Selective SMARCA2 Degradation Promotes Leukemic Differentiation and Synergizes with CDK9 Inhibition to Potently Induce Cancer Cell Death in Preclinical Models of Acute Myeloid Leukemia.

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Background

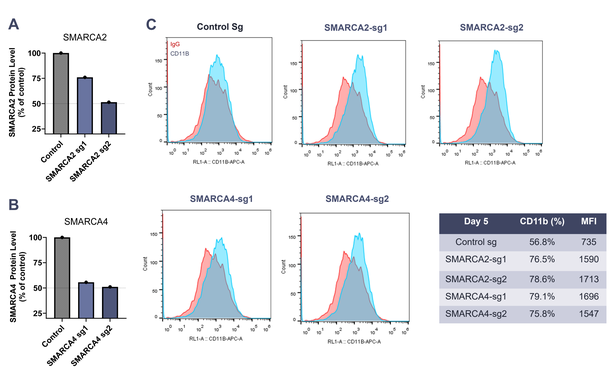
- SWI/SNF (BAF) nucleosome remodeling complexes play an important role in regulating gene expression by maintaining chromatin accessibility.[1]
- Mammalian SWI/SNF complexes contain two mutually exclusive, functionally redundant ATPase subunits, **SMARCA2** and **SMARCA4**.[1]
- Deregulated SWI/SNF activity has been linked to the pathogenesis of several malignancies including myeloid disorders like AML and MDS.[2]
- In AML/MDS, deregulated BAF activity has been associated with differentiation block, allowing the accumulation of immature leukemic stem cells in the myeloid compartment.[2]
- Small molecule inhibitors targeting both BAF ATPase subunits have shown promising clinical activity, inducing differentiation of leukemic stem cells in AML/MDS patients, with dose-limiting on-target toxicities.[3]
- In genetic ablation studies, dual loss of SMARCA2 and SMARCA4 has been associated with pronounced cytotoxicity in multiple cell types while selective ablation of SMARCA2 is tolerated due to functional compensation by SMARCA4.[4,5]
- In the present study, we sought to investigate PRT3789, a highly selective clinical-stage SMARCA2 degrader, as a differentiation agent in AML/MDS and evaluate potential PRT3789 combinations to potently suppress leukemic cell growth in preclinical models.

Key Findings

- Here we show that PRT3789 induces robust leukemic cell differentiation in various AML preclinical models and combines with CDK9 inhibitor PRT2527 to potently inhibit leukemic cell growth *in vivo*.

Results

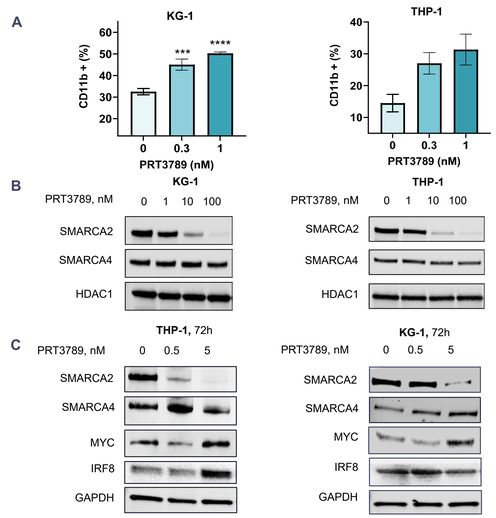
Figure 1. Selective genetic depletion of SMARCA2 or SMARCA4 is associated with increased differentiation of AML cell lines



Day 5	CD11b (%)	MFI
Control sg	56.8%	735
SMARCA2-sg1	76.5%	1590
SMARCA2-sg2	78.6%	1713
SMARCA4-sg1	79.1%	1696
SMARCA4-sg2	75.8%	1547

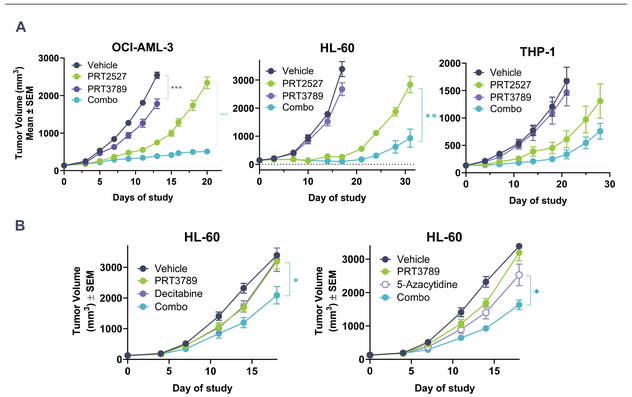
(A, B) Quantification of SMARCA2/4 protein levels as measured by western blot in THP-1 cells following Cas9-sgRNA-mediated genetic depletion of SMARCA2 or SMARCA4 **(C)**. Flow cytometry assessing surface expression of myeloid differentiation marker CD11b 5-days post depletion of SMARCA2 or SMARCA4.

Figure 2. Selective SMARCA2 degradation by PRT3789 induces differentiation in AML cell lines



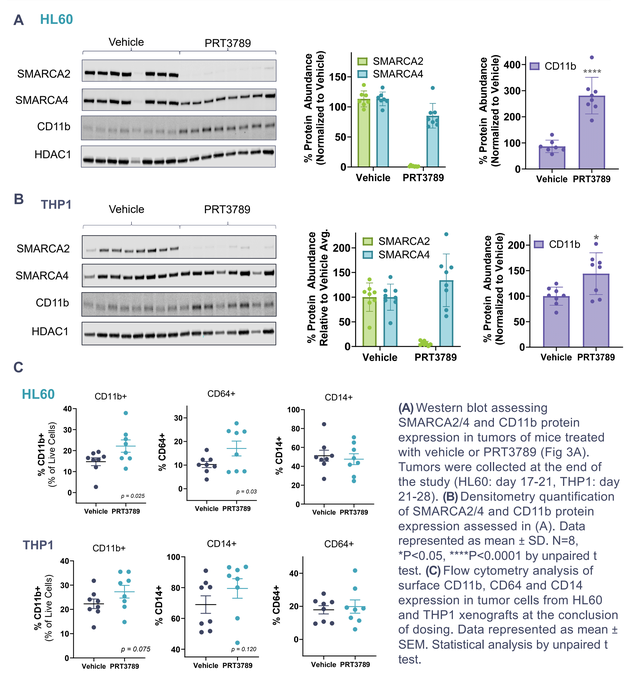
(A) Flow cytometry analysis of differentiation marker CD11b in AML cells following treatment with PRT3789 for 3-5 days. Data represented as mean ± SD. N=3, ***P<0.001, ****P<0.0001 by unpaired t test **(B)** Western blot confirming potent and selective degradation of SMARCA2 with no effect on SMARCA4 levels in AML cells following overnight treatment with PRT3789. **(C)** Western blot demonstrating induction of MYC and IRF8, two key transcriptional regulators of myeloid differentiation, following selective SMARCA2 degradation by PRT3789.

Figure 3. PRT3789 combines with CDK9 inhibition to potently inhibit AML growth *in vivo*



(A) PRT3789, administered subcutaneously (200 mg/kg, Q3D), combines with CDK9 inhibitor PRT2527, administered intravenously (15 mg/kg, QW, b.i.d.) to potently inhibit tumor growth in multiple AML cell line-derived xenograft (CDX) models, including regressions. Data represented as mean ± SEM. N=8, * P<0.05, **P<0.001, ***P<0.001 by Mann-Whitney U test. **(B)** PRT3789, administered, subcutaneously combines with hypomethylating agents to potently inhibit tumor growth in AML xenografts. Decitabine and 5-azacytadine were dosed as follows—Decitabine, 0.4 mg/kg, *i.p.* qd 4on/10off; 5-azacytadine, 5 mg/kg, *i.v.* Q3D. Data represented as mean ± SEM. N=8, * P<0.05, **P<0.001, ***P<0.001 by Mann-Whitney U test. All treatments were well tolerated.

Figure 4. PRT3789 selectively degrades SMARCA2 *in vivo* and induces expression of differentiation markers in AML xenografts



(A) Western blot assessing SMARCA2/4 and CD11b protein expression in tumors of mice treated with vehicle or PRT3789 (Fig 3A). Tumors were collected at the end of the study (HL60: day 17-21, THP1: day 21-28). **(B)** Densitometry quantification of SMARCA2/4 and CD11b protein expression assessed in (A). Data represented as mean ± SD. N=8, *P<0.05, ****P<0.0001 by unpaired t test. **(C)** Flow cytometry analysis of surface CD11b, CD64 and CD14 expression in tumor cells from HL60 and THP1 xenografts at the conclusion of dosing. Data represented as mean ± SEM. Statistical analysis by unpaired t test.

Figure 5. PRT3789 induces blast differentiation in primary AML patient samples *ex vivo* and inhibits clonogenic growth

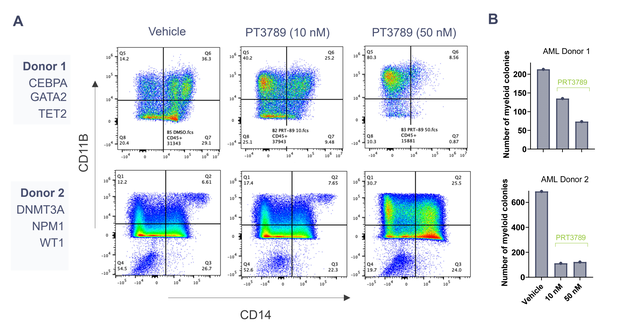
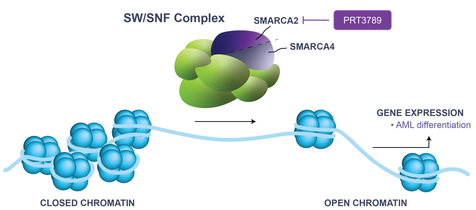


Figure 5. Expression of myeloid differentiation markers CD11b and CD14, in primary AML samples following treatment with PRT3789. Bone marrow mononuclear cells from AML/MDS patients were cultured in a methylcellulose colony forming assays and treated with varying doses of PRT3789 or vehicle. Clonogenic growth was assessed at the end of the study (B) and colonies assessed for expression of myeloid cell markers by flow cytometry (A).

Summary

1. Selective degradation of SMARCA2 by PRT3789 induces differentiation in AML cells *in vitro* and *in vivo*.
   - Selective degradation of SMARCA2 induces expression of MYC and IRF8, two key modulators of myeloid cell differentiation.
2. PRT3789 combines with CDK9 inhibitor PRT2527 to potently inhibit AML tumor growth in vivo.
   - Increased MYC expression may sensitize AML cells to CDK9 inhibition.
   - PRT3789 and PRT2527 combinations were well tolerated with no bodyweight loss.
3. PRT3789 induces CD11b expression on AML patient leukemic blasts and potently represses ex vivo clonogenic growth.
4. Taken together these findings highlight the promise of PRT3789 as a differentiation agent in myeloid malignancies, and in combination with PRT2527 for the treatment of relapsed/refractor AML/MDS.
   - PRT2527 is currently being evaluated in Phase I clinical trials for relapsed/refractory hematologic malignancies (NCT05665530).
   - PRT3789 is currently being evaluated in Phase I clinical trials for advanced or metastatic solid tumors with SMARCA4 mutations (NCT05639751)

Selective SMARCA2 degradation by PRT3789 promotes AML differentiation



References

1. Centore, R.C., 2020. Trends in Genetics, 36(12), pp.936-950.
2. Andrades, A., 2023. Molecular cancer, 22(1), p.39.
3. DiNardo, C.D., 2023. Blood, 142, p.4284.
4. Maher, J., 2023. Toxicology and Applied Pharmacology, 475, p.116627.
5. Hoffman, G.R., 2014. PNAS, 111(8), pp.3128-3133.

Acknowledgements

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