# Elucidating the Molecular Mechanism of Action of the First-in-Human SMARCA2 Selective Degrader PRT3789

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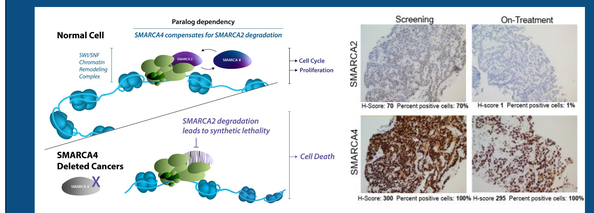
## Background

- The SWI/SNF family of chromatin-remodeling complexes is frequently dysregulated in multiple tumor types, resulting in aberrant expression of genes. SMARCA4-deficient cells become highly dependent on the other catalytic subunit, SMARCA2 (BRM), for their survival[1]. Therefore, selective degradation of SMARCA2 has therapeutic potential in these cancers.[2]
- PRT3789 is a first-in-human SMARCA2 degrader that can selectively induce deep SMARCA2 degradation in preclinical and clinical studies.
- PRT3789 is currently under evaluation in Phase 1 and Phase 2 studies in patients with advanced solid tumors with loss of SMARCA4 (NCT05639751 and NCT06682806).




## Key Findings

- PRT3789 is a first-in-human SMARCA2 degrader that can selectively induce deep SMARCA2 degradation in preclinical and clinical studies.[3]
- Mechanistic studies suggested that PRT3789 induces unique ubiquitination on lysine 1405 residue in SMARCA2.
- Although SMARCA2 and SMARCA4 share high sequence similarity, our studies revealed that the extended loop containing K1405 in SMARCA2 plays a key role in the selective SMARCA2 degradation induced by PRT3789.
- SMARCA2/4 resynthesis studies in multiple cancer cell lines indicated that SMARCA4 is recovered 2-3 times faster than SMARCA2.
- A targeted protein degradation approach has the potential to drug previously undruggable targets. In addition, it can be used to enhance selectivity between proteins with high homology through optimizing of binding, ternary complex formation, ubiquitination rates, and taking advantage of differences in their resynthesis rates.

**References**

1. Fernando, TM, et al., Nat Commun. 2020;11:5551.
2. Ito K, et al., Cancer Res. 2021;81(13_Supplement):1139.
3. Yap, T. A., et al. European Journal of Cancer 211 (2024): 114530.

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**Disclosures**

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

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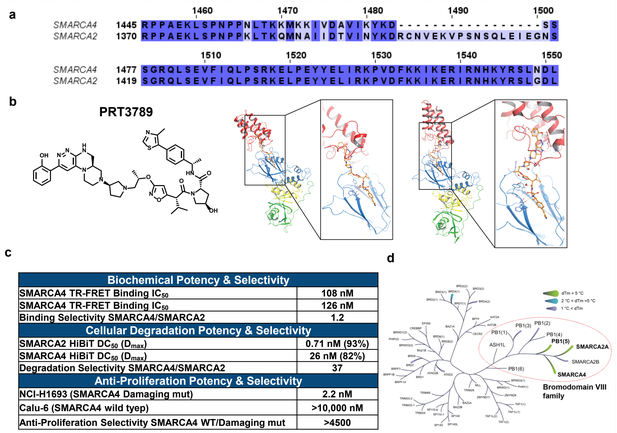
## Figure 1. PRT3789 is a potent SMARCA2 Selective Degrader that binds to the bromodomain of SMARCA2 and SMARCA4

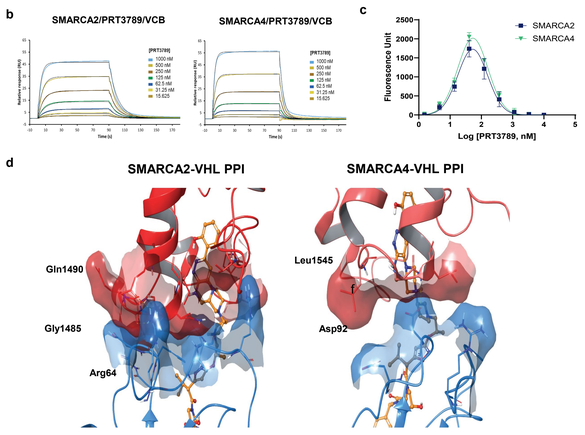



a. Bromodomain sequence alignment between SMARCA2 and SMARCA4 showed high similarity (74% identity)
b. Structure of PRT3789 and x-ray structure of PRT3789 human SMARCA2 and SMARCA4 complexed with human VHL:Elongin C:Elongin B (VCB).
c. PRT3789 profile in cell free and cellular analysis
d. PRT3789 is selective for bromodomain subfamily VIII in a panel of Bromo domain DSF assays.

## Figure 2. Biophysical characterization of ternary complex kinetics of PRT3789 revealed higher ternary complex stability for SMARCA2 than SMARCA4

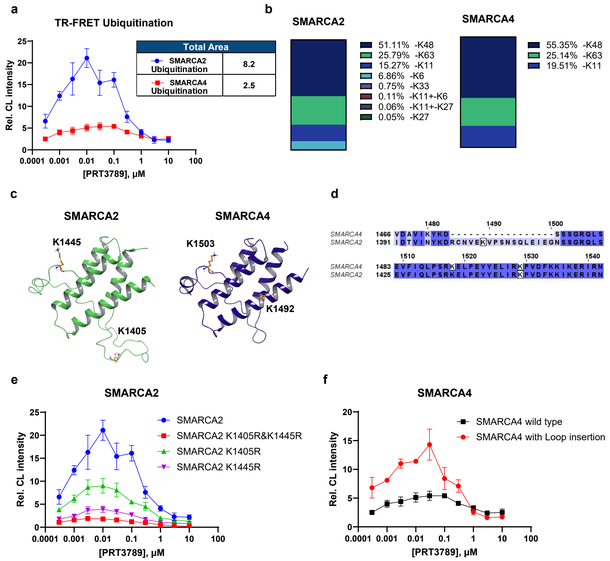
a

| Target | Binding Mode | SPR | | |
|---|---|---|---|---|
| | | $k_{on}$ X$10^5$ ($M^{-1}s^{-1}$) | $k_{off}$ x$10^{-2}$ ($s^{-1}$) | $K_d$ (nM) |
| SMARCA2 | Binary | 6.8 ± 0.7 | 3.2 ± 0.7 | 46.9 ± 5.2 |
| | Ternary | 1.8 ± 0.2 | 7.8 ± 1.0 | 437 ± 72 |
| SMARCA4 | Binary | 5.9 ± 0.7 | 2.9 ± 0.8 | 49.3 ± 7.0 |
| | Ternary | 1.8 ± 0.1 | 17.1 ± 1.9 | 960 ± 114 |



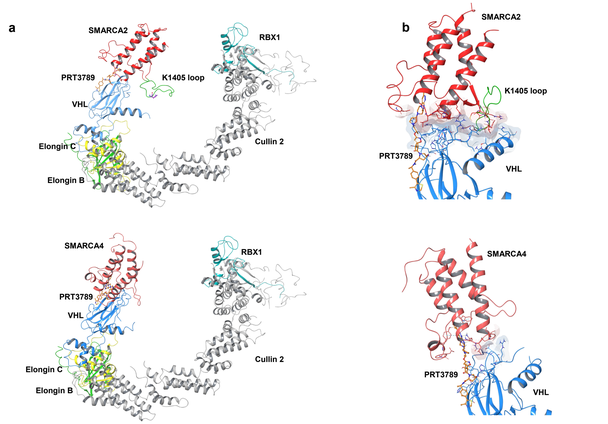

a. PRT3789 binding data for PRT3789 (binary) and PRT3789:VHL complexes (ternary) binding to immobilized SMARCA2 or SMARCA4 (isolated bromodomain)
b. SPR sensorgrams for PRT3789 induced SMARCA2:VCB and SMARCA4:VCB complexes reveal different binding kinetics suggesting SMARCA2:PRT3789:VHL complex dissociate slower than SMARCA4 complex
c. TR-FRET proximity assay for SMARCA2 or SMARCA4 and VCB ternary complex formation
d. Protein-protein interfaces (PPIs) between SMARCA2/4 and VHL. For SMARCA4, the PPI with VHL is stabilized by a H-bond between Leu1545 with Asp92 in VHL. While the SMARCA2 bromodomain forms more extensive interactions with VHL in the structure, as demonstrated by a 9-fold increase in PPI area

## Figure.3 PRT3789 induces SMARCA2 selective polyubiquitination



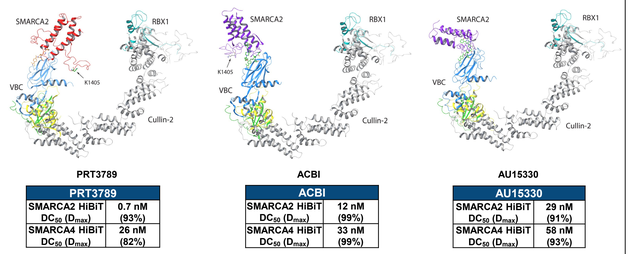

a. PRT3789 induced more ubiquitination in SMARCA2 than SMARCA4 as shown by a TR-FRET assay
b. Mass spectrometry revealed PRT3789 polyubiquitinates and creates more diverse ubiquitin chains with SMARCA2, compared to SMARCA4
c. Ubiquitination proteomics identified K1405 and K1445 were ubiquitinated in SMARCA2, while K1492 and K1503 were the sites in SMARCA4. Among these Lys residues, K1405 is unique to SMARCA2
d. 3D structure shows the positions of ubiquitinated lysine residues
e. Mutation of K1405 or K1445 or both Lys residues to Arg abolished ubiquitination in SMARCA2
f. Insertion of SMARCA2 K1405 loop in SMARCA4 enhanced the ubiquitination

## Figure 4. Structural analysis revealed K1405 loop not only provides unique a lysine residue for ubiquitination but also stabilizes SMARCA2:VHL ternary complex




a. Integration of E2/E3 complexes into the x-ray ternary structures of SMARCA2 and SMARCA4 show the relative position of the SMARCA2 bromodomain in the PRT3789-induced ubiquitination complex. These models revealed that all of the ubiquitinated lysine residues are facing toward the E2 ubiquitin conjugate enzyme (RBX1).
b. The extended K1405 loop in SMARCA2 provided additional PPI area (increased from 271 to 435 Å$^2$) with an additional interaction with VHL Arg69, compared to the 28 Å$^2$ in the SMARCA4:VHL complex.

## Figure 5. PRT3789 orients the K1405 loop towards the E2 ubiquitin-conjugating enzymes (RBX1). In comparison, non-selective SMARCA2 and SMARCA4 dual degraders, ACBI1 and AU15330, orientate this loop far away from RBX1

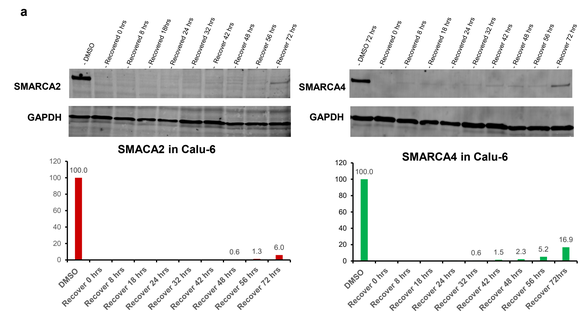



| PRT3789 | |
|---|---|
| SMARCA2 HiBiT $DC_{50}$ ($D_{max}$) | 0.7 nM (93%) |
| SMARCA4 HiBiT $DC_{50}$ ($D_{max}$) | 26 nM (82%) |

| ACBI | |
|---|---|
| SMARCA2 HiBiT $DC_{50}$ ($D_{max}$) | 12 nM (99%) |
| SMARCA4 HiBiT $DC_{50}$ ($D_{max}$) | 33 nM (99%) |

| AU15330 | |
|---|---|
| SMARCA2 HiBiT $DC_{50}$ ($D_{max}$) | 29 nM (91%) |
| SMARCA4 HiBiT $DC_{50}$ ($D_{max}$) | 58 nM (93%) |

## Figure 6. SMARCA4 resynthesis is at least 2 times faster than SMARCA2 as demonstrated in multiple cell lines




| Target protein resynthesis rate | |
|---|---|
| SMARCA2 $k_{resyn}$ (recovery half-time) | 0.03 $h^{-1}$ (23 hour) |
| SMARCA4 $k_{resyn}$ (recovery half-time) | 0.06 $h^{-1}$ (11 hour) |

a. Western blot time course of SMARCA2 and SMARCA4 resynthesis in Calu-6 human lung cancer cell line. The cells were treated with potent SMARCA2/4 dual degrader or DMSO for 24 hours and then washed three times before allowing to recover for up to 72 hours.
b. SMARCA2 and SMARCA4 resynthesis in Hela SMARCA2 and SMARCA4 HiBiT cell lines. The cells were treated with potent SMARCA2/4 dual degrader or DMSO for 24 hours and then washed three times before allowing to recover for 26 hours.

## Conclusions

- PRT3789 selectively degrades SMARCA2 over SMARCA4 by 37-fold and over 4500-fold greater antiproliferative selectivity observed in SMARCA4-deficient cells compared to SMARCA4 wild-type cells.
- PRT3789 induces a more stable ternary complex between SMARCA2 and VHL than SMARCA4 and VHL.
- K1405 loop in SMARCA2 not only provides unique lysine residue for ubiquitination but also stabilizes the SMARCA2:VHL complex to facilitate more efficient ubiquitination on SMARCA2.
- SMARCA2 resynthesis rate is 2-3 times slower than SMARCA4, thereby contributing to the SMARCA2 selective degradation by PRT3789.