PRT2527, a novel highly selective cyclin-dependent kinase 9 (CDK9) inhibitor, is active in preclinical models of prostate cancer

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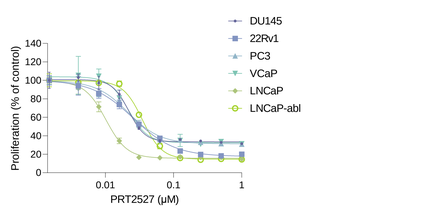
Background

- Transcriptional addiction is a common feature and a therapeutic vulnerability in many cancers.
- Transcription-associated cyclin-dependent kinases (CDKs), like CDK9, are exploitable therapeutic targets for developing novel treatment strategies for transcriptionally-addicted cancers.
- CDK9 interacts with the positive transcription elongation factor b (P-TEFb), phosphorylates RNA polymerase II at Serine 2, and promotes transcriptional activation.
- CDK9 cooperates with multiple transcription factors, like c-Myc, NF-κB and the androgen receptor (AR). CDK9 stabilizes AR-associated proteins in prostate cancer, and CDK9 inhibition can overcome transcriptional addiction and AR-dependency and inhibit the downstream transcriptional programs driving tumorigenesis, stemness and treatment resistance.
- This study evaluates the novel and highly selective CDK9 inhibitor PRT2527 in preclinical models of prostate cancer, assessing the effects on cell proliferation, stem-like tumor cells, 3D organoid development, and tumor growth in mice, along with the drug's ability to inhibit the anticipated molecular targets both in vitro and in vivo.

1 Effects of PRT2527 on proliferation, organoids formation and stem-like cell compartment



Cell line	DU145	22Rv1	PC3	VCaP	LNCaP	LNCaP-abl
IC50 ± SD (nM)	33 ± 3.5	37 ± 3.6	34 ± 2.1	22 ± 5.8	11 ± 1.5	54 ± 11.6

Figure 1. Anti-proliferative activity of PRT2527 in prostate cancer cell lines. Bottom, table of IC_{50} calculated for the different cell lines. Data are mean ± SD.

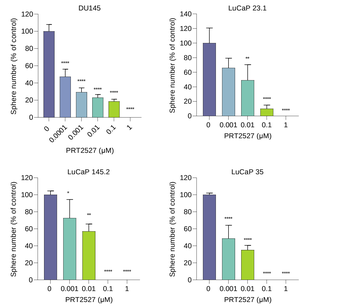


Figure 2. PRT2527 inhibits tumorigenic stem-like cells in tumor-sphere forming assays *in vitro*. Data are mean ± SD. * p ≤ 0.05; ** p ≤ 0.01; **** p ≤ 0.0001.

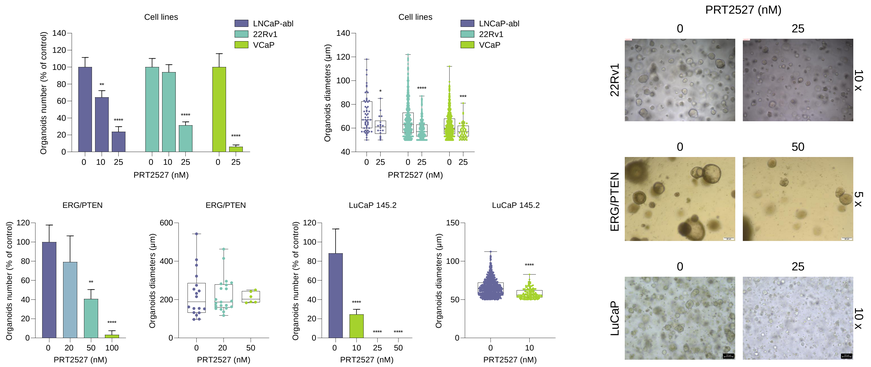


Figure 3. PRT2527 reduces growth of 3D organoid cultures of prostate cancer cell lines (top panels), mouse-derived (ERG-PTEN) and PDX-derived (LuCaP 145.2) organoids (bottom panels). Right, representative images of organoid cultures. Data are mean ± SD. * p ≤ 0.05; ** p ≤ 0.01; **** p ≤ 0.0001.

PRT2527 inhibits the proliferation of androgen-dependent (i.e., LNCaP, VCaP) and androgen-independent (i.e., DU145, PC3, 22Rv1, LNCaP-abl) prostate cancer cell lines, tumor-sphere formation by stem-like tumor cells, and in vitro growth of tumor organoids from cell lines, patient-derived xenografts (PDXs) and ERG/PTEN transgenic mice.

2 Effects of CDK9 inhibition on molecular targets *in vitro*

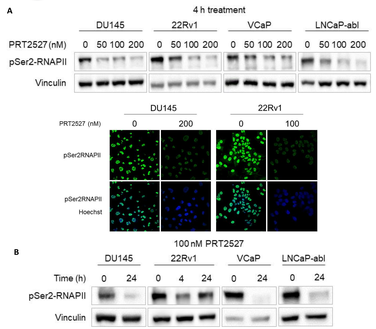


Figure 4. Inhibition of phosphorylation of RNA Polymerase II (pSer2 RNAPII) by PRT2527. Cells were examined at 4 h (A) and 24 h (B) of treatment.

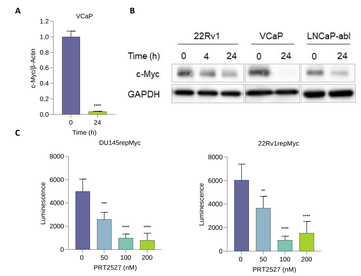


Figure 5. Inhibition of c-Myc expression (A-B) and transcriptional activity (C) in prostate cancer cells. Data are mean ± SD. ** p ≤ 0.01; **** p ≤ 0.0001.

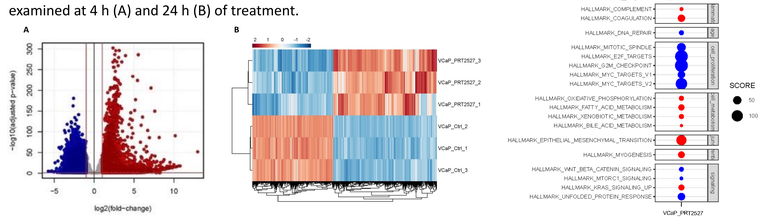


Figure 6. Broad transcriptional changes occurring upon PRT2527 treatment. A) Volcano plot showing transcriptional changes upon PRT2527 treatment. **B)** Upregulated and downregulated genes upon treatment with PRT2527. **C)** Major changes in the Hallmark cellular pathways upon PRT2527 treatment.

PRT2527 reduces pSer2 RNAPII, and c-Myc expression and transcriptional activity, and induces broad changes in the cell transcriptome leading to downregulation of multiple oncogenic and pro-tumorigenic target genes

3 Effects of PRT2527 on growth of patient-derived prostate tumor xenografts

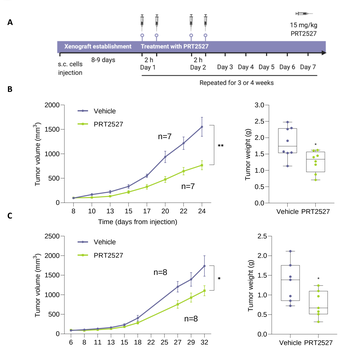


Figure 7. Growth of patient-derived xenografts upon systemic treatment with PRT2527. A) Treatment schedule. **B)** LuCaP 35. **C)** LuCaP 145.2. Data are mean ± SD. * p ≤ 0.05; ** p ≤ 0.01.

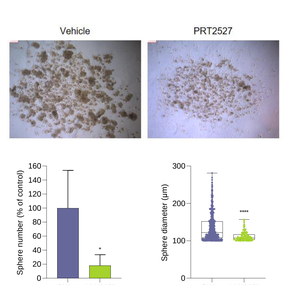


Figure 8. Systemic treatment of PDX-bearing mice with PRT2527 reduces the fraction of stem-like tumor cells in tumor xenografts. Stem-like tumor cell content was evaluated by ex vivo tumor-sphere forming assay. Data are mean ± SD. * p ≤ 0.05; *** p ≤ 0.001.

PRT2527 reduces growth of patient-derived xenografts in mice and the fraction of tumor-initiating stem-like cells in *ex vivo* assays.

4 Effects of PRT2527 on molecular targets *in vivo*

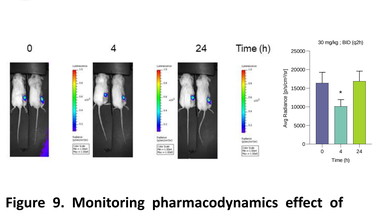


Figure 9. Monitoring pharmacodynamics effect of PRT2527 by c-Myc reporter luciferase activity in mice bearing DU145repMYC xenografts. Mice received 30 mg/kg, BID, (q2h). Data are mean ± SD. * p ≤ 0.05.

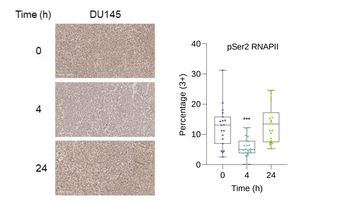


Figure 10. PRT2527 reduces phosphorylation of RNA Polymerase II at Serine 2 (pSer2RNAPII) in DU145 tumor xenografts. Mice received 15 mg/kg, BID, (q2h) for 2 days. Data are mean ± SD. *** p ≤ 0.001.

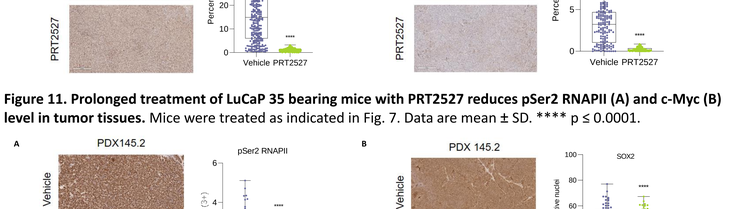


Figure 11. Prolonged treatment of LuCaP 35 bearing mice with PRT2527 reduces pSer2 RNAPII (A) and c-Myc (B) level in tumor tissues. Mice were treated as indicated in Fig. 7. Data are mean ± SD. **** p ≤ 0.0001.

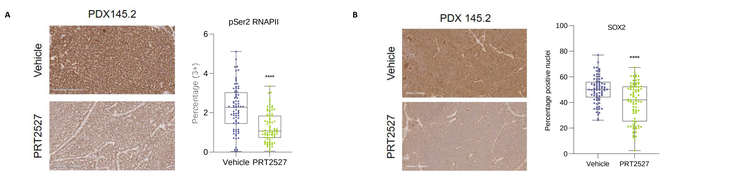


Figure 12. Prolonged treatment of LuCaP 145.2 bearing mice with PRT2527 reduces pSer2 RNAPII (A) and SOX2 (B) level in tumor tissue. Mice were treated as indicated in Fig. 7. Data are mean ± SD. **** p ≤ 0.0001.

PRT2527 inhibits pSer2 RNAPII and leads to reduced expression and function of oncogenic transcription factors like c-Myc and Sox2 in patient-derived xenograft models.

Summary and Conclusions

- PRT2527 has potent anticancer activity (IC_{50}, ≤50 nM) towards androgen-dependent and independent prostate cancer cell lines. PRT2527 also strongly suppresses the growth of 3D tumor organoids derived from cell lines, patient-derived xenografts and transgenic mouse models.
- PRT2527 is very effective in blocking stem-like tumor cells *in vitro* in tumor-spheroid assays and *in vivo* upon systemic treatment of tumor-bearing mice.
- *In vitro* PRT2527 reduces phosphorylation of pSer2 RNAPII and expression of c-Myc, common CDK9 targets, and leads to broad transcriptomic changes in prostate cancer cells. Consistently, *in vivo* treatment with PRT2527 inhibits pSer2 RNAPII, c-Myc, and Sox2 in tumor xenografts.
- Treatment *in vivo* with PRT2527 reduces the growth of patient-derived xenografts with distinct characteristics, such as LuCaP 35 (castration-sensitive adenocarcinoma) and LuCaP 145.2 (castration-resistant neuroendocrine).
- Collectively, these data demonstrate the potent pharmacodynamics and antitumor activity of PRT2527 in multiple models of castration-sensitive and castration-resistant prostate cancer. PRT2527 is currently in phase 1 studies in solid tumors, including advanced prostate cancer, and may represent an interesting addition to the therapeutic options for these patients.

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