TNG908 is an MTAP^{null}-selective PRMT5 inhibitor that drives tumor regressions in *MTAP*-deleted xenograft models across multiple histologies

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ABSTRACT

TNG908 is an investigational PRMT5 inhibitor with a novel MTA-cooperative binding mechanism designed to leverage the synthetic lethal interaction between PRMT5 inhibition and *MTAP* deletion. *MTAP* deletion occurs in 10-15% of all human cancer representing multiple histologies. MTA is a negative regulator of PRMT5 that accumulates as a result of *MTAP* deletion. TNG908 selectively binds the PRMT5-MTA complex driving selective inhibition of PRMT5 in MTAP-null cancers, which is postulated to create a large therapeutic index relative to PRMT5 inhibitors currently in clinical development. 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 lineage in a large, diverse cell line panel. *In vitro* mechanistic studies confirm that MTAP^{null}-selective PRMT5 inhibitors can selectively target MTAP^{null} cancer cells in either an admixture of MTAP^{null} and MTAP^{WT} cells, or with intracellular MTA accumulation 2-5X relative to basal levels in MTAP^{WT} cells. Oral administration of TNG908 drives dose-dependent, MTAP^{null}-selective antitumor activity in multiple xenograft models, including tumor regressions in models representing glioblastoma, non-small cell lung cancer (adenocarcinoma and squamous), 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. Preclinical studies suggest clinical combinations that leverage PRMT5 biology and/or concurrent oncogenic driver mutations, such as KRAS^{G12C}. In summary, TNG908 is a novel, potent 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

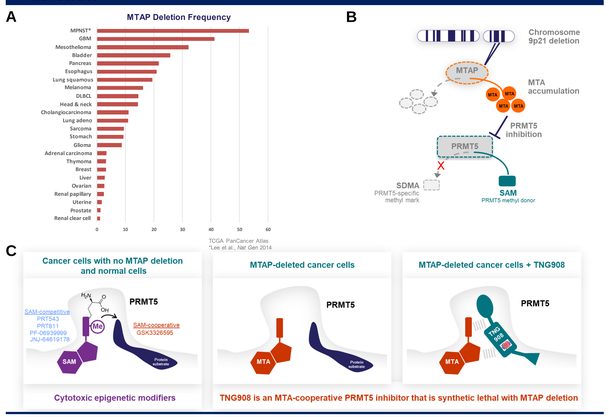


Figure 1: *MTAP*-deletion is a common genetic event in human cancer. (A) *MTAP* deletion frequency in a subset of human cancers (Cerami et al 2012; Gao et al 2013; Lee et al 2014). (B) Biological rationale for sensitivity of MTAP^{null} cells to PRMT5 perturbation. (C) Differentiating strategy between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

MTAP^{null}-selective PRMT5i are efficacious in a cellular admixture, and with minimal MTA

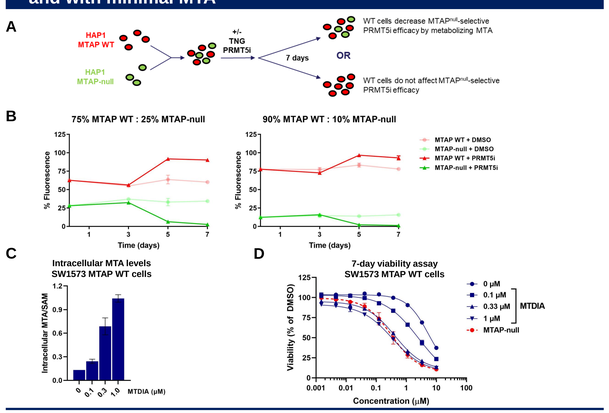
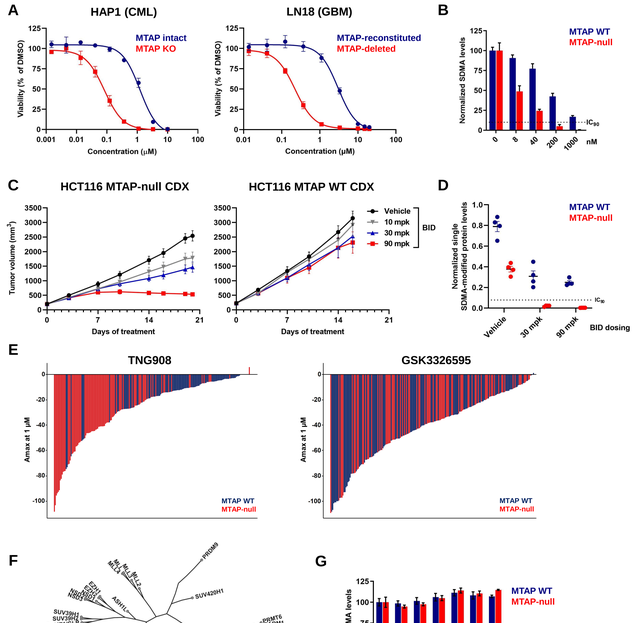


Figure 2: MTAP^{null}-selective PRMT5 inhibitors selectively target MTAP^{null} cells in heterogeneous cell populations and with minimal MTA accumulation. (A) Experimental schematic. In brief, HAP1 MTAP WT cells with stable RFP expression were mixed at various ratios with HAP1 MTAP^{null} cells with stable GFP expression. The admixtures were cultured +/- an MTAP^{null}-selective 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 MTAP^{null}-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 an MTAP^{null}-selective PRMT5 inhibitor



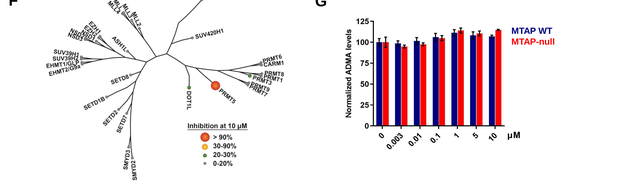
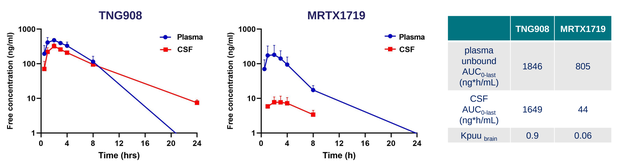


Figure 3: TNG908 is MTAP^{null}-selective *in vitro* and *in vivo*. (A) Antiproliferative activity of TNG908 in MTAP-isogenic cell lines engineered by either CRISPR-mediated MTAP gene knockout (HAP1) or by reconstituting exogenous MTAP in an endogenous MTAP-deleted cell line (LN18). Data are represented 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 lineages including NSCLC, PDAC, bladder, CNS, and heme malignancies were profiled with either TNG908 or GSK3326595, a non-MTAP^{null}-selective 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) Dendrogram demonstrating biochemical selectivity of TNG908 for PRMT5 in a histone methyltransferase panel. (G) Pharmacodynamic activity of TNG908 to inhibit Type I PRMTs 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.

TNG908 is brain-penetrant in non-human primates



	TNG908	MRTX1719
plasma unbound $AUC_{0\text{-last}}$ (ng*h/mL)	1846	805
CSF $AUC_{0\text{-last}}$ (ng*h/mL)	1649	44
$Kpuu_{brain}$	0.9	0.06

Figure 4: TNG908 is brain-penetrant in non-human primates. Following an oral administration of 10 mg/kg TNG908 or MRTX1719 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. MRTX1719 CSF concentration, on the other hand, was approximately 6% of its free plasma concentration.

TNG908 is efficacious in MTAP-null xenograft models across histologies

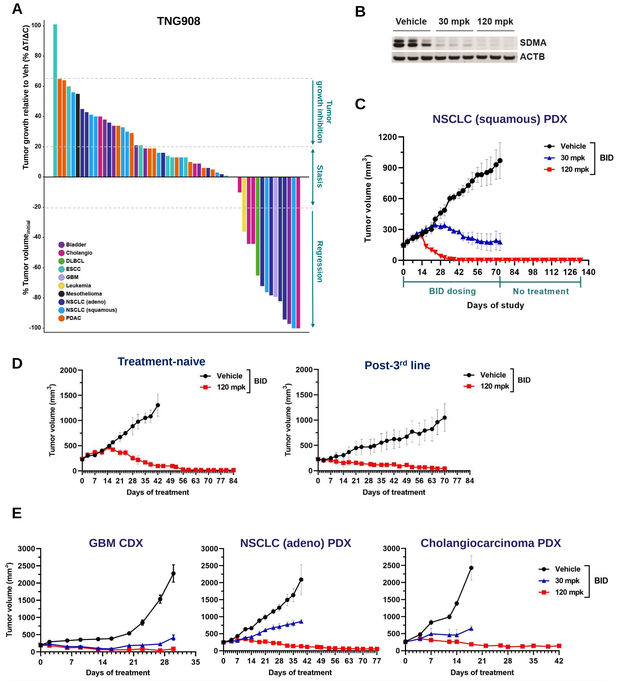


Figure 5: TNG908 is efficacious in MTAP-deleted xenograft models across clinically relevant lineages. (A) Waterfall plot demonstrating activity of TNG908 dosed at 120 mpk BID in MTAP-deleted CDX and PDX models representing the indicated tumor histologies. (B) Representative terminal PD analysis of a 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. (D) TNG908 antitumor activity in NSCLC (adeno) PDX models generated from the same patient either pre-treatment or after three lines of therapy. (E) TNG908 antitumor activity in 2 selected PDX models, and the U-87 MG GBM cell line-derived xenograft model.

TNG908 and KRAS^{G12C} inhibitor combination treatment drives tumor regression *in vivo*

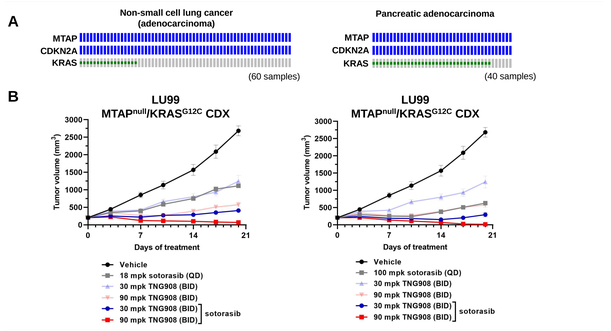


Figure 6: TNG908 and sotorasib combination treatment in an MTAP-null/KRAS^{G12C} NSCLC xenograft model drives tumor regression. (A) TCGA analysis demonstrating co-occurrence of MTAP-deletion and KRAS mutation (Cerami et al 2012 and Gao et al 2013). (B) The LU99 MTAP-null/KRAS^{G12C} mutant NSCLC xenograft model was treated for 21 days with single agent TNG908 or sotorasib, or a combination of TNG908 and sotorasib. Strong single agent TNG908 activity is driven in the LU99 model at 30-120 mpk BID. Sotorasib doses were chosen to be clinically relevant and were adjusted in combination with TNG908 to deliver equivalent exposures to single agent. n=8 mice per group, and data are presented as mean ± SEM.

MAT2A and PRMT5 inhibition are synergistic

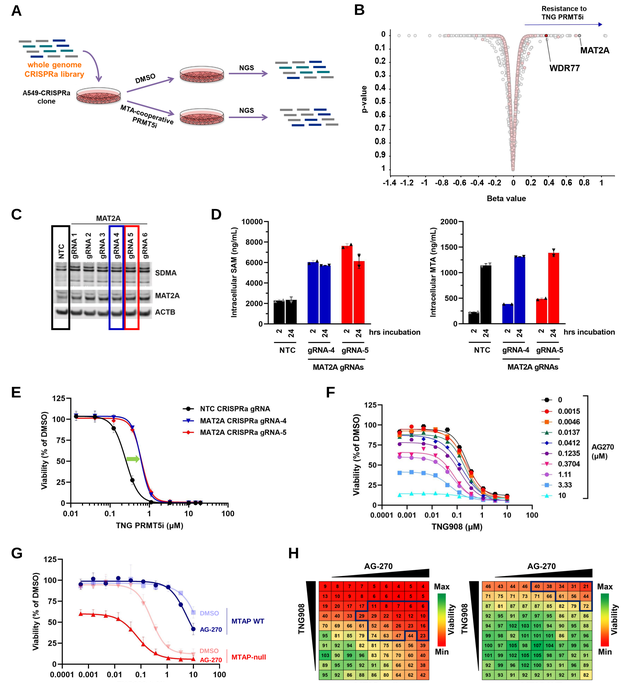


Figure 7: MAT2A and PRMT5 inhibition are synergistic in MTAP-null cells. (A) Experimental schematic. In brief, an A549 MTAP-null cell line clone with the stable expression of CRISPRa components (dCas9 SAM system) was infected with a whole genome library and cultured for 14 days +/- treatment with a 100X MTAP^{null}-selective PRMT5 inhibitor before being subjected to NGS and downstream analysis. (B) CRISPRa gRNA depletion or enrichment in cells treated +/- TNG PRMT5i. MAT2A and WDR77 guides were among the most significantly enriched with TNG PRMT5i treatment. (C-E) The stable A549 dCas9 cell line was transduced with lentiviral CRISPRa guides either encoding a non-targeting control (NTC), or different MAT2A-targeting guides. Immunoblot analysis (C), LC-MS/MS confirmation of intracellular MTA and SAM levels (D), and a 14-day CellTiter-Glo assay conducted with a 100X MTAP^{null}-selective PRMT5i (E). (F) 7-day CellTiter-Glo analysis of MTAP-null LN18 cell line treated with a combination matrix of TNG908 and MAT2A inhibitor, AG-270. (G) Demonstration of MTAP^{null}-selective viability effects of TNG908 + AG-270 combination treatment in a 7-day CellTiter-Glo assay using LN18 MTAP-isogenic cell lines. (H) HSA synergy analysis of 7-day CellTiter-Glo assay using TNG908 + AG-270 combination treatment in LN18 MTAP-isogenic cell lines (G).

SUMMARY

- **MTA-cooperative PRMT5 inhibitors are efficacious in an admixture of MTAP WT and MTAP-null cells, and require minimal MTA accumulation for efficacy and selectivity**
- **TNG908 demonstrates 15X selectivity for MTAP-null cells in MTAP-isogenic cell lines representing different cancer lineages, and is MTAP^{null}-selective in a large cancer cell line panel**
- **TNG908 is MTAP-selective *in vivo*, and drives tumor regressions as a single agent in MTAP-null xenograft models representing multiple tumor histologies**
- **TNG908 is brain-penetrant and efficacious in MTAP-null GBM xenograft models**
- **Treatment of KRAS^{G12C}-mutant lung adenocarcinoma with TNG908 and a KRAS^{G12C} inhibitor may be of clinical benefit in lung cancers with concurrent *MTAP* deletion and KRAS^{G12C} mutation**
- **MAT2A and PRMT5 inhibition are synergistic, and may provide a beneficial therapeutic rationale**

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