Potent SMARCA2 targeted protein degraders induce genetic synthetic lethality in SMARCA4 deleted cancer

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INTRODUCTION

- SWI/SNF complexes play an important role in controlling gene expression by remodeling chromatin.
- SMARCA2 (BRM) and SMARCA4 (BRG1) are the core subunits of the SWI/SNF complexes which contain ATPase domain and DNA binding bromodomains.
- SMARCA4 protein expression is lost in some cancers due to damaging mutations (e.g. nonsense, frameshift deletion, splice site mutations) and SMARCA4-deleted cancer cells are highly dependent on its paralog gene SMARCA2 for their survival.
- Patients with homozygously deleted *SMARCA4* cancer show worse prognosis, compared to patients with *SMARCA4* WT expressing cancer, and are not likely to benefit from currently available targeted therapy/immunotherapy.
- We demonstrate that targeting SMARCA2 using SMARCA2 selective degraders induce strong synthetic lethality in SMARCA4-deleted cancer cells.

RESULTS

SMARCA4 DELETED CANCERS ARE DEPENDENT ON ITS PARALOG GENE SMARCA2 FOR SURVIVAL

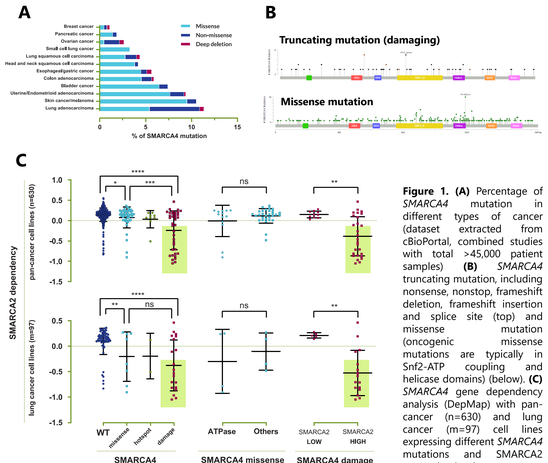
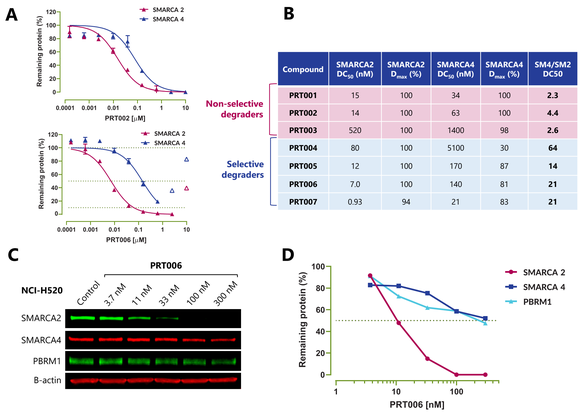


Figure 1. (A) Percentage of *SMARCA4* mutation in different types of cancer (dataset extracted from cBioPortal, combined studies with total >45,000 patient samples) **(B)** *SMARCA4* truncating mutation, including nonsense, nonstop, frameshift deletion, frameshift insertion and splice site (top) and missense mutation (oncogenic missense mutations are typically in Snf2-ATP coupling and helicase domains) (below). **(C)** *SMARCA4* gene dependency analysis (DepMap) with pan-cancer (n=630) and lung cancer (n=97) cell lines expressing different *SMARCA4* mutations and SMARCA2 expression levels.

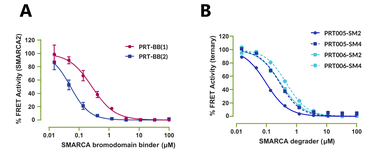
IDENTIFICATION OF POTENT AND SELECTIVE SMARCA2 DEGRADERS



	Compound	SMARCA2 DC_{50} (nM)	SMARCA2 D_{max} (%)	SMARCA4 DC_{50} (nM)	SMARCA4 D_{max} (%)	SM4/SM2 DC50
Non-selective degraders	PRT001	15	100	34	100	2.3
	PRT002	14	100	63	100	4.4
	PRT003	520	100	1400	98	2.6
Selective degraders	PRT004	80	100	5100	30	64
	PRT005	12	100	170	87	14
	PRT006	7.0	100	140	81	21
	PRT007	0.93	94	21	83	21

Figure 2. (A) SMARCA2 and SMARCA4 degradation potency of SMARCA degraders analyzed by in-cell western assay using SMARCA4 WT NCI-H520 lung cancer cells. **(B)** Summary table for SMARCA2 and SMARCA4 DC_{50} and D_{max} of SMARCA degraders. **(C)** Western blot shows selective degradation of SMARCA2 (200 kDa) by PRT006 treatment. **(D)** SMARCA2, SMARCA4 and PBRM1 WB bands were quantitated and normalized by β-actin.

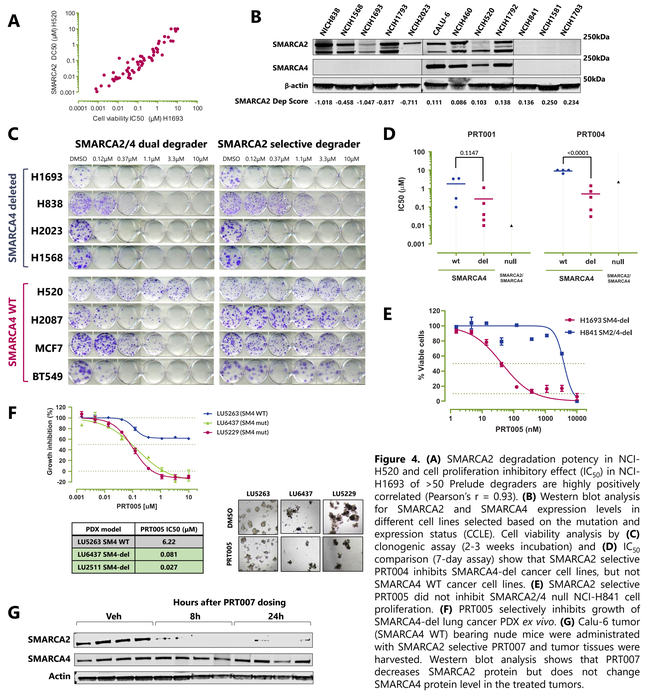
SELECTIVE SMARCA2 DEGRADERS FORM BINARY/TERNARY COMPLEX WITH BOTH SMARCA2 AND SMARCA4



		Binary FRET SM4/SM2 (fold)	Ternary FRET SM4/SM2 (fold)
Non-selective degraders	PRT001	1.1	0.95
	PRT002	1.1	2.4
Selective degraders	PRT005	1.3	2.9
	PRT006	1.4	1.5

Figure 3. (A) Binary TR-FRET competition assay with biotin-conjugated SMARCA bromodomain binder shows potent binding activity of novel bromodomain binders to SMARCA2 bromodomain protein. **(B)** Ternary complex TR-FRET competition assay with biotin-conjugated SMARCA bromodomain binder with novel SMARCA degraders demonstrates potent ternary complex formation with modest selectivity for SMARCA2 over SMARCA4. **(C)** Summary table for binary and ternary TR-FRET IC_{50} selectivity (fold change).

SMARCA2 DEGRADERS SELECTIVELY INHIBIT PROLIFERATION OF SMARCA4-DEL NSCLC CANCER CELL LINES AND PATIENT-DERIVED LUNG CANCER CELLS



PDX model	PRT005 IC50 (μM)
LU5263 SM4 WT	6.22
LU6437 SM4-del	0.081
LU2511 SM4-del	0.027

Figure 4. (A) SMARCA2 degradation potency in NCI-H520 and cell proliferation inhibitory effect (IC_{50}) in NCI-H1693 of >50 Prelude degraders are highly positively correlated (Pearson's r = 0.93). **(B)** Western blot analysis for SMARCA2 and SMARCA4 expression levels in different cell lines selected based on the mutation and expression status (CCLE). Cell viability analysis by **(C)** clonogenic assay (2-3 weeks incubation) and **(D)** IC_{50} comparison (7-day assay) show that SMARCA2 selective PRT004 inhibits SMARCA4-del cancer cell lines, but not SMARCA4 WT cancer cell lines. **(E)** SMARCA2 selective PRT005 did not inhibit SMARCA2/4 null NCI-H841 cell proliferation. **(F)** PRT005 selectively inhibits growth of SMARCA4-del lung cancer PDX *ex vivo*. **(G)** Calu-6 tumor (SMARCA4 WT) bearing nude mice were administrated with SMARCA2 selective PRT007 and tumor tissues were harvested. Western blot analysis shows that PRT007 decreases SMARCA2 protein but does not change SMARCA4 protein level in the treated tumors.

SMARCA2 DEGRADERS DOWNREGULATE CELL CYCLE AND PROLIFERATION GENE SIGNATURES IN SMARCA4-DEL NSCLC CELLS

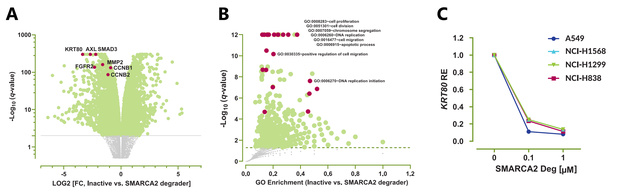


Figure 5. SMARCA4-del NCI-H1693 cells were treated with PRT001 or PRT003 for 3 days. Global mRNA expression was analyzed by NGS (Illumina HiSeq). **(A)** A volcano plot displays Log2 (fold change, vs. inactive degrader) and adjusted *p*-value (*q*-value). *KRT80*, *AXL* and other key genes downregulated by PRT003 were highlighted in red. **(B)** Gene ontology analysis (GeneSCF v.1.1-p2, goa_human GO list, biological processes) shows negatively regulated cell cycle, cell proliferation, apoptosis related gene signatures. **(C)** Quantitative RT-PCR confirms that PRT001 downregulates *KRT80* mRNA expression in multiple SMARCA4-del NSCLC cell lines.

GLOBAL CELLULAR PROTEOMICS PROFILE WITH SMARCA2 SELECTIVE DEGRADERS

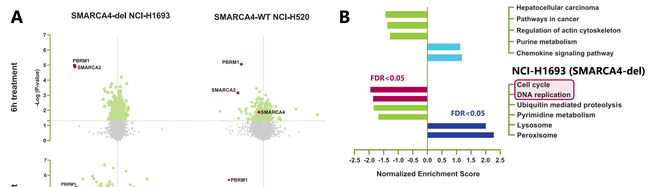
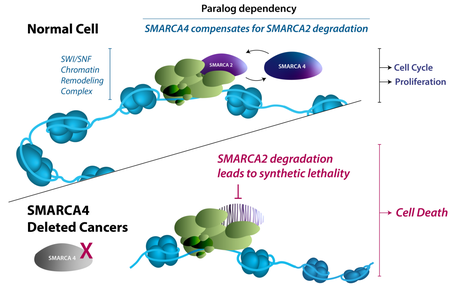


Figure 6. (A) SMARCA4-del NCI-H1693 or SMARCA4 WT NCI-H520 cells were treated with SMARCA2 selective PRT006 for 6h or 36h. Global cellular proteome analyzed by TMT-based mass spectrometry demonstrates that PRT006 shows significant selectivity for SMARCA2 and PBRM1 (red). **(B)** Protein set enrichment analysis shows that SMARCA2 selective PRT006 regulates cell cycle and DNA replication pathway proteins only in NCI-H1693, but not in NCI-H520 cells, after 36h treatment.

MODEL OF SMARCA2 DEGRADER-INDUCED SYNTHETIC LETHALITY IN SMARCA4-DEL CANCERS



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CONCLUSIONS

- **Our validation studies confirmed the concept of strong synthetic lethality of targeting SMARCA2 in SMARCA4 deleted cancers, consistent with previous reports by other groups.**
- **Both the SMARCA4 deletion mutation and SMARCA2 expression could be essential biomarkers for patient selection strategy of targeting SMARCA2.**
- **Selective SMARCA2 degradation over SMARCA4 does not require high selectivity of binary/ternary complex formation for SMARCA2, presumably due to SMARCA2 selective ternary conformations that are competent for ubiquitination and subsequent degradation.**
- **Our SMARCA2 degraders selectively decreases SMARCA2 protein levels and significantly inhibits growth of SMARCA4-del NSCLC and PDX cells.**
- **Selective degradation of SMARCA2 over SMARCA4 was confirmed in an in vivo xenograft model.**
- **In vivo efficacy studies with SMARCA2 selective degraders in SMARCA4-del NSCLC xenograft/PDX models are on going.**