Unbiased in vitro and in vivo drug anchor screens identify mechanisms of resistance and sensitization for MTA-cooperative PRMT5 inhibitors in MTAP-deleted cancer models

Steven Lombardo[1,2], Matthew R Tonini[1,2], Lauren Grove[1], Silvia Fenoglio[1], James Tepper[1], Binzhang Shen[1], Zachary Decker[1], Hannah Stowe[1], Shangtao Liu[1], Samuel R Meier[1], Ashley H Choi[1], Tenzing Khendu[1], Yi Yu[1], Kevin M Cottrell[1], John P Maxwell[1], Jannik N Andersen[1], Alan Huang[1], Kimberly J Briggs[1], and Teng Teng[1]

[1]Tango Therapeutics [2]These authors made equal contributions

Abstract # B098

INTRODUCTION

Homozygous deletion of the MTAP gene is one of the most common genetic alterations in cancer, affecting 10-15% of all human cancer. Clinical MTA-cooperative PRMT5 inhibitors, including TNG908 and TNG462, were developed to leverage the well-characterized synthetic lethal interaction between PRMT5 inhibition and MTAP deletion. Indeed, the first disclosed clinical data for an MTA-cooperative PRMT5 inhibitor demonstrated the ability of TNG908 to selectively inhibit PRMT5 in MTAP-deleted tumors while sparing adjacent normal, MTAP-proficient cells in patients. To further explore the mechanism of action of MTA-cooperative PRMT5 inhibitors in MTAP-deleted cells, we employed multiple CRISPR-based editing platforms (CRISPRn, CRISPRi and CRISPRa) to conduct unbiased MTA-cooperative PRMT5 inhibitor anchor screens in vitro and in vivo. A panel of cancer models representing multiple histologies revealed potential PRMT5 inhibitor sensitization and resistance pathways that also include mechanisms that are specific to MTA-cooperative inhibitors. Specifically, the results of these screens identify 1) the effect of MTA/SAM metabolism on MTA-cooperative PRMT5 inhibitors, and 2) identification of potential sub-stratification strategies for patients with MTAP-deleted cancer, and 3) the activity of PRMT5 in anti-apoptotic pathways and synergy between PRMT5 and BCLxL inhibition.

MTA-cooperative PRMT5 inhibitors are synthetic lethal with MTAP deletion

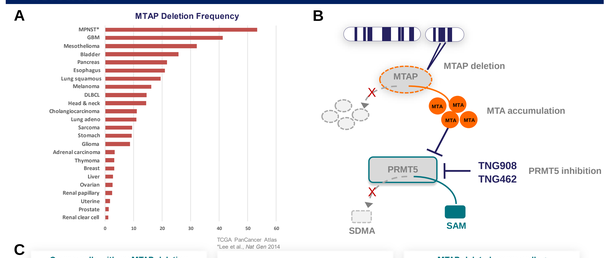
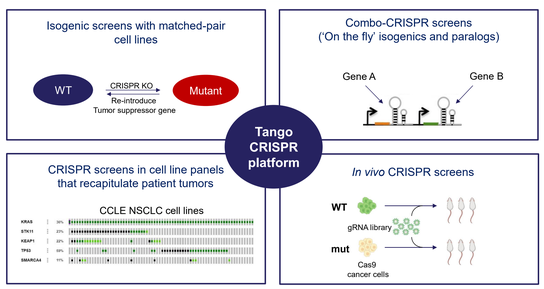


Figure 1: TNG908 and TNG462 are synthetic lethal MTA-cooperative PRMT5 inhibitors. (A) *MTAP* deletion frequency in a subset of human cancers (Cerami et al 2012; Gao et al 2013; Lee et al 2014). (B) Biological rationale for sensitivity of MTAP-deleted cells to PRMT5 perturbation. (C) Differentiating strategy between non-MTA-cooperative PRMT5 inhibitors and TNG908 or TNG462.

Leveraging the Tango CRISPR platform for PRMT5 inhibitor in vitro and in vivo anchor screens



CDX model	Lineage	Format	Library
A549	Lung	*In vitro*	Whole Genome CRISPRn
A549	Lung	*In vitro*	Druggable Genome Paralog Combo CRISPRn
A549	Lung	*In vitro*	Whole Genome CRISPRi
A549	Lung	*In vitro*	Whole Genome CRISPRa
SW1573	Lung	*In vitro*	Whole Genome CRISPRn
LN18	GBM	*In vitro*	Whole Genome CRISPRn
Miapaca2	PDAC	*In vitro*	Whole Genome CRISPRn
RT112/84	Bladder	*In vitro*	Whole Genome CRISPRn
MC38 MTAP KO	Mouse colon	*In vivo syngeneic*	Focused context and target genes CRISPRn
A549	Lung	*In vivo (CRISPR StAR)*	Tumor Suppressor/Collateral Damage genes CRISPRn
SW1573	Lung	*In vivo (CRISPR StAR)*	Tumor Suppressor/Collateral Damage genes CRISPRn

Figure 2: Leveraging Tango CRISPR platform for PRMT5 inhibitor anchor screens. Top: Scheme representing Tango CRISPR platforms for target discovery and translational biology. Bottom: Summary table of completed MTA-cooperative PRMT5 inhibitor anchor screens including CRISPR nuclease (CRISPRn) platform for gene knockout, CRISPR interference (CRISPRi) platform for gene knockdown and CRISPR activation (CRISPRa) platform for gene upregulation.

BCLxL and PRMT5 inhibition are synergistic

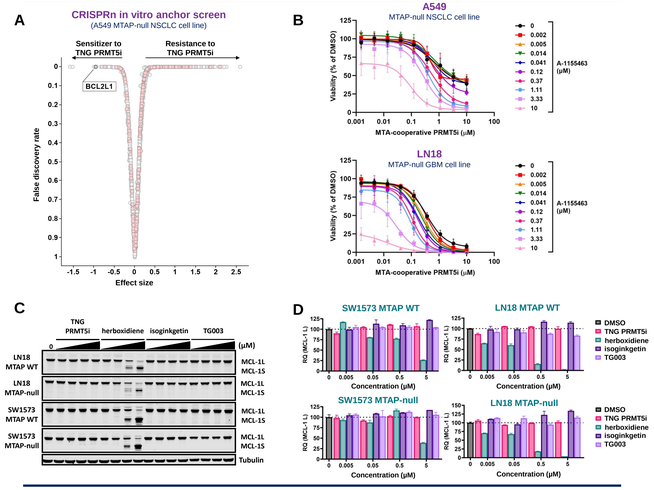


Figure 3: MTA-cooperative PRMT5 and BCLxL inhibitors are synergistic in MTAP-null cells in an MCL1-independent manner. (A) CRISPRn gRNA depletion or enrichment in A549 cells treated +/- an MTA-cooperative PRMT5 inhibitor tool molecule (TNG PRMT5i). sgRNAs targeting BCLxL (encoded by BCL2L1) were among the most significantly depleted with TNG PRMT5i treatment. (B) In vitro demonstration of synergy between an MTA-cooperative PRMT5i tool molecule and a selective BCLxL inhibitor, A-1155463, in LN18 and A549 cell lines. (C) Immunoblot analysis of MCL1 protein levels in LN18 or SW1573 MTAP-isogenic cell lines (generated by expression of an exogenous MTAP cDNA in MTAP-deleted parental cell lines) treated with either an MTA-cooperative PRMT5i or the indicated splicing inhibitors. (D) Real-time PCR quantification of MCL1 isoform levels in LN18 or SW1573 MTAP-isogenic cell lines used in (C) treated with either an MTA-cooperative PRMT5i tool molecule or the indicated splicing inhibitors.

PTEN loss may sensitize MTAP-deleted glioblastoma to TNG908

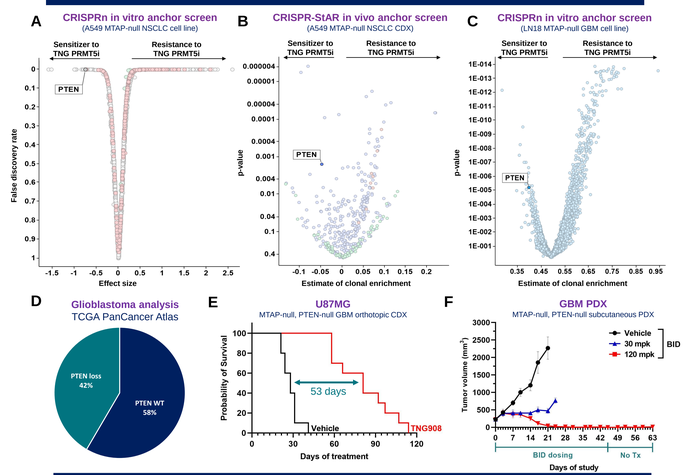


Figure 4: PTEN loss is common in glioblastoma and may sensitize MTAP-deleted tumors to TNG908. (A) CRISPRn gRNA depletion or enrichment in A549 MTAP-null cells treated +/- an MTA-cooperative PRMT5i in vitro tool molecule (TNG PRMT5i) in vitro. PTEN was among the most significantly depleted with TNG PRMT5i treatment. (B) CRISPRn gRNA depletion or enrichment in A549 MTAP-null cells treated +/- an MTA-cooperative PRMT5i in vivo tool molecule (TNG PRMT5i) in vivo with the CRISPR-StAR system. PTEN was among the most significantly depleted with TNG PRMT5i treatment. (C) CRISPRn gRNA depletion or enrichment in LN18 MTAP-null GBM cells treated +/- an MTA-cooperative PRMT5i in vitro tool molecule (TNG PRMT5i) in vitro. PTEN was among the most significantly depleted with TNG PRMT5i treatment. (D) Concurrent MTAP-deletion and PTEN loss in 166 MTAP-deleted glioblastoma samples (Cerami et al., 2012 and Gao et al., 2013). Loss is defined as putative driver mutations or homozygous deletion. (E) Overall survival determined in an 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. (F) Efficacy of TNG908 in 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.

Positive regulators of polyamine metabolism pathway modulate cellular response to PRMT5 inhibition

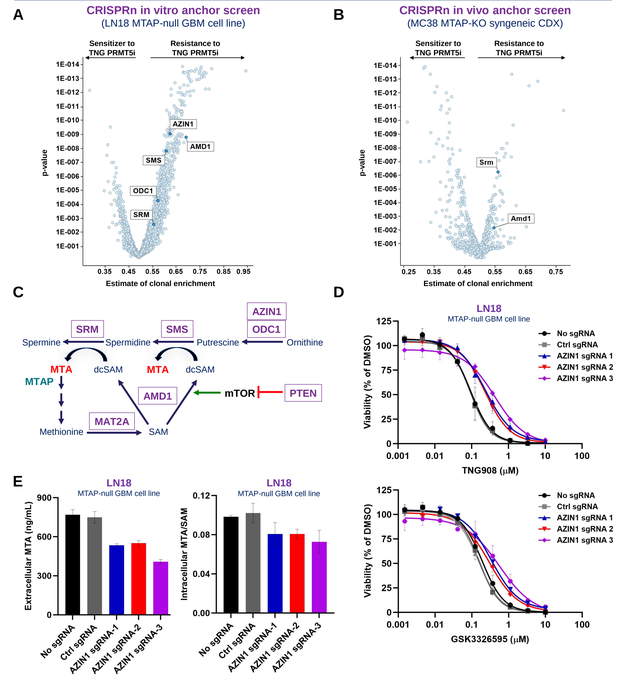


Figure 5: Positive regulators of polyamine metabolism are potential resistance mechanisms for PRMT5 inhibitors. (A) CRISPRn gRNA depletion or enrichment in LN18 MTAP-null cells treated +/- an MTA-cooperative PRMT5i in vitro tool molecule (TNG PRMT5i). Positive regulators of polyamine metabolism pathway such as AZIN1, AMD1, SMS, ODC1 and SRM were among the most significantly enriched with TNG PRMT5i treatment. (B) CRISPRn gRNA depletion or enrichment in MC38 MTAP-KO syngeneic xenograft model treated +/- an MTA-cooperative PRMT5i in vivo tool molecule (TNG PRMT5i) in vivo. Positive regulators of polyamine metabolism pathway such as Amd1 and Srm were among the most significantly enriched with TNG PRMT5i treatment. (C) Pathway illustration of polyamine metabolism process and the potential connection to PTEN//mTOR pathway (Adapted from Casero et al., Nat Rev Cancer 2018 and Zabala-Letona, A., Nature 2017). Anchor screen hits are designated by purple boxes. (D) In vitro 7-day viability assay of LN18 cell line with or without AZIN1 KO in response to TNG908 or GSK3326595, a SAM-cooperative PRMT5i. (E) LC-MS/MS confirmation of extracellular or intracellular MTA levels in LN18 cell line with or without AZIN1 KO. Extracellular MTA levels are normalized to cell number. Intracellular MTA levels are normalized to intracellular SAM levels. (F) In vitro 7-day viability assay of putrescine in combination with TNG908 or GSK3326595 in the LN18 cell line. (G) In vitro 7-day viability assay in the HAP1 MTAP-isogenic cell line pair with treatment of TNG908 +/- putrescine. (H) Immunoblot analysis of HAP1 MTAP WT cancer cell lines treated with a combination of putrescine and TNG908 for 72hrs. At doses where synergy was observed in 7-day CTG assays, SDMA levels were unchanged by putrescine addition suggesting that MTA may not be the only alteration driving the synergy or decreased potency shown in (D).

MAT2A and PRMT5 inhibition are synergistic

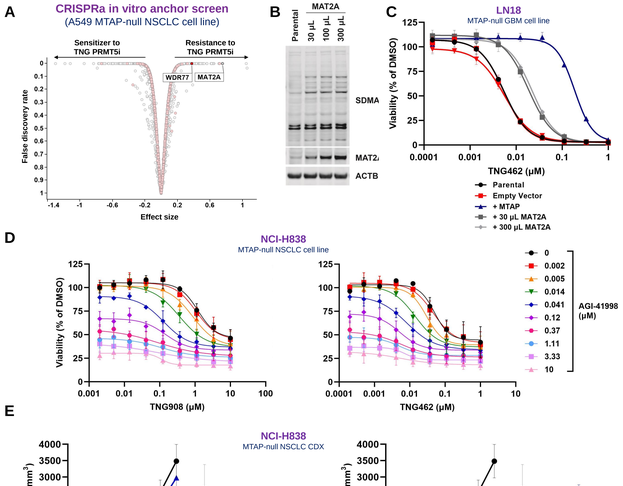


Figure 6: MTA-cooperative PRMT5 and MAT2A inhibitors are synergistic in MTAP-null cells. (A) CRISPRa whole genome gRNA depletion or enrichment in a A549 MTAP-null cell line clone with stable expression of CRISPRa components (dCas9 SAM system) cells treated for 14 days +/- an MTA-cooperative PRMT5i tool compound. MAT2A and WDR77 guides were among the most significantly enriched with TNG PRMT5i treatment (B) Immunoblot analysis of LN18 MTAP-null cancer cell lines engineered to express either an empty vector control, or different levels of MAT2A cDNA. (C) In vitro 7-day viability assay of LN18 cell line in response to TNG462. (D) In vitro demonstration of synergy between TNG908 or TNG462 and a MAT2A inhibitor, AGI-41998, in the NCI-H838 MTAP-null NSCLC cell line. (E) In vivo demonstration of synergy between TNG462 and a MAT2A inhibitor, AGI-41998, in the NCI-H838 MTAP-null NSCLC CDX model.

SUMMARY

- **Unbiased in vitro and in vivo MTA-cooperative PRMT5 inhibitor anchor screens were conducted using Tango's CRISPR platform to identify potential predictors of sensitivity or resistance to TNG908 and TNG462**
  1. **BCLxL inhibition is synergistic with TNG MTA-cooperative PRMT5 inhibitors**
  2. **PTEN loss may sensitize MTAP-deleted tumors to TNG MTA-cooperative PRMT5 inhibitors**
  3. **Polyamine metabolism pathway affects cellular response to PRMT5 inhibition**
  4. **MAT2A and MTA-cooperative PRMT5 inhibition are synergistic, but PRMT5 inhibitor as a single agent, at well-tolerated doses, is sufficient to achieve maximum efficacy**
- **TNG908 (NCT05275478) and TNG462 (NCT05732831) are actively enrolling MTAP-deleted patients in Phase 1/2 clinical trials**

ACKNOWLEDGEMENTS and REFERENCES

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Visit Tango posters B018 to learn more about modulation of RNA splicing by TNG908, C048 for more on the CRISPR-StAR in vivo screening platform, and B054 to learn about TNG348, a USP1 inhibitor