TNG908 is a brain-penetrant, MTA-cooperative PRMT5 inhibitor for the treatment of MTAP-deleted cancer

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Abstract # 233

ABSTRACT

TNG908 is a clinical stage MTA-cooperative PRMT5 inhibitor that leverages the synthetic lethal interaction between PRMT5 inhibition and *MTAP* deletion. TNG908 was discovered using structure-based design following an initial high-throughput screening campaign. TNG908 is 15X selective for MTAP-null cell lines over isogenic MTAP WT cell lines and has marked selectivity for *MTAP*-deleted cancer cell lines independent of histology in a large, diverse cell line panel. Oral administration of TNG908 drives dose-dependent, selective antitumor activity in *MTAP*-deleted xenograft models, including durable tumor regressions in models representing glioblastoma, non-small cell lung cancer (adenocarcinoma and squamous), mesothelioma, cholangiocarcinoma, urothelial carcinoma, and others. TNG908 is brain-penetrant as exposure in the cerebrospinal fluid (CSF) approximates free, unbound plasma exposure in non-human primate studies. These data combined with strong preclinical activity in glioblastoma models strongly position TNG908 as a potential treatment of *MTAP*-deleted glioblastoma or solid tumor CNS metastases. A Phase 1/2 clinical trial (NCT05275478) is currently enrolling to assess safety, tolerability, and efficacy in patients with advanced or metastatic *MTAP*-deleted solid tumors, including non-small cell lung cancer (adenocarcinoma and squamous), mesothelioma, cholangiocarcinoma, malignant peripheral nerve sheath tumor, and in a histology-agnostic cohort. In summary, TNG908 is a clinical stage, potent, brain-penetrant PRMT5 inhibitor with excellent drug-like properties and strong preclinical activity in multiple xenograft models that has the potential for histology-agnostic clinical development in *MTAP*-deleted solid tumors.

MTA-cooperative PRMT5 inhibitors are synthetic lethal with *MTAP* deletion

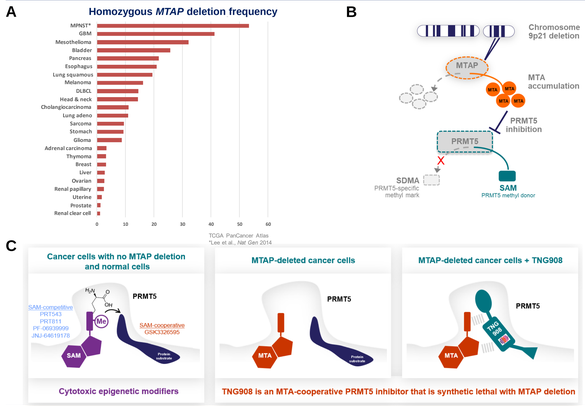


Figure 1: *MTAP* deletion is a common genetic event in human cancer. (A) *MTAP* deletion frequency in a subset of human cancers (Cerami et al 2012; Gao et al 2013; Lee et al 2014). (B) Biological rationale for sensitivity of *MTAP*-deleted cells to PRMT5 perturbation. (C) Differentiating strategy between non-MTA-cooperative PRMT5 inhibitors and TNG908.

MTA-cooperative PRMT5i are efficacious in a cellular admixture and with ≥2X MTA accumulation

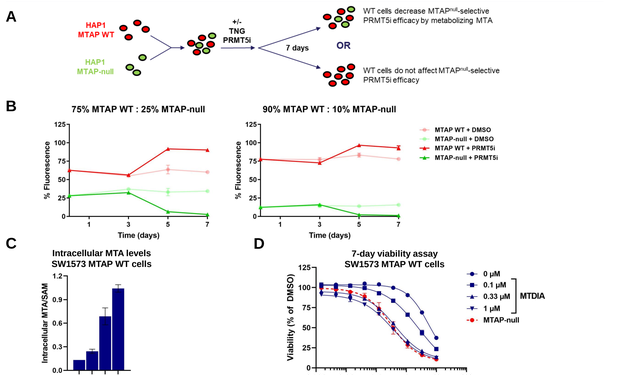


Figure 2: MTA-cooperative PRMT5 inhibitors selectively target *MTAP*-deleted cells in heterogeneous cell populations and with minimal MTA accumulation. (A) Experimental schematic. In brief, HAP1 MTAP WT cells with stable RFP expression were mixed at various ratios with HAP1 MTAP-null cells with stable GFP expression. The admixtures were cultured +/- an MTA-cooperative PRMT5 inhibitor for 7 days and then the RFP:GFP ratio was determined by flow cytometry. (B) Flow cytometry analysis of the results from experiment denoted in (A). n=2 replicates, and data are presented as mean ± SD. (C) LC-MS/MS-based quantification of intracellular MTA in SW1573 MTAP WT cell line treated for 48 hrs with MTDIA, an MTAP inhibitor. (D) 7-day CellTiter-Glo viability assay demonstrating selectivity and efficacy are maintained if MTA levels are increased ~2x, and maximal when MTA levels are increased 5x relative to untreated MTAP WT cell line.

TNG908 is 15X selective for *MTAP*-deleted cancer cells

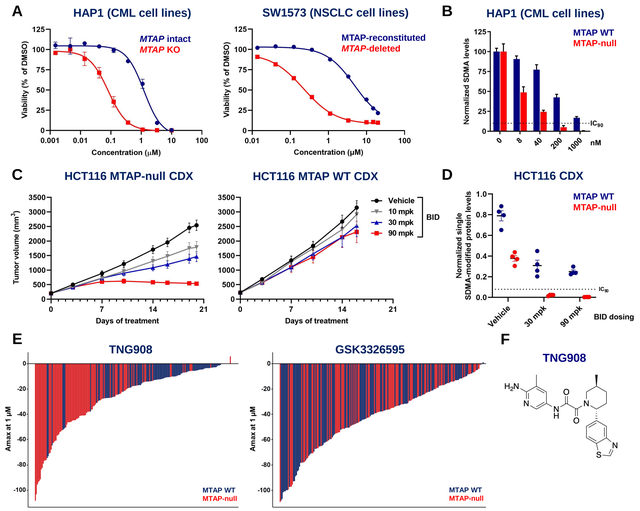


Figure 3: TNG908 is selective for *MTAP*-deleted cells *in vitro* and *in vivo*. (A) Antiproliferative activity of TNG908 in MTAP-isogenic cell lines engineered by either CRISPR-mediated MTAP gene knockout (HAP1) or by reconstituting exogenous MTAP in an endogenous *MTAP*-deleted cell line (SW1573). Data are represented as mean ± SD. (B) Pharmacodynamic activity of TNG908 to inhibit PRMT5 in the HAP1 MTAP-isogenic cell line pair. The data are normalized to a DMSO control for each cell line and presented as mean ± SD. (C) Antitumor activity in HCT116 MTAP-isogenic xenograft models with TNG908 dosed as indicated. n=8 mice per group. Data are represented as mean ± SEM. (D) Terminal pharmacodynamic analysis of tumors from the HCT116 MTAP-isogenic xenograft models dosed with TNG908 in panel (C). A single SDMA-modified substrate was quantified by immunoblot and normalized to a loading control from tumors processed 8 hrs post-last dose. n=4 tumors per group. Data are represented as mean ± SEM. (E) 199 cancer cell lines representing multiple cancer histologies including NSCLC, PDAC, bladder, CNS, and heme malignancies were profiled with either TNG908 or GSK3326595, a non-MTA-cooperative PRMT5 inhibitor, in a 7-day CellTiter-Glo assay. The maximum effect at 1 μM (10X the GI_{50}) for each cell line is reported for each compound, and the cell lines are colored by MTAP status. GSK3326595 and TNG908 are equipotent in MTAP-null cancer cell lines *in vitro*. (F) TNG908.

TNG908 drives strong, histology-agnostic antitumor responses

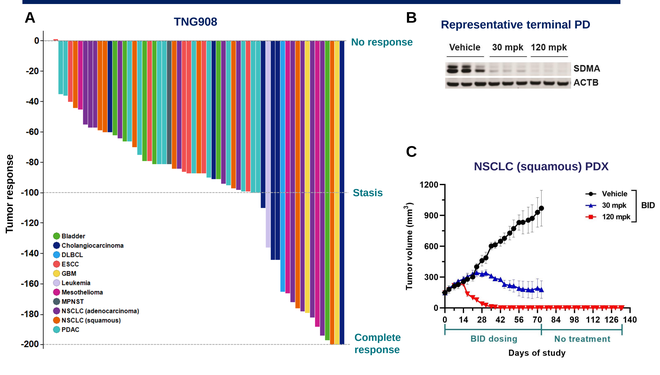
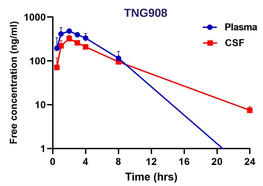


Figure 4: TNG908 is efficacious in *MTAP*-deleted xenograft models across clinically relevant histologies. (A) Waterfall plot demonstrating activity of TNG908 dosed at 120 mpk BID in *MTAP*-deleted CDX and PDX models representing the indicated tumor histologies. -%TGI is reported for tumors with Tumor $Volume_{final} \geq$ Tumor $Volume_{initial}$ (values -100 to 0). %Tumor $Volume_{initial}$ -100 is reported for models with Tumor $Volume_{final} <$ Tumor $Volume_{initial}$ (values -200 to -100). "Stasis" is defined as 100% TGI and "Complete response" is defined as %Tumor $Volume_{initial}$ equal to -100%. (B) Representative terminal PD analysis of a TNG908-treated PDX tumor dosed at the indicated levels BID. Tissue harvested 8 hours post-last dose. (C) NSCLC (squamous) PDX tumor-bearing mice were dosed with TNG908 for the indicated time period, and then the mice that had been treated with 120 mpk BID TNG908 were monitored for 60 days after dosing was ended. In these mice, the tumors did not regrow after the cessation of dosing.

TNG908 is brain-penetrant in non-human primates



	TNG908
plasma unbound $AUC_{0\text{-last}}$ (ng*h/mL)	1846
CSF $AUC_{0\text{-last}}$ (ng*h/mL)	1649
$Kpuu_{brain}$	0.9

Figure 5: TNG908 is brain-penetrant in non-human primates. Following an oral administration of 10 mg/kg TNG908 to cisterna magna ported cynomolgus monkeys (n=3/group), serial samples of cerebrospinal fluid (CSF, a surrogate for free brain concentration) and plasma were collected. TNG908 CSF concentration closely approximated free TNG908 plasma concentration.

TNG908 is selective for *MTAP*-deleted GBM cell lines *in vitro*

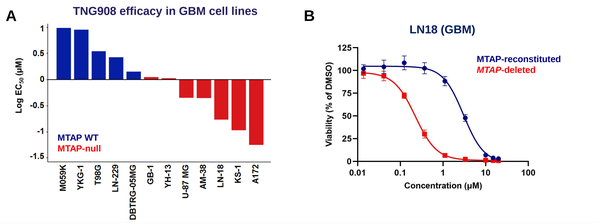


Figure 6: TNG908 is efficacious and selective for MTAP-null cell lines representing glioblastoma *in vitro*. (A) 12 glioblastoma cancer cell lines (5 MTAP WT and 7 MTAP-null) were treated for 7-days with a 9-point dose titration of TNG908 and antiproliferative activity was determined by CellTiter-Glo assay. For each cell line a variable-slope (four parameter) curve was fit and the concentration at which half-maximal efficacy was determined and plotted on the y-axis in (A). (B) Antiproliferative activity of TNG908 in glioblastoma MTAP-isogenic cell line engineered by reconstituting exogenous MTAP in an endogenous *MTAP*-deleted cell line (LN18). Data are represented as mean ± SD.

MTAP deletion and MGMT methylation are independent events and MGMT status does not predict response to TNG908 *in vitro*

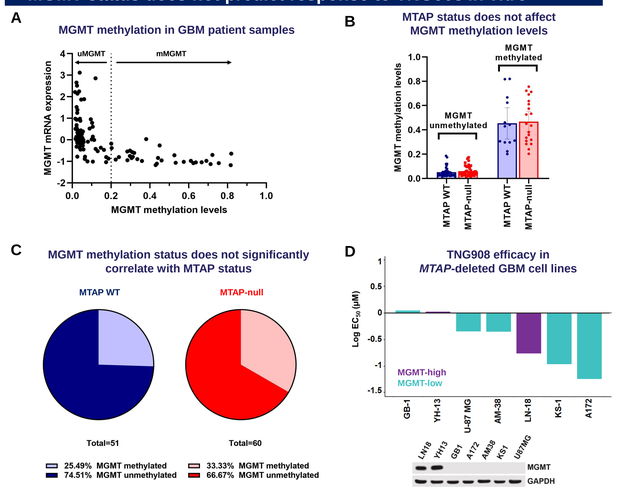


Figure 7: MGMT methylation status does not significantly correlate with MTAP status in GBM patient samples, nor predict response to TNG908 in *MTAP*-deleted GBM cell lines. (A) 111 glioblastoma samples from TCGA Firehouse Legacy were profiled for MGMT methylation (HM27 and HM450) and expression status (z-scores relative to diploid samples; RNA Seq V2 RSEM). MGMT methylation threshold was defined as >0.2 for further analyses. (B) MGMT methylation levels in GBM samples from (A) were segregated by MTAP status. The degree of MGMT methylation is not influenced by MTAP status. (C) MGMT status from GBM samples in (A) were segregated by MTAP status. MGMT methylation status does not significantly correlate with MTAP status. (D) 7-day antiproliferative assay data from *MTAP*-deleted GBM cell lines from Figure 6A were color-coded by MGMT status according to MGMT immunoblot.

TNG908 drives strong antitumor responses in subcutaneous and orthotopic *MTAP*-deleted GBM xenograft models

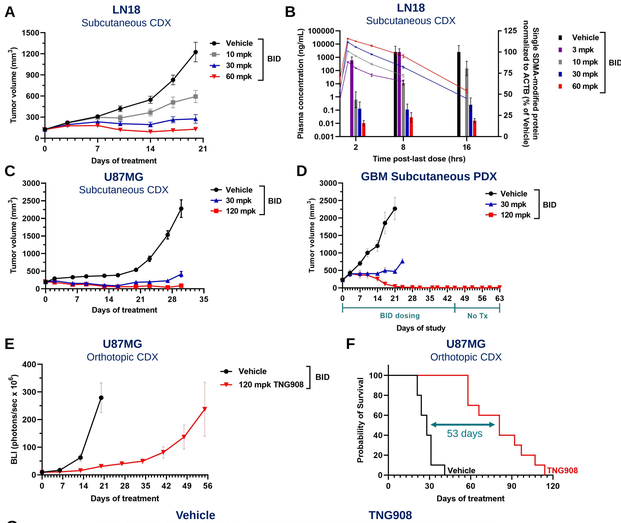


Figure 8: TNG908 is efficacious in subcutaneous and orthotopic *MTAP*-deleted glioblastoma xenograft models. (A and B) Efficacy (A) and activity in a 7-day PK/PD study (B) of TNG908 in the subcutaneous LN18 MTAP-null GBM CDX model. n=8 mice per group. (C and D) Efficacy of TNG908 in the subcutaneous U87MG MTAP-null GBM CDX model (C) or a subcutaneous MTAP-null GBM PDX model (D) n=5 mice per group. The GBM PDX model was dosed BID for the indicated time, and then tumor volumes were monitored for the indicated time period after completion of dosing. 4/5 mice were cured. (E-G) Orthotopic U87MG MTAP-null GBM CDX model either treated with vehicle or 120 mpk BID TNG908 (n=10 mice per group). Of note, rodent TNG908 brain Kpuu ~0.15. Weekly bioluminescent data until the first mouse from the group was lost due to tumor burden (E), overall survival with the 53-day median survival benefit relative to vehicle indicated (F), or selected bioluminescent images at the indicated timepoints (G). Where applicable, data are plotted as mean ± SEM.

SUMMARY

- **MTA-cooperative PRMT5 inhibitors are efficacious in an admixture of MTAP WT and MTAP-null cells and are efficacious and selective with only 2X MTA accumulation**
- **TNG908 demonstrates 15X selectivity for *MTAP*-deleted cells in MTAP-isogenic cell lines representing multiple cancer histologies, and is selective for *MTAP*-deleted cells in a large cancer cell line panel**
- **TNG908 is selective for *MTAP*-deleted tumors *in vivo*, and drives tumor regressions as a single agent in *MTAP*-deleted xenograft models representing clinically relevant tumor histologies**
- **TNG908 is brain-penetrant and efficacious in subcutaneous and orthotopic *MTAP*-deleted GBM xenograft models**
- ***MTAP* deletion does not enrich in either MGMT-methylated or unmethylated GBM patient samples, nor does MGMT status predict response to TNG908 *in vitro***
- **TNG908 is a clinical stage, potent, brain-penetrant PRMT5 inhibitor with excellent drug-like properties and strong preclinical activity in multiple xenograft models that has the potential for histology-agnostic clinical development in *MTAP*-deleted solid tumors including glioblastoma**

ACKNOWLEDGEMENTS and REFERENCES

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