SMARCA2 degraders promote differentiation and inhibit proliferation in AML models

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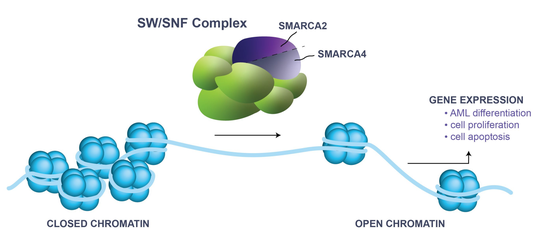
Background

- The success of ATRA and decitabine, in a subset of AML patients, has proven that inducing differentiation can play a critical role in long-term durable responses(1)
- Recently, targeting SWI/SNF chromatin remodeling complexes has also been shown to regulate key leukemic gene-expression signatures and induce AML differentiation(2)
- Small molecule inhibitors as well as gene knockdown of ATP-dependent SWI/SNF subunits, SMARCA2 (BRM) and SMARCA4 (BRG1), are associated with re-direction of oncogenic transcriptional regulation to drive cellular differentiation and apoptosis in AML models(3,5)
- PRT3789, a SMARCA2 selective degrader, has shown synthetic lethality in SMACRA4 deleted cancers in our previous pre-clinical studies and being tested in Phase I clinical trial (NCT05639751) in human(4)
- In the present study, we demonstrate the pronounced effects of PRT3789 on cell proliferation and cell differentiation in pre-clinical AML models.

Objectives

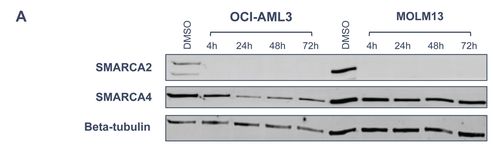
To determine whether PRT3789-induced SMARCA2 degradation inhibits AML cell proliferation by inducing differentiation

SMARCA2 and SMARCA4 Regulate Chromatin Accessibility and Gene Expression in AML cells



A schematic representation of the SWI/SNF complex highlights its role in the regulation of multiple signaling pathways related to AML cell proliferation and differentiation. The SWI/SNF complex binds to DNA and Histones, alters their structure and makes DNA accessible to multiple transcription machineries.

Figure 1. PRT3789 promotes SMARCA2 protein degradation in AML cells

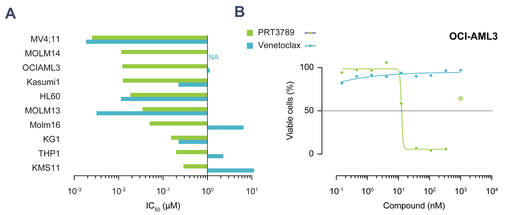


B

AML cell line	SMARCA2 DC50	SMARCA4 DC50	Selectivity
OCI-AML3	0.16 nM	3.0 nM	19-fold
MOLM13	0.11 nM	2.5 nM	23-fold

A) Western blot analysis shows SMARCA2 and SMACRA4 degradation in a time-course when treated with PRT3789. **B**) SMARCA2 and SMARCA4 DC_{50} and selectivity from the dose response curves are shown.

Figure 2. PRT3789 broadly inhibits proliferation of both Venetoclax sensitive and resistant AML cells *in Vitro*



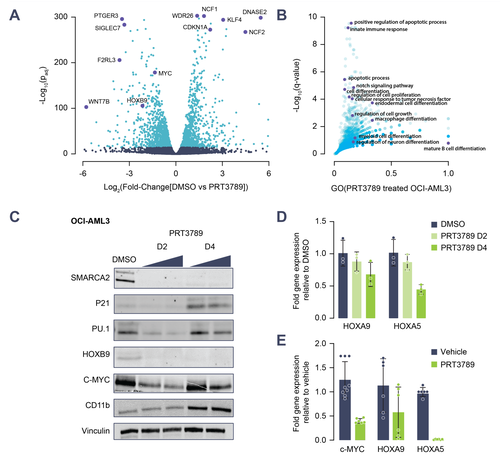
A) IC_{50} values of PRT3789 and Venetoclax tested in AML cell lines are shown. Each cell line was treated with PRT3789 for 6 days and 9-point dose-dependent curves were generated. IC_{50} values of Venetoclax (ABT-199) were extracted from cancerrxgene.org. **B**) The dose-response curves of PRT3789 and Venetoclax in OCI-AML3 cells are shown.

Table 1. Major driver gene alteration, PRT3789 absolute IC_{50} and max inhibition and Venetoclax sensitivity of AML cell lines tested

Cell lines	Driver mutations	PRT3789 IC_{50} (nM)	Max inhibition (%)	Venetoclax sensitivity
MV4;11	FLT3-ITD, KMT2A-AFF1 fusion	7	89	Sensitive
MOLM14	FLT3-ITD, TET2, NPM1, WT1	12	99	NA
OCI-AML3	FLT3-ITD, TET2, NPM1 Fs ins, NRAS Q61L, IDH2	13	94	Resistant
KASUMI1	TP53 R248Q, KIT N822K, RAD21 FS ins, SMARCA4 FS del, RUNX1-RUNX1T1 fusion	14	92	Sensitive
HL60	FLT3-ITD, NPM1, CEBPA, IDH1/2, DNMT3A, KIT	18	99	Sensitive
MOLM13	CNL ss, MLH1 Fs ins, MLLT3-KMT2A fusion	14	98	Sensitive
MOLM16	TP53 V173M/C238S, NF1 ss, EPCAM FS ins	263	52	Resistant
KG1	FLT3-ITD, NPM1, T53, NRAS G12D, KIT,	152	100	Sensitive
THP1	TP53 (P248R), FLT3-ITD, NRAS Q61L, NOTCH1	20	100	Resistant
KMS11	TP53, KRASG12V, NRAS Q61K, BRAF V600E	566	79	Resistant

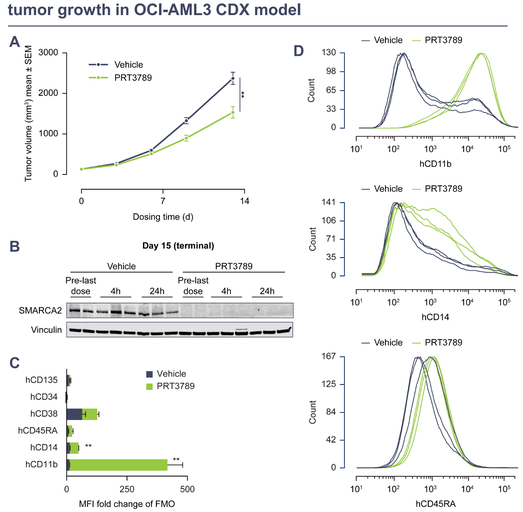
Table 1) Main driver gene alterations in AML cell lines tested for PRT3798 and Venetoclax sensitivity. The gene mutation data was analyzed in CCLE 2019 database.

Figure 3. PRT3789 treatment regulates the gene expression profile in OCI-AML3 cells



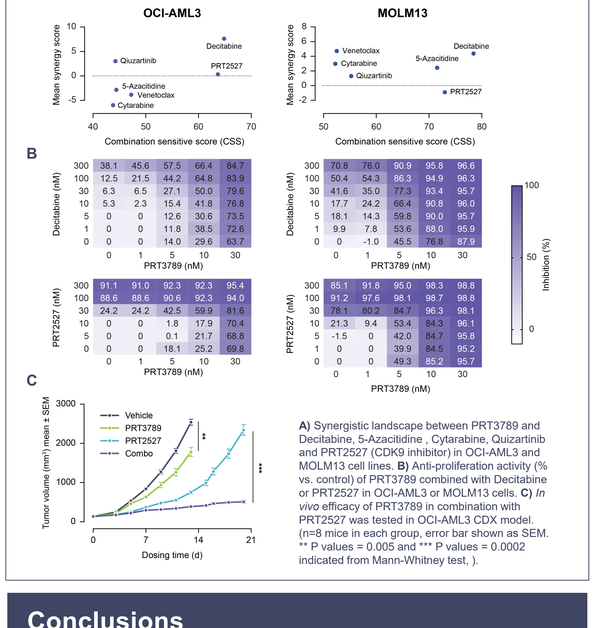
A) Volcano plot demonstrating an overview of the differential expression of genes in PRT3789 treated OCIAML3 cells. **B)** WebGestalt pathway analysis of gene expression profile data showing key enriched pathway gene sets post PRT3789 treatment. **C)** Immunoblot of total protein lysates of OCI-AML3 cells treated with PRT3789 for 2 days or 4 days. **D)** Bar graphs showing *HOXA9* and *HOXA5* expression (normalized with DMSO and *GAPDH*) from the total RNA extracted from OCI-AML3 cells post PRT3789 treatment **E)** Bar graphs showing a set of *HOXA9*, *HOXA5* and *c-Myc* gene expression from the total RNA extracted from the tumor tissue collected at the end of the *in vivo* efficacy study.

Figure 4. PRT3789 induces cell differentiation and suppresses tumor growth in OCI-AML3 CDX model



A) *In vivo* efficacy of PRT3789 was tested in OCI-AML3 CDX model. (n=8 mice in each group, error bar shown as SEM, **: a P value =0.005 indicated Mann-Whitney test) **B)** Immunoblot of SMARCA2 and SMARCA4 protein levels in tumor tissues collected at pre, 4 and 24 h post the last treatment from dose. **C,D)** Flow cytometry analysis of the tumor tissues collected. All tissues (vehicle and treated groups) were stained and analyzed for a panel of cell surface markers CD11b, CD14, CD45RA, CD38, and CD135 24 hours post the last dose. The fold change of mean fluorescent intensity (n=3 for each group, error bar shown as SEM) has significantly increased with P value of hCD11b =0.008 and hCD14=0.002).

Figure 5. PRT3789 with Decitabine or PRT2527 (Prelude CDK9 inhibitor) show synergistic inhibitory effects in AML models



A) Synergistic landscape between PRT3789 and Decitabine, 5-Azacitidine , Cytarabine, Quizartinib and PRT2527 (CDK9 inhibitor) in OCI-AML3 and MOLM13 cell lines. **B)** Anti-proliferation activity (% vs. control) of PRT3789 combined with Decitabine or PRT2527 in OCI-AML3 or MOLM13 cells. **C)** *In vivo* efficacy of PRT3789 in combination with PRT2527 was tested in OCI-AML3 CDX model. (n=8 mice in each group, error bar shown as SEM. ** P values = 0.005 and *** P values = 0.0002 indicated from Mann-Whitney test,).

Conclusions

- PRT3789, a potent and selective SMARCA2 degrader, shows good selectivity vs. SMARCA4 in AML cells *in vitro* and *in vivo*.
- PRT3789 inhibits AML cell proliferation in a broad panel of AML cell lines. PRT3789 also shows anti-proliferation activity in Venetoclax (a BCL-2 inhibitor) resistant AML cell lines.
- PRT3789 induces AML cell differentiation markers including CD11b, CD45RA, and CD14. The in-depth molecular mechanisms by which PRT3789 promotes AML cell differentiation requires further investigation.
- PRT3789 monotherapy suppresses tumor growth in the OCI-AML3 CDX model at a well-tolerated dose. When used in combination with PRT2527 (a CDK9 small molecule inhibitor), PRT3789 exhibits prolonged and robust tumor growth inhibition, compared to monotherapy in the OCI-AML3 CDX model.

References

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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.