Clinical Biomarkers Based on PK/PD Modeling to Guide the Development for a First-in-Class, Highly Selective SMARCA2 (BRM) Degrader, PRT3789

Min Wang[1], Andrew Moore[1], Alex Grego[1], Miles Cowart[1], Joseph Rager[1], Gina Paris[1], Koichi Ito[1], Naveen Babbar[1], Sandy Geeganage[1], Peggy Scherle[1], Neha Bhagwat[1].

[1] Prelude Therapeutics Incorporated, Wilmington, DE; contact: nbhagwat@preludetx.com

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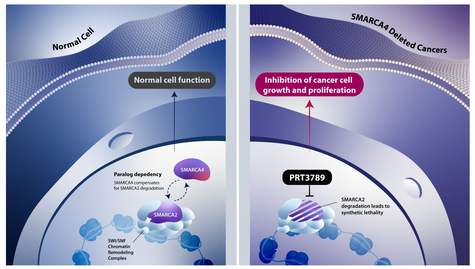
Background

- The SWI/SNF family of chromatin-remodeling complexes is frequently dysregulated in multiple tumor types, resulting in aberrant expression of genes. Damaging mutations resulting in loss of function of one of its core catalytic subunits SMARCA4 (BRG1) occur in multiple tumor types, including in 5-10% of non-small cell lung cancer (NSCLC)[1].
- SMARCA4-deleted cells become highly dependent on the other catalytic subunit, SMARCA2 (BRM), for their survival[2]. Therefore, selective degradation of SMARCA2 has therapeutic potential in these cancers, which we have previously shown in preclinical models using novel SMARCA2 degraders[3].
- PRT3789 is a first-in-class, potent and selective SMARCA2 degrader that is currently under evaluation in a Phase 1 study in patients with advanced solid tumors with loss of SMARCA4 (NCT05639751)

Figure 1. SMARCA2 Degradation Leads to Synthetic Lethality in SMARCA4 Deleted Cancers



Objectives

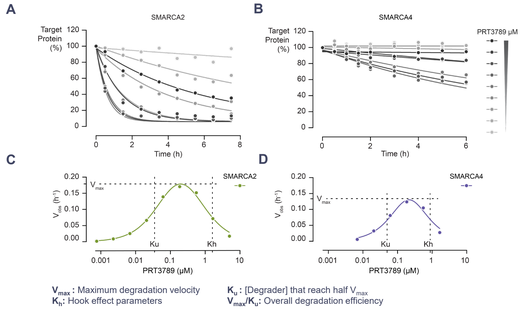
- Establish degradation kinetic parameters of PRT3789 and correlate with *in vivo* preclinical studies
- Develop a suite of biomarker assays to assess target engagement and downstream effects of SMARCA2 protein degradation

Key Findings

- PRT3789 was determined to be a highly efficient degrader of SMARCA2 with minimal effects on SMARCA4 using a kinetic model
- Calculated predictions from the mathematical model correlated closely with observed *in vivo* PD
- We demonstrated sensitive and quantitative measurement of SMARCA2 protein expression and function in PBMCs using immunoassays and quantitative PCR. SMARCA2 and SMARCA4 protein levels in tumor tissues was assessed by immunohistochemistry

Results

Figure 2. Characterization of kinetic parameters of SMARCA2/4 degradation

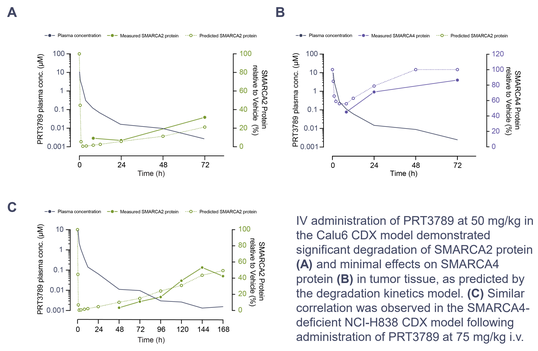


V_{max} : Maximum degradation velocity
K_H: Hook effect parameters

K_U : [Degrader] that reach half V_{max}
V_{max}/K_U: Overall degradation efficiency

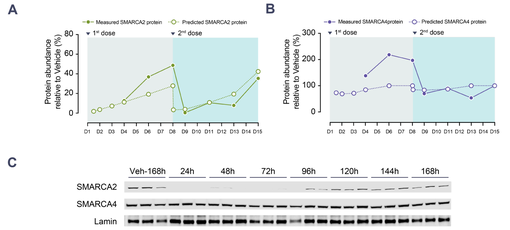
HeLa HiBit cells were incubated with increasing concentrations of PRT3789 and **(A)** SMARCA2; and **(B)** SMARCA4 protein levels were measured by luminescence at indicated timepoints. 1st order rate equation was used to fit time course data. The dose-dependent curves for **(C)** SMARCA2; and **(D)** SMARCA4 are fitted using an enzyme activator model.

Figure 3. Degradation kinetics and PK parameters predict in vivo PD in multiple tumor models



IV administration of PRT3789 at 50 mg/kg in the Calu6 CDX model demonstrated significant degradation of SMARCA2 protein **(A)** and minimal effects on SMARCA4 protein **(B)** in tumor tissue, as predicted by the degradation kinetics model. **(C)** Similar correlation was observed in the SMARCA4-deficient NCI-H838 CDX model following administration of PRT3789 at 75 mg/kg i.v.

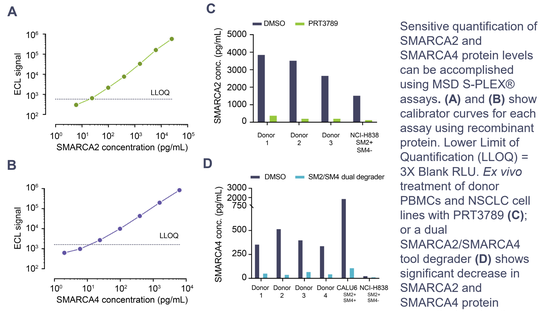
Figure 4. PRT3789 demonstrates potent degradation of SMARCA2 with minimal effect on SMARCA4 in PBMCs *in vivo* in rats



PRT3789 was administered by IV bolus once weekly for two doses (QWx2) in male Sprague-Dawley rats and blood was collected at indicated timepoints. SMARCA2 and SMARCA4 protein was assessed in PBMCs by immunoblotting. Protein degradation as predicted by mathematical modeling closely matched observed SMARCA2 **(A)**; and SMARCA4 **(B)** protein reduction in PBMCs. A representative image is shown in **(C)**

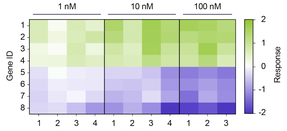
Biomarker Assays

Figure 5. SMARCA2 and SMARCA4 MSD assay for protein quantification



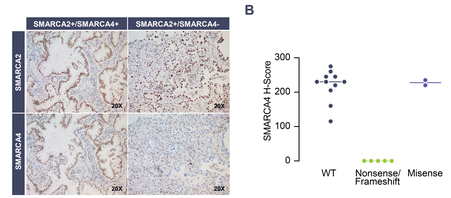
Sensitive quantification of SMARCA2 and SMARCA4 protein levels can be accomplished using MSD S-PLEX® assays. **(A)** and **(B)** show calibrator curves for each assay using recombinant protein. Lower Limit of Quantification (LLOQ) = 3X Blank RLU. *Ex vivo* treatment of donor PBMCs and NSCLC cell lines with PRT3789 **(C)**; or a dual SMARCA2/SMARCA4 tool degrader **(D)** shows significant decrease in SMARCA2 and SMARCA4 protein respectively using the MSD S-PLEX® assays

Figure 6. qPCR to assess SMARCA2 transcriptional targets



An 8-gene panel was identified based on dose-dependent response observed with *ex vivo* treatment of PBMCs with PRT3789. Heat map displays fold change relative to DMSO (log_2). A secondary, qPCR-based assay was developed for assessment of SMARCA2 transcriptional targets in peripheral blood that may be associated with response to SMARCA2 degradation by PRT3789.

Figure 7. SMARCA2 and SMARCA4 IHC Assay



An immunohistochemistry assay for the assessment of SMARCA2 and SMARCA4 protein expression in tumor tissue was developed on the Leica BOND III platform using commercially available antibodies. The IHC assay demonstrated discrete and robust nuclear tumor staining with a dynamic intensity range and limited cytoplasmic background for both SMARCA2 and SMARCA4. **A)** Representative images of SMARCA4 expressing and null tumor samples. **B)** Loss of SMARCA4 protein expression in tumor cells by IHC was associated with nonsense and frameshift mutations in the SMARCA4 gene.

Conclusions

- We deeply characterized the degradation kinetic parameters of PRT3789 based on *in vitro* assays
- The calculated predictions from the mathematical model correlated closely with observed in vivo PD in preclinical tumor xenografts and PK/PD models.
- We demonstrate the sensitive assessment of SMARAC2 protein degradation and effects on downstream gene expression in PBMCs.

References

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2. Fernando, TM, et al., Nat Commun. 2020;11:5551.
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Acknowledgments

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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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