MTA-cooperative PRMT5 inhibitors are efficacious in MTAP-deleted malignant peripheral nerve sheath tumor models

Xiaochun Zhang[1], Dana C Borcherding[1], Minjie Zhang[2], Himanshi Bhatia[1], Yang Lyu[1], Kevin He[1], Liuzhan Yang[1], Kuangying Yang[1], Heather DiBenedetto[2], Alice Tsai[2], Alan Huang[2], John P Maxwell[2], Kevin M Cottrell[2], Kimberly J Briggs[2], and Angela C Hirbe[1]

[1] Washington University in St. Louis School of Medicine, [2] Tango Therapeutics, Boston, MA

Division of Oncology
Department of Internal Medicine

Introduction

OBJECTIVES: Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive ¬¬sarcomas with limited treatment options and poor survival rates, necessitating the development of novel therapeutics. Approximately 25-50% of MPNST harbor loss of the enzyme methylthioadenosine phosphorylase (MTAP) due to a passenger deletion driven by loss of the proximal tumor suppressor gene, *CDKN2A*. PRMT5 was identified as a selective dependence in *MTAP*-deleted cells due to the accumulation of the substrate methylthioadenosine (MTA), which is itself an endogenous PRMT5 inhibitor. TNG908 and TNG462 are clinical stage MTA-cooperative PRMT5 inhibitors that demonstrate selectivity for *MTAP*-deleted cells over *MTAP*-intact cells of 15X and 45X, respectively. Previous reports show both molecules drive durable tumor regressions in xenograft models of various MTAP-deleted cancer histologies. Here, our objectives are to examine the activity of TNG908 and TNG462 in preclinical MPNST models.

METHODS: The proliferation effects of MTA-cooperative PRMT5 inhibitors, TNG908 or TNG462, and a SAM-cooperative PRMT5 inhibitor, GSK3326595, on *MTAP*-deleted and *MTAP*-intact MPNST cell lines were determined using CellTiter-Glo (CTG) assays. TNG908 and TNG462 were further profiled in two *MTAP*-deleted MPNST patient-derived xenograft (PDX) models, WU-356 and WU-386.

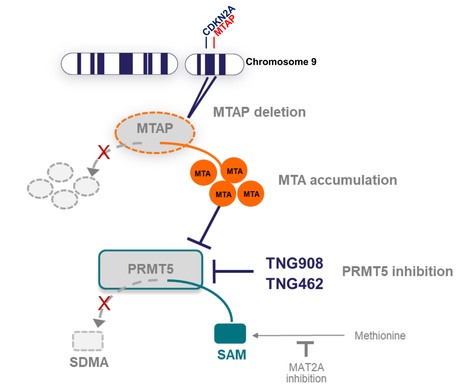
RESULTS: Incubation with the MTA-cooperative PRMT5 inhibitors, TNG908 and TNG462, selectively decreased the proliferation of *MTAP*-deleted MPNST cell lines relative to *MTAP*-intact MPNST cell lines. TNG908 and TNG462 drove dose-dependent antitumor activity including tumor regressions in the *MTAP*-deleted MPNST PDX models, WU-356 and WU-386, at well-tolerated doses.

CONCLUSIONS: The clinical stage MTA-cooperative PRMT5 inhibitors TNG908 (NCT05275478) and TNG462 (NCT05732831) are efficacious in MPNST models *in vitro* and *in vivo* and are therefore promising therapeutic agents for patients with *MTAP*-deleted MPNST.

Methods

- MPNST cell-lines (JH-2-079 and JH-2-009) were plated in 96-well plates at 1000 and 100 cells/well, respectively. The next day, cells were treated with MTA-cooperative PRMT5 inhibitors, TNG908 or TNG462, for 7 to 14 days. Assay length and cell density at plating were determined by doubling time of each cell line.
- After 7- to 14-day drug treatment, the proliferation effects of MTA-cooperative PRMT5 inhibitors on MTAP-deleted and MTAP-intact MPNST cell lines were determined using CellTiter-Glo (CTG) assays.
- Tumor samples were obtained from patients with NF1- or sporadic MPNST.
- We queried our previously published WGS data to determine MTAP status.
- Immunodeficient NRG mice were implanted with one of two MTAP-deleted MPNST patient-derived xenograft (PDX) lines, WU-356 and WU-386. When tumors reached a volume of 50-150 mm3, mice were treated with the indicated doses of TNG908, TNG462, or vehicle control. Mice were dosed with drugs twice per day by oral gavage for 3-6 weeks.

Background



- **MTAP deletion causes MTA to accumulate.**
- **MTA binds to and inhibits PRMT5.**
- **MTA-cooperative PRMT5 inhibitors selectively bind to the PRMT5-MTA complex.**
- **TNG908 and TNG462 can fully inhibit PRMT5 activity in MTAP-deleted cancer cells while sparing normal cells.**
- **TNG908 MTA-cooperative proof-of-mechanism demonstrated in phase 1 update.**

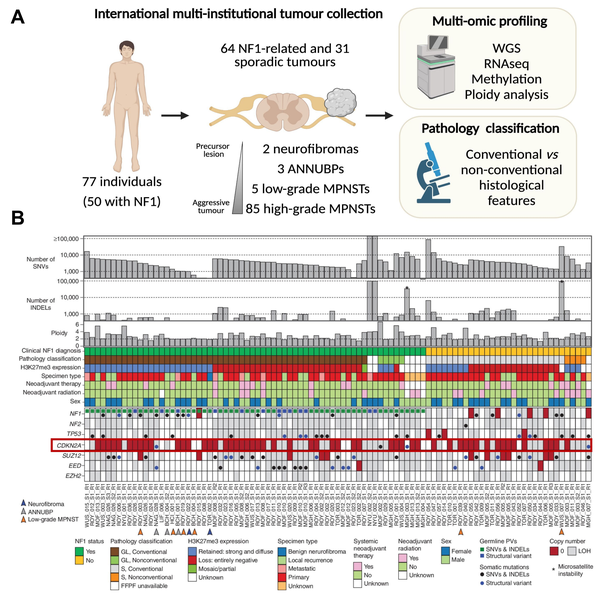


Figure 1. Genomic and pathology classification of MPNSTs. A) Overview of the experimental design and technologies utilized in this study. The GeM cohort consists of 95 tumor samples from 90 tumors (61 NF1-related and 29 sporadic), including 7 tumor samples without FFPE available for pathology review and confirmation of main tumor diagnosis. B) Clinical information, histopathologic features and mutations in driver genes in the GeM cohort. Precursor lesions are marked by arrowheads. Sample labels not marked by an arrowhead correspond to high-grade MPNSTs. ANNUBP, atypical neurofibromatous neoplasms of uncertain biological potential; CN, copy number; GL, germline; INDEL, insertions and deletion; PV, pathogenic variant; RNA-seq, RNA sequencing; S, sporadic; SNV, single-nucleotide variants.

Results

	HAP1 MTAP-null potency	Selectivity in MTAP-isogenic cell lines
TNG908	**110 nM**	**15X**
TNG462	**4 nM**	**45X**
GSK3326595	**120 nM**	**No selectivity**

Table 1. Potency of MTA-cooperative PRMT5 inhibitors in the HAP1 MTAP-isogenic cell line pair relative to GSK3326595, a SAM-cooperative PRMT5 inhibitor, in 7-day viability assays.

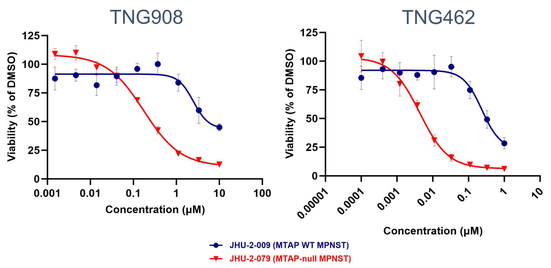


Figure 2. MTA-cooperative PRMT5 inhibitors are efficacious and selective in MTAP-deleted MPNST cell lines. MTAP wild-type MPNST cells (JH-2-009) and MTAP-null MPNST JH-2-079 cells were treated with the indicated doses of MTA-cooperative PRMT5 inhibitors, TNG908 or TNG462, for 7 to 14 days. Cell viability was examined by CellTiter-Glo assay. Mean ± SEM.

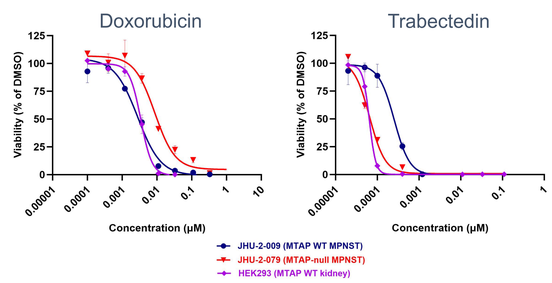


Figure 3. Common chemotherapeutic drugs are effective in MPNST cell lines but are not selective for cancer cells. A) JH-2-009 (MTAP wild-type MPNST), HEK293 (MTAP wild-type kidney) and JH-2-079 (MTAP-null MPNST) cells were treated with the indicated doses of doxorubicin for 7 to 14 days, and cell viability was determined by CellTiter-Glo assay. B) JH-2-009, HEK293 and JH-2-079 cells were incubated with trabectedin for 7 to 14 days and cell viability was determined by CellTiter-Glo assay. Mean ± SEM.

Sample	CDKN2A Somatic structural variant (sSV)	MTAP sSV
JH-2-002 PDX	Homozygous microdeletion	Heteozygous microdeletion
JH-2-002 Tumor	Homozygous microdeletion	Heteozygous microdeletion
JH-2-031 PDX	NA	Normal
JH-2-031 Tumor	NA	Normal
JH-2-055-b PDX	Homozygous microdeletion	Heteozygous microdeletion
JH-2-055-b Tumor	NA	Normal
WU-356 PDX	Homozygous microdeletion	Homozygous microdeletion
WU-356 Tumor	Homozygous microdeletion	Homozygous microdeletion
WU-368 PDX	Homozygous microdeletion	Homozygous microdeletion
WU-368 Tumor	Homozygous microdeletion	Homozygous microdeletion
WU-436 PDX	Homozygous microdeletion	Homozygous microdeletion
WU-436 Tumor	Homozygous microdeletion	Homozygous microdeletion
JH-2-079 PDX	Homozygous microdeletion	Heteozygous microdeletion
JH-2-079 Tumor	Homozygous microdeletion	Heteozygous microdeletion
WU-487 PDX	Partial homozygous microdeletion	Heteozygous microdeletion
WU-487 Tumor	Partial homozygous microdeletion	Heteozygous microdeletion
MN-2 PDX	Homozygous microdeletion	Normal
MN-2 Tumor	NA	Normal
WU-386 PDX	Homozygous microdeletion	Homozygous microdeletion
WU-386 Tumor	Homozygous microdeletion	Homozygous microdeletion
WU-561 PDX	Partial homozygous microdeletion	Heteozygous microdeletion
WU-561 Tumor	Partial homozygous microdeletion	Heteozygous microdeletion
WU-225 PDX	NA	Normal
WU-225 Tumor	NA	Normal
JH-2-023 PDX	Homozygous microdeletion	Normal
JH-2-023 Tumor	Homozygous microdeletion	Normal

Table 2. *CDKN2A* is lost in 9/13 NF1-MPNST PDX and *MTAP* is lost in 4/13 PDX lines.

Results(continued)

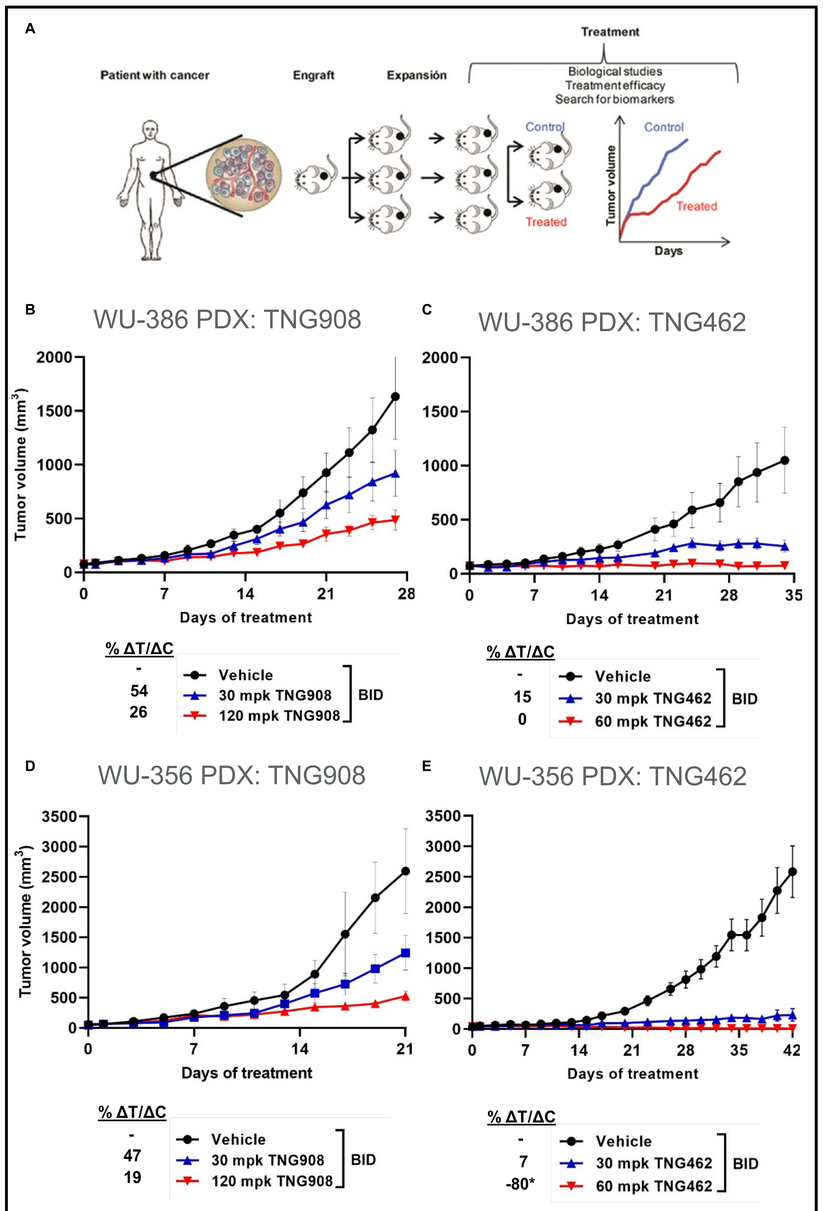


Figure 4. The PRMT5 inhibitors, TNG908 and TNG462, dramatically reduce tumor growth in two MPNST PDX mouse models. A) Diagram of MPNST PDX mouse model propagation and drug treatments. Mice bearing (B-C) WU-386 or (D-E) WU-356 PDX tumors (both NF1-mutated and MTAP-deleted) were treated with TNG908 or TNG462 for 3 to 6 weeks at the indicated doses. The tumor growth curves of different treatment groups are shown as mean +/- SEM. All treatments were well tolerated.

Conclusion

- Homozygous MTAP loss is observed in conjunction with CDKN2A in 25% of MPNST.
- Incubation with the MTA-cooperative PRMT5 inhibitors, TNG908 and TNG462, selectively decreased the proliferation of MTAP-deleted MPNST cell lines relative to MTAP-intact MPNST cell lines.
- TNG908 and TNG462 are clinical stage MTA-cooperative PRMT5 inhibitors with significant anti-tumor activity in two MTAP-deleted MPNST PDX models.
- TNG462 is the first drug in which we have been able to demonstrate tumor regression in MPNST PDX models.
- TNG908 and TNG462 are currently being evaluated in Phase 1/2 studies in MTAP-deleted solid tumors including MPNST.

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