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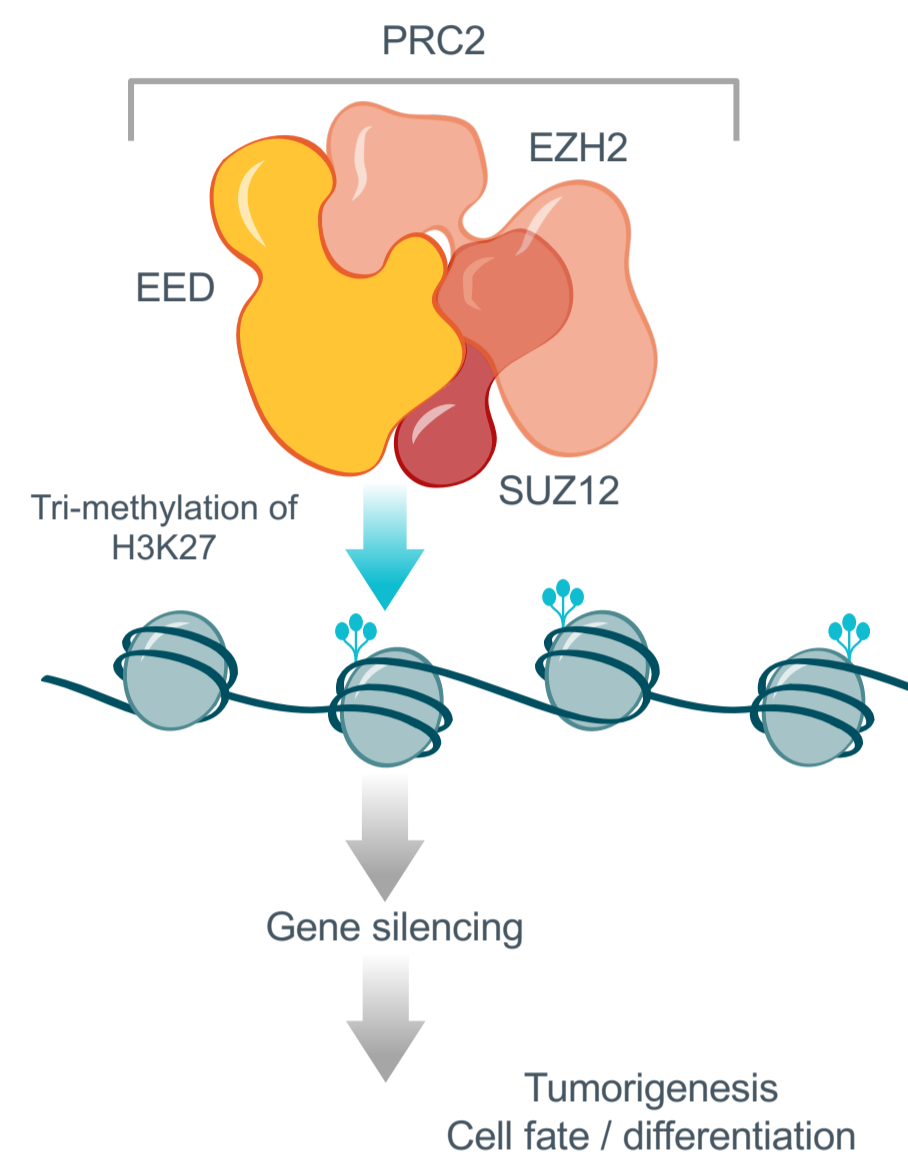
Background

Polcomb repressive complex 2 (PRC2) tri-methylates histone H3 at lysine 27 (H3K27me3) leading to transcriptionally silenced genes.

ORIC-944 is a potent, oral, highly selective allosteric small molecule inhibitor of PRC2 via targeting the embryonic ectoderm development (EED) subunit, and is in early clinical development as a monotherapy for patients with metastatic prostate cancer.

We previously reported strong tumor growth inhibition with ORIC-944 in enzalutamide-resistant 22Rv1 prostate cancer xenografts (Daemen et al, AACR Annual Meeting 2021).

Here, to devise and implement a biomarker strategy to inform ORIC-944 dose selection in the ongoing phase 1 study (NCT05413421), we undertook preclinical pharmacology studies and assay development.



1. ORIC-944 Induced Dose-dependent H3K27me3 Reduction in Murine Skin Punch Biopsies In Vivo

ORIC-944 depleted H3K27me3 in the epidermis of mice after 7 days

ORIC-944 significantly reduced H3K27me3 in the skin epidermal layer

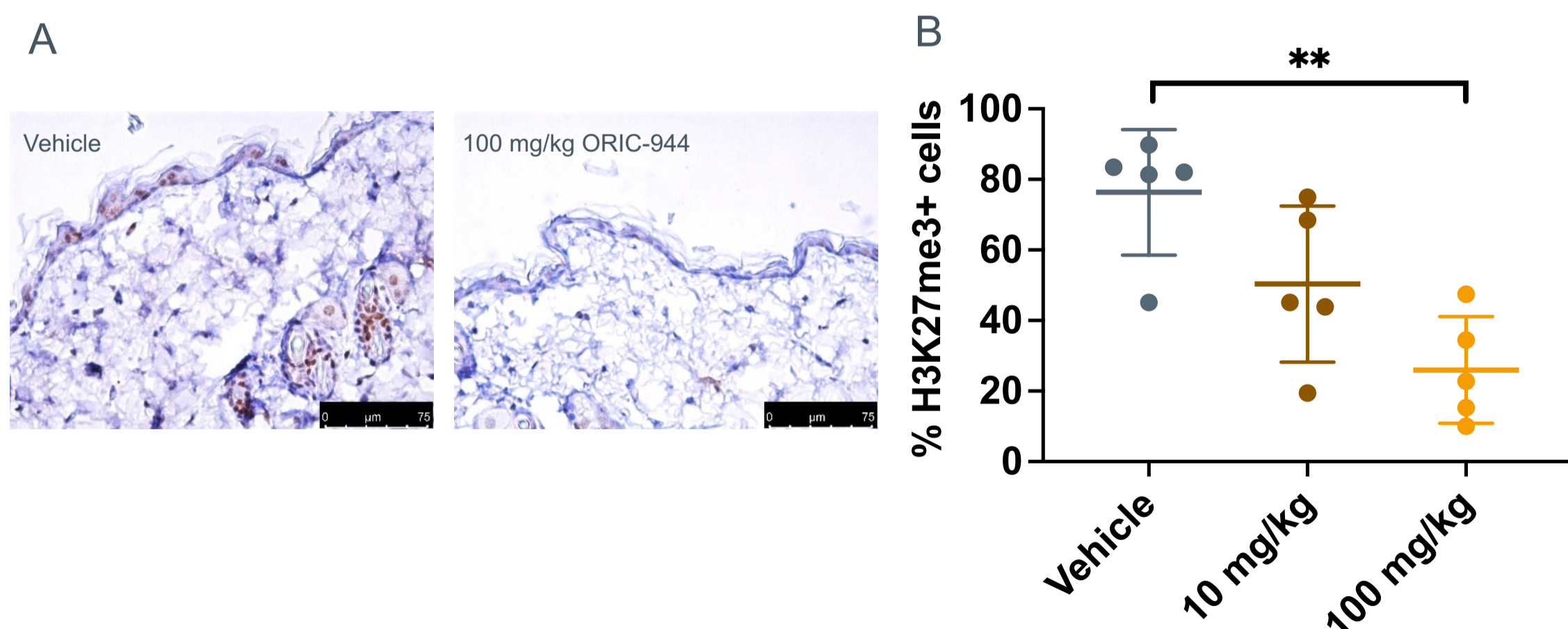


Figure 1. Non-tumor bearing male mice were treated with Vehicle, 10 mg/kg or 100 mg/kg ORIC-944 PO QDx7. H3K27me3 was evaluated by IHC in dorsal skin (epidermal layer) collected 4 hours after dose suspension. **A.** Representative IHC images of skin from non-tumor bearing mice treated QDx7. H3K27me3 antibody ab192985. **B.** Semi-quantitative image analysis of percentage of H3K27me3 positive cells relative to total cell count in selected regions of dorsal skin on day 7. Solid lines show the group mean, error bars indicate SD, with n=5/group. One-way ANOVA, followed by Tukey's multiple comparison test, was used to compare ORIC-944 groups to Vehicle. **, p<0.01 vs. Vehicle.

2. ORIC-944 Reduced H3K27me3 in Murine Peripheral Blood Monocytes In Vivo

ORIC-944 reduced H3K27me3 to background levels in monocytes from mice after 21 days

ORIC-944 reduced H3K27me3 levels ~90% in monocytes

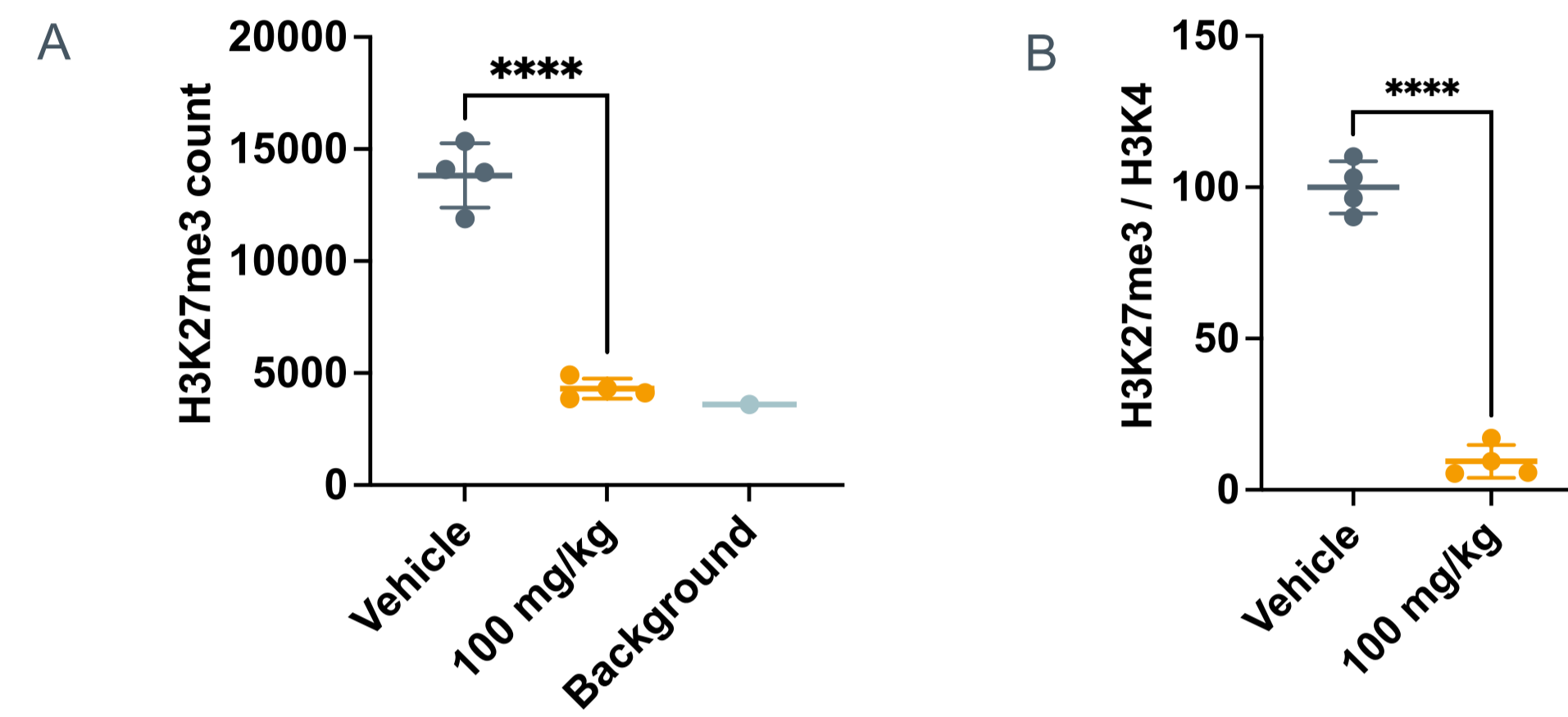


Figure 2. Whole blood was collected from non-tumor bearing mice treated with Vehicle or 100 mg/kg ORIC-944 QDx21, 4 hours after dose suspension. Blood was RBC-lysed, monocytes were extracted, counted and aliquoted at 15,000 cells/condition/replicate. Cells were spun down, lysed and histone extraction was performed followed by alphaLISA for H3K27me3 and H3K4. Shown is average alphaLISA count for **A.** H3K27me3, and **B.** H3K27me3 normalized to H3K4 after background subtraction. Error bars indicate SD, with n=4/group. Significant difference in treatment group vs. Vehicle, unpaired t-test. ****, p<0.0001 vs. Vehicle.

3. ORIC-944 Induced a Time- and Dose-dependent H3K27me3 Reduction in Cell Free Nucleosomes In Vivo

ORIC-944 decreased cf-H3K27me3 levels after 7 days in a dose-dependent manner

ORIC-944 decreased cf-H3K27me3 levels with 100 mg/kg in a time-dependent manner

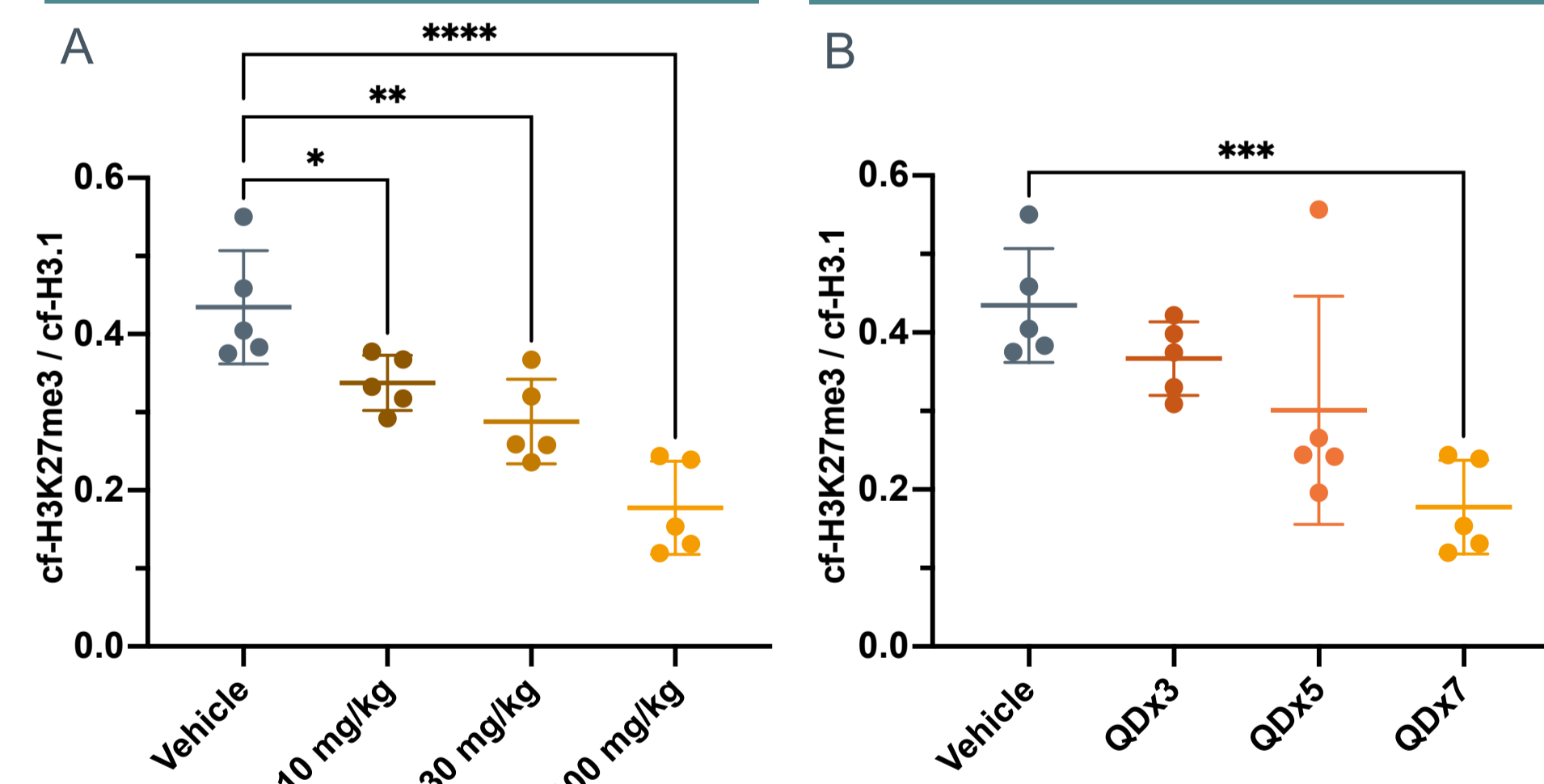


Figure 3. Since dying tumor cells release nucleosomes to circulation, cell-free (cf)-nucleosomal H3K27me3 levels normalized to cf-H3.1 were assessed in plasma using a magnetic bead-based sandwich immunoassay (Belgian Volition SRL). 22Rv1-tumor bearing mice were treated with Vehicle QDx7, 100 mg/kg ORIC-944 QDx3, QDx5 or QDx7, and 10 or 30 mg/kg ORIC-944 QDx7. Shown are average cf-nucleosomal H3K27me3 levels normalized to cf-H3.1, error bars indicate SD, with n=5/group. One-way ANOVA, followed by Dunnett's multiple comparison test, was used to compare ORIC-944 groups to Vehicle. *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 vs. Vehicle. **A.** Normalized cf-H3K27me3 levels significantly decreased with increasing dose (one-way ANOVA test for linear trend, p<0.0001). **B.** Normalized cf-H3K27me3 levels significantly decreased with increasing treatment duration (p=0.0024).

4. H3K27me3 Modulation in cf-nucleosomes was Tumor Specific In Vivo

ORIC-944-induced reduction in cf-H3K27me3 levels was selectively observed in 22Rv1-tumor bearing mice

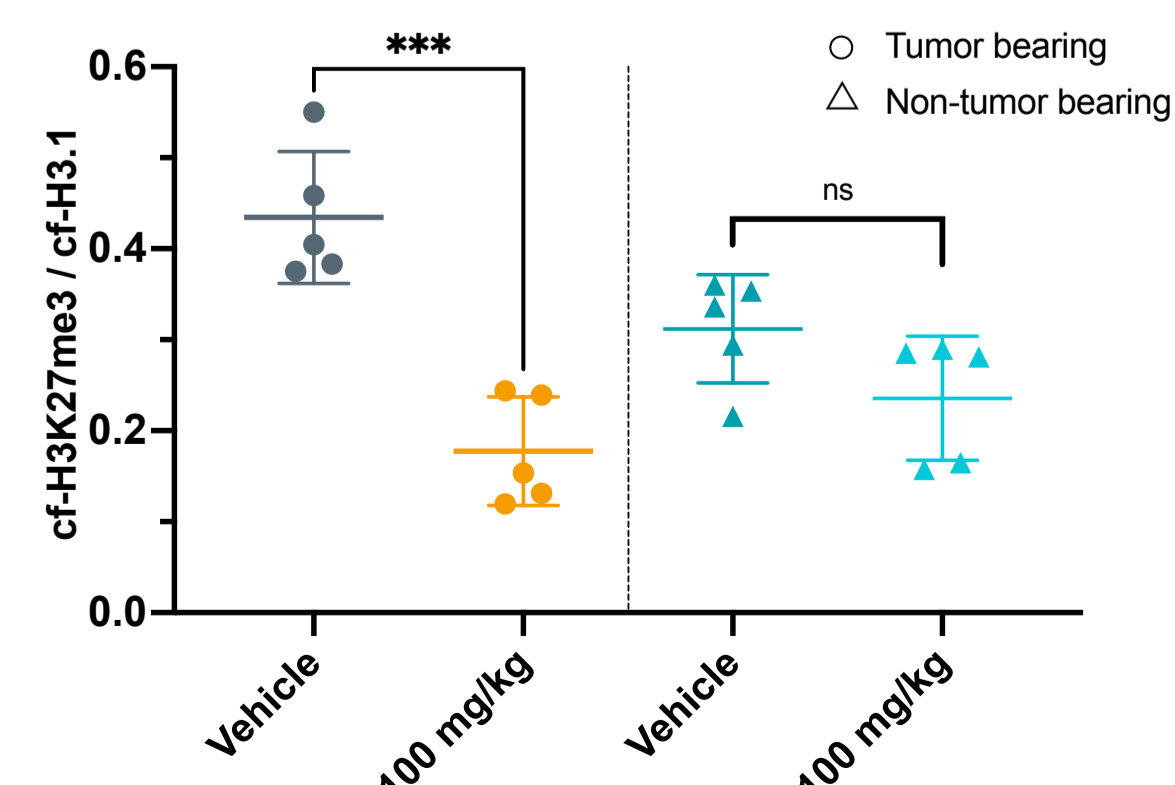


Figure 4. 22Rv1-tumor bearing and non-tumor bearing mice were treated with Vehicle or 100 mg/kg ORIC-944 QDx7. Average cf-nucleosomal H3K27me3 levels normalized to cf-H3.1 are shown, error bars indicate SD, with n=5/group. Unpaired Welch's t-test was used to compare ORIC-944 to Vehicle separately for tumor and non-tumor bearing mice. ns, not significant; ***, p<0.001 vs. Vehicle.

5. Putative PRC2 Target Genes Revealed with Time-dependent Transcriptional Profiling of Xenografts Dosed with ORIC-944

ORIC-944 treatment and drug withdrawal acutely affected genes in 22Rv1 tumors

H3K27me3 levels in tumor decreased in a time-dependent manner, with a rebound following dose suspension

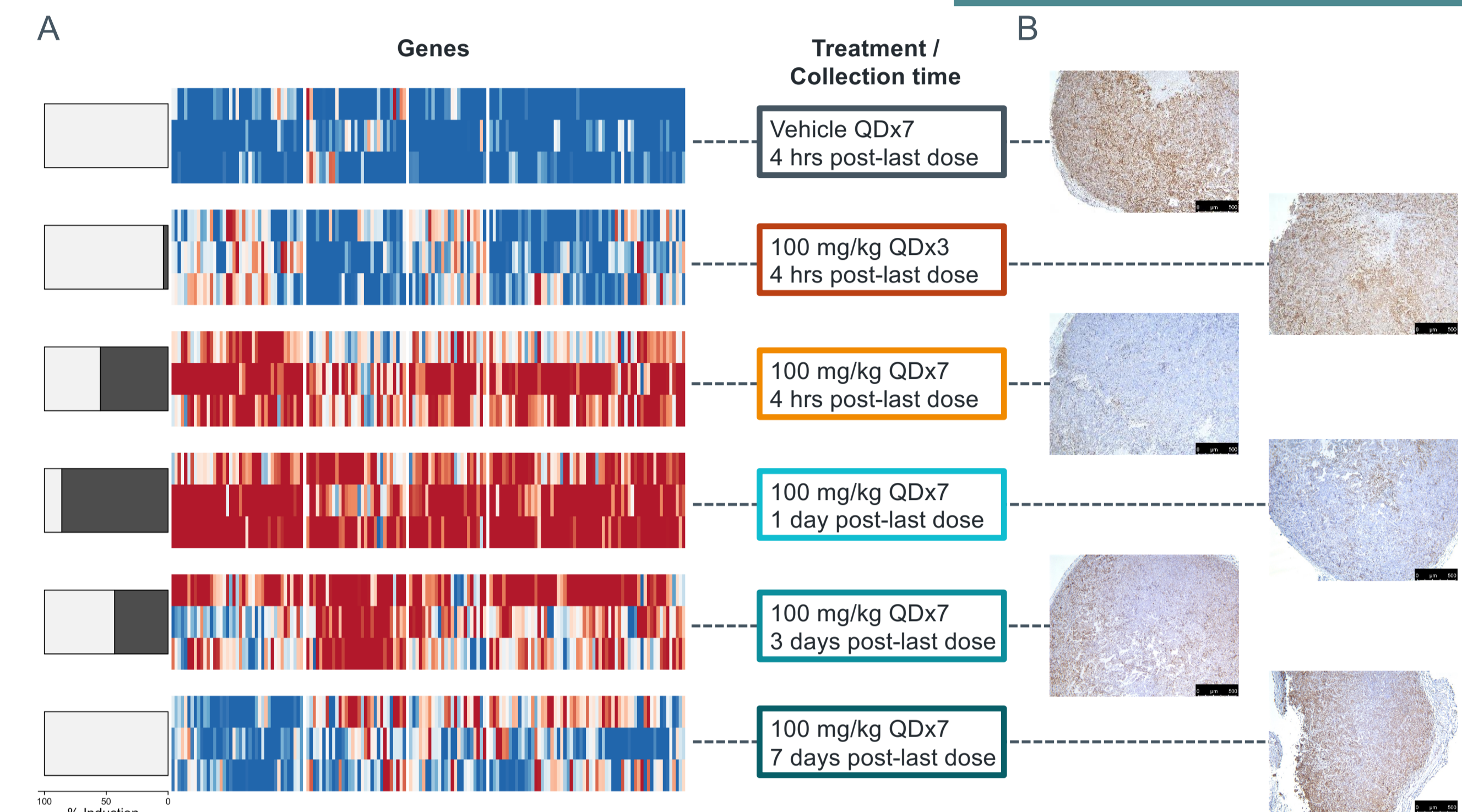


Figure 5. Putative PRC2 target genes were identified in 22Rv1 xenografts by focusing on those genes with H3K27me3 binding in the promoter, whose expression was de-repressed by ORIC-944 in a time-dependent manner and then re-silenced after dose suspension. 22Rv1 tumor-bearing mice were treated with Vehicle QDx7 or 100 mg/kg ORIC-944 QDx3 or QDx7. Tumors were collected 4 hours, 1, 3 or 7 days after dose suspension, and profiled with RNA-sequencing (Q2 Solutions) and H3K27me3 ChIP-sequencing (Active Motif, Inc.), with n=3/group. **A.** Heatmap displays z-scored log2(TPM + .01) expression of genes (columns) in 22Rv1 xenograft tumors (rows), with percentage of genes significantly induced in each group relative to Vehicle shown in the barplot. **B.** Representative IHC images of 22Rv1 xenograft tumors. H3K27me3 antibody ab192985.

6. Biomarker Plan for the Ongoing Phase 1 Study

The biomarker plan is focused on early, non-invasive readouts of target engagement, with opportunity to capture dose-dependency

- H3K27me3 levels are being evaluated in:
 - the stratum spinosum of skin using a proprietary IHC assay
 - blood-derived monocytes using an alphaLISA test
 - plasma-derived cf-nucleosomes using Volition's Nu.Q® immunoassay
- Modulation in the expression of the putative PRC2 target genes is being assessed in blood-derived peripheral blood mononuclear cells by RNA-sequencing

Conclusions

This comprehensive biomarker strategy enables the assessment of target engagement and captures exposure-dependent pharmacodynamics in the ongoing phase 1 study evaluating ORIC-944 as a potential best-in-class PRC2 inhibitor for the treatment of patients with advanced prostate cancer (NCT05413421).

