MRTX1719, an MTA-cooperative PRMT5 Inhibitor, Stabilizes the Ternary PRMT5-MTA Complex and Leads to Synthetic Lethality in *MTAP* deleted Cancers

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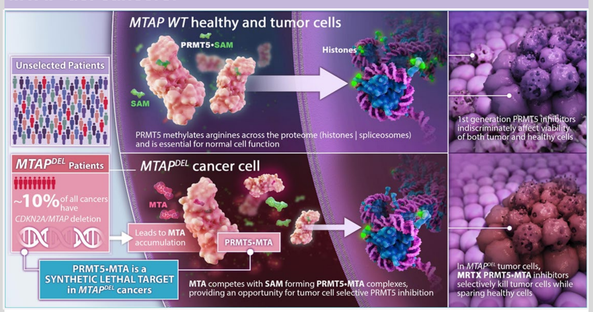
Abstract # 2778

BACKGROUND

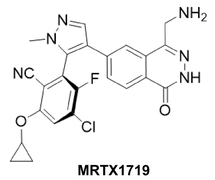
- PRMT5 is a methyltransferase that adds symmetric dimethyl arginine marks to proteins, regulating several essential cellular functions.
- Functional genomics studies have shown that cancer cell lines with a homozygous deletion of the *MTAP* gene (*MTAP* del) have an increased dependency on PRMT5 activity.
- MTAP is an enzyme responsible for metabolizing MTA as part of the methionine salvage pathway.
- In *MTAP* del cells, MTA accumulates and partially inhibits PRMT5 activity by directly competing with SAM, the universal methyl donor and PRMT5 substrate. The increased concentration of MTA within *MTAP* del cells also increases the concentration of the synthetically lethal target, PRMT5-MTA.
- MTA-cooperative inhibitors are hypothesized to exhibit an increased therapeutic index compared to first generation PRMT5 inhibitors by preferentially inhibiting PRMT5 in *MTAP* del cancer cells while sparing the essential function of PRMT5 in normal cells.
- MRTX1719, an MTA-cooperative PRMT5 inhibitor preferentially and potently binds PRMT5 in the presence of MTA and selectively inhibits *MTAP* del cancer models.
- In a biochemical activity assay MRTX1719 has five-fold selectivity for the PRMT5-MTA complex. In cell based pharmacodynamic and functional assays, MRTX1719 demonstrates > 70-fold selectivity for *MTAP* del HCT116 cells over *MTAP* WT HCT116 cells.

The PRMT5-MTA complex is a synthetic lethal target in *MTAP* del cancers.



RESULTS

MRTX1719 selectively inhibits PRMT5 in the presence of MTA.

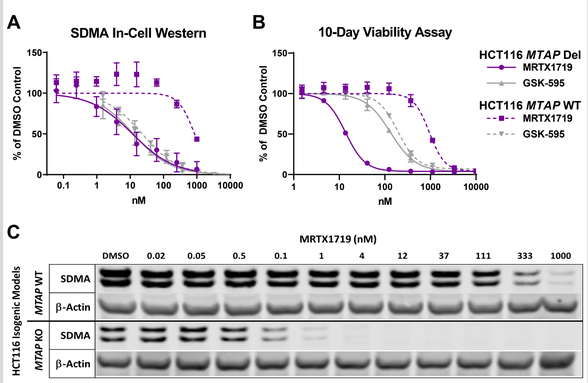


MRTX1719

MRTX1719 was evaluated in biochemical assays that measure the inhibition of the PRMT5/MEP50 complex enzymatic activity using SAM as a methyl donor and a histone 4 peptide substrate. The assay was performed with or without MTA to determine the half-maximal inhibitory concentration (IC_{50} nM) in conditions intended to model elevated MTA levels present in *MTAP* del tumor cells compared to *MTAP* WT cells.

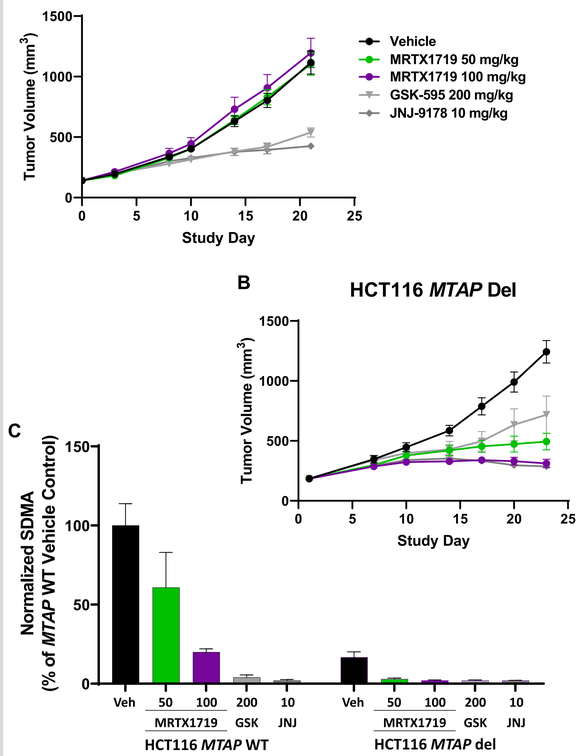
Assay			MRTX1719	GSK-595	JNJ-9178
Biochemical	PRMT5-MTA		**3.6**	ND	ND
	PRMT5		20	ND	ND
Cellular Activity HCT116 Isogenic Cell Lines	SDMA In-Cell Western	*MTAP* del	**8**	25	2
		MTAP WT	653	27	3
	10-Day Viability	*MTAP* del	**12**	164	5
		MTAP WT	890	200	5

MRTX1719 demonstrates greater than 70-fold *in vitro* selectivity of PRMT5-mediated SDMA modification and cell viability in HCT116 *MTAP* del compared to *MTAP* WT cells.



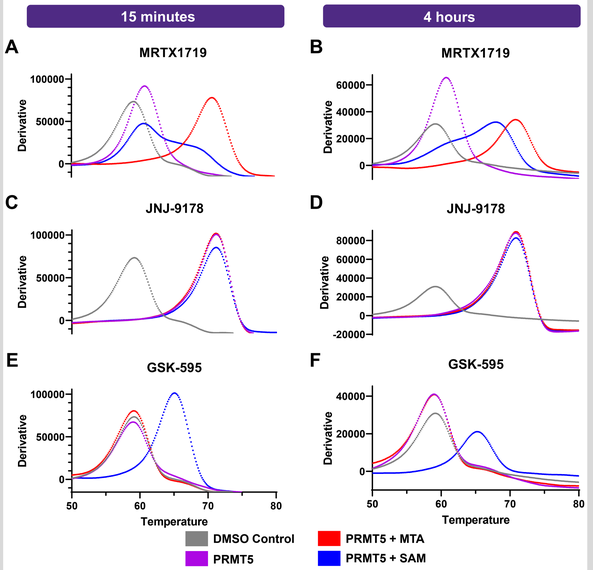
Dose response curves of MRTX1719 and GSK-595 were used to determine IC_{50} values in SDMA In-Cell (SYM11 antibody) Westerns (**A**) and 10-day viability assays (**B**) in *MTAP* del and *MTAP* WT HCT116 cell lines. (**C**) Cropped SDMA SDS-PAGE Western blot showing protein bands that correlate with the molecular weight of SmD3 in *MTAP* del and WT HCT116 cell lines following 4 days of treatment with a range of concentrations of MRTX1719. β-Actin was used as a loading control.

MRTX1719 exhibits selective dose-dependent tumor growth inhibition and PRMT5-dependent SDMA modification in HCT116 *MTAP* del tumor xenografts *in vivo*.



MRTX1719, GSK-595 or JNJ-9178 were administered QD via daily oral gavage at the doses indicated to mice bearing established HCT116 *MTAP* WT (**A**) or *MTAP* del (**B**) cell line-derived tumor xenografts. Dosing was initiated when tumors were ~150 mm^3. Data are shown as mean tumor volume ± SEM. (**C**) End of study vehicle and drug treated tumors from the above studies were collected four hours post dose and SDMA was analyzed by immunoblot. Data shown represent the average of 3 tumors per treatment group ± SEM.

MRTX1719 preferentially binds to the PRMT5-MTA complex with a differentiated binding mechanism compared to first generation PRMT5 inhibitors.



(**A**) Thermal shift experiments show MRTX1719 demonstrates rapid binding to PRMT5-MTA whereas MRTX1719 exhibited only partial binding to PRMT5-SAM after a 15-minute incubation. (**B**) At 4 hours, MRTX1719 binding partially stabilizes the PRMT5-SAM complex, while the ternary complex of MRTX1719-PRMT5-MTA displays increased stability. JNJ-9178 (**C and D**) shows no selectivity between complexes and GSK-595 (**E and F**) only stabilizes the PRMT5-SAM complex preventing synthetically lethal targeting of PRMT5 in *MTAP* del cancer cells. All compounds were tested at 1 μM.

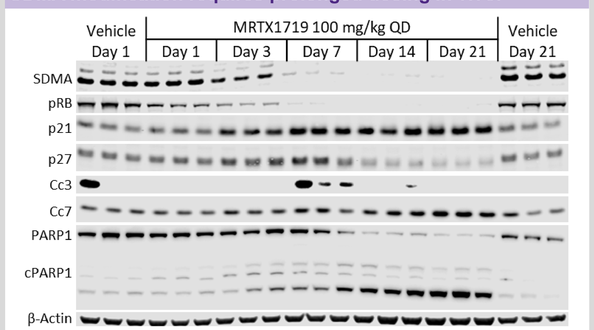
Transient MRTX1719 exposure is sufficient to inhibit PRMT5 mediated SDMA activity *in vitro* and requires extended timepoints to observe maximal pharmacodynamic inhibition.

MTAP:	KO	WT	WT
MTA:	-	+ 20 mM	-
3 hr Treatment	> 1000	> 10000	> 10000
3 hr Treatment + 69 hr Washout	44	92	> 10000
72 hr Treatment	3.4	35	323

IC_{50} (nM): 10 100 1000

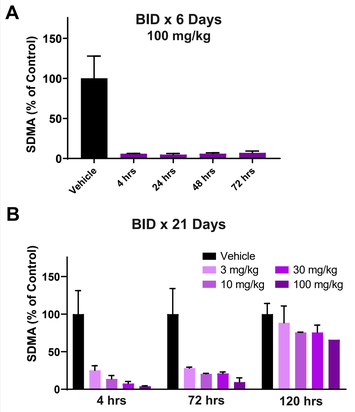
Isogenic HCT116 cell lines, as indicated, were incubated with serial dilutions of MRTX1719 for 3 hrs, 3 hrs followed by a 3-day washout, or continuously for 3 days and IC_{50} values were subsequently determined in an SDMA in-cell western. Treatment of *MTAP* del or *MTAP* WT cells plus MTA for 3 hours followed by a 3-day washout resulted in significant reduction of SDMA with only a moderate reduction in efficacy compared to continuous treatment with MRTX1719 for 3 days.

Maximal pharmacodynamic modulation of PRMT5-mediated SDMA modification requires prolonged dosing *in vivo*.



Western blot analysis of SDMA, proliferation and apoptosis biomarkers from LU99 cell line derived tumor xenografts treated for indicated durations with vehicle or MRTX1719 at 100 mg/kg QD.

Durable pharmacodynamic modulation of PRMT5 mediated SDMA modification post dosing *in vivo*.



MRTX9768, a structurally related MTA-cooperative PRMT5 tool compound, was administered QD via daily oral gavage at the doses indicated to mice bearing established LU99 cell line derived tumor xenografts. Dosing was initiated when tumors were ~150 mm^3. End of study vehicle and drug treated tumors were collected at the indicated timepoints post last dose and SDMA was analyzed by immunoblot. Data shown represent average per group ± SEM.

Smith CR et al. *Cancer Res* 1 July 2021; 81 (13_Supplement): LB003

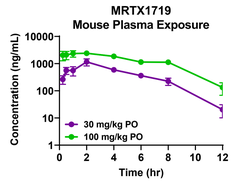
Reduced potency of MRTX1719 against *MTAP* WT hematopoietic cells suggests an improved therapeutic index compared to first generation PRMT5 inhibitors

	Human Cancer Cell Lines HCT116 *MTAP* Isogenic Pair		*MTAP* WT Human Hematopoietic Cells	
	10-Day Viability IC_{50}		7-Day HemaTox IC_{50} (nM)	
	MTAP del	*MTAP* WT	Erythroid	Myeloid
MRTX1719	12	890	545	559
GSK-595	164	200	62	58
JNJ-9178	5	5	3	2

- MRTX1719 demonstrated reduced activity and ~50-fold lower potency in the HemaTox™ viability assays compared to the activity in the *MTAP* del cell line assay.
- In contrast, JNJ-9178 and GSK-595 demonstrated ~2-3 fold increased potency in the HemaTox™ assays compared to either the *MTAP* WT or *MTAP* del cell line assays.

Preclinical pharmacokinetic properties of MRTX1719

Pharmacokinetic Parameter	Mouse
Cl_{total} (mL/min/kg)	83
Vd_{ss} (L/kg)	6.3
$t_{1/2}$ (h)	1.5
F(%)	80



Plasma pharmacokinetic analysis in CD-1 mice following a single dose of MRTX1719.
IV: 3 mg/kg in 20% SBE-B-CD / 50 mM citric acid pH 5.
PO: 30 or 100 mg/kg in 0.5% (MC 4000 cps) / 0.2% Tween 80 in water.

CONCLUSIONS

- First generation PRMT5 inhibitors target apo-PRMT5 and/or the PRMT5-SAM complex. In contrast, MRTX1719 requires the presence of MTA to preferentially target the PRMT5-MTA complex and inhibit the methyltransferase activity of PRMT5.
- Full inhibition of PRMT5 activity by MRTX1719, results in significant tumor growth inhibition of *MTAP* del cancer models.
- Tight binding to PRMT5-MTA, reduced binding to PRMT5-SAM and prolonged pharmacodynamic effects post drug exposure (*in vitro* and *in vivo*), suggest MRTX1719 will exhibit a significantly improved therapeutic window for the treatment of *MTAP* del cancer patients.
- MRTX1719 is well-positioned to test the PRMT5 synthetic lethality hypothesis in *MTAP* del cancers by maximizing the anticipated therapeutic index via sparing *MTAP* WT normal cells and enabling maximal, if not complete, inhibition of PRMT5 in *MTAP* del tumors.

ACKNOWLEDGEMENTS

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- StemCell Technologies for HemaTox viability assays.
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