PRMT5 inhibition regulates alternative splicing and DNA damage repair pathways in SF3B1 R625C/G expressing uveal melanoma cells

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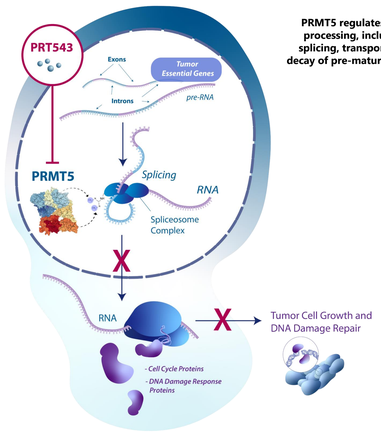
INTRODUCTION

PRMT5 (Protein arginine methyltransferase 5)

- PRMT5 is a predominant Type II PRMT that catalyzes symmetric dimethylation of protein arginine residues (sDMA) and regulates multiple essential biological processes to promote cancer growth.
- Previous studies have shown that PRMT5 is a critical molecule for pre-mRNA processing, including RNA splicing.
- Mechanistically, PRMT5 directly methylates arginine residues of several splicing factors such as Small nuclear ribonucleoprotein (SNRPB and SNRPD3) and Serine and arginine rich splicing factor 1 (SRSF1), which contributes to spliceosome assembly and promotes canonical splicing of many essential genes in cancer cells.
- In the present study, we examined the effects of PRT543, a potent and selective PRMT5 inhibitor, on alternative splicing in uveal melanoma which frequently express hotspot mutations on one of spliceosome subunits, *Splicing factor 3b subunit 1 (SF3B1)*.



PRMT5 regulates RNA processing, including splicing, transport, and decay of pre-mature mRNA.

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).

RESULTS

PRT543 INHIBITS GROWTH OF SF3B1 R625C/G EXPRESSING UVEAL MELANOMA

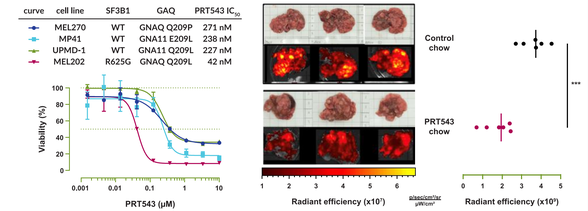


Figure 1. (A) PRT543 inhibits cell viability of uveal melanoma cell lines expressing WT SF3B1 (MEL270, MP41, UPMD1) and R625G hotspot mutation (MEL202) in a concentration-dependent manner. Following 10-day incubation in vitro, the cell viability was measured by ATPlite. A summary table shows PRT543 IC_{50} (nM) and *SF3B1/GAQ* mutation status of each cell line. **(B)** UM001-td-Tomato cells (SF3B1 R625C) were injected into spleens of NSG mice. After 10-week treatment with PRT543-containing chow, IVIS ex vivo imaging detected the reduced liver metastatic growth of UM001 in the PRT543 treated mice (***P < 0.001 vs. Control chow by t test).

PRT543 DOWNREGULATES SPLICEOSOME TARGET GENES AND DNA DAMAGE RESPONSE GENES IN UVEAL MELANOMA CELLS

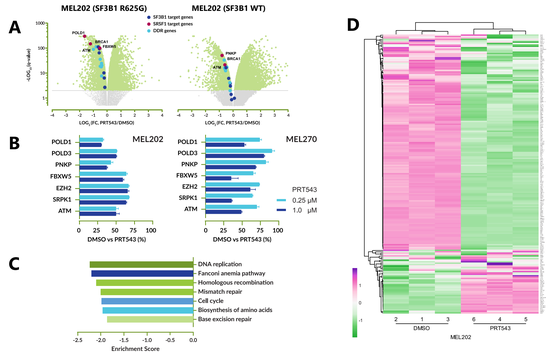


Figure 2. **(A)** Volcano plots of global RNA expression data (Illumine Hi-Seq) with PRT543 treated MEL202 and MEL270 uveal melanoma cells. SF3B1 target genes (H3B-8800 targets), SRSF1 target genes and DNA damage response (DDR) genes are highlighted as indicated. **(B)** PRT543 decreases transcript levels of indicated key genes (percentage vs. DMSO control) in MEL202 and MEL270 cells. **(C)** Unbiased gene set enrichment analysis demonstrates that PRT543 significantly downregulates the multiple DDR/DNA replication pathways (FDR<0.05) in MEL202 cells. **(D)** Cluster analysis of HRD gene signature shows PRT543 treatment increases HRD signature in MEL202 cells.

PRT543 PROMOTES ALTERNATIVE SPLICING IN UVEAL MELANOMA CELLS

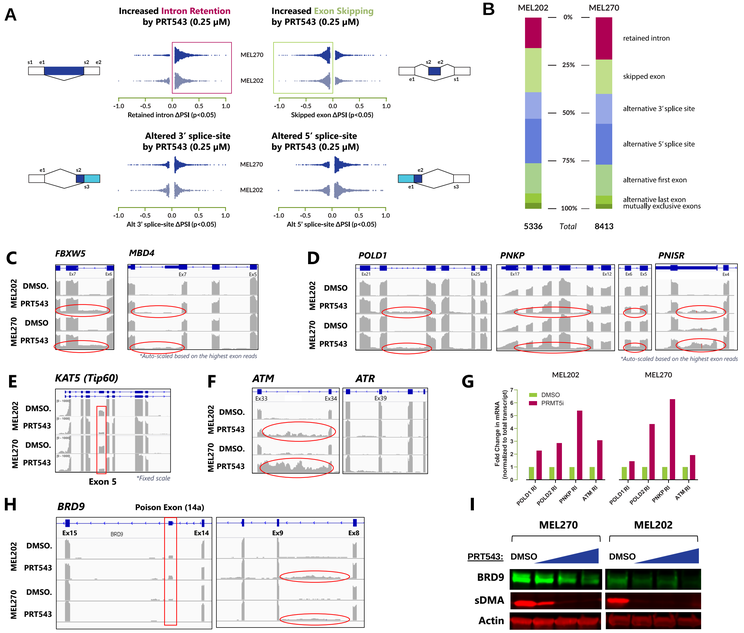


Figure 3. (A) PRT543 promotes alternative splicing events in MEL202 and MEL270 cells analyzed by RNA-seq data (100M per sample, Illumine Hi-Seq). ΔPSI (percentage of splice-in) was calculated by SUPPA2 (genes with $p<0.05$ were plotted). **(B)** Percentage of alternatively spliced genes (ΔPSI $p<0.05$) in PRT543 treated MEL202 and MEL270 cells. PRT543 increases **(C)** intron retention of *FBXW5* and *MBD4* (SF3B1 target genes), **(D)** intron retention of *POLD1*, *PNKP*, and *PNISR* (SRSF1 target genes), **(E)** exon skipping (exon 5) of *KAT5* (*Tip60*) and **(F)** intron retention of *ATM* and *ATR* in both MEL202 and MEL270 cells. **(G)** Quantitative RT-PCR validated increased intron retention of SRSF1 target genes and *ATM* in PRT543-related PRMT5 inhibitor C220 treated MEL202 and MEL270 cells. **(H)** PRT543 decreased BRD9 poison exon (14a) in MEL270 cells, but not in MEL202 cells. PRT543 increased BRD9 intron retention (ex8-ex9) and **(I)** decreased BRD9 protein levels in both MEL202 and MEL270 cells.

PRT543 DECREASES DDR RELATED PROTEIN LEVELS IN UVEAL MELANOMA CELLS

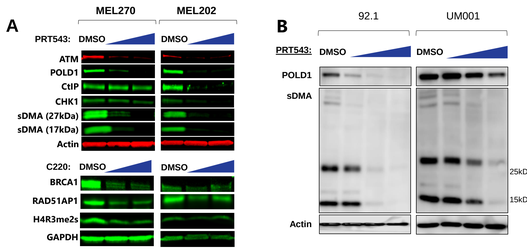
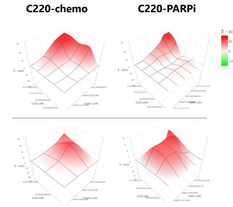


Figure 4. Western blot analysis demonstrates the decreased DDR/DNA replication protein levels, particularly ATM, POLD1 and BRCA1, in PRT543 or related PRMT5 inhibitor C220 treated **(A)** MEL270, MEL202 and **(B)** 92.1 and UM001 cells. Reduced levels of H4R3me2s, a marker for DDR gene regulation, are also confirmed in C220 treated MEL270 and MEL202 cells.

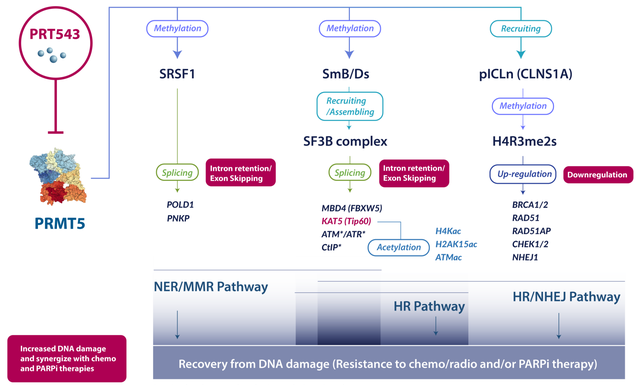
PRMT5 INHIBITION SYNERGIZES WITH CHEMOTHERAPY AND PARP INHIBITION IN UVEAL MELANOMA *IN VITRO*



	Combination	Synergy.score	Most.syn.score
MEL270	chemo - C220	16	21
	PARPi – C220	3.9	12
MEL202	chemo - C220	8.3	11
	PARPi - C220	2.8	7.7

Figure 5. Combination of C220 and chemotherapy (DNA alkylating agent) or PARP inhibitor demonstrates synergistic anti-proliferative effects in MEL270 or MEL202 cells. Following 10-day incubation, cell viability was measured by ATPlite. Synergy scores were calculated using SynergyFinder (https://synergyfinder.fimm.fi/). Average of two experiments were shown in a summary table.

A POTENTIAL MODEL FOR PRT543 MECHANISM OF ACTION



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CONCLUSIONS

- PRT543 shows more potent anti-proliferative activity in SF3B1 R625C/G uveal melanoma cells than SF3B1 WT cells.
- PRT543 downregulates expression of spliceosome target genes and DDR/DNA replication responsible genes by promoting alternative splicing and downregulating H4R3me2s (also described in **poster #1185**).
- PRT543 shows synergistic effects with DNA damaging chemotherapy drugs and PARP inhibitors in both SF3B1 WT and R625C/G expressing uveal melanoma cells.
- On going studies investigate PRT543 *in vivo* activities and PARPi/chemotherapy combination effects on growth of uveal melanoma using PDX models (both SF3B1 WT and SF3B1 R625C mutation).