MRTX1719: A First-in-class MTA-cooperative PRMT5 Inhibitor that Selectively Elicits Antitumor Activity in *MTAP/CDKN2A* Deleted Cancer Models

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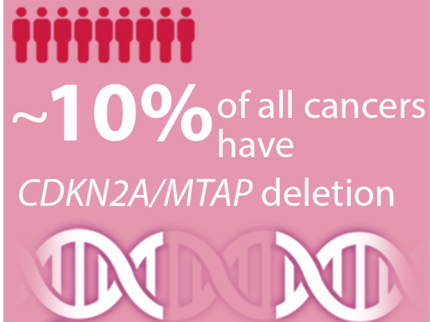
Targeting the PRMT5•MTA Complex: a Synthetic Lethal, Precision Medicine Approach for the Treatment of *MTAP* Deleted Cancers

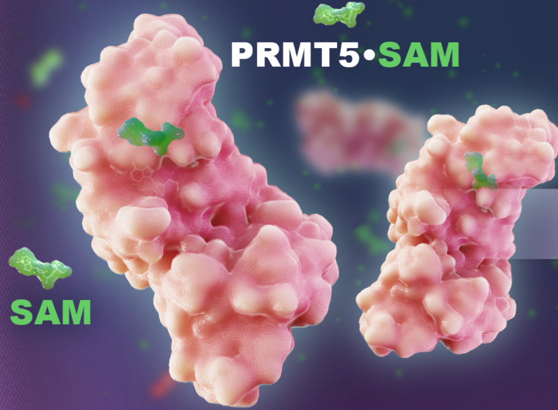
- PRMT5 was a top hit in large scale functional genomics screens that demonstrated shRNA-mediated PRMT5 inhibition selectively inhibited *MTAP* del cancer cell line viability[1]
- *MTAP* is proximal to and co-deleted with *CDKN2A*, the most commonly deleted gene in human cancer; *MTAP* deletion increases 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. Since PRMT5 is critical for the viability of cancer and normal cells, this may result in a narrow therapeutic index[2]
- MRTX1719 selectively binds the PRMT5•MTA complex which is elevated in *MTAP* del cancer cells and is therefore positioned to have an improved therapeutic index based on the concept of synthetic lethality
- MRTX1719 was identified as a development candidate which selectively binds the PRMT5•MTA complex

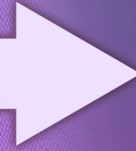
[1]Mavrakis, K, et al, Science, 2016; Marjon, K, et al, Cell Reports, 2016; Kryukov, G, et al, Science, 2016
[2]EPZ666 (GSK) – References in 1; JNJ – Brehmer D (Janssen), ASCO, 2017.

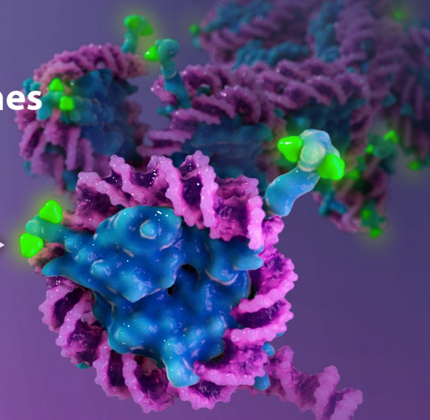
Precision Medicine for *MTAP*^{DEL} cancers by targeting the PRMT5•MTA complex



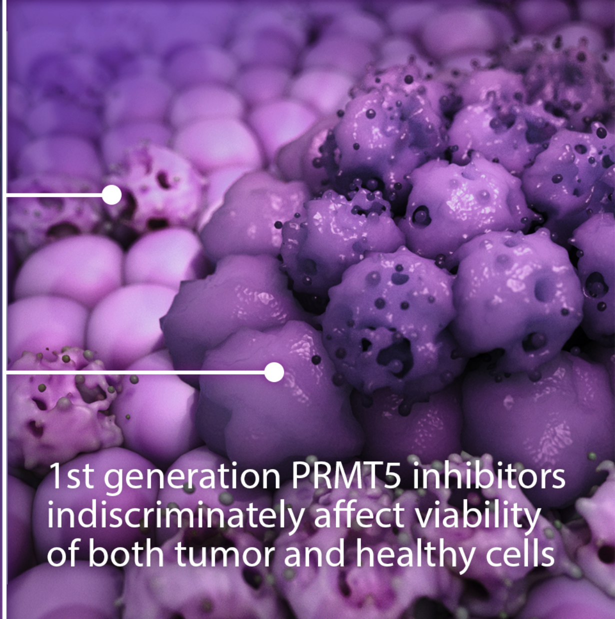
MTAP WT healthy and tumor cells



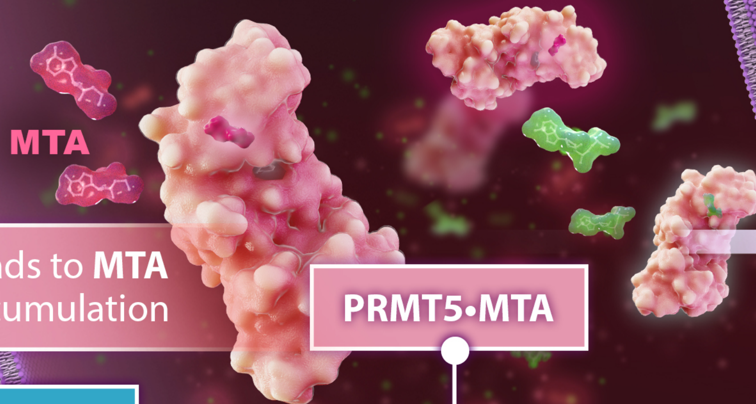




PRMT5 methylates arginines across the proteome (histones | spliceosomes) and is essential for normal cell function

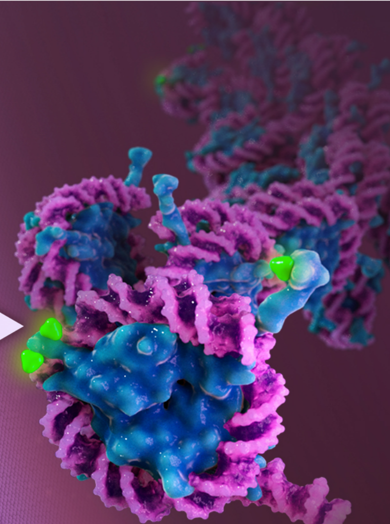


MTAP^{DEL} cancer cell



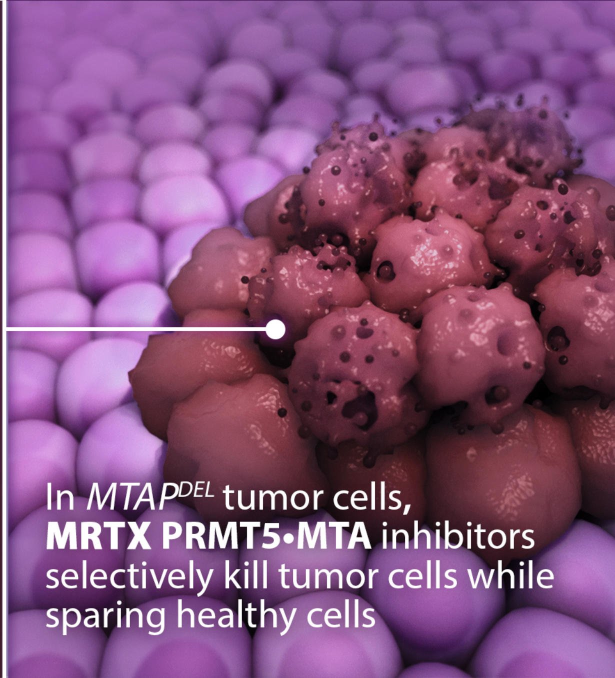
Leads to **MTA** accumulation

PRMT5•MTA



PRMT5•MTA is a SYNTHETIC LETHAL TARGET in *MTAP*^{DEL} cancers

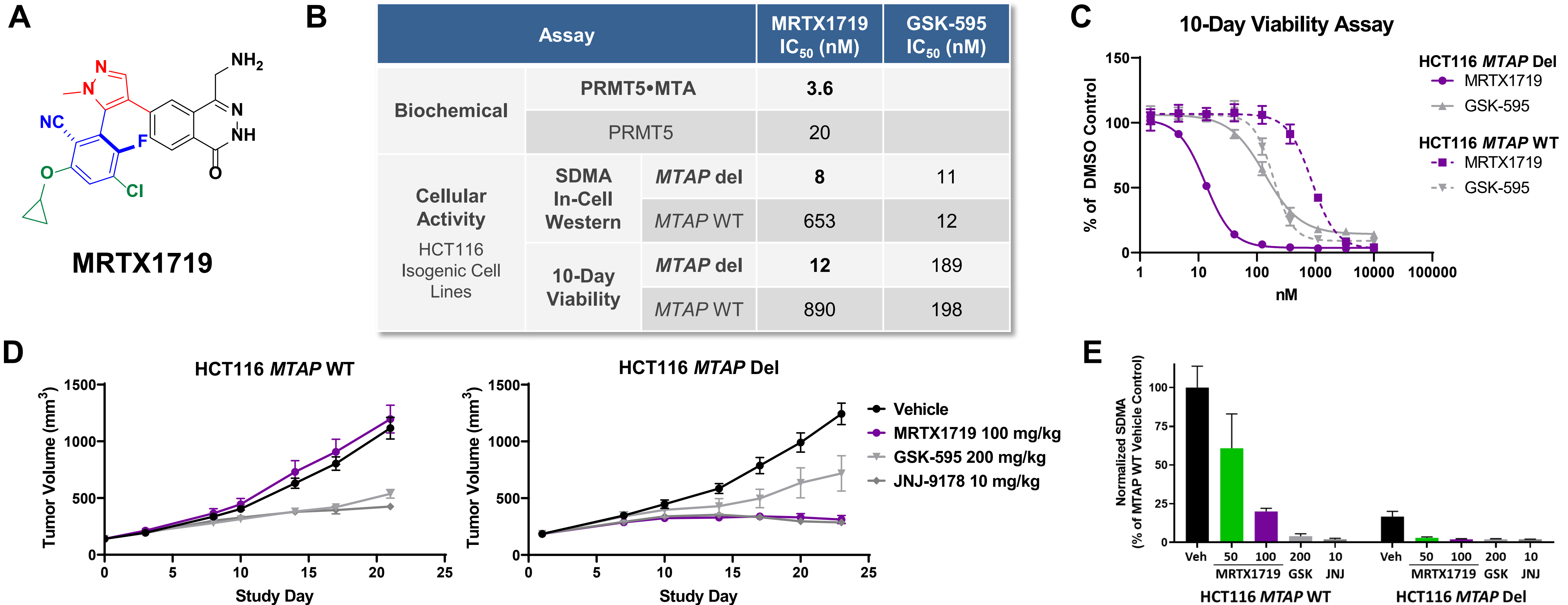
MTA competes with **SAM** forming **PRMT5•MTA** complexes, providing an opportunity for tumor cell selective PRMT5 inhibition



MRTX1719: A PRMT5 Inhibitor Selectively Targeting *MTAP* Deleted Cancers

- MRTX1719 is a potent, selective inhibitor of the PRMT5•MTA complex with favorable drug-like properties that selectively targets *MTAP* del cancers
- MRTX1719 is differentiated from previously described PRMT5 inhibitors that inhibit PRMT5 in *MTAP* WT normal tissue, impact hematopoietic cell viability and may exhibit a narrow TI
- MRTX1719 inhibition leads to dysregulated RNA splicing, decreased pRb, decreased proliferation and increased apoptosis
- MRTX1719 elicits strong antitumor activity across a panel of *MTAP* del CDX and PDX models and induces tumor regression in a subset of models as a single agent, including lung and mesothelioma models
- Additional mechanistic and combination studies are underway to inform patient populations for clinical development

MRTX1719 Selectively Inhibits the PRMT5•MTA Complex, *MTAP* Deleted Cell Lines and *MTAP* Deleted Xenograft Tumor Growth



Assay			MRTX1719 IC_{50} (nM)	GSK-595 IC_{50} (nM)
Biochemical	PRMT5•MTA		**3.6**	
	PRMT5		20	
Cellular Activity HCT116 Isogenic Cell Lines	SDMA In-Cell Western	*MTAP* del	**8**	11
		MTAP WT	653	12
	10-Day Viability	*MTAP* del	**12**	189
		MTAP WT	890	198

A. A co-crystal structure of the fragment hit (**shown in black**) with PRMT5 confirmed MTA binding and suggested vectors for further elaboration. Addition of methylpyrazole increased binding to the PRMT•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 anti-proliferation in *MTAP* del cells. Further optimization resulted in improvement in antiproliferative activity and high bioavailability in rodent and non-rodent species. **B.** Compounds were tested in a PRMT5/MEP50 enzymatic assay in which PRMT5 uses SAM to add symmetric dimethyl groups to arginine residues (SDMA) within an H4 peptide (1-15) with and without MTA (Reaction Biology Corp). PRMT5 cellular activity was measured using a 4-day In-Cell Western assay to measure SDMA marks using the SYM11 antibody and normalized using DRAQ5 in HCT116 *MTAP* deleted and WT cell lines. Cell viability was measured using a 10-day Cell Titer Glo assay (Promega) in HCT116 *MTAP* del and *MTAP* WT cell lines. **C.** Graph depicting the dose-concentration curves for MRTX1719 and GSK-595 in the 10-day viability assay in HCT116 *MTAP* del and *MTAP* WT cell lines. **D.** MRTX1719, GSK-595 and JNJ-9178 were administered via daily oral gavage to immunocompromised mice bearing established HCT116 *MTAP* del or *MTAP* WT xenograft tumors at the indicated doses QD. Data are shown as mean tumor volume (n=10/group) +/- standard error of the mean (SEM). **E.** Tumors from the tumor growth inhibition study (**D**) were collected at the end of the study four hours after the last dose and analyzed by western blot and densitometry for SDMA levels. Representative bands were analyzed from 3 tumor lysates per treatment group.

MRTX1719 Inhibits Growth & SDMA Modification in *MTAP* Deleted Xenograft Tumors with Minimal SDMA Modulation/Viability Effects in Bone Marrow

A LU99 In Vivo TGI

MRTX1719 — JNJ-9178

B

MRTX1719 — JNJ-9178

C LU99 In Vivo PD Timecourse

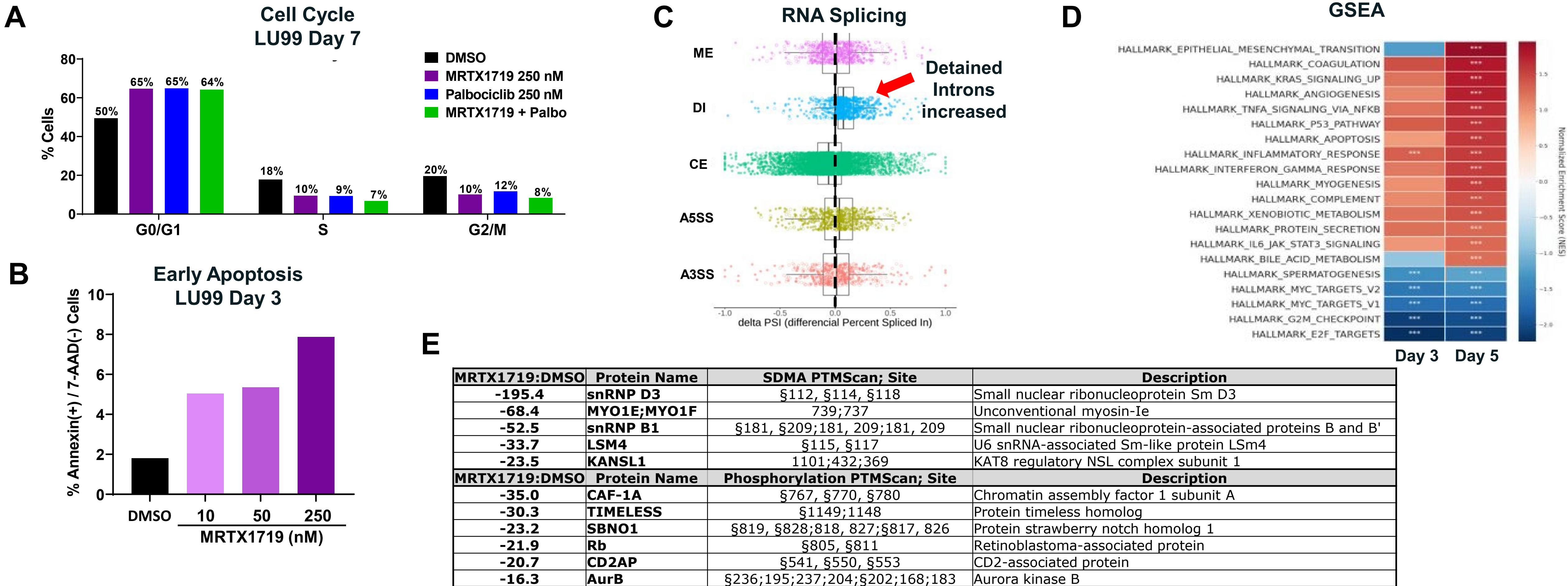
Tumor SDMA — Bone Marrow SDMA

D

	Human Cancer Cell Lines HCT116 *MTAP* Isogenic Pair		Human Bone Marrow *MTAP* WT	
	10-Day Viability IC_{50} (nM)		7-Day HemaTox IC_{50} (nM)	
	MTAP del	*MTAP* WT	Erythroid	Myeloid
MRTX1719	12	890	545	559
JNJ-9178	5	5	3	2
GSK-595	189	198	62	58

A. MRTX1719 (right) and JNJ-9178 (left) were administered via daily oral gavage to immunocompromised mice bearing established *MTAP* del LU99 xenograft tumors at the indicated doses QD. Data are shown as mean tumor volume (n=5/group) +/- standard error of the mean (SEM). **B.** Tumors and bone marrow from the tumor growth inhibition study (**A**) were collected at the end of the study four hours after the last dose and analyzed by western blot and densitometry for SDMA levels. Representative bands were analyzed from 3 or 5 tumor lysates per treatment group. **C.** MRTX1719 and GSK-595 were administered via daily oral gavage to immunocompromised mice bearing established LU99 xenograft tumors at the indicated doses QD for the indicated number of weeks. Tumor (left) and bone marrow (right) were collected at the end of the study four hours after the last dose and analyzed by western blot and densitometry for SDMA levels. Representative bands were analyzed from 3 tumor lysates per treatment group. **D.** MRTX1719, JNJ-9178 and GSK-595 were run in human erythroid and myeloid 7-day HemaTox assays (STEMCELL Technologies) and were compared to IC_{50}s from 10-day *MTAP* del and *MTAP* WT cell line viability assays.

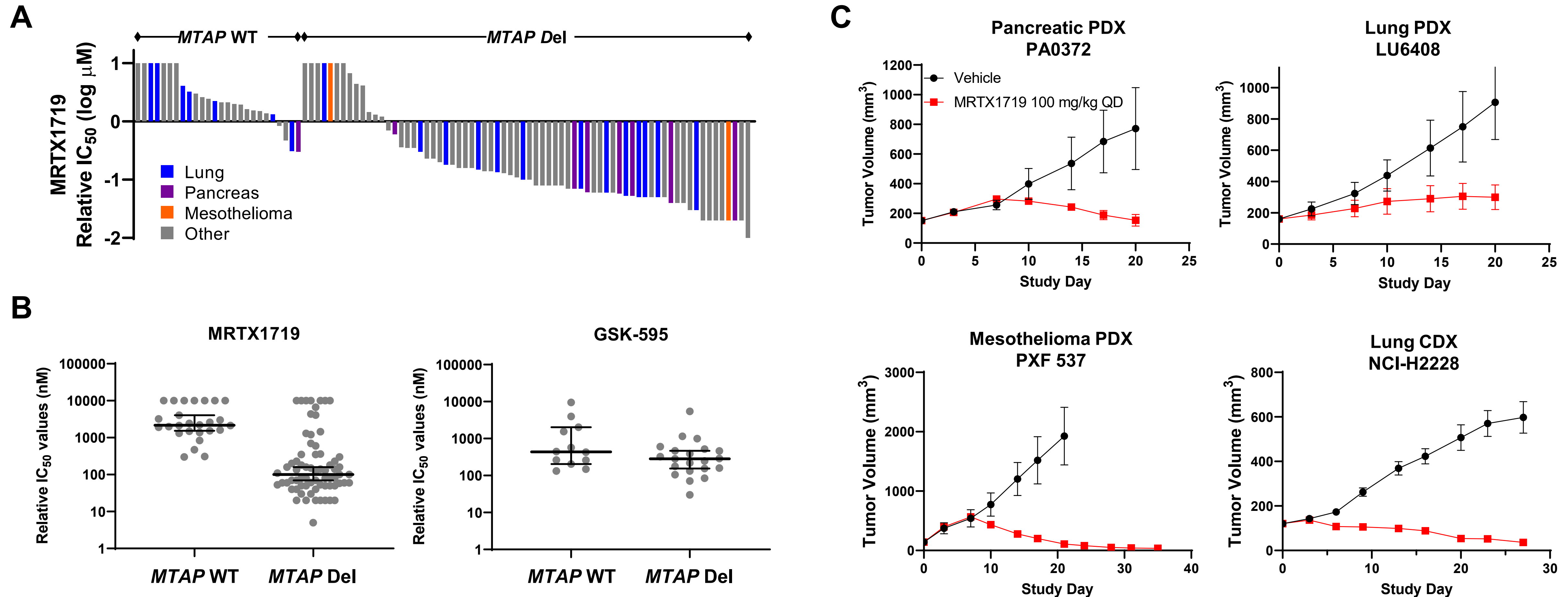
MRTX1719 Treatment Decreases Proliferation, Increases Apoptosis and Leads to Dysregulated RNA Splicing



MRTX1719:DMSO	Protein Name	SDMA PTMScan; Site	Description
-195.4	snRNP D3	§112, §114, §118	Small nuclear ribonucleoprotein Sm D3
-68.4	MYO1E;MYO1F	739;737	Unconventional myosin-Ie
-52.5	snRNP B1	§181, §209;181, 209;181, 209	Small nuclear ribonucleoprotein-associated proteins B and B'
-33.7	LSM4	§115, §117	U6 snRNA-associated Sm-like protein LSm4
-23.5	KANSL1	1101;432;369	KAT8 regulatory NSL complex subunit 1
MRTX1719:DMSO	**Protein Name**	**Phosphorylation PTMScan; Site**	**Description**
-35.0	CAF-1A	§767, §770, §780	Chromatin assembly factor 1 subunit A
-30.3	TIMELESS	§1149;1148	Protein timeless homolog
-23.2	SBNO1	§819, §828;818, 827;§817, 826	Protein strawberry notch homolog 1
-21.9	Rb	§805, §811	Retinoblastoma-associated protein
-20.7	CD2AP	§541, §550, §553	CD2-associated protein
-16.3	AurB	§236;195;237;204;§202;168;183	Aurora kinase B

A. LU99 cells were treated with 250 nM MRTX1719 (665B), 250 nM palbociclib or the combination for 7 days and cell cycle distribution analysis was performed using 7-AAD staining on a Guava flow cytometer **B.** LU99 cells were treated with MRTX1719 at the concentrations indicated for 3 days and apoptotic cells were analyzed for surface Annexin V positive, 7-AAD negative staining. **C.** RNAseq data was generated from LU99 cells treated with MRTX1719 or DMSO for 3 days and transcripts were analyzed for altered RNA splicing using rMATS. Transcripts with detained introns were increased in MRTX1719-treated vs DMSO-treated cells. ME – Mutually exclusive exon usage, DI – Detained introns, CE – Skipped exons, A3SS – Alternative 3' splice site, A5SS – Alternative 5' splice site. **D.** Gene signature enrichment analysis (GSEA) was performed on RNAseq data from 3 or 5-day MRTX1719-treated LU99 cells compared to DMSO treated. "***" indicates FDR < 0.25. **E.** Fold changes (1st column) in post translational modifications that were down regulated in selected trypsin-digested peptides from MRX1719 vs DMSO-treated LU99 cell lysates as measured by LC-MS/MS following antibody enrichment using an SDMA motif antibody (top) or the multi-pathway kit that includes phosphorylation (Cell Signaling Technology). Site denotes amino acid. "§" indicates known site of modification.

MRTX1719 Demonstrates Broad Activity *in vitro* and *in vivo* and Induces Regression in a Subset of Cell Line- and Patient-derived Xenograft Models



A. MRTX1719 in vitro activity across a panel of *MTAP* WT and *MTAP* del cell line models (5-day viability assay, Crown Biosciences). **B.** Dot plots showing median with 95% confidence intervals of IC_{50} values for MRTX1719 (left) and GSK-595 (right) in *MTAP* WT and *MTAP* del cell line models. A smaller cohort of models was tested with GSK-595. Median values: MRTX1719 - *MTAP* del - 100 nM; *MTAP* WT - 2.2 µM; GSK-595 - *MTAP* del - 284 nM; *MTAP* WT - 437 nM. Of note, in internal studies, IC_{50} values were 3-4-fold lower in the preferred 10-day viability assay format (e.g. MRTX1719 LU99 5-day viability IC_{50} – 72 nM; 10-day viability IC_{50} – 20 nM (n=13, both formats)) **C.** MRTX1719 was tested in a panel of cell line- and patient derived-xenograft tumor models at 50 or 100 mg/kg administered by oral gavage QD and a range of activity was observed including minimal response, tumor growth delay, tumor stasis and tumor regression. Individual tumor growth plots from selected models shown in which average tumor volume is plotted +/- SEM (n=3/treatment group).

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