Preclinical characterization of SMARCA2-selective monovalent direct degraders

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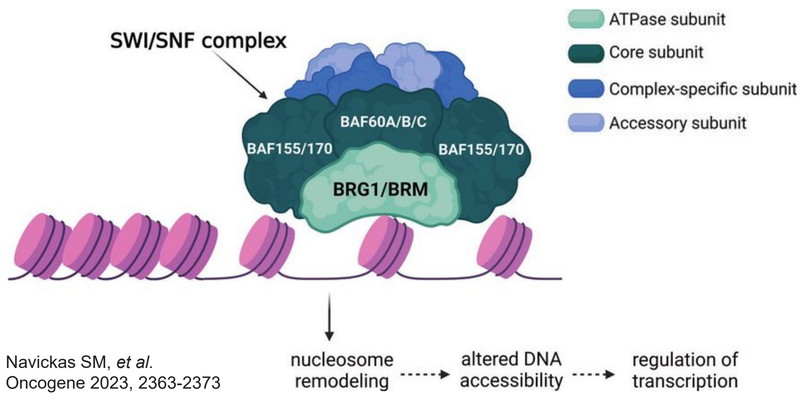
83

Background

SMARCA2 and SMARCA4 are essential, yet redundant, catalytic subunits of human BAF complexes, which are involved in controlling gene expression through the remodeling of chromatin structure. In a subset of solid tumors, SMARCA4 is commonly mutated, rendering SMARCA4-deficient cancer cells dependent on SMARCA2 for viability. This synthetic lethal relationship may provide effective and safe treatment options for patients with SMARCA4-deficient tumors through the development of SMARCA2-selective degraders. To enable degrader discovery, we utilized a monovalent direct degrader strategy where small molecules are designed to bind the target protein and induce its degradation through the recruitment of an E3 ligase complex. The work detailed here highlights the characterization of our selective and orally bioavailable monovalent direct degraders of SMARCA2.



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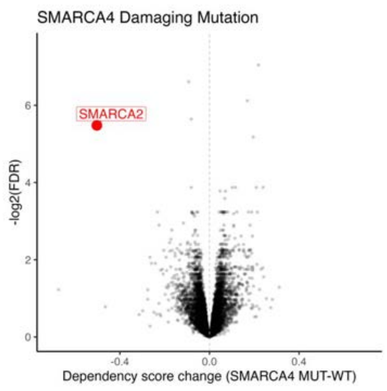
	Tumor Type	Mutation Frequency
	Uterine	13%
	NSCLC	10%
	Bladder	8%
	Melanoma	7%
	CRC	6%
	Gastric	6%
	Esophageal	5%

*cBioPortal includes SMARCA4 mutations and deep deletions



Plexium's direct degrader approach

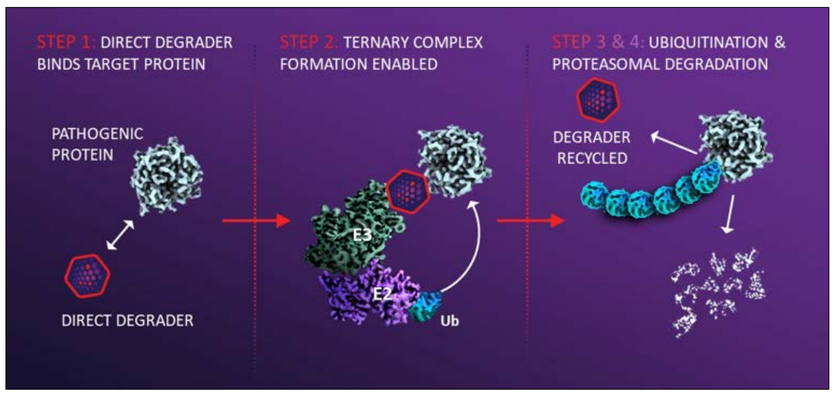
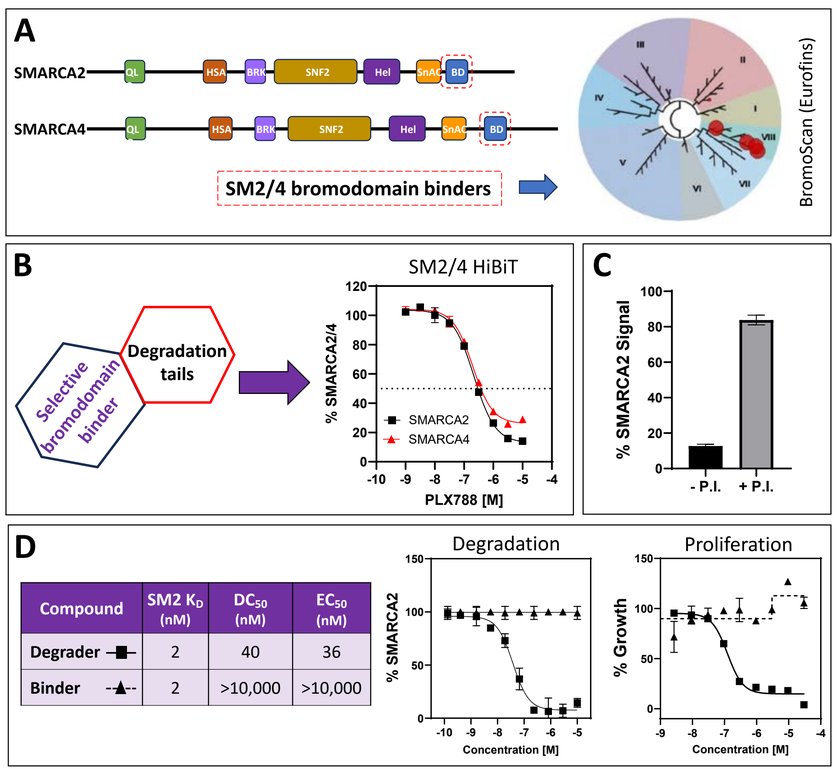


Figure 1: Overview of the Plexium Direct Degrader design strategy. Small molecules are designed to bind to the protein of interest and elicit its degradation through induced interactions with an E3 ligase complex. Prospective degraders can be designed in an E3-agnostic manner and screened for activity in cell-based assays which expose compounds to the repertoire of cellular E3 ligases, or alternatively, E3-specific degrader design principles can be applied to known POI binders to produce small molecule direct degraders.

SMARCA2 direct degrader design and discovery



Compound	SM2 K_D (nM)	DC_{50} (nM)	EC_{50} (nM)
Degrader	2	40	36
Binder	2	>10,000	>10,000

Figure 2: A. SMARCA2 and SMARCA4 share similar protein domain architecture and highly homologous bromodomains. Potent and selective bromodomain binders (as shown in the BromoScan chart) were selected as starting points for degrader design. **B.** Compound libraries were designed by adding small MW "degradation tails" to selective bromodomain binders and screened for SMARCA2/4 degradation in HiBiT cell lines. An early, non-selective hit (PLX788) is shown. **C.** SMARCA degradation is proteasome mediated. The SMARCA4-deficient cell line, SK-MEL-5, was treated with PLX788 for 8h ± 100nM bortezomib (proteasome inhibitor, P.I.) and SMARCA2 levels were quantified by immunofluorescence (IF). **D.** SMARCA2/4 bromodomain binding is not sufficient for anti-proliferative activity. SK-MEL-5 cells were treated with a binder/non-degrader versus a binder/degrader. Only the degrader impacted tumor cell viability. Degradation was monitored by immunofluorescence (IF) after 24h of compound treatment. Cell viability was monitored by Cell Titer Glo (CTG), with growth inhibition curves determined after 72h of compound treatment.

SMARCA2 direct degrader series optimization

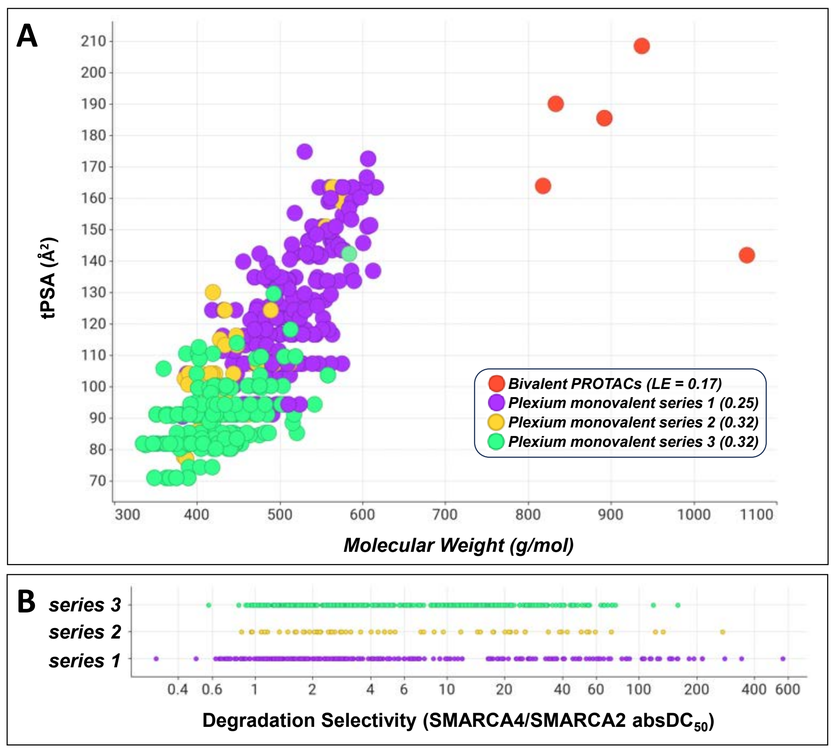


Figure 3: A. Plexium monovalent direct degraders are small molecules with good druglike properties. Scatter plot highlights molecular weight and polar surface area (tPSA) values of Plexium degraders across three series versus select PROTACs. Ligand efficiencies (LE) were calculated from SMARCA2 HiBiT DC_{50}s with representative compounds. **B.** SMARCA2/4 DC_{50} plot illustrating that selectivity is achievable and optimizable across molecular series. Data points in **A** and **B** were limited to compounds with SM2 absDC$_{50}$ < 1µM; Dmax > 60%.

Potent and selective SMARCA2 degradation leads to selective anti-tumor activity

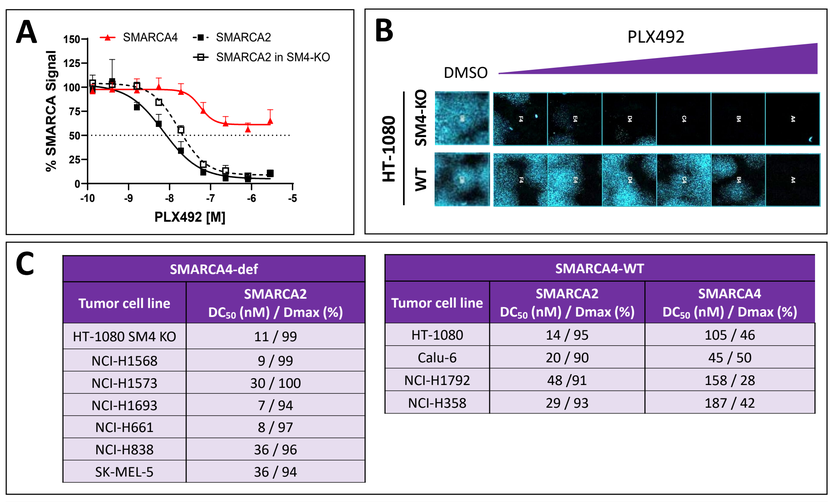


Figure 4: A. PLX492 (series 2) is a selective degrader of SMARCA2. Curves represent degradation profiles of PLX492 in HT-1080 and HT-1080 SMARCA4 knockout (SM4-KO) cells. Degradation was monitored by IF after 24h of compound treatment. **B.** PLX492 potently inhibits proliferation in the SM4-KO cell line and minimally impacts WT cells, demonstrating the synthetic lethal relationship of SMARCA2 and SMARCA4. Clonogenic assays were for 10-14 days with 5 – 5000nM PLX492. **C.** Tables showing PLX492 DC_{50} and Dmax values for select SM4-def vs SM4-WT tumor cell lines. Values determined by IF after 24h of compound treatment. **D.** Waterfall plot showing anti-proliferative activity of PLX492 in 7-day CTG growth assays (Crown Biosciences). Green bars = SM4-def and/or SM4 RNA low cell lines. **E.** PLX492 Omniscreen IC_{50} data comparing SM4-WT cell lines to tumor lines harboring SM4 damaging mutations and/or low levels of SM4 RNA. **F.** Majority of cell lines sensitive to PLX492 are also sensitive to SM2 loss via CRISPR (DepMap). Green dots = SM4-def and/or SM4 RNA low cell lines.

Omics analyses identifies transcriptional targets

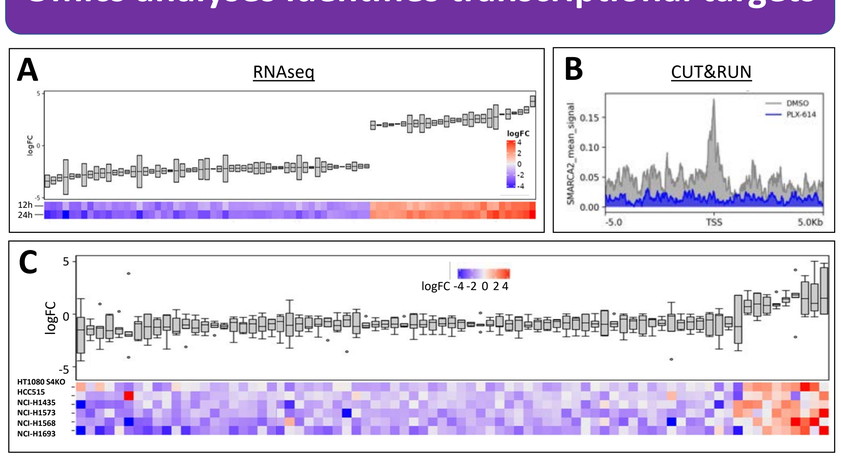


Figure 5: A. Heat maps showing time-dependent regulation of gene expression in HT-1080 SM4-KO cells treated with tool compound, PLX614, for 12 and 24h. Graph highlights the most significantly modulated genes. **B.** CUT&RUN experiments (Epicypher) demonstrate the loss of SMARCA2 complex binding at transcriptional starts sites (TSS). Graph depicts combined SMARCA2 peaks for the down-regulated genes shown in panel **A**. **C.** Gene expression profiles resulting from SMARCA2 loss are highly correlative across a small panel of SMARCA4-def NSCLC cell lines. Graph depicts RNAseq data from cells treated with compound PLX639 for 24h. PLX614 and PLX639 = series 3 SMARCA2-selective degraders.

PLX492 elicits potent and selective anti-tumor activity *in vivo*

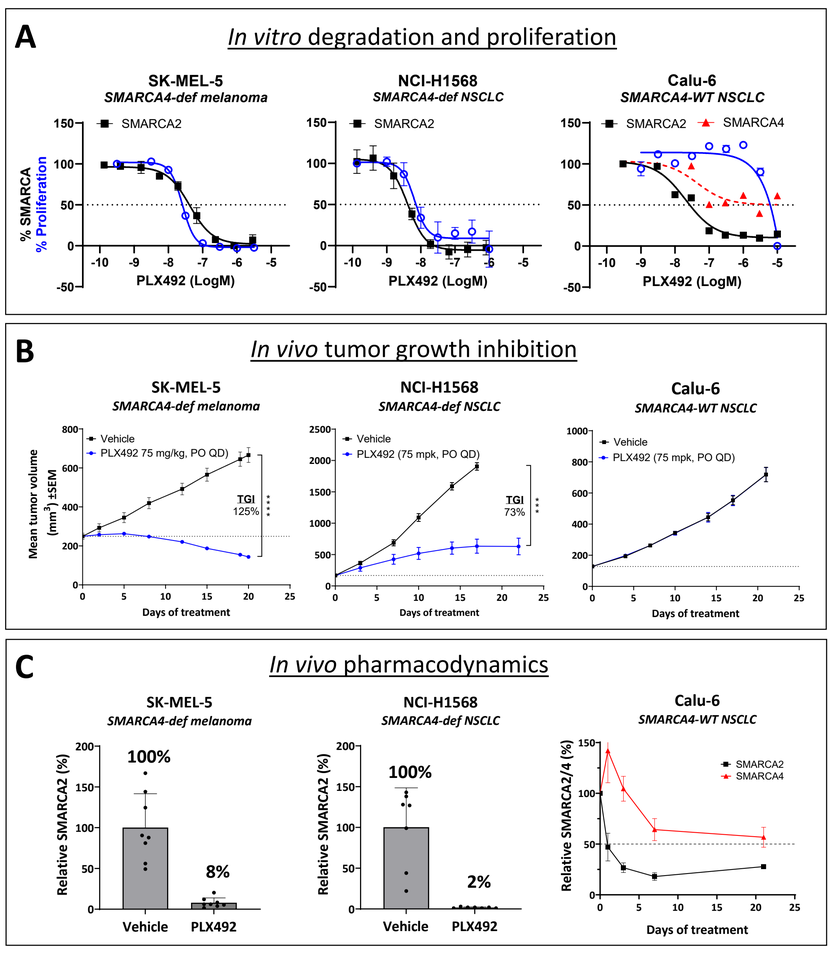
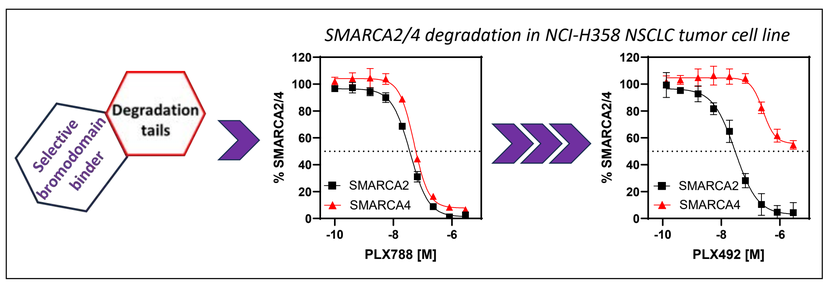


Figure 6: A. *In vitro* degradation and proliferation profiles of PLX492 in SMARCA4-def versus SMARCA4-WT tumor cell lines. Depicted are the SM4-def lines SK-MEL-5 and NCI-H1568, and the SM4-WT line Calu-6. SMARCA4-def cell lines (left and middle panel) show strong correlations between SMARCA2 loss and anti-proliferative phenotypes. In the SM4-WT line (Calu-6; right panel), PLX492 potently degrades SMARCA2 while only partially degrading SMARCA4, with minimal impact on cell viability. Degradation was monitored by IF after 24h of compound treatment; proliferation monitored by CTG after 7 days. **B.** PLX492 elicits significant tumor growth inhibition in SM4-def models at well tolerated doses. No impact on tumor growth was observed in the control SM4-WT model (right panel). Athymic nude mice bearing SK-MEL-5, NCI-H1568, or Calu-6 xenografts were treated with daily oral doses of PLX492 (75mg/kg) for 20-22 days. Tumor volumes were measured twice weekly. **C.** PLX492 potently and selectively degrades SMARCA2 in tumor tissue. Tumors excised after 20-22 days of compound treatment were monitored for SMARCA2/4 protein levels via Simple Western analyses (Bio-Techne). SM4-def tumors treated with PLX492 showed near-complete loss of SMARCA2 protein. In the SM4-WT model, SMARCA2/4 PD measurements demonstrate that selectivity is maintained over the course of 21 consecutive daily doses of PLX492. Tumors excised after 1, 3, 7, and 21 days of compound treatment show deep degradation of SMARCA2 and partial degradation of SMARCA4 (Dmax < 50%).

Summary



- Direct degraders of SMARCA2/4 were designed by combining degradation tails developed at Plexium to known Class VIII bromodomain binders
- Medicinal chemistry efforts enabled the development of selective SMARCA2 degraders with good druglike properties, exemplified here by PLX492
- PLX492 shows potent and selective SMARCA2 degradation across a panel of tumor cell lines and elicits selective *in vitro* antitumor activity in cell lines harboring SMARCA4 damaging mutations
- Selective SMARCA2 degradation modulates gene expression via transcriptional regulation in sensitive cell lines, enabling the identification of robust biomarkers
- PLX492 elicits potent antitumor activity *in vivo* at well-tolerated doses using SMARCA4-def xenograft models; no anti-tumor activity observed with control SMARCA4-WT model
- Insights gained from early degrader series have enabled the rapid advancement and design of our SMARCA2 direct degrader development candidate

Acknowledgments

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