PRMT5 inhibition epigenetically regulates DNA repair pathways in cancer cells and sensitizes to chemotherapy and PARP inhibition

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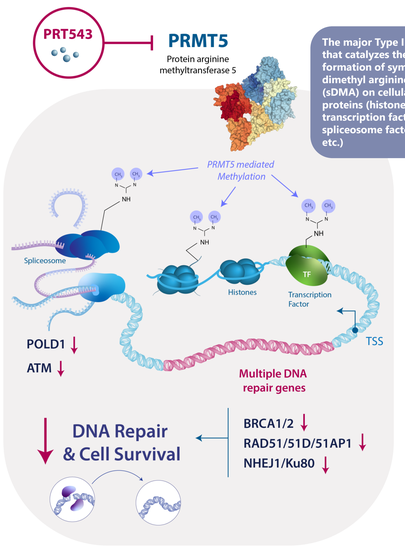
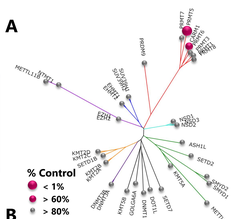
Poster #: 1185

INTRODUCTION



- Genetic defects of DNA damage repair (DDR) pathways in cancer cells induces synthetic lethality with DNA damaging chemotherapy and PARP inhibitors.
- Resistance to these therapies can occur due to circumvention and/or restoration of the inactivated DDR genes.
- PRMT5 promotes expression of DNA damage repair and DNA replication responsible genes by several mechanisms including RNA processing and epigenetic regulation.
- We show that pharmacological inhibition of PRMT5 with our potent and selective small molecule inhibitor PRT543 downregulates multiple genes involved in the DDR/DNA replication pathways, resulting in the sensitization of cancer cells to chemotherapy drugs and PARP inhibitors.

PRT543 - A POTENT AND SELECTIVE PRMT5 INHIBITOR



Intro Figure: (A) Biochemical selectivity analysis of PRT543 against 37 human methyltransferases. Percent control represents remaining enzymatic activity in the presence of 10 µM PRT543 relative to DMSO control. **(B)** PRT543 shows anti-proliferative activity in a broad range of cancer cells following 10 days of treatment. The log IC_{50} values (µM) are shown. *[Bhagwat N. et al. Caner Res 2020;80 (16 Suppl): Abstract nr2915]*

RESULTS

PRMT5 TRANSCRIPT LEVELS POSITIVELY CORRELATE TO MULTIPLE DDR GENE EXPRESSION LEVELS IN CANCER CELL LINES

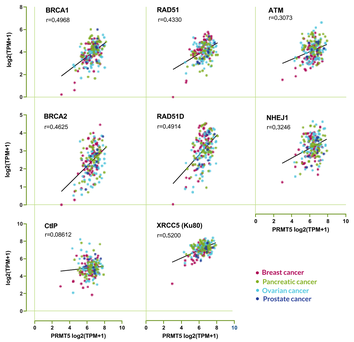


Figure 1. Correlation of PRMT5 and DDR related gene expression in cancer cell lines (breast, ovarian, pancreatic and prostate cancer) was analyzed by using data extracted from the CCLE (total n=188). The RNA-seq TPM data were transformed to Log2, using a pseudo-count of 1. Pearson's correlation coefficient (r) values are shown in each graph.

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).

PRMT5 INHIBITION PROMOTES ALTERNATIVE SPLICING AND DOWNREGULATES EPIGENETIC MARKS, ASSOCIATED WITH REDUCED DDR PATHWAY GENE SIGNATURE

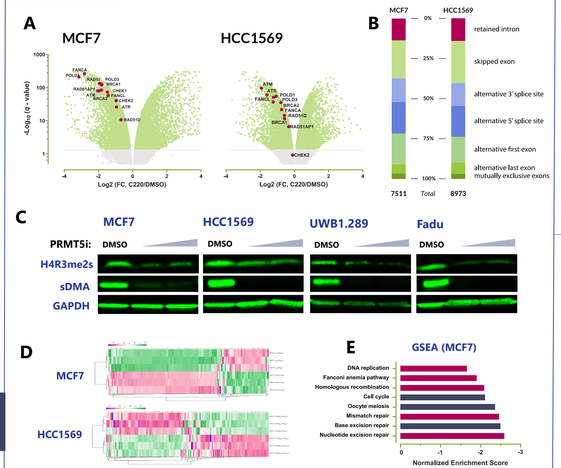


Figure 2. (A) Global gene expression changes in PRT543-related PRMT5 inhibitor (C220) treated MCF7 (HR-proficient) and HCC1569 (HR-deficient) breast cancer cells are shown in volcano plots. Key DDR genes are highlighted in red. **(B)** Percentage of alternatively spliced genes (ΔPSI p<0.05) in C220 treated MCF7 or HCC1569 cells. **(C)** Western blot analysis shows that C220 reduces sDMA and H4R3me2s, global PRMT5 and PRMT5-pICln complex substrate, respectively. **(D)** Cluster analysis of HRD gene signature (*Sun et al. 2018*) using C220 treated MCF7 and HCC1569 expression data. **(E)** Unbiased gene set enrichment analysis (pathway, KEGG) demonstrates that C220 downregulates the DDR/DNA replication related gene signatures (highlighted in red).

PRT543 DOWNREGULATES EXPRESSION OF DDR GENES IN HR-PROFICIENT AND HR-DEFICIENT BREAST AND OVARIAN CANCER CELL LINES

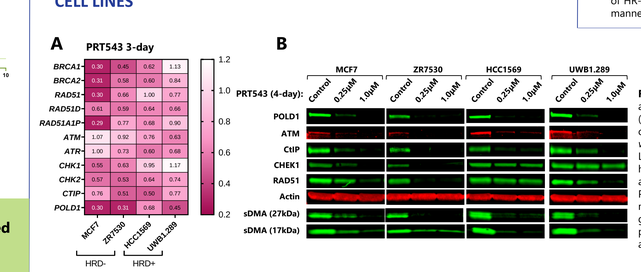


Figure 3. HR-proficient (MCF7 and ZR7530) and HR-deficient (HCC1569 and UWB1.289) cancer cells were treated with PRT543 *in vitro*. **(A)** Quantitative RT-PCR Log2 (FC, vs. DMSO) shown in a heatmap and **(B)** western blot analysis demonstrates that PRT543 reduces expression of multiple DDR/DNA replication genes and proteins in both HR-proficient and HR-deficient breast and ovarian cancer cells.

PRT543 PROMOTES DNA DAMAGE IN BREAST AND OVARIAN CANCER CELLS

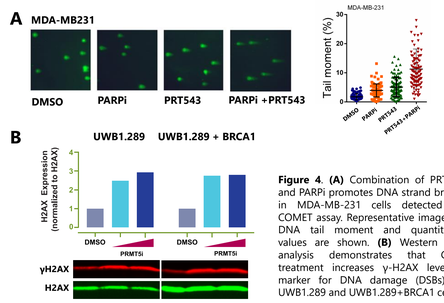


Figure 4. (A) Combination of PRT543 and PARPi promotes DNA strand breaks in MDA-MB-231 cells detected by COMET assay. Representative images of DNA tail moment and quantitated values are shown. **(B)** Western blot analysis demonstrates that C220 treatment increases γ-H2AX level, a marker for DNA damage (DSBs), in UWB1.289 and UWB1.289+BRCA1 cells.

PRT543 SYNERGIZES WITH CHEMOTHERAPY AND PARP INHIBITOR IN VITRO

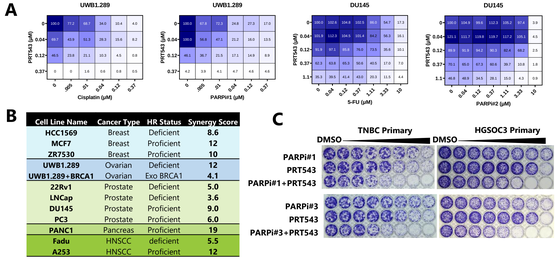


Figure 6. (A) Combination of PRT543 and chemotherapeutic agent or PARP inhibitor demonstrates synergistic anti-proliferative effects in UWB1.289 and DU145 cancer cells, following 10-day incubation. Heatmaps indicate percentage of viable cells vs. DMSO control. **(B)** Synergy scores of PRT543 and PARPi#1 in various cell lines calculated in SynergyFinder. **(C)** Combination of PRT543 and PARP inhibitors demonstrates synergistic anti-proliferative effects in primary culture of triple negative breast cancer and high-grade serous ovarian cancer cells.

PRT543 INHIBITS PROLIFERATION OF HR-DEFICIENT CANCER CELLS

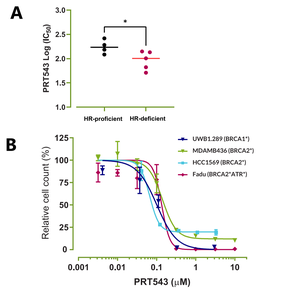


Figure 5. (A) PRT543 preferably inhibits proliferation of HR-deficient cancer cells vs. HR-proficient cancer cells demonstrated by a comparison of *in vitro* IC_{50} values (*P < 0.05 vs. DMSO by *t* test). **(B)** PRT543 inhibits proliferation of HR-deficient cancer cells in a concentration-dependent manner *in vitro*, following 10-day incubation.

PRT543 SYNERGIZES WITH PARP INHIBITOR IN HR-DEFICIENT CANCER XENOGRAFT MODEL IN VIVO

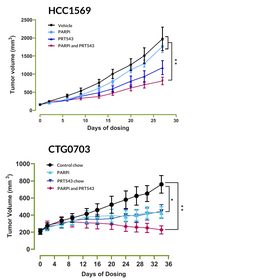


Figure 7. Oral administration of PRT543 and PARP inhibitor suppresses growth of **(A)** HRD+ breast caner CDX (HCC1569) and **(B)** HRD+ ovarian caner PDX at well-tolerated doses. Data represent ± SEM. * $P < 0.05$, ** $P < 0.01$ vs. vehicle by Mann-Whitney U test.

PRT543 SUPPRESSES GROWTH OF PARP INHIBITOR-RESISTANT CANCER CELLS

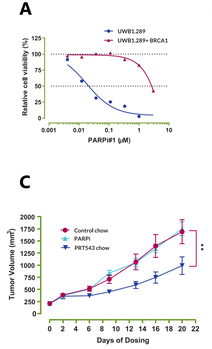


Figure 8. (A) Overexpression of BRCA1 renders UWB1.289 cells resistant to PARPi, while remaining responsive to PRT543 **(B)** *in vitro* in a dose-dependent manner, following 10-day incubation. **(C)** Oral administration of PRT543 suppresses growth of HR-deficient (*BRCA1/2*-del) breast PDX tumor that is insensitive to PARPi treatment. Data represent ± SEM. ** $P < 0.01$ vs. vehicle by Mann-Whitney U test.

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CONCLUSIONS

- **PRT543 downregulates a DDR/DNA replication gene signature and protein expression *in vitro* by regulating RNA splicing (also see Poster#1138 for details) and H4R3me2s, associated with increased DNA damage in cancer cells.**
- **PRT543 preferably inhibits proliferation of HR-deficient cancer cells.**
- **Combination of PRT543 and chemotherapy drugs or PARP inhibitors show synergistic effects in both HR-proficient and HR-deficient cancer cells.**