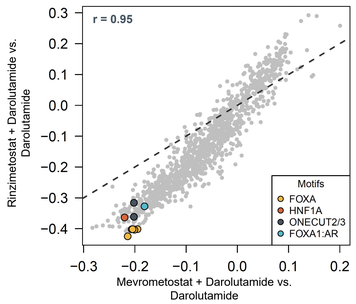


Figure 2. Addition of rinzimetostat vs. mevrometostat to darolutamide results in accessibility changes to the same key transcription factor binding sites in LNCaP (CSPC) xenografts. Every point is the TOBIAS-calculated score of motif accessibility change for consolidated motifs. Shown in color are lineage-associated transcription factor motifs with reduced accessibility following treatment with PRC2i. r = Pearson correlation.

3. EED and EZH1/2 Inhibitors Combined with AR Inhibitors Enhance Antiproliferative Effects in Prostate Cancer Cell Lines In Vitro

Cell Growth Inhibition with Combination PRC2i and ARPI in CSPC

Cell Growth Inhibition with Combination PRC2i and ARPI in CRPC

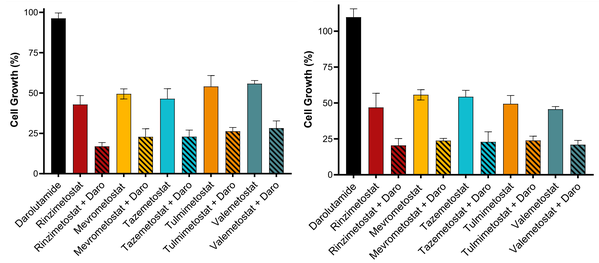
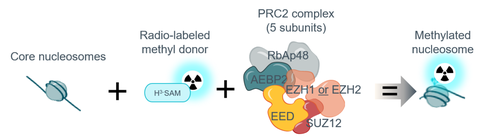


Figure 3. Left. Cell viability measured by CellTiterGlo of LNCaP (CSPC) cells treated with the dose of PRC2i at which 50% cell growth was achieved as a single agent or the same dose in combination with darolutamide (1.1μM) for 14 days. Cells were re-treated at day 7. **Right.** Cell viability measured by CellTiterGlo of castration resistant (CRPC) C4-2 cells treated with the dose of PRC2i at which 50% cell growth was achieved as a single agent or the same dose in combination with darolutamide (1.1μM) for 14 days. Cells were re-treated at day 7.

4. Rinzimetostat Inhibits PRC2 Complexes Harboring Either EZH1 or EZH2

PRC2 Biochemical Assay



PRC2 complex	Ratio of Biochemical Potency EZH1/EZH2 IC50 (nM)				
	Rinzimetostat EED inhibitor	Mevrometostat EZH2 inhibitor	Tazemetostat EZH2 inhibitor	Tulmimetostat EZH1/2 inhibitor	Valemetostat EZH1/2 inhibitor
EZH1 WT	7.3	34	545	2.4	8.1
EZH2 WT	3.9	1.9	2.9	2.1	1.4
Ratio EZH1/2	**2x**	**18x**	**188x**	**1x**	**6x**

Figure 4. Top. Schematic overview of biochemical HotSpot assay executed using five PRC2 proteins with either EZH1 or EZH2 protein. 10nM methyltransferase concentration on chicken nucleosomes. **Bottom.** Table of potency results of each PRC2 inhibitor on PRC2 complexes including EZH1 or EZH2.

5. Rinzimetostat Retains Activity When EZH1 Is Upregulated, While EZH1/2 Inhibitor Potency Is Reduced

EZH1 Is Expressed in Prostate Cancer, and May Compensate for EZH2 Inhibition

EZH1 Overexpressed Prostate Cancer Cell Lines

EZH1 Reduces Viability Effects of EZH2 Inhibitors, but Not Rinzimetostat

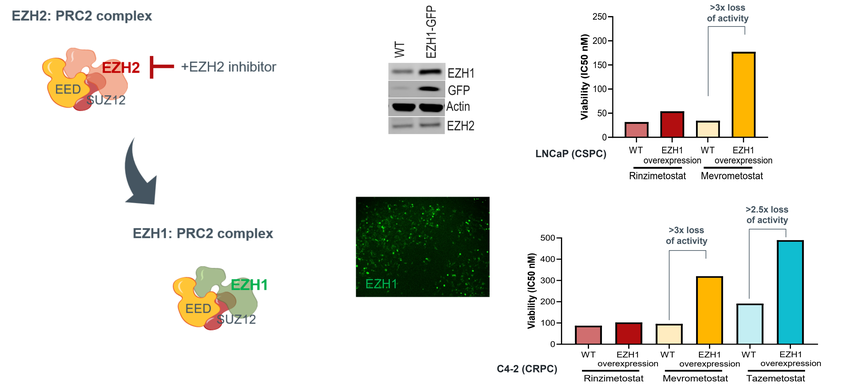


Figure 5. Left. Schematic diagram illustrating the concept that upon EZH2 inhibition, EZH1 is able to compensate within the PRC2 complex to retain PRC2 activity in prostate cancer cells. **Middle.** EZH1 was transiently overexpressed in LNCaP (CSPC) and C4-2 (CRPC) cells. EZH1 expression was confirmed via western blot and detection of immunofluorescent visualization of the protein in vitro. LNCaP shown. **Right.** Cell viability of prostate cancer cell lines as measured by CellTiterGlo in response to indicated PRC2i treatment for 14 days in the presence or absence of EZH1 overexpression. LNCaP (upper panel) and C4-2 (lower panel).

6. Rinzimetostat Retains Activity in Clinically Acquired EZH1/2 Inhibitor Mutational Settings

H3K27me3 in EZH2 Y666N Mutant Prostate Cancer Cells

Methyltransferase Activity of EED H213R Mutation in Biochemical Assay

H3K27me3 in EED H213R Mutant Prostate Cancer Cells

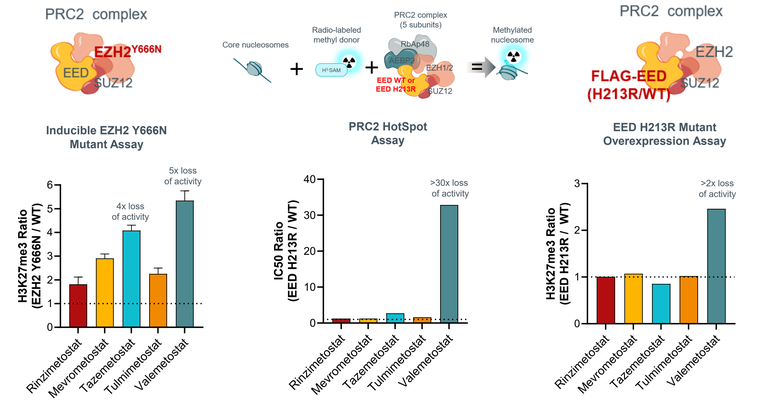


Figure 6. Left. Y666N mutation was identified in a patient with rhabdoid sarcoma following relapse on tazemetostat (ref: Kazansky Y *et al.*, Cancer Disc 2024) and in a T-cell lymphoma patient following acquired resistance to valemetostat (annotated as Y661N; ref: Yamagishi *et al.*, Nature 2024). EZH2 Y666N mutant dox-inducible LNCaP cells were treated with doxycycline (1 μg/mL) for 2 days, followed by 5 days of drug treatment. PRC2 activity was assessed by H3K27me3 western blot and band intensities were quantified using Image Studio 6.0. **Middle and Right.** EED H213R mutation was identified from a T-cell lymphoma patient following acquired resistance to valemetostat (ref: Yamagishi *et al.*, Nature 2024). **Middle.** PRC2 HotSpot assay was performed using recombinant EED WT or EED H213R. Shown is the IC50 ratio of H213R relative to WT. **Right.** LNCaP cells overexpressing EED H213R or WT protein treated with the respective PRC2 inhibitor for 5 days. PRC2 activity was assessed by H3K27me3 western blot and band intensities were quantified using Image Studio 6.0.

7. Rinzimetostat Has Potential Best-in-Class Drug Properties

Head-to-head Preclinical Studies of Clinical Comparators

Clinical Compound	Solubility	Mouse Oral Bioavailability	Half-life in Mice	CYP Inhibition IC50	CYP Induction	Clinical Half-life, Dosing
Rinzimetostat EED inhibitor	11.7 mg/ml	64%	3-5 hr PO 2.5 hr IV	>10 uM	Clean	~20 hrs QD
Mevrometostat EZH2 inhibitor	0.5 mg/ml	7.6%	1.2 hr PO 2.4 hr IV	>10 uM	Clean	<4 hrs* BID
Tazemetostat EZH2 inhibitor	6.9 mg/ml	34%	0.8 hr PO 2.3 hr IV	1-4 uM + time-dependent inhibition	Strong 3A4	~3 hrs* BID
Tulmimetostat EZH1/2 inhibitor	4.9 mg/ml	11%	0.5 hr PO 1.9 hr IV	>10 uM	Moderate 3A4	~6 hrs* QD
Valemetostat EZH1/2 inhibitor	>11 mg/ml	25%	2.8 hr PO 2.5 hr IV	>10 uM	Clean	~11 hrs* QD

Figure 7. ORIC data on file. Thermodynamic solubility at pH 2 room temperature: *Published Data. Clinical half-life estimated for mevrometostat based on published Phase 1 data. Clinical half-life for tulmimetostat estimated for RP2D of 375 mg QD. Valemetostat clinical half-life, Keam *et al.*, Drugs 2022

CONCLUSIONS

- PRC2 inhibition, targeting either EED or EZH1/2, leads to transcriptional and chromatin effects across prostate cancer contexts that mechanistically support the rationale for ARPI combination therapy
- PRC2 inhibitors combine with ARPI enhancing antiproliferative effects in prostate cancer cell lines
- Differential potency of PRC2 inhibitors on EZH1 vs EZH2 complexes impacts activity in resistant contexts, providing advantages for EED targeting:
  - *Rinzimetostat, but not EZH1/2 inhibitors, inhibits compensatory bypass activity of EZH1 in vitro*
  - *Rinzimetostat overcomes clinically observed EZH1/2 inhibitor acquired resistance mechanisms*
- Rinzimetostat has improved solubility, oral bioavailability, half-life, and CYP profile compared to comparator compounds

➢ Rinzimetostat is a potential best-in-class PRC2i with advantageous EED targeting and optimized properties

Himalayas-1 Phase 3 study of rinzimetostat in combination with darolutamide initiating in 2026