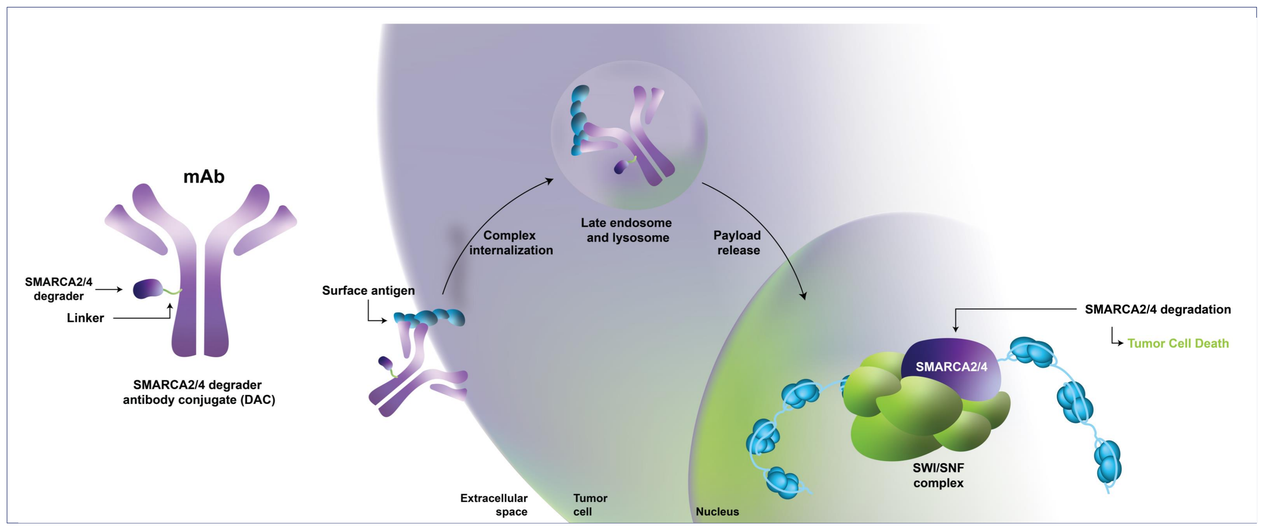
Discovery of first-in-class precision antibody drug conjugates with a potent SMARCA2/4 dual degrader payload

Carter J, Agarwal A, Sivakumar M, Reichelderfer A, Park J, Osinubi O, Holmes R, Fultang N, Schwab A, Rager J, Stahl N, Cote J, Crossan A, Chen C, Pawley S, Wang M, Geeganage S, Lee S, Combs A, Scherle P, Buesking A and Ito K

Prelude Therapeutics Incorporated, Wilmington, DE
contact: abuesking@preludetx.com

Background

- SMARCA2 and SMARCA4 are the core catalytic subunits of SWI/SNF complexes and play a key role in controlling chromatin remodeling and gene expression.
- Targeting SWI/SNF complexes with small molecules or targeted protein degraders demonstrates anti-tumor activity in preclinical models and early clinical trials have been initiated in multiple cancer types.
- Because either SMARCA2 or SMARCA4 is necessary for normal cellular functions, maximal suppression of both SMARCA2/4 proteins simultaneously is unlikely to be tolerated.
- Degrader Antibody Conjugates (DACs) represent a new frontier in advancing the scientific and clinical potential of antibody drug conjugates (ADCs).
- We developed DACs with potent SMARCA2/4 dual degraders as payloads on tumor specific antibodies to achieve maximal target degradation in tumors and spare healthy tissues.

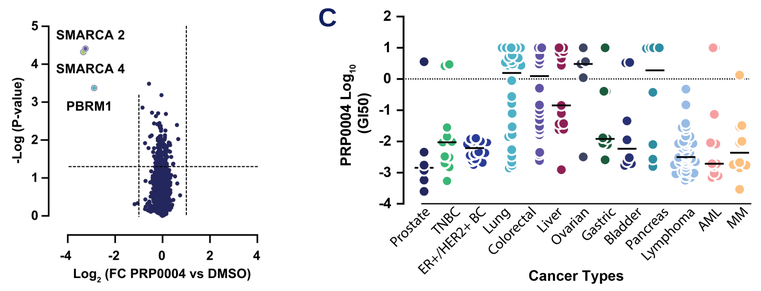


Results

Figure 1. Identification of Selective SMARCA2/4 Dual Degraders with Potent Anti-Cancer Activity

A

Cellular Potency & Selectivity			
Payload	PRP 0004	PRP 0005	PRP 0006
SMARCA2 DC_{50} (nM)	0.37	0.26	0.04
SMARCA4 DC_{50} (nM)	2.72	1.18	0.09
Fold Selectivity SMARCA4/SMARCA2	7	5	2

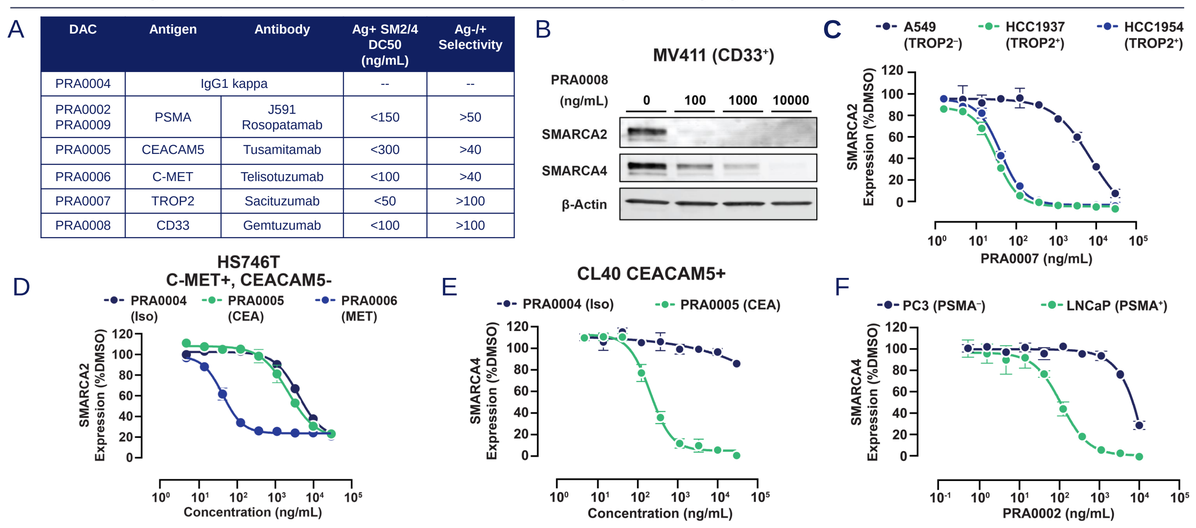


(A) SMARCA2/4 degradation potency of 3 payloads in a HeLa HiBiT cell-based assay. (B) Global proteomics analysis following treatment of LNCaP human prostate cancer cells with 25 nM PRP0004 for 1h. (C) GI_{50} of a panel of cancer cell lines treated with PRP0004, assessed by CellTiter-Glo® assay.

Figure 2. Conjugation of Clinically-Validated Antibodies to SMARCA2/4 Degrader Payloads Drives Antigen-Dependent Internalization and Target Engagement

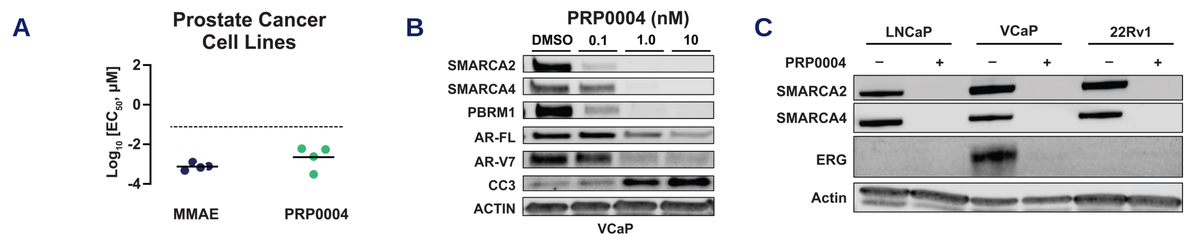
A

DAC	Antigen	Antibody	Ag+ SM2/4 DC50 (ng/mL)	Ag-/+ Selectivity
PRA0004	IgG1 kappa		--	--
PRA0002 PRA0009	PSMA	J591 Rosopatamab	<150	>50
PRA0005	CEACAM5	Tusamitamab	<300	>40
PRA0006	C-MET	Telisotuzumab	<100	>40
PRA0007	TROP2	Sacituzumab	<50	>100
PRA0008	CD33	Gemtuzumab	<100	>100



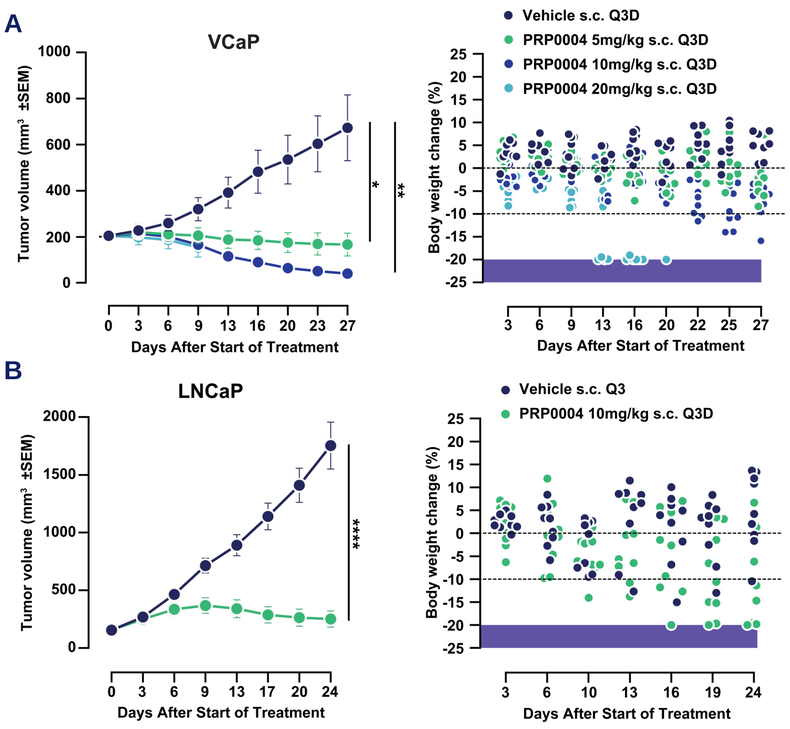
(A) Summary of SMARCA2/4 degradation potency and antigen (Ag)-selectivity for a panel of generated DACs. (B) Western blot for SMARCA2/4 in CD33+ MV411 cells treated with PRA0008 for 24h. (C-F) In-cell western blots showing expression of SMARCA2 or SMARCA4 after treatment of cancer cell lines that are positive or negative for TROP2 (C), C-MET/CEACAM5 (D, E), or PSMA (F) with indicated DACs for 24h. (G) Internalization (Incucyte® Fabfluor-pH labeling) of PRA0002 in PSMA+ LNCaP, but not PSMA- PC3 cells.

Figure 3. PRP0004 Induces Apoptosis and Regulates the Expression of Key Oncoprotein Drivers in Prostate Cancer Cells



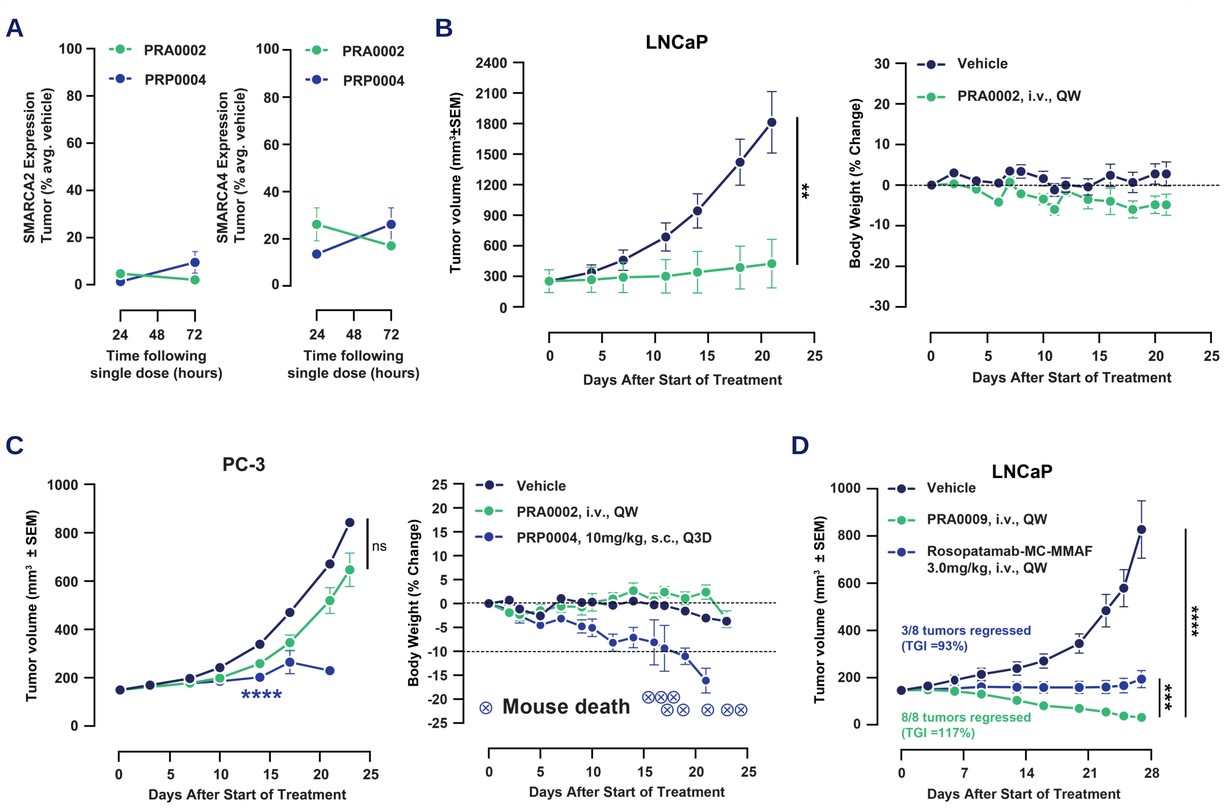
(A) EC_{50} of human prostate cancer cell lines treated with PRP0004 or MMAE for 7-days (CellTiter-Glo® assay). (B) Western blot showing the expression of SMARCA2/4, AR-FL, AR-V7, and cleaved-caspase 3 (CC3) in VCaP cells treated with PRP0004 for 3 days. (C) Western blot showing the expression of ERG following treatment with PRP0004 in cells that express a *TMPRSS2-ERG* fusion.

Figure 4. Payload PRP0004 Induces *In Vivo* Tumor Regressions in Prostate Cancer Xenografts But is Limited by a Narrow Therapeutic Index



(A) Payload PRP0004 administered s.c. Q3D demonstrated dose-dependent tumor growth inhibition in the human prostate cancer VCaP CDX model. At higher doses, PRP0004 induced tumor regressions but caused time and dose-dependent body weight loss and mouse deaths. *P<0.05 **P<0.01 versus vehicle (T-test) (B) Payload PRP0004 administered s.c. Q3D induced significant tumor growth inhibition in the human prostate cancer LNCaP CDX model, while leading to delayed body weight loss and mouse death. ****P<0.0001 versus vehicle (T-test).

Figure 5. Anti-PSMA SMARCA2/4 DACs Demonstrate Robust *In Vivo* Target Engagement and Significant Antigen-Selective Tumor Growth Inhibition While Being Well-Tolerated.



(A) SMARCA2/4 protein expression was analyzed in DAC PRA0002 and payload PRP0004-treated LNCaP tumors at the indicated time points following a single dose. Graphs are quantitation of western blots. (B) Weekly i.v. administration of PRA0002 was well-tolerated and demonstrated significant tumor growth inhibition (89%) of PSMA+ LNCaP tumors. (C) Weekly i.v. administration of PRA0002 did not induce significant tumor growth inhibition in PSMA- PC3 tumors, in comparison to PRP0004 which was efficacious, but caused mouse body weight loss and death (D) Weekly i.v. administration of PRA0009 demonstrated tumor regression and significantly better efficacy compared to a PSMA cytotoxic ADC (Rosopatamab-MC-MMAF, DAR2) in LNCaP tumors.

Conclusions

- PRP0004 is a potent SMARCA2/4 degrader that robustly inhibits cancer growth and induces cell death.
- Conjugation of PRP0004 to clinically-validated antibodies yielded DACs demonstrating proof-of-concept for achieving potent and antigen-selective internalization and target engagement in multiple cancer types.
- Prostate cancer was amongst the most sensitive indications to PRP0004, which downregulated prostate cancer drivers, rationalizing the use of PSMA-targeting antibodies for further proof-of-concept studies.
- PRP0004 was highly efficacious in prostate cancer xenografts but displayed a narrow therapeutic window.
- Anti-PSMA SMARCA2/4 DACs demonstrated robust target engagement and antigen-dependent efficacy in xenograft models while being well-tolerated.
- This data highlights the potential of this first-in-class precision antibody drug conjugate approach utilizing a SMARCA2/4 degrader payload to achieve maximal target degradation in tumors and spare healthy tissues while expanding the reach of SMARCA2/4 degraders to patients without SMARCA4 mutations.

References

1. Fernando, T.M. et al. Functional characterization of SMARCA4 variants identified by targeted exome-sequencing of 131,668 cancer patients. Nat Commun 11, 5551 (2020).
2. Ito K et al. Abstract 1139: Potent SMARCA2 targeted degraders induce genetic synthetic lethalityin SMARCA4 deleted cancer. Cancer Res 1 July 2021; 81 (13_Supplement): 1139.
3. Hulse M et al. Abstract 3263: Preclinical characterization of PRT3789, a potent and selective SMARCA2 targeted degrader. Can Res 2022, 82 (12_Supplement): 3263
4. PRT3789 Monotherapy and in Combo w/Docetaxel in Participants w/Advanced or Metastatic Solid Tumors w/SMARCA4 Mutation. NCT05639751. https://clinicaltrials.gov/study/NCT05639751
5. Centore et al. Mammalian SWI/SNF Chromatin Remodeling Complexes: Emerging Mechanisms and Therapeutic Strategies. Trends Genet. 12, 936-950 (2020)
6. Hong et al. Degrader-Antibody Conjugates: Emerging New Modality. J Med Chem 1, 140-148 (2023)

Acknowledgments

This study was funded by Prelude Therapeutics Incorporated.
Editorial Support was provided by Endosymbiont GmbH.
We thank Karl Blom, Ken Ray, and Raul Leal for their analytical support.

Disclosures

All authors are or were employees of Prelude Therapeutics Incorporated, Wilmington, DE and may own equity in the Company.