Biochemical characterization of TNG908 as a novel, potent MTA-cooperative PRMT5 inhibitor for the treatment of MTAP-deleted cancers

Abstract # 75

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ABSTRACT

TNG908 is a clinical stage MTA-cooperative PRMT5 inhibitor that leverages the synthetic lethal interaction between PRMT5 inhibition and *MTAP* deletion. PRMT5 is a type II arginine methyltransferase that regulates multiple essential cellular functions via symmetric dimethylation of arginine in target proteins. SAM is an essential co-factor for PRMT5, serving as the methyl donor when bound to a PRMT5-substrate protein complex. MTA is structurally similar to SAM but lacks the amino-carboxy terminus, therefore functions as an intrinsic inhibitor of PRMT5 when bound to a PRMT5-substrate protein complex. MTA is rapidly metabolized by MTAP in normal cells but accumulates in MTAP-deleted cancer cells to levels 10-100 times greater than MTAP WT cells. MTA-PRMT5 complexes are the predominant form in MTAP-deleted cancer cells and present a unique and selective precision oncology target. We have discovered small molecules that exhibit MTA-cooperative PRMT5 binding and selectively kill MTAP-deleted cancer cells compared to MTAP WT cells. Here we present biochemical and orthogonal binding assay data to demonstrate that our clinical candidate, TNG908, is a potent, reversible, peptide substrate competitive inhibitor with a novel MTA-cooperative binding mechanism that binds selectively to the MTA-PRMT5 complex, with 15X selectivity for MTAP-deleted cell lines vs isogenic MTAP WT cell lines. We further propose a working model describing the mechanism of inhibition by MTA-cooperative binding of PRMT5 by TNG908.

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

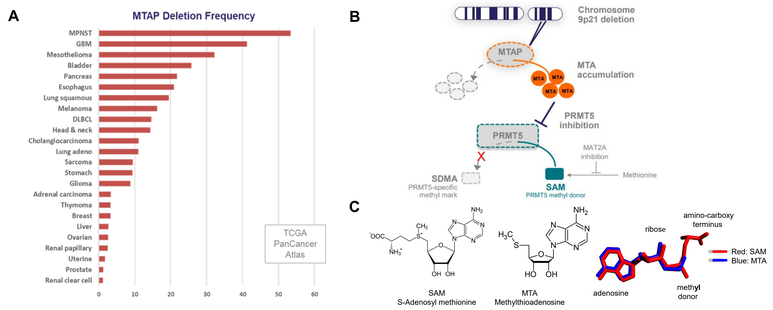


Figure 1: *MTAP*-deletion is a common genetic event in human cancer. (A) *MTAP* deletion frequency in a subset of human cancers (Lee et al, 2014). (B) Biological rationale for sensitivity of MTAP^{null} cells to PRMT5 perturbation. (C) SAM is a cofactor and MTA is an inhibitor of PRMT5. (D) Crystal structure of PRMT5:MEP50 + MTA + TNG908. (E) TNG908 is an MTA cooperative PRMT5 inhibitor that can differentiate from non-MTAP^{null}-selective PRMT5 inhibitors.

PRMT5 catalyzed reaction and assays used to characterize TNG908

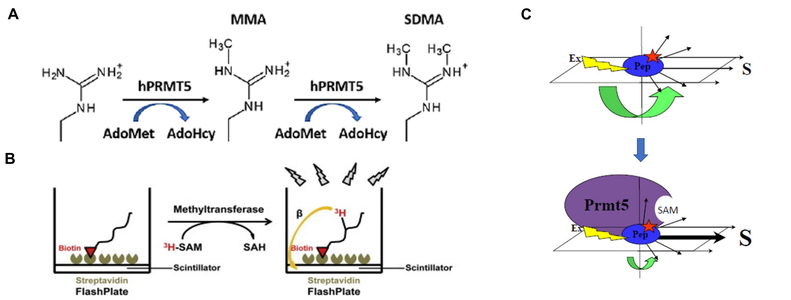
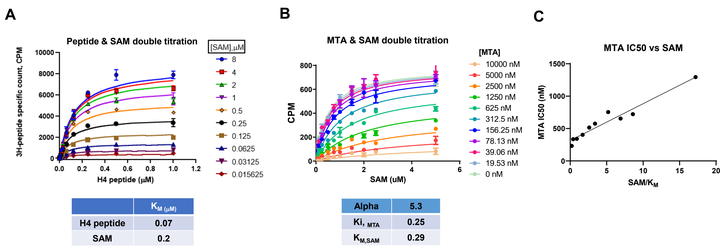


Figure 2: PRMT5 catalyzed reaction and assays. (A) PRMT5 catalyzed SDMA reaction (Eddershaw et al 2020). (B) Biochemical FlashPlate assay using ^{3}H-SAM and biotinylated H4 peptide substrate. (C) Binding FP assay using a fluorophore labeled H4 peptide.

Steady state K_M measurement and MTA as a SAM competitive inhibitor of PRMT5

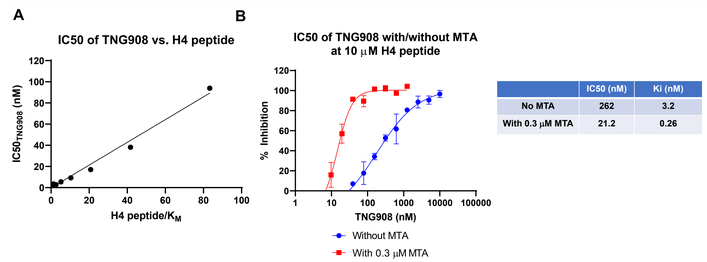


	K_M (uM)
H4 peptide	0.07
SAM	0.2

Alpha	5.3
Ki, $_{MTA}$	0.25
$K_{M,SAM}$	0.29

Figure 3: (A) Steady state K_M of H4 peptide and SAM. (B) MTA is a SAM competitive inhibitor by MTA/SAM double titration. (C) IC_{50} of MTA increase with increase of SAM concentration.

TNG908 competes with H4 peptide and displays enhanced inhibition of PRMT5 in the presence of MTA

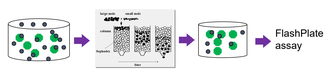


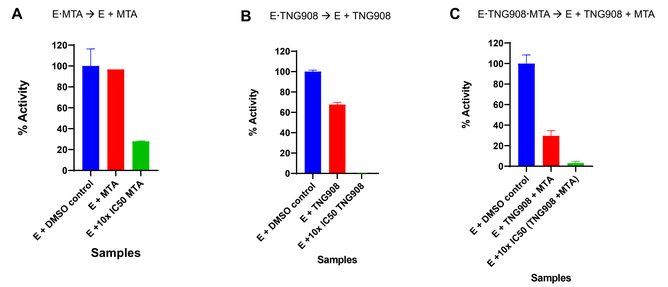
	IC50 (nM)	Ki (nM)
No MTA	262	3.2
With 0.3 μM MTA	21.2	0.26

Figure 4: (A) TNG908 is a H4 peptide competitive inhibitor of PRMT5. (B) TNG908 inhibits PRMT5 and shows enhanced potency with MTA-PRMT5 complex (average of N=3).

PRMT5 activity recovery assay demonstrates enhanced TNG908 binding to MTA-PRMT5 complex

- PRMT5:inhibitor complexes were pre-formed by incubation of PRMT5 with saturating MTA, TNG908 or TNG908 + MTA
- PRMT5:inhibitor complex was then separated from the excess inhibitor by a size exclusion column
- The amount of free enzyme recovered over time from E·I complex was monitored to assess reversibility from inhibition



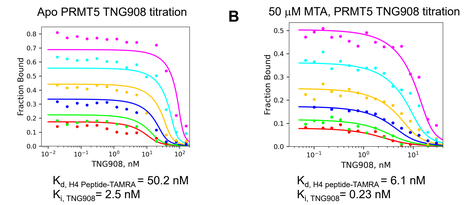


Estimated K_d of inhibitory species were calculated using quadratic equation based upon the percentage of recovered PRMT5 activity and are in line with those from biochemical and orthogonal FP assays

	% activity recovered	Kd (nM) calculated
MTA	>98%	300
TNG908	68%	7.5
TNG908 + MTA	30%	0.65

Figure 5: (A) PRMT5 activity recovery from PRMT5:MTA binary complex. (B) PRMT5 activity recovery from PRMT5:TNG908 binary complex.(C) PRMT5 activity recovery from PRMT5:TNG908:MTA ternary complex.

Presence of MTA induces cooperative binding of TNG908 and H4 peptide substrate to PRMT5



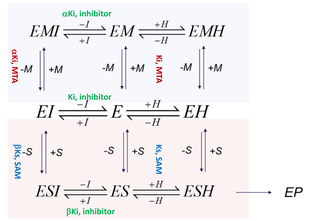
$K_{d,\ H4\ Peptide\text{-}TAMRA}$ = 50.2 nM
$K_{i,\ TNG908}$ = 2.5 nM

$K_{d,\ H4\ peptide\text{-}TAMRA}$ = 6.1 nM
$K_{i,\ TNG908}$ = 0.23 nM

	Without MTA	With MTA	Fold
$K_{d,TNG908}$ (nM)	2.5	0.23	10.8
$K_{d,H4\ peptide}$ (nM)	50.2	6.1	8.2

Figure 6: (A) TNG908 (0-500 nM) and PRMT5 (110, 66.6, 44.4, 29.6, 17.7 and 13.2 nM) double titration at 25 nM H4 peptide without MTA. (B) TNG908 (0-100 nM) and PRMT5 (18.8, 12.5, 8.3, 5.6, 3.7 and 2.5 nM) double titration at 25 nM H4 peptide with MTA (representative data of N=3).

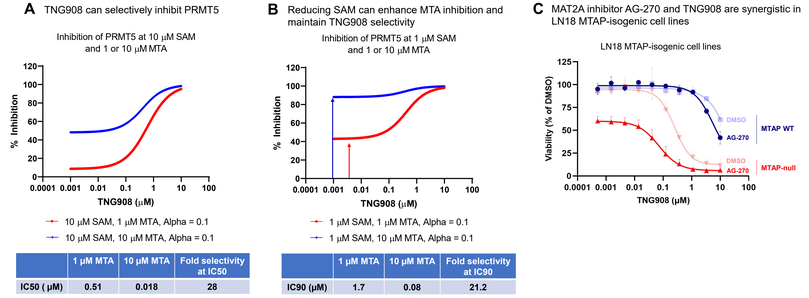
Proposed mechanistic model for cooperative binding between TNG908 and MTA



- M: MTA
- H: H4 peptide
- S: SAM
- I Inhibitor
- P: Product

Figure 7: Working mechanistic model for cooperative binding between TNG908 and MTA. α: Inhibitor:MTA cooperativity. β: Inhibitor:SAM cooperativity.

Simulation of TNG908 inhibition of PRMT5:MTA complex demonstrates importance of MTA:SAM ratio to potency and MTAP-null selectivity



	1 μM MTA	10 μM MTA	Fold selectivity at IC50
IC50 (μM)	0.51	0.018	28

	1 μM MTA	10 μM MTA	Fold selectivity at IC90
IC90 (μM)	1.7	0.08	21.2

Figure 8: Simulation of TNG908 inhibition under different SAM and MTA concentrations using experimentally determined parameters, assuming β =1. (A) TNG908 can selectively inhibit PRMT5 under specified conditions. (B) Reducing SAM concentration increases MTA occupancy in PRMT5, raising basal inhibition level, and maintainsTNG908 selectivity at IC90 concentration. (C) 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 (Briggs et al 2022; Konteatis et al 2021).

Summary

- **Biochemical and binding studies demonstrated that TNG908 is a potent PRMT5 inhibitor with MTA cooperative binding**
- **Mechanistic model and simulation support increased potency of TNG908 selectively in MTAP-deleted cells relative to MTAP WT cells**
- **The simulation also support the cellular data that an MAT2A inhibitor would be synergistic with TNG908 in MTAP null cancers**

Reference

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William Lee et al (2014), Nat. Genetics, 46, 1227-1232. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors.
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