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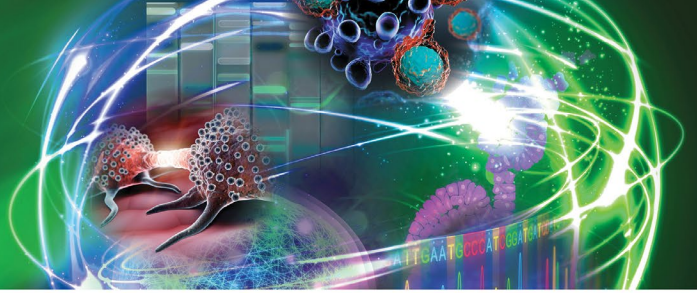


Fragment-based discovery of MRTX9768, a synthetic lethal-based inhibitor designed to bind the PRMT5•MTA complex and selectively target *MTAP*^{DEL} tumors

Matthew A. Marx, Senior Vice President, Drug Discovery
Mirati Therapeutics, San Diego, CA

Abstract LB003

Disclosure Information

Matthew Marx

I have the following financial relationships to disclose:

Stockholder in: Mirati Therapeutics
Employee of: Mirati Therapeutics

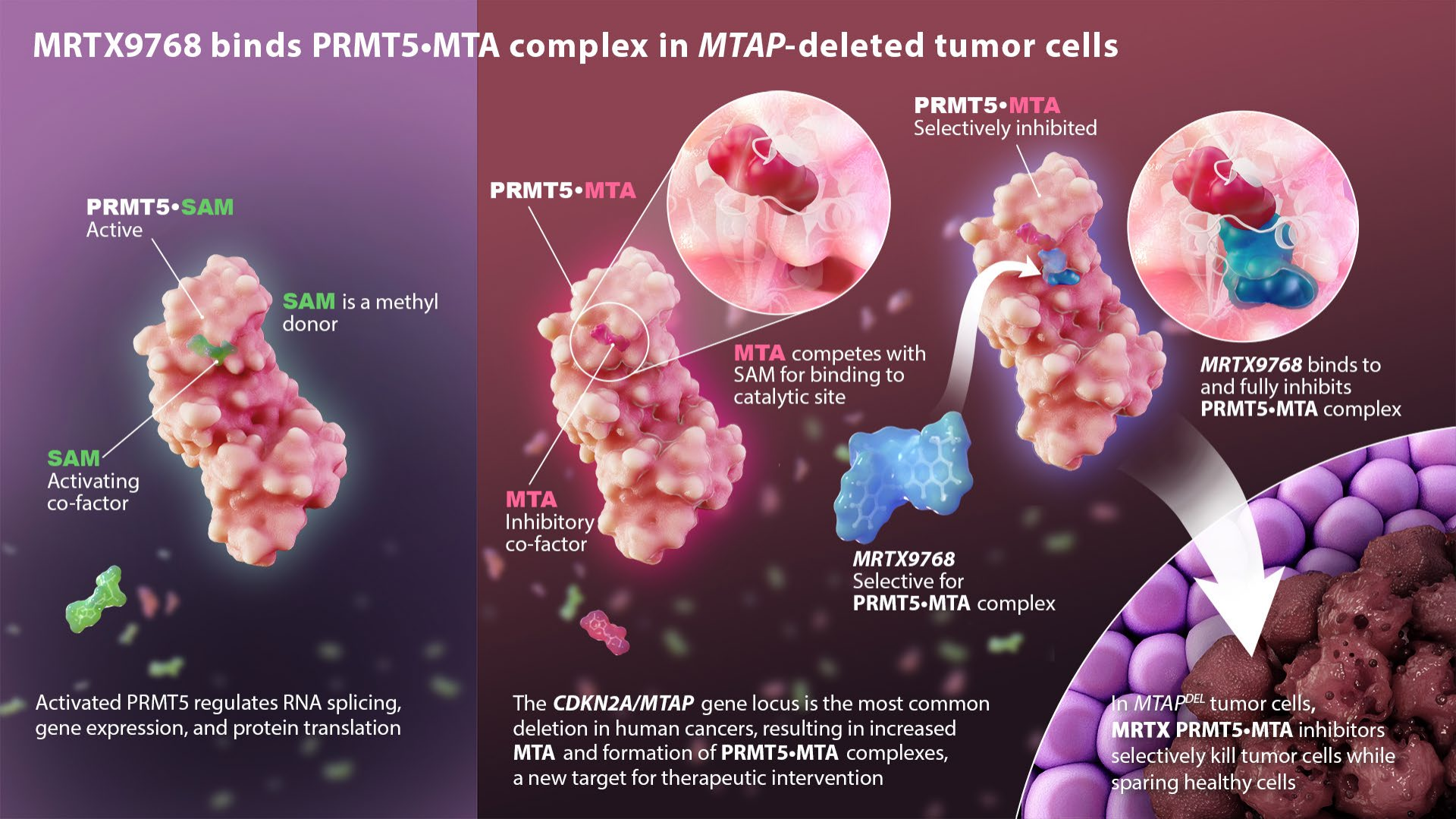
I will not discuss off label use and/or investigational use in my presentation.

First-in-class Approach Targeting the PRMT5•MTA Complex

- PRMT5 was the top hit in large scale functional genomics screens that demonstrated shRNA-mediated PRMT5 inhibition selectively inhibited *MTAP*^{DEL} cancer cell line viability[1]
- *MTAP* is proximal to and co-deleted with *CDKN2A*, the most commonly deleted gene in human cancer; *MTAP* deletion increases cellular concentrations of its substrate, MTA
- MTA binds to and partially inhibits PRMT5, creating a novel, *MTAP*^{DEL} cancer cell-specific target, the PRMT5•MTA complex
- Current clinical PRMT5 inhibitors do not bind PRMT5•MTA and do not exhibit selectivity for *MTAP*^{DEL} cancers, resulting in "head to toe" inhibition of PRMT5 and the possibility of a low therapeutic index[2]
- MAT2A was also identified as a synthetic lethal target for *MTAP*^{DEL} cancers as an indirect approach to inhibit PRMT5 by depleting its substrate, SAM; Early clinical data suggests maximal MAT2A inhibition only leads to partial PRMT5 inhibition in tumors[3]
- MRTX9768 is a small molecule proof of concept for selective inhibition of the PRMT5•MTA complex in *MTAP*^{DEL} cancer cells with prospects for an improved therapeutic index based on the concept of synthetic lethality

[1] Mavrakis, K, Science, 2016; Kryukov, G, Science, 2016; Marjon, Cell Reports, 2016; [2] Barbash L (GSK), AACR, 2017; JNJ – Brehmer D (Janssen), AACR, 2017;
[3] Heist, R, AACR, 2019 poster (investor.agios.com) - 65-74% plasma SAM decrease → ~37% tumor SDMA inhibition.

MRTX9768 binds PRMT5•MTA complex in *MTAP*-deleted tumor cells



Identification of the First Known Selective Binder of the PRMT5•MTA Complex

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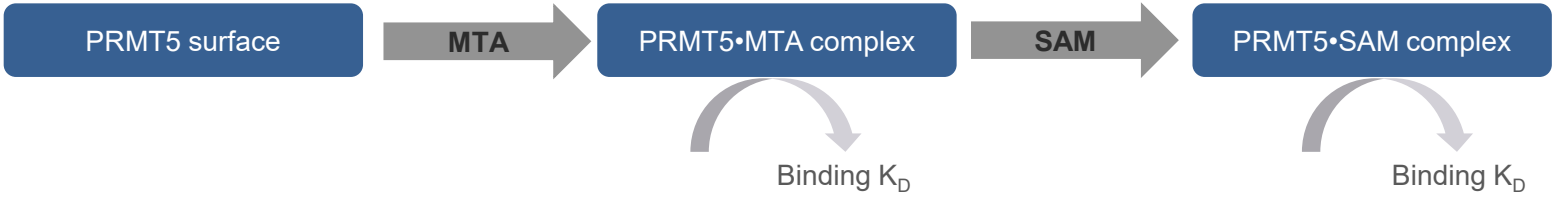
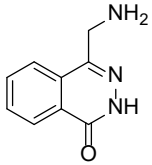
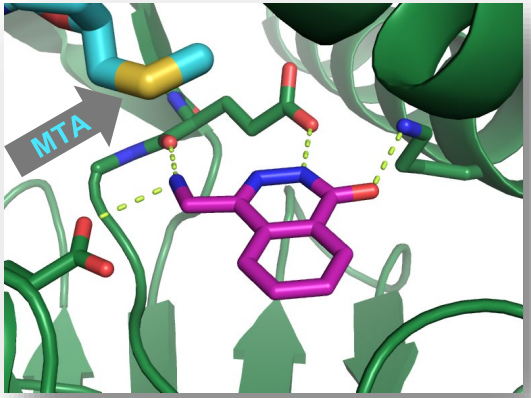
- Fragment library screened by SPR for binding to PRMT5•MTA and PRMT5•SAM complexes

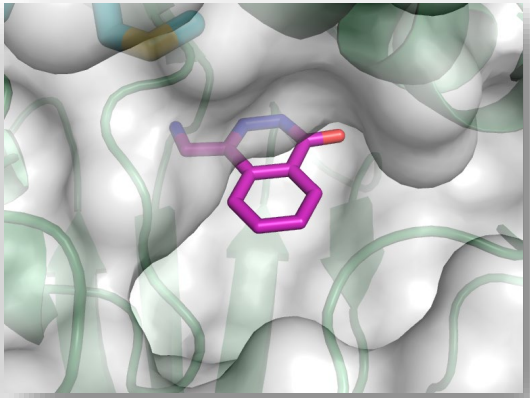


- Mirati fragment hit – First x-ray structure of ligand bound to the PRMT5•MTA complex

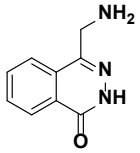


Complex	Binding K_D (μM)
PRMT5•MTA	10.2
PRMT5•SAM	50.7

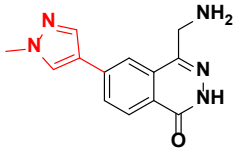




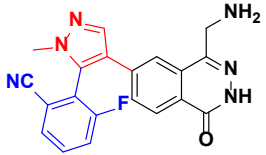
PRMT5•MTA Inhibitor Optimization to MRTX9768



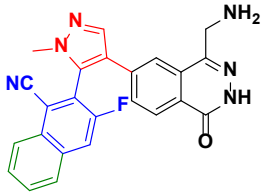
Compound 1
PRMT5/MTA
Binding K_D 10.2 μM



Compound 2
Binding K_D 57 nM



Compound 3
*$MTAP^{DEL}$ Cell Prolif IC_{50} = 761 nM



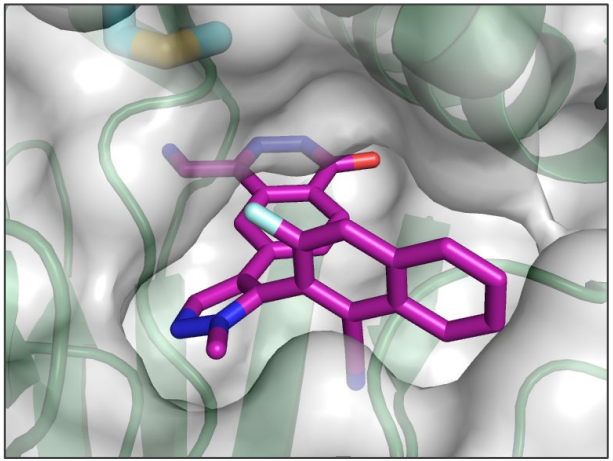
MRTX9768
*$MTAP^{DEL}$ Cell Prolif IC_{50} = 10 nM

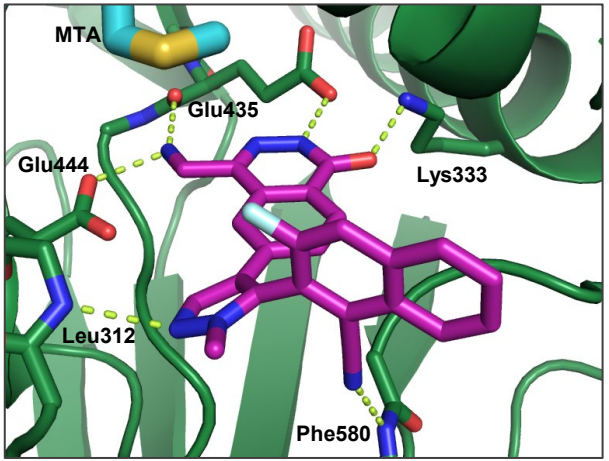
- A co-crystal structure of the fragment hit in PRMT5 confirmed MTA binding and suggested vectors for further elaboration
- Addition of methylpyrazole increased binding to the PRMT5•MTA complex by making a key hydrogen bond to Leu312 backbone N-H
- Addition of cyanofluorophenyl increased potency through an additional interaction with Phe580 backbone N-H and demonstrated selective inhibition of viability in *$MTAP^{DEL}$* cells
- Further optimization resulted in improvement in antiproliferative activity and high bioavailability in rodent and non-rodent species

***$MTAP^{DEL}$* Cell Prolif assay: 10 Day Cell Titer Glo assay in CRISPR/Cas9-engineered *$MTAP^{DEL}$* HCT116 (CRC) cells**

MRTX9768 has a Favorable ADME Profile and Binds the PRMT5•MTA Complex

ADME/PK property		MRTX9768
In vitro	PPB Fu (H | C | D | M)	0.14 | 0.10 | 0.07 | 0.13
	CYP Inhibition	CYP3A4 IC_{50} 6.2 µM
	CYP TDI	acceptable
	CYP induction	acceptable
	Hep Eh (H | C | D | M)	0.50 | 0.50 | 0.69 | 0.68
In vivo	Cl Eh (C | D | M)	0.55 | 0.67 | 0.86
	%F (C | D | M)	28 | 56 | 54





>50% bioavailability in mice and dogs

Moderate to high clearance

Co-crystal structure with PRMT•5MTA

Key polar interactions with protein driving optimization of compound binding and potency

*** PO dose 30 mg/kg in CD-1 mouse and beagle dog, 10 mg/kg in cynomolgus monkey**

MRTX9768: First-in-class Selective Inhibitor of the PRMT5·MTA Complex

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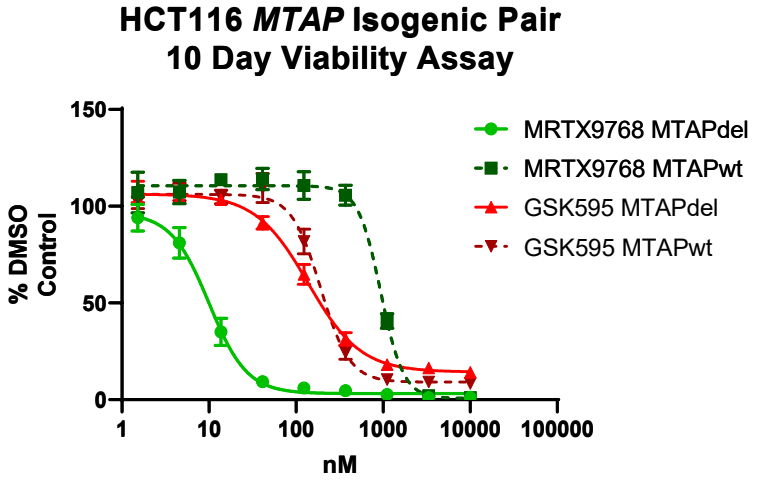
Assay	MRTX9768
PRMT5•MTA *MTAP*^{DEL} SDMA cell activity	3 nM
Selectivity over *MTAP*^{WT} cells (SDMA)	188-fold
PRMT5•MTA *MTAP*^{DEL} cell proliferation	10 nM
Selectivity over *MTAP*^{WT} cells (proliferation)	104-fold



- MRTX9768 binds selectively to the PRMT5•MTA complex with a very slow off rate (K_D determination ongoing)
  - Tight binding leads to prolonged PD effects in vivo
- MRTX9768 demonstrates selective inhibition of SDMA marks and cellular viability in *MTAP*-deleted tumor cells
- A representative clinical compound is not selective for *MTAP*-deleted cells. These findings indicate differential inhibition of PRMT5 in *MTAP*^{wt} (normal) and *MTAP*-deleted (tumor) cells by these two classes of agents and suggest the potential for increased therapeutic index compared with first generation PRMT5 inhibitors

Cell activity and proliferation assays: 96 hr SDMA In-Cell Western and 10 Day Cell Titer Glo assays, respectively, in *MTAP*^{DEL} (PRMT5•MTA) and *MTAP*^{WT} HCT116 (CRC) cells

In Vitro Cell Viability Screen Demonstrates Selective Sensitivity of Cell Lines Harboring *MTAP*^{DEL} to PRMT5•MTA Inhibition

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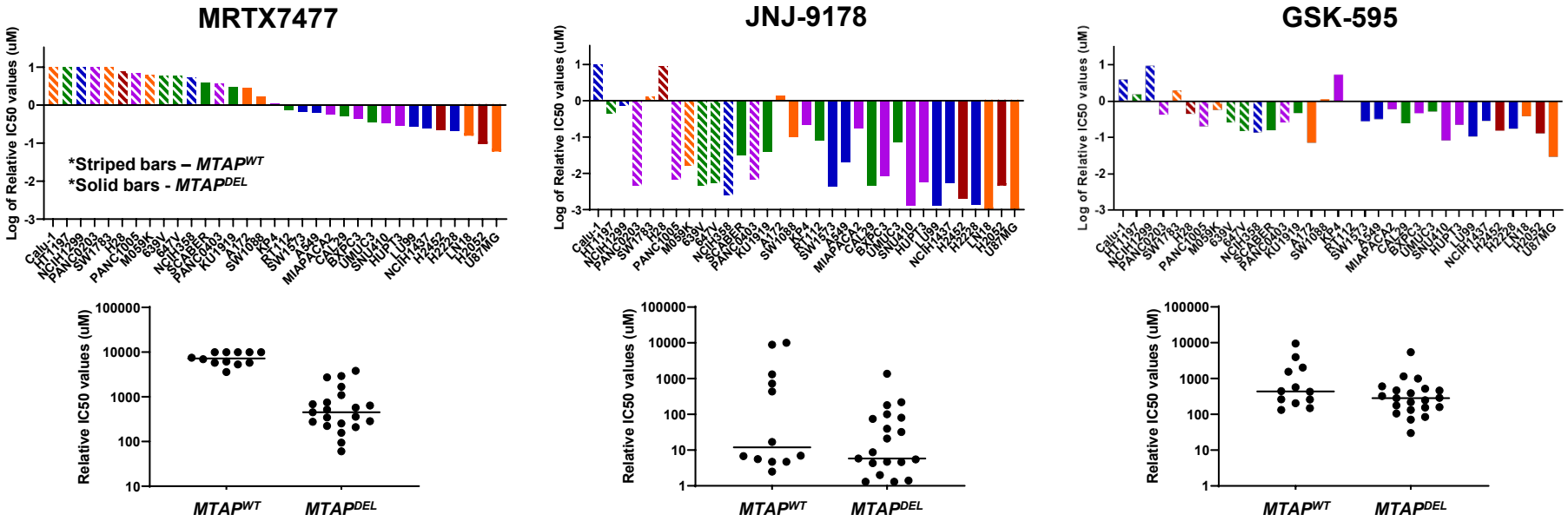

- MRTX7477 selectively inhibits growth of *MTAP*^{DEL} cell lines in 5-day viability assay
- JNJ-9178 and GSK-595 inhibit cellular viability independent of *MTAP* gene status

Sustained PD Inhibition Suggests Durable Target Occupancy

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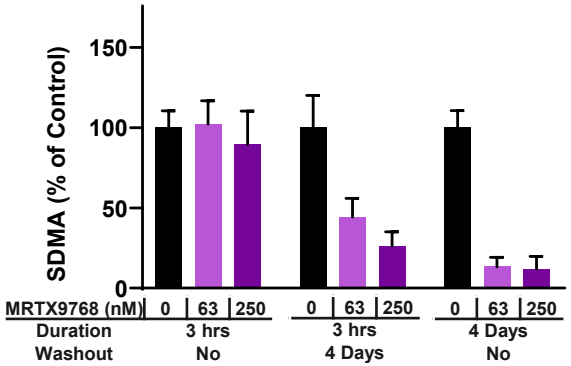
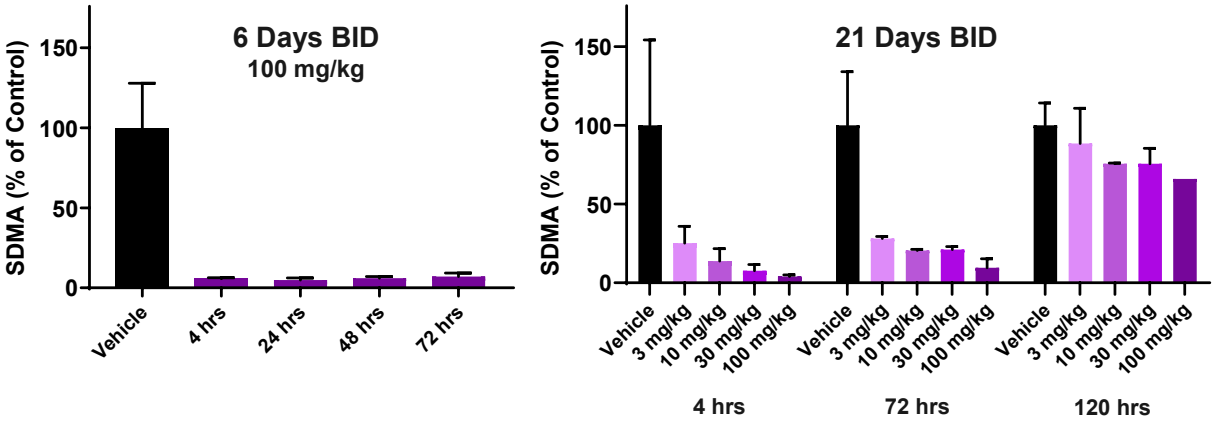
LU99 In Vitro SDMA Washout MRTX9768



Tumor SDMA Time Course Post Last MRTX9768 Dose



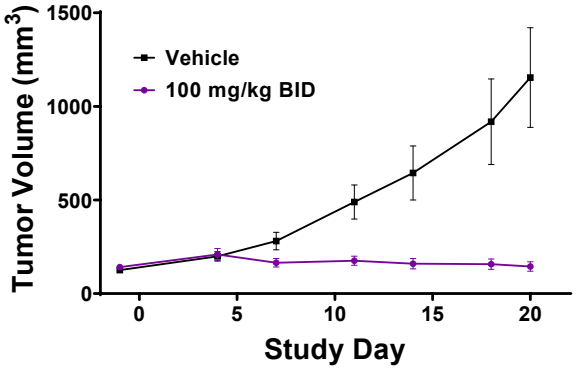
- In vitro SDMA inhibition maintained after 3-hr drug treatment followed by 4-day washout
- In vivo SDMA inhibition maintained 3 days after dosing is stopped
- Data suggest MRTX inhibitors exhibit tight binding and prolonged PRMT5•MTA occupancy

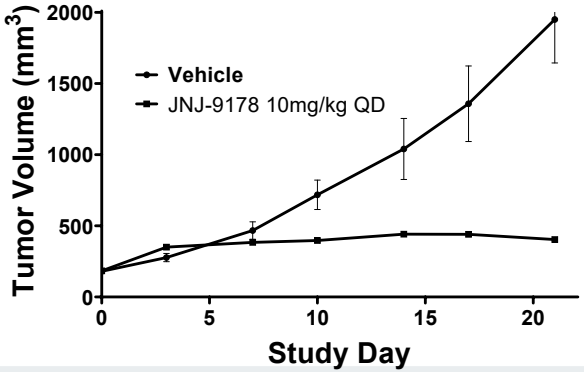
MRTX9768 Treatment Results in Strong In Vivo Efficacy and PD Target Modulation

MRTX9768

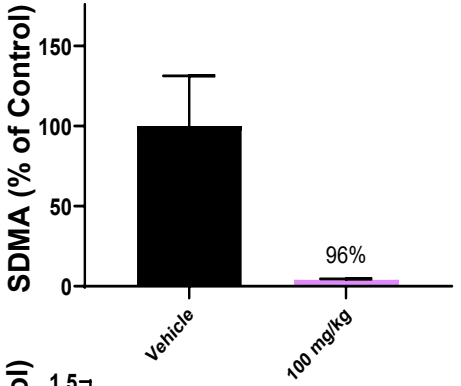
JNJ-9178

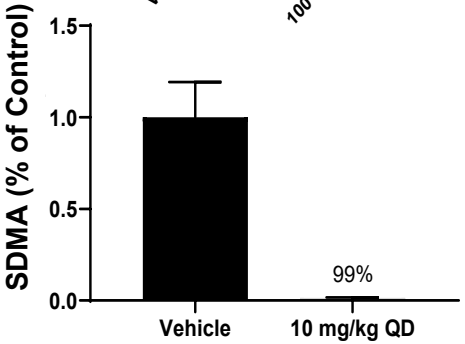
LU99 TGI





Tumor SDMA (4h after final dose)



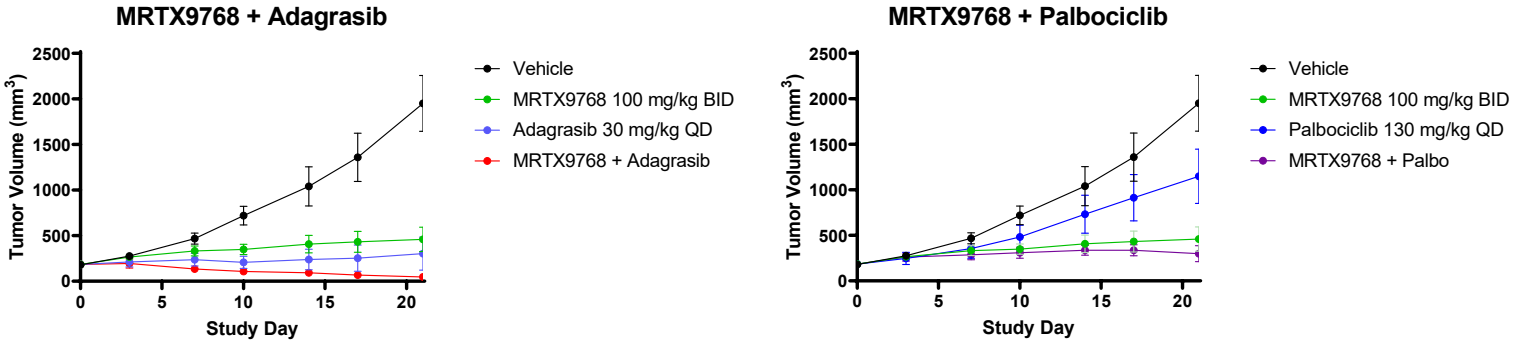


Hematology Changes Observed in Mice for Clinical Agents are Not Observed for MRTX9768

Compound	HCT116 $MTAP^{DEL}$ / $MTAP^{WT}$ prolif. IC_{50} (nM)	Dose (QD, mg/kg)	Multiple of efficacious daily dose	AUC_{d14} (µg*h/mL)	Red blood cells	Platelets	Reticulocytes
JNJ-64619178	5 / 7	30 N=3	3x (10 mg/kg QD)	32	**7.12**	**839**	**67.1**
GSK-3326595	189 / 237	300 N=2	1.5x (100 mg/kg BID)	60	**7.13**	1,212	**76.5**
MRTX9768	10 / 815	1,000 N=4	**5x (100 mg/kg BID)**	143	8.75	1,141	243
				Reference ranges	7.94-10.52	1,032-1,850	236-440

- JNJ: Large decreases in platelets and reticulocytes
- GSK: Large decrease in reticulocytes
- Preclinical findings for clinical agents consistent with myelosuppression reported in GSK and JNJ clinical trials (anemia, neutropenia and thrombocytopenia)
- MRTX9768: No changes in RBC parameters when administered well above efficacious concentrations (1000 mg/kg)

Increased Tumor Growth Inhibition Observed in Combination with Adagrasib or Palbociclib in LU99 Xenograft Model



LU99 model; KRAS^{G12C} mut, CDKN2A/MTAP^{DEL}

- Preclinical evaluation of targeted therapy and chemotherapy combinations ongoing
  - Adagrasib: *KRAS^{G12C}* prevalent in lung adenocarcinoma cancers harboring *MTAP^{DEL}*
  - Palbociclib: CDK4/6 may act as co-driver in cancers harboring co-deletion of *CDKN2A/MTAP*
- Near complete response in MRTX9768 plus MRTX849 (adagrasib, KRAS G12C inh)-treated tumors
- Increased anti-tumor activity in MRTX9768 plus palbociclib-treated tumors

Summary

- We have described the first known preclinical approach specifically targeting the PRMT5•MTA complex in *MTAP*^{DEL} cancers, a deletion identified in approximately 10% of all human cancers
- MRTX9768 provides >100-fold selectivity in mechanistic and cell viability assays when evaluated in the HCT116 cell line with and without an MTAP deletion; current clinical agents do not target the PRMT5•MTA complex specifically, and are nonselective inhibitors of cellular viability
- Preclinical antitumor efficacy and tumor PD of MRTX9768 are comparable to a representative clinical PRMT5 inhibitor
- MRTX9768 shows no changes to hematological parameters in mice at multiples of the efficacious dose; current clinical agents show evidence of myelosuppression
- Targeting the PRMT5•MTA complex specifically may provide an opportunity for deeper and more sustained target inhibition in *MTAP* deleted tumors and improved therapeutic index relative to current inhibitors of PRMT5 and MAT2A

Mirati PRMT5•MTA Discovery Team

Ruth Aranda
David Briere
Aaron Burns
James Christensen
Jeff Clarine
Lars Engstrom
Robin Gunn
Jill Hallin
Anthony Ivetac
John Ketcham
Lana Kulyk
Jade Laguer
Dave Lawson
James Medwid
Matthew Marx
Krystal Moya
Natalie Nguyen
Peter Olson
Lisa Rahbaek
Chris Smith
Nicole Thomas
Darin Vanderpool
Laura Waters

Past employees:
Nidhi Arora
Thomas Bobinski