Combination therapy with selective SMARCA2 (BRM) degraders for treatment of SMARCA4 (BRG1)-deficient cancers

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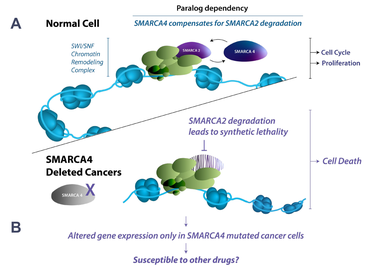
Background

Model of SMARCA2 degrader induced synthetic lethality in SMARCA4-del cancers



A) SMARCA2 and SMARCA4 are the core catalytic subunits of the SWI/SNF complexes, which play an important role in controlling gene expression by remodeling chromatin. SMARCA4 is mutated in multiple cancers and SMARCA4-deficient cancer cells can become highly dependent on SMARCA2 for their survival[1]. Therefore, targeting SMARCA2 in SMARCA4-deleted cancers using selective SMARCA2 degraders induces synthetic lethality while sparing SMARCA4 wild-type normal cells. **B)** SMARCA2 protein degradation in a SMARCA4-deficient tumor background leads to global gene dysregulation, potentially making these tumors vulnerable to other therapy combinations.

Results

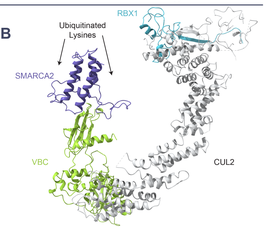
Figure 1. PRT3789 inhibits SMARCA4-deficient tumor growth

A

Assay	PRT3789
HeLa SM2 HiBiT DC_{50} (nM)	0.73
HeLa SM4 HiBiT DC_{50} (nM)	26
Selectivity SM4/SM2 (HiBiT)	40X
Selectivity Cell Proliferation	>1000

Rat PK (IV)	PRT3789
C_0 (µM)	1.9
$t_{1/2}$ (h)	5.2
CL (mL/min/kg)	66

B



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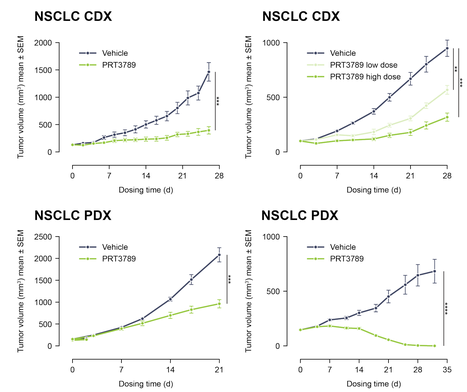


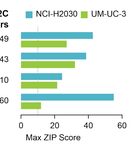
A,B) PRT3789, a highly potent and selective SMARCA2 protein degrader, inhibits proliferation of SMARCA4-del/knockout cancer cell lines, but not SMARCA4 WT cancer cell lines. **C)** PRT3789 monotherapy significantly inhibits growth and induces regression of SMARCA4-del NSCLC PDX and CDX models at well tolerated doses. **P<0.01 ***P<0.001, ****P<0.0001 versus vehicle (two-tailed Mann-Whitney test).

Figure 2. PRT3789 synergizes with KRAS G12C, SHP2 and MEK inhibitors *in vitro*

A

Genie v13 NSCLC[2]	Total NSCLC %	Overlap % in total NSCLC	Overlap % in SMARCA4 damaging/del	Tendency (q-value)
SMARCA4 damaging/del*†	5	-	100	-
KRAS G12C	12	0.75	16	Co-occurrence (<0.001)

B



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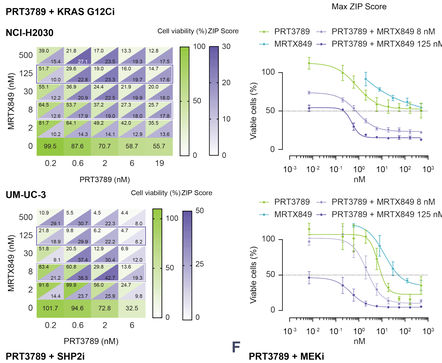

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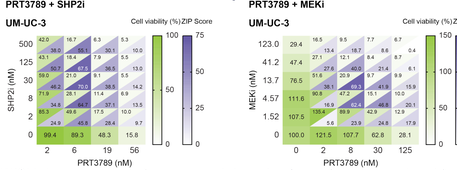
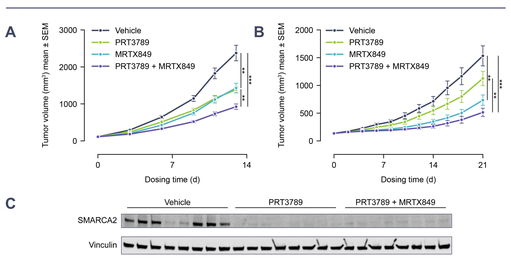


F

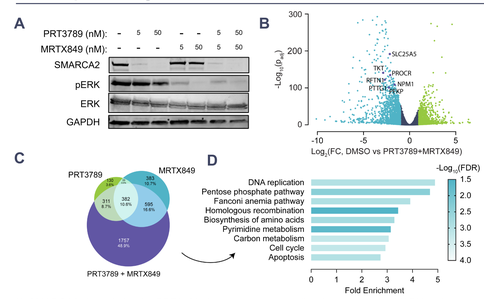
A) SMARCA4 damaging mutations (nonsense, nonstart, nonstop, frameshift, truncating, splice site) and deep deletions are found in approximately 5% of 14,000+ NSCLC patient samples and significantly co-occur with KRAG G12C mutations (AACR project GENIE v13[2]). PRT3789 and KRAS G12C inhibitors **B)** demonstrate excellent synergy (ZIP scores larger than 10: the interaction between two drugs is likely to be synergistic[3]) in **C)** the lung cancer cell line NCI-H2030 (KRAS G12C mutation/SMARCA4 low-expression) and **D)** the urinary bladder cancer cell line UM-UC-3 (KRAS G12C mutation/SMARCA4 del) **E, F)** PRT3789 also exhibits excellent synergy with other MAPK pathway inhibitors, including SHP2 and MEK inhibitors. % viability vs DMSO controls in 7-day cell titer glo assay.

Figure 3. PRT3789 combination with KRAS G12C inhibitor shows enhanced efficacy in SMARCA4/KRAS G12C mutant cancers *in vivo*



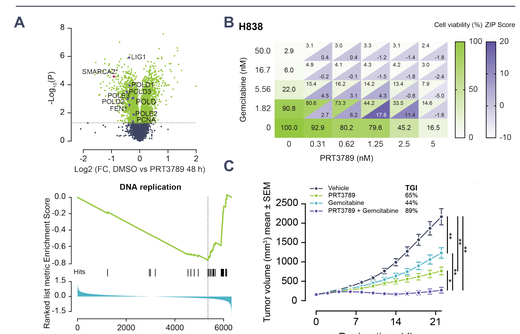
A) PRT3789 + KRAS G12C inhibitor (MRTX849) combination therapy significantly inhibits tumor growth in a SMARCA4-del/KRAS G12C UM-UC3 CDX model, at well tolerated doses. **B)** PRT3789 + KRAS G12C inhibitor (MRTX849) combination therapy increased TGI versus each constituent monotherapy in a SMARCA4-low expression/KRAS G12C NCI-H2030 CDX model, at well tolerated doses **C)** Western blot of UM-UC-3 tumor samples post PRT3789 dose demonstrated complete degradation of SMARCA2 protein in PRT3789 monotherapy and combination groups. **P<0.01 ***P<0.001, ****P<0.0001 versus vehicle (two-tailed Mann-Whitney test).

Figure 4. PRT3789 and KRAS G12Ci inhibitor induces unique transcriptional signatures



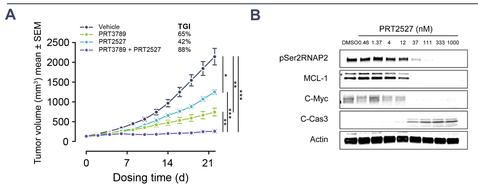
A) SMARCA2 degradation by PRT3789 does not appear to directly regulate the MAPK pathway as determined by western blot analysis of phospho-ERK (T202/Y204) levels 24 h post dose in H2030 cells. **B)** Volcano plots display Log_2 (fold change vs DMSO) gene expression and adjusted P value (Q value) in UM-UC-3 (KRAS G12C mutation/SMARCA4 del) cells treated with PRT3789+MRTX849 for 48 hours. Genes that are uniquely regulated in the UM-UC-3 combination groups vs each monotherapy are labelled. **C)** PRT3789 and MRTX849 combination treatment regulates expression of 1757 unique genes. **D)** KEGG analysis of these unique genes regulated by PRT3789+MRTX849 combination (Shiny GO 0.77).

Figure 5.PRT3789 downregulates base excision repair (BER), DNA replication proteins and synergizes with NSCLC SOC chemotherapy *in vitro* and *in vivo*



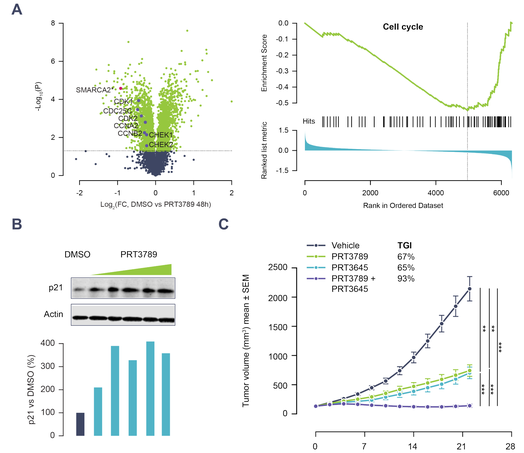
A) Global proteomics revealed that PRT3789 downregulates cell cycle protein signatures[4]. Volcano plots display Log2 (fold change vs DMSO) protein expression and adjusted -LogP value in SMARCA4-del NCI-H1693 cells treated with PRT3789 for 48 hours. Key BER proteins downregulated by PRT3789 treatment were labelled. **B)** PRT3789 + Gemcitabine combination therapy demonstrated synergy in vitro in the SMARCA4-del H838 NSCLC cell line in a 7-day cell titer glo assay. % viability vs DMSO controls. ZIP scores calculated using SynergyFinder 2.0[2]**(C)** PRT3789 + Gemcitabine combination therapy resulted in TGI of 89% in the SMARCA4-del H838 NSCLC CDX model. *P<0.05 **P<0.01 ***P<0.001, versus vehicle (two-tailed Mann-Whitney test). TGI, mean tumor growth inhibition vs vehicle.

Figure 6. PRT3789 combination with the CDK9 inhibitor PRT2527 shows enhanced efficacy *in vivo*



A) PRT3789 +CDK9 inhibitor PRT2527 combination therapy significantly inhibits tumor growth in the SMARCA4-del H838 NSCLC CDX model at well tolerated doses. **B)** PRT2527 regulates expression of several immediate early genes driving oncogenesis and resistance, including MCL1[5]. The MCL1 inhibitor PRT1419 has previously been shown to combine with PRT3789 and induce regression the SMARCA4-del H838 NSCLC CDX model[6]. *P<0.05 **P<0.01 ***P<0.001, versus vehicle (two-tailed Mann-Whitney test). TGI, mean tumor growth inhibition vs vehicle.

Figure 7. PRT3789 downregulates cell cycle proteins and combines with the Next generation CDK4/6 inhibitor PRT3645 *in vivo*



A) Global proteomics revealed that PRT3789 downregulates cell cycle protein signatures[4]. Volcano plots display Log2 (fold change vs DMSO) protein expression and adjusted -LogP value in SMARCA4-del NCI-H1693 cells treated with PRT3789 for 48 hours. Key cell cycle proteins downregulated by PRT3789 treatment were labelled. **B)** SMARCA4-del NCI-H838 cells treated with PRT3789 for 48 hours led to induction of p21 protein. **(C)** PRT3789 + the CDK4/6 inhibitor PRT3645 combination therapy induced tumor regression in the SMARCA4-del H838 NSCLC CDX model at well tolerated doses. *P<0.05 **P<0.01 ***P<0.001, versus vehicle (two-tailed Mann-Whitney test). TGI, mean tumor growth inhibition vs vehicle.

Conclusions

- Targeting SMARCA2 in SMARCA4-deficient cancers with PRT3789 monotherapy significantly inhibits growth and induces regression of SMARCA4-del NSLCL PDX and CDX models at well tolerated doses.
- PRT3789 combines synergistically with agents that target the MAPK pathway, including KRAS G12C, SHP2 and MEK inhibitors.
- PRT3789 combines in vivo with KRAS G12C inhibitor, NSCLC SOC chemotherapy, CDK4/6 and CDK9 inhibitors to inhibit tumor growth and induce regression of SMARCA4-del CDX models

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Acknowledgments

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Disclosures

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