Preclinical Characterization of PRT7732: A Highly Potent, Selective, and Orally Bioavailable Targeted Protein Degrader of SMARCA2

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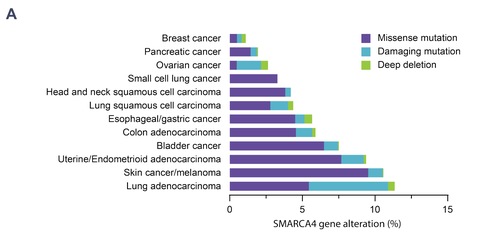
Background

- SMARCA2 (BRM) and SMARCA4 (BRG1) are the two mutually exclusive catalytic core subunits of SWI/SNF complexes that play an important role in controlling gene expression by remodeling chromatin (1).
- SMARCA4 has been shown to be mutated in multiple cancers, including up to 10-12% of non-small cell lung cancer (2).
- The SMARCA4-deficient cancer cells are highly dependent on the paralog gene SMARCA2 for their survival (3,4,5).
- We have identified PRT7732: A highly potent, selective, and orally bioavailable targeted protein degrader of SMARCA2 that induces synthetic lethality in SMARCA4-deficient cancers.

Introduction

Synthetic lethal relationship between targeting SMARCA2 and SMARCA4-deficiency in cancer

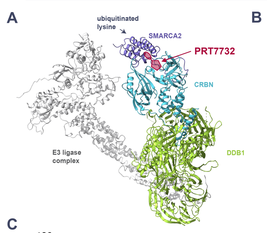


A) Percentage of SMARCA4 mutations in different types of cancer. Datasets are from mixed studies (2021).
B) Cell lines with SMARCA4 damaging mutation or low expression show high SMARCA2 gene dependency scores [3, 5], suggesting the synthetic lethal relationship of targeting SMARCA2 and SMARCA4-deficiency.
C) Model of SMARCA2 degradation induced synthetic lethality in SMARCA4 deficient cancers

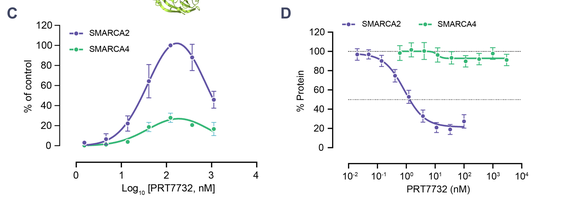
Key Findings

- Identified potent, selective and orally bioavailable SMARCA2 degrader PRT7732
- PRT7732 exhibits >3000-fold selectivity for SMARCA2 over SMARCA4 in cell-based assays, with DC_{50} values in cancer cell lines in the low nanomolar range
- PRT7732 shows favorable pharmacokinetic properties and safety profiles
- Oral administration of PRT7732 demonstrates robust efficacy in human SMARCA4-deficient lung cancer models in mice
- Oral administration of PRT7732 resulted in near-total degradation of SMARCA2 protein levels with complete selectivity over SMARCA4 protein *in vivo*

Figure 2. Identification of PRT7732, a potent and selective orally bioavailable SMARCA2 degrader candidate

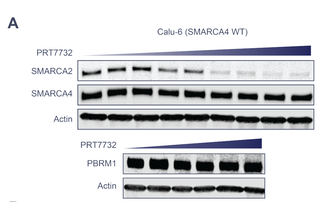


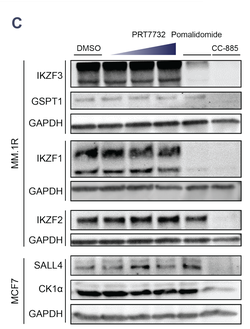
Biochemical Potency & Selectivity	
SMARCA2 TR-FRET ternary complex EC_{50} (nM)	30
SMARCA4 TR-FRET ternary complex EC_{50} (nM)	29
Selectivity SMARCA4/SMARCA2	1.0
Cellular Potency & Selectivity	
SMARCA2 DC_{50} (nM)	0.98
SMARCA4 DC_{50} (nM)	>3000
Selectivity SMARCA4/SMARCA2	>3000



A) 3D structure of SMARCA2 bromodomain and CRBN/DDB1 E3 ligase complex (PDB: 6TTU and 6BNB).
B) PRT7732 profile summary analyzed in biochemical and cell-based assays.
C) TR-FRET proximity assay for SMARCA2 or SMARCA4 and CRBN/DDB1 ternary complex formation.
D) PRT7732 demonstrates potent and selective degradation of SMARCA2 over SMARCA4, analyzed in a Hela HiBiT cell-based assay (PRT7732 tested concentrations: 0.02 nM ~ 100 nM for SMARCA2, 0.6 nM ~ 3 μM for SMARCA4).

Figure 3. PRT7732 shows excellent degradation potency and selectivity for SMARCA2 in human cancer cell lines



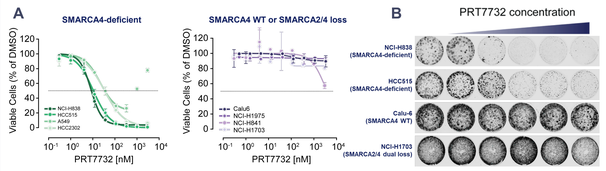


B

	Cell line	SMARCA2 DC_{50} (nM) / D_{max} (%)	SMARCA4 DC_{50} (nM)
SMARCA4 WT	Calu-6	0.57 / 89	> 250
	NCI-H1975	3.8 / 61	> 250
	NCI-H358	2.8 / 66	> 250
	HT1080 WT	2.5 / 95	> 250
SMARCA4-del	NCI-H838	0.72 / 98	NA
	HCC515	3.2 / 91	NA
	A549	1.5 / 92	NA
	NCI-H1793	0.040 / 100	NA
	NCI-H1693	1.8 / 100	NA
	HT1080-SM4del	0.11 / 100	NA

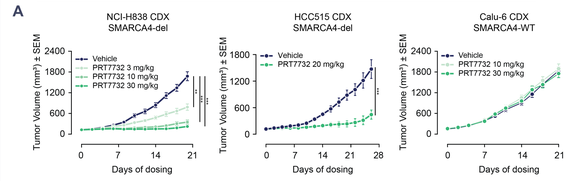
A) Western blot for SMARCA2, SMARCA4 and PBRM1 was performed using Calu-6 human lung cancer cell line. The cells were treated with PRT7732 (3.2 pM ~ 250 nM) for 24h.
B) Western blot for SMARCA2 and SMARCA4 was performed in 10 human cancer cell lines. The SMARCA2 signals were normalized by GAPDH or actin control and DC_{50} and D_{max} were determined by Prism (GraphPad).
C) No off-target effects of PRT7732 (10 nM ~ 1.0 μM) on known IMiD neosubstrates were detected by western blot.

Figure 4. PRT7732 demonstrates synthetic lethality in SMARCA4-deficient human cancer cells *in vitro*



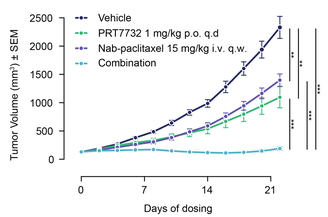
PRT7732 inhibited proliferation of SMARCA4-deficient cancer cell lines, but not SMARCA4 WT or SMARCA2/SMARCA4 dual loss human lung cancer cell lines analyzed.
A) CellTiter-Glo® assay, 7-day treatment (PRT7732: 0.3 nM~ 3 μM). Dose-response curves were plotted using Prism (GraphPad).
B) Clonogenic assay, 2~3-week treatment (PRT7732: 1 nM ~ 10 μM).

Figure 5. Daily oral administration of PRT7732 demonstrates significant anti-tumor activity in SMARCA4-deficient cancer xenograft models and had no effect in SMARCA4-WT model



A) Daily oral administration of PRT7732 demonstrated significant tumor growth inhibition in SMARCA4-deficient NCI-H838 and HCC515 CDX models in a dose-dependent manner, but no effect was seen in the SMARCA4-WT Calu-6 CDX model. *P<0.05 **P<0.01 ***P<0.001, versus vehicle (two-tailed Mann-Whitney test).
B) Tumor PD (SMARCA2 protein) was analyzed in samples from NCI-H838 efficacy studies by Western blot. The quantitated values were plotted using Prism (GraphPad).

Figure 6. Low dose oral administration of PRT7732 in combination with nab-paclitaxel induces tumor regression in NCI-H838 SMARCA4-deficient lung cancer xenograft model



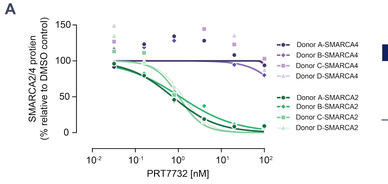
Oral administration of PRT7732 low dose 1 mg/kg in combination with nab-paclitaxel (Abraxane®) induced tumor regression in the NCI-H838 tumor model in mice at well-tolerated doses. *P<0.05 **P<0.01 ***P<0.001, versus vehicle (two-tailed Mann-Whitney test).

Table 1. PRT7732 demonstrates good oral PK profile across preclinical species with clean safety pharmacology

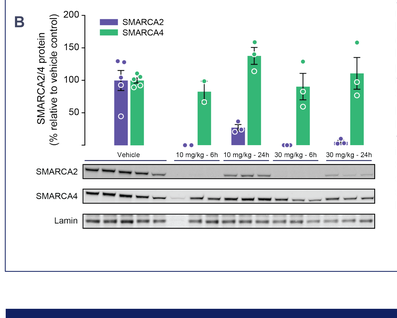
Safety Pharmacology	
hERG IC_{50} (μM)	> 30
CYP1A2, 2C9, 2C8, 2B6, 2D6, 3A4 IC_{50} (μM)	> 10, no TDI
PXR binding assay	Clean
Safety 47 panel	Clean
Pharmacokinetics	**Mouse / Rat/ Dog / Cyno**
F%	35 / 18 / 36 / 30

Safety pharmacology studies show no significant findings. Single dose PK studies show good oral bioavailability.

Figure 7. *Ex Vivo* and *in vivo* PRT7732 treatment demonstrates near absolute selectivity for SMARCA2 degradation in human and rat PBMCs



Ex Vivo human PBMC	
SMARCA2 DC_{50} (nM)	0.50
SMARCA4 DC_{50} (nM)	> 100 nM



A) Purified peripheral blood mononuclear cells (PBMCs) from four healthy human donors were cultured *ex vivo* overnight in the presence of PRT7732. SMARCA2 and SMARCA4 protein levels were determined using the Jess™ Automated Western Blot System (ProteinSimple).
B) Rats were dosed orally with PRT7732 daily for 4 days and PBMCs were collected at the timepoints indicated following the last dose. SMARCA2 and SMARCA4 levels were determined by Western blot. The quantitated values were plotted using Prism (GraphPad).

Conclusions

- We have identified development candidate PRT7732 that selectively degrades SMARCA2 over SMARCA4 by >3000-fold
- PRT7732 shows excellent potency and selectivity for SMARCA2 degradation both *in vitro* and *in vivo*
- PRT7732 shows strong anti-proliferation activity in SMARCA4-deficient cells, while sparing SMARCA4 WT cells, demonstrating synthetic lethality in SMARAC4-deficient cancers
- Oral administration of PRT7732 demonstrates robust anti-tumor activity in SMARCA4-deficient human lung cancer models *in vivo*
- Low dose oral administration of PRT7732 in combination with NSCLC SOC chemotherapy induces tumor regression in a SMARCA4-deficient human lung cancer model *in vivo*
- *In vivo* PD studies in mouse xenograft models and rats indicate daily dosing of PRT7732 leads to complete SMARCA2 degradation, while sparing SMARCA4
- PRT7732 has completed IND-enabling studies and is on track to enter Phase 1 clinical trials in the second half of 2024

References

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Acknowledgments

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Disclosures

Authors are or were employees of the Company at the time of research, and may own equity in the Company.