PRMT5 inhibition downregulates MYB and NOTCH1 signaling, key molecular drivers of Adenoid Cystic Carcinoma

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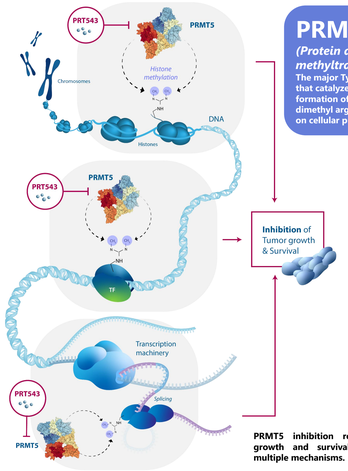
INTRODUCTION



PRMT5
(Protein arginine methyltransferase 5)
The major Type II PRMT that catalyzes the formation of symmetric dimethyl arginine (sDMA) on cellular proteins

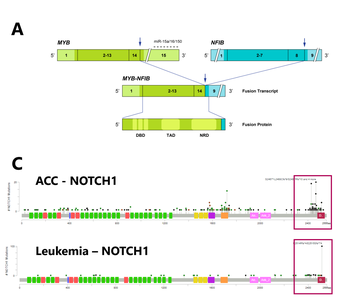
PRMT5 inhibition regulates cancer growth and survival pathways by multiple mechanisms.

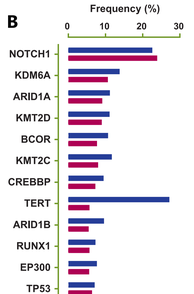
- Adenoid cystic carcinoma (ACC) is a rare cancer of secretory glands, characterized by slow and unpredictable growth, unrelenting relapse and high rates of metastasis. Poor response to chemotherapy and targeted drugs has resulted in there being no approved therapies to date, which contributes to poor prognosis with less than a 20% 5-year survival rate in patients with high-risk ACC.
- A vast majority of ACC tumors are known to have recurrent chromosomal translocation t(6;9) or t(8;9) resulting in *MYB/MYBL1-NFIB* fusions, or high levels of MYB protein expression.
- A subset of ACC tumors expresses *NOTCH1* active mutations (20-25%) and hyperactive NOTCH signaling in both primary and recurrent/metastatic tumors.
- A previous report demonstrated that an inhibitor of PRMT5 (protein arginine methyltransferase) shows favorable responsiveness in patients with advanced ACC in a phase I clinical trial (METEOR-1: A phase I study of GSK3326595).
- Here, we investigate mechanistic aspects as to how PRMT5 inhibition potentially regulates the unique molecular drivers of ACC tumor growth.

RESULTS

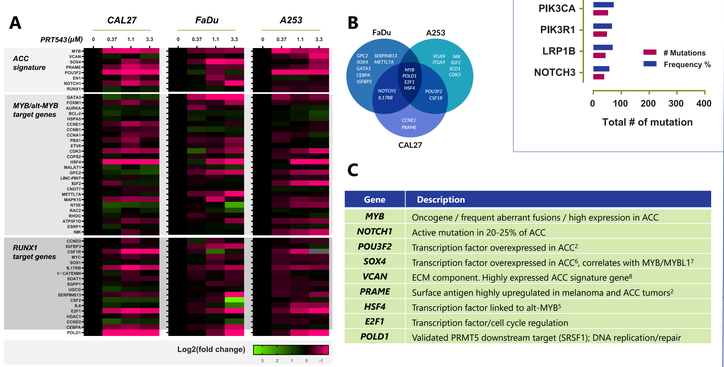
ACC EXPRESSES UNIQUE MOLECULAR DRIVERS

Figure 1. (A) A diagram showing t(6;9) chromosomal translocation and MYB-NFIB fusion transcript frequently expressed in ACC.
(B) Common mutated genes found in ACC analyzed with 1045 ACC patient sample dataset (extracted from cBioPortal).
(C) *NOTCH1* mutation expressed in patient ACC and CLL (cBioPortal). Red box indicates frameshift mutations known as a *NOTCH1* hotspot gain-of-function (GOF) mutations.





PRT543 DOWNREGULATES ACC GENE SIGNATURE, INCLUDING MYB AND NOTCH1



C

Gene	Description
MYB	Oncogene / frequent aberrant fusions / high expression in ACC
NOTCH1	Active mutation in 20-25% of ACC
POU3F2	Transcription factor overexpressed in ACC[2]
SOX4	Transcription factor overexpressed in ACC[6], correlates with MYB/MYBL1[7]
VCAN	ECM component. Highly expressed ACC signature gene[8]
PRAME	Surface antigen highly upregulated in melanoma and ACC tumors[2]
HSF4	Transcription factor linked to alt-MYB[5]
E2F1	Transcription factor/cell cycle regulation
POLD1	Validated PRMT5 downstream target (SRSF1); DNA replication/repair

Figure 2. (A) PRT543 regulates expression of ACC signature, MYB and altered-MYB target genes and RUNX1 target genes in A253, Fadu and CAL27 HNSCC cells analyzed by quantitative RT-PCR. Data are shown in heatmaps (Log2FC). **(B)** Venn-diagram indicates several downregulated genes (>2-fold) are overlapped within three HNSCC cell lines. **(C)** Key ACC related genes.

PRT543 DOWNREGULATES EXPRESSION OF MYB TARGET GENES AND ALTERNATIVELY SPLICED MYB (9A/10A) IN CANCER CELLS

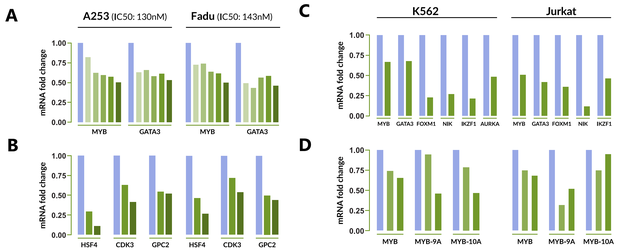


Figure 3. PRT543 3-day treatment downregulates expression of **(A)** *MYB* and MYB target *GATA3* and **(B)** Alt-MYB (TSS2, uniquely found in ACC) target genes *HSF4, CDK3* and *GPC2* in A253 and Fadu cells detected by quantitative RT-PCR. PRT543 IC_{50} values (10-day in vitro assay) are indicated. PRT543 downregulates expression of **(C)** MYB target genes and **(D)** *MYB* alternative transcripts *MYB-9A* and *MYB-10A* (c-term truncated like *MYB* fusion transcript frequently expressed in ACC) in K562 and Jurkat cells detected by quantitative RT-PCR.

PRT543 DOWNREGULATES NOTCH1 PATHWAY GENES IN NOTCH1-ACTIVE CANCER CELLS

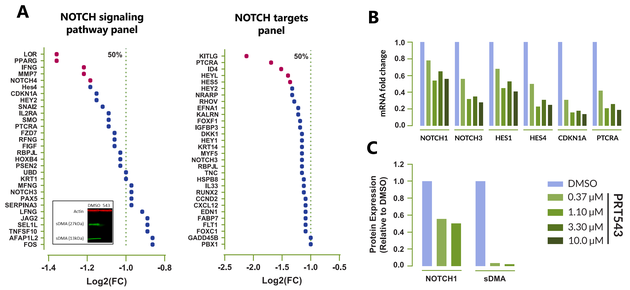


Figure 4. (A) PRT543 4-day treatment downregulates expression of NOTCH signaling pathway and NOTCH target genes in NOTCH1-active HPB.ALL T-cell ALL cells analyzed by targeted RT-PCR array (RT2 Profiler). Decreased sDMA in PRT543 treated cells was detected by western blot. **(B)** PRT543 4-day treatment decreases expression of selected NOTCH pathway genes in HPB.ALL cells validated by quantitative RT-PCR. **(C)** PRT543 decreases NOTCH1 protein expression in HPB.ALL cells detected by western blot.

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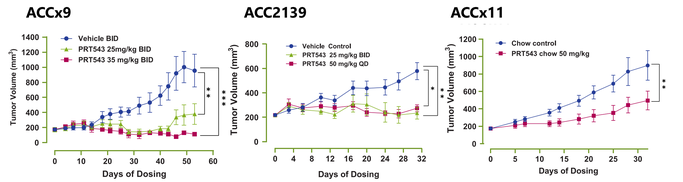
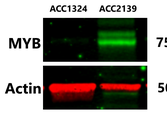
PRT543 INHIBITS GROWTH OF MYB-FUSION/NOTCH1 ACTIVE ACC PDX IN VIVO



B

	ACCx9	ACC2139	ACCx11
MYB fusion	*MYB-NFIB*	*MYB-NFIB*	*MYB-NFIB*
NOTCH1	I1680N (GOF) ICN1 high (IHC) High *NOTCH3* mRNA	E2154Pfs* (GOF) High *NOTCH3* mRNA	Tandem dup 3′ ICN1 high (IHC) High *NOTCH3* mRNA
TGI	>100% (p<0.001)	95% (p<0.001)	56% (p<0.01)



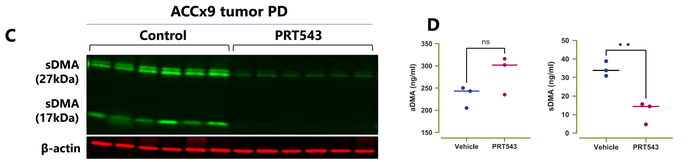


Figure 5. (A) Oral administration of PRT543 or PRT543-containing chow demonstrates significant anti-tumor activity in ACCx9, ACC2139 and ACCx11. Data represent mean ± SEM. * P < 0.05, ** P < 0.01, *** P < 0.005, **** P< 0.001 vs. vehicle by Mann-Whitney U test. **(B)** Summary table for ACC PDX models, including *MYB* and *NOTCH1* status, intracellular cleaved NOTCH1 (ICN1) status and tumor growth inhibition (TGI, %). Western blot shows MYB overexpression in MYB-NFIB expressing ACC (ACC2139), compared to WT MYB expressing ACC (MYB1324). **(C)** ACCx9 tumor tissues harvested from PRT543 treated mice show decreased sDMA analyzed by western blot. **(D)** PRT543 decreases sDMA, but not aDMA, in plasma collected from ACC2139 PDX tumors measured by mass spectrometry.

CONCLUSIONS

- **PRT543 regulates ACC gene signature including MYB and NOTCH1 in cancer cell lines.**
- **PRT543 regulates expression of MYB, altered MYB and MYB downstream genes in leukemia cell lines that express high MYB.**
- **PRT543 downregulates key NOTCH signaling and target genes in NOTCH1 active leukemia cells.**
- **PRT543 oral administration demonstrates significant inhibitory activity on a subset of ACC PDX growth in vivo.**
- **Ongoing studies aim to identify potential biomarkers for PRT543 sensitive subsets of ACC.**

PRT543 is a potent and selective PRMT5 inhibitor currently under evaluation in a Phase I clinical trial in patients with advanced solid tumors (HRD, ACC and spliceosome mutation expressing tumors) and hematologic malignancies (NCT03886831).