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PRMT5 Inhibitor PRT543 Displays Potent Antitumor Activity in U2AF1^{S34F} and RBM10^{LOF} Spliceosome-Mutant Non-Small Cell Lung Cancer *In Vitro* and *In Vivo*

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Disclosure Information

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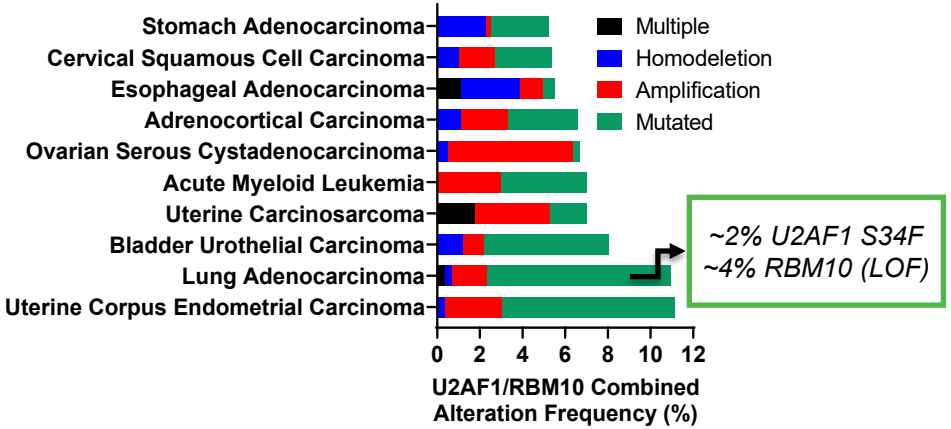
Jack Carter

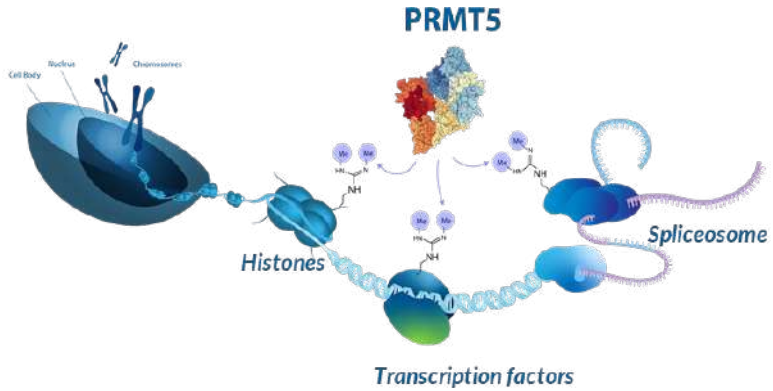
I have the following relevant financial relationships to disclose:

- Employee of: Prelude Therapeutics, Inc.
- Stockholder in: Prelude Therapeutics, Inc.

This study was funded by Prelude Therapeutics, Inc.

Background



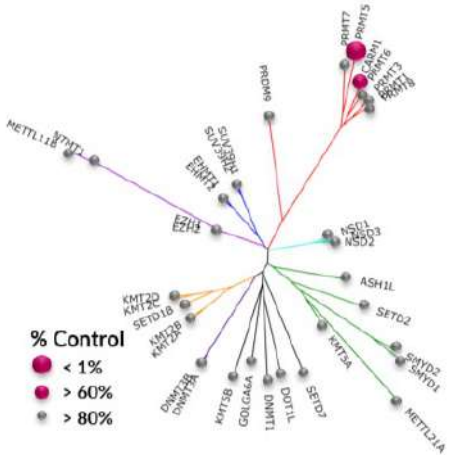


- PRMT5 catalyzes the formation of symmetric dimethyl arginine (sDMA) on protein substrates[1]
- PRMT5 is a multifaceted regulator of tumor growth via epigenetic, transcriptional, and splicing regulation[1]
- Recurrent mutations in splicing factors (e.g., *SF3B1, SRSF2, U2AF1*) are potential biomarkers for PRMT5 in leukemias[2]
- *U2AF1* and *RBM10* are important splicing regulators[3,4] with recurrent hotspot and damaging mutations in solid tumors, including ~10% combined alteration in lung cancer[5,6]

PRMT5, protein arginine methyltransferase 5; *U2AF1*, U2 small nuclear RNA auxiliary factor 1; *RBM10*, RNA binding motif protein 10.

1. Chen Y, et al. *Biomed Pharmacother*. 2021;144:112252. 2. Fong JY, et al. *Cancer Cell*. 2019;36:194-209.e9; 3. Esfahani M, et al. *Nature Comm*. 2019;10:5712. 4. Cao Y, et al. *Front Oncol*. 2021;11:603932. 5. Mutation data sourced from *The Cancer Genome Atlas*. Available at https://www.cancer.gov/tcga (accessed March 11, 2022); and 6. Cerami E, et al. *Cancer Discovery*. 2012;2:401-404.

Objective



Biochemical selectivity of PRT543 against 37 methyltransferases[1]

Assay	PRT543 IC_{50} (nM)
Biochemical (PRMT5/MEP50)	10.0
Cell proliferation	44.5
sDMA in-cell Western blot	46.0

The objective of this preclinical study was to evaluate the *in vitro* and *in vivo* efficacy of PRT543 in non-small cell lung cancer harboring recurrent hotspot or damaging mutations in *U2AF1* or *RBM10* and explore potential mechanisms of action.

MEP50, methylosome protein 50.

1. Bhagwat N, et al. Presented at AACR 2020, April 24-29, 2020, Virtual. Abstract 2915.

PRT543 Regulated Global Alternative Splicing in *U2AF1 S34F* and *RBM10-fs* Mutant NSCLC *In Vitro*

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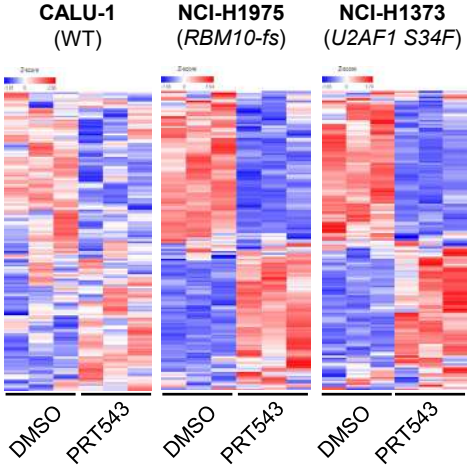

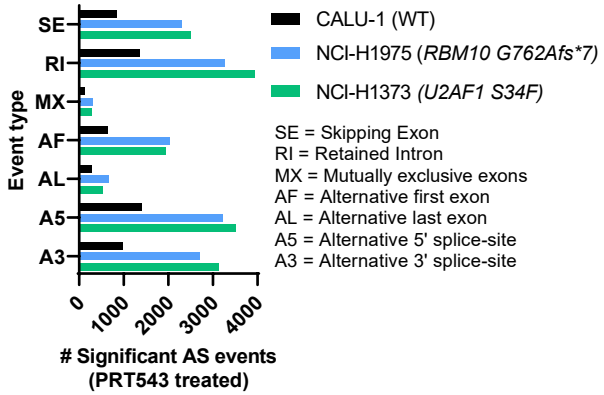

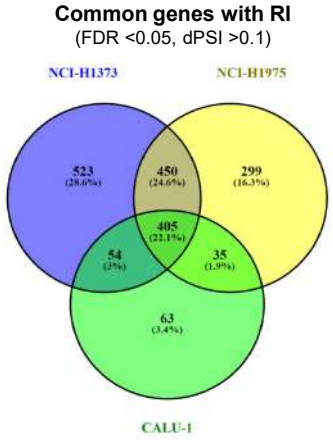

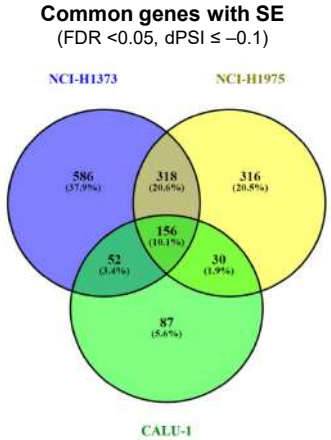

- PRT543 had a greater effect on global gene expression in cell lines with *U2AF1 S34F* or *RBM10* damaging mutations
- PRT543 preferentially induced global AS (e.g., retained introns, skipped exons) in splicing-mutant cell lines
- Overlap of AS events also point to a conserved set of genes dependent on PRMT5 function in NSCLC

n=3.
AS, alternative splicing; dPSI, delta percent-spliced-in

PRT543 Induced Intron Retention of DNA Repair Genes in Splicing-Mutant NSCLC *In Vitro*

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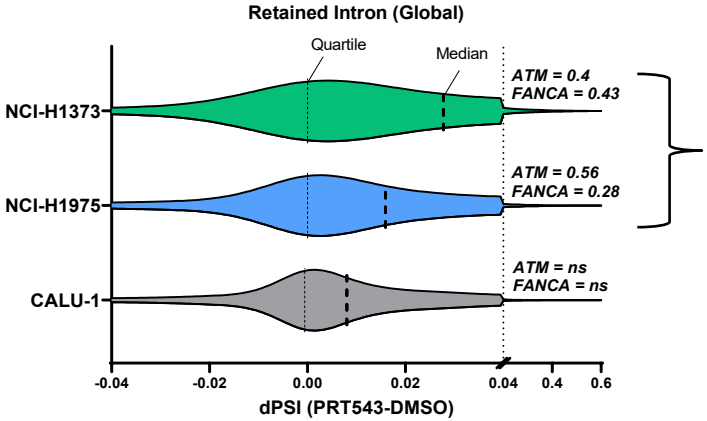
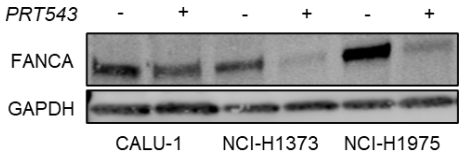
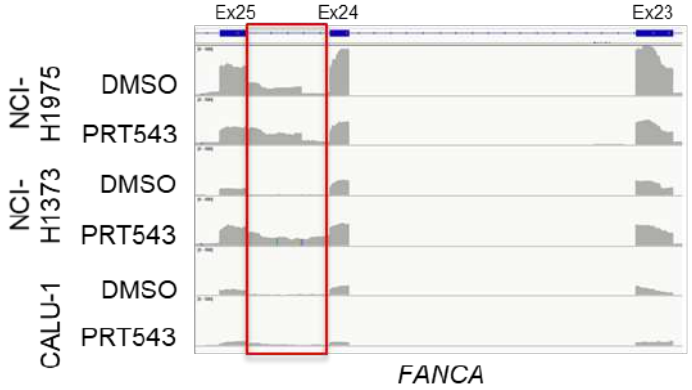


KEGG Pathway (Top 5)

Homologous recombination
Replication and repair
Genetic information processing
AMPK signaling
Fanconi anemia pathway



- DNA replication and repair pathways are enriched across common genes with PRT543-induced intron retention in splicing-mutant cells
- PRT543 preferentially induced intron retention and decreased protein expression of FANCA in splicing-mutant cells



FANCA, Fanconi anemia complementation group A.
FANCA FDR <0.05. KEGG pathway analysis (common genes from H1373/H1975 with RI events FDR <0.05, dPSI >0.1, with KEGG score P<0.005, >5 altered genes).

PRT543 Selectively Inhibited Growth of NSCLC With *U2AF1 S34F* or *RBM10* Mutations *In Vitro*

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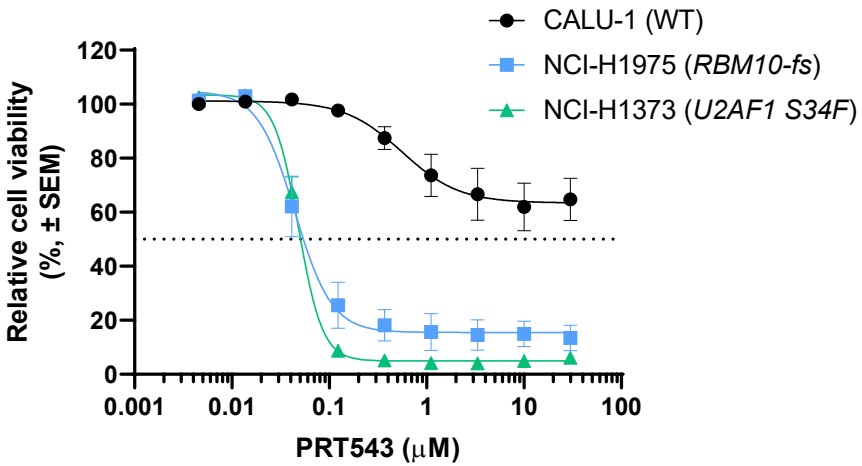

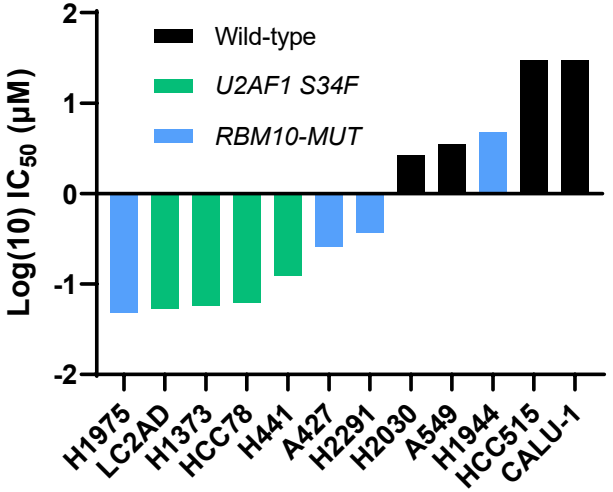

- PRT543 potently and selectively inhibited growth of *U2AF1 S34F* and *RBM10*-mutant NSCLC cell lines *in vitro*

n ≥2

PRT543 Significantly Inhibited Tumor Growth in *U2AF1 S34F*-Mutant NSCLC *In Vivo*

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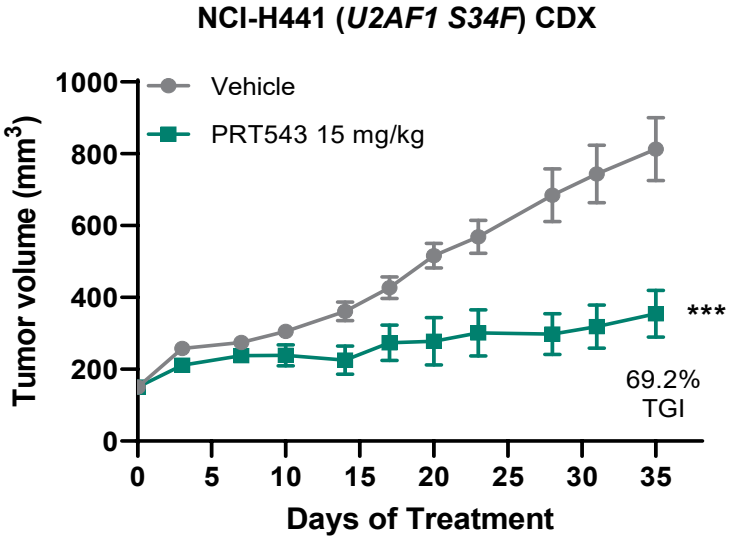

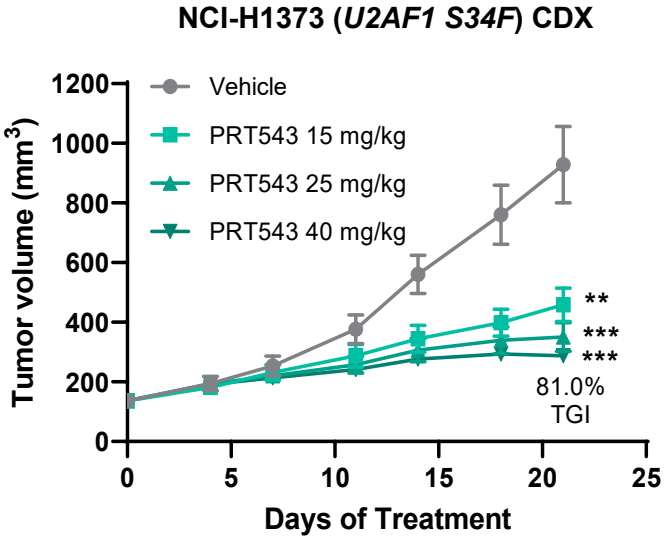

- PRT543 induced significant tumor growth inhibition in NSCLC CDX models harboring *U2AF1 S34F* hotspot mutations, at well-tolerated doses

PRT543 given orally as chow. Data represent mean ± SEM; n=8/group; **P<0.01, ***P<0.001 versus vehicle.

PRT543 Significantly Inhibited Tumor Growth in *RBM10*-Mutant NSCLC *In Vivo*

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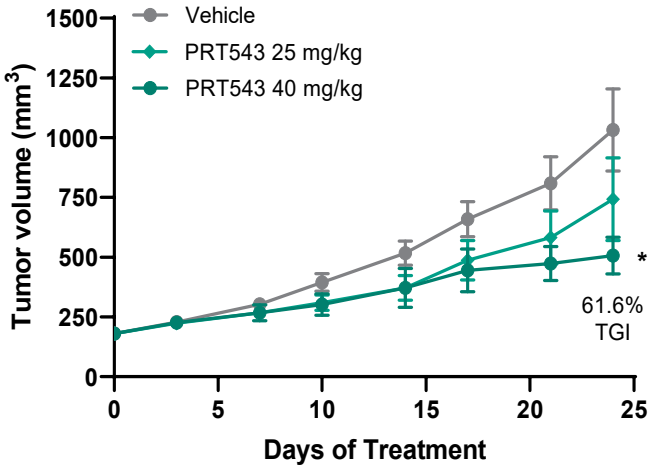
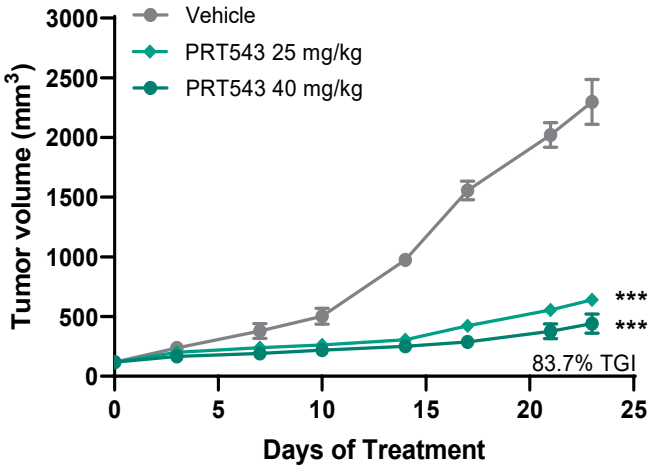
A427 (*RBM10 missense*) CDX



NCI-H1975 (*RBM10 G762Afs*7*) CDX



- PRT543 induced significant tumor growth inhibition in NSCLC CDX models with *RBM10* mutations, at well-tolerated doses, and with increased sensitivity in the NCI-H1975 model containing a damaging mutation

PRT543 given orally as chow. Data represent mean ± SEM; n=8/group; *P<0.05, ***P<0.001 versus vehicle.

PRMT5 Inhibitors Improved the *In Vitro* and *In Vivo* Efficacy of Docetaxel in *RBM10*-Mutant Cells

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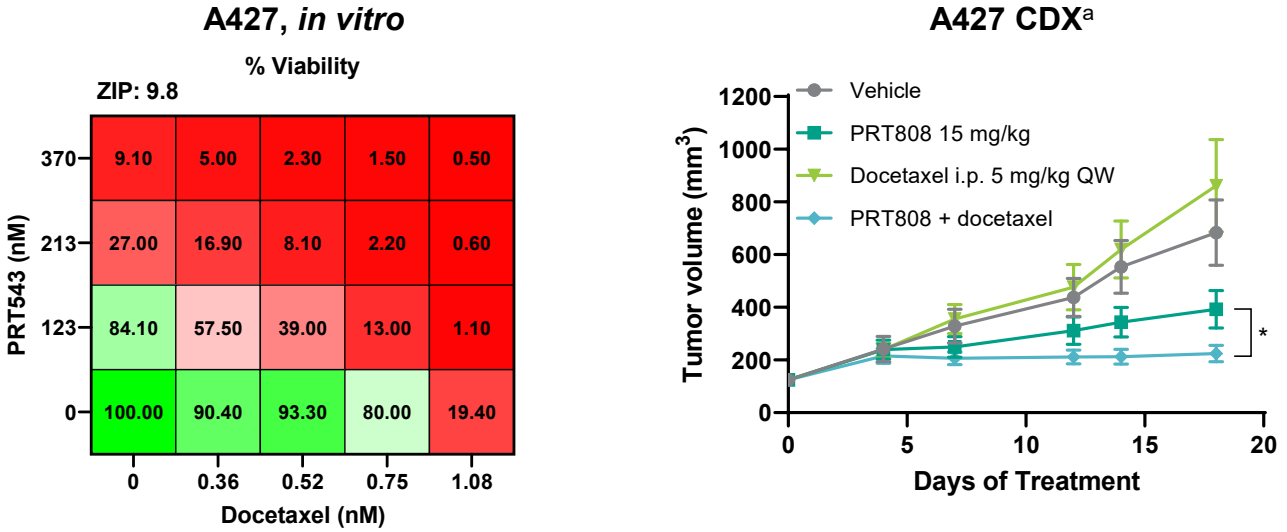

- PRT543 improved the *in vitro* efficacy of docetaxel in *RBM10* mutant lung cancer cells
- PRMT5 inhibitor, PRT808, improved the *in vivo* efficacy of docetaxel in a *RBM10* mutant NSCLC CDX model, at well-tolerated doses

[a]PRT808 given orally as chow. Data represent mean ± SEM; n=8/group; *P<0.05.

PRT543 Improved the *In Vivo* Efficacy of Cisplatin in NSCLC with *RBM10* Damaging Mutation

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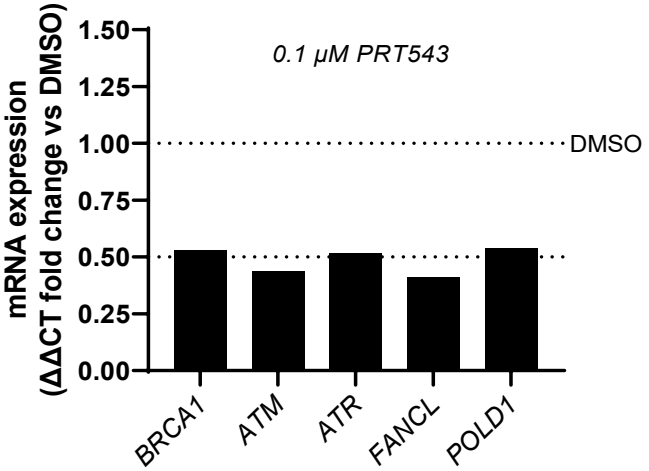
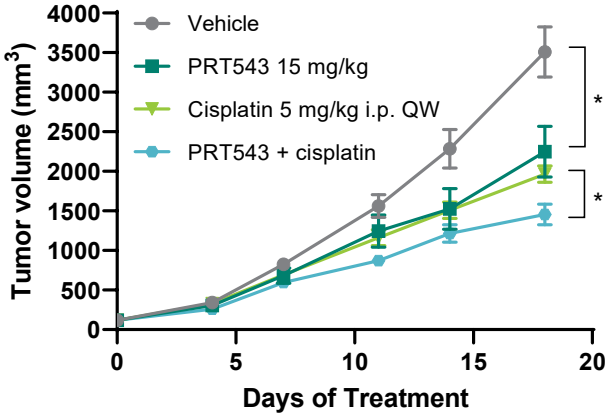
NCI-H1975, *in vitro*



NCI-H1975 CDX[a]



- PRT543 downregulated the expression of genes associated with DNA damage repair
- PRT543 improved the *in vivo* efficacy of cisplatin in a NSCLC CDX model harboring a *RBM10* damaging mutation, at well-tolerated doses

[a]PRT543 given orally as chow. Data represent mean ± SEM; n=8/group; *P<0.05.

Conclusions

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- PRT543 selectively inhibited the growth of lung cancer models with *U2AF1 S34F* or *RBM10* mutations *in vitro* and *in vivo*
- *U2AF1 S34F* and *RBM10* mutations are potential biomarkers for PRMT5 inhibitors in lung cancer
- PRT543 preferentially regulated global AS in lung cancer cell lines harboring *U2AF1 S34F* and *RBM10* damaging mutations
- PRT543 commonly induced intron retention within DNA replication and repair pathways, including *ATM* and *FANCA,* in splicing-mutant cell lines
- PRMT5 inhibitors improved the efficacy of standard-of-care agents in splicing-mutant lung cancer models *in vitro* and *in vivo*

Acknowledgments

- *In vivo* studies were run with the assistance of CrownBio
- Genomics studies were run with the assistance of GeneWiz
- Editorial assistance was provided by Russell Craddock, PhD, of Parexel International, Uxbridge, UK and was funded by Prelude Therapeutics, Inc.