Evaluation of the impact of homozygous MTAP truncations on the activity and selectivity of MTA-cooperative PRMT5 inhibitors

Abstract #4631

Matthew R. Tonini, Andre Mignault, Douglas A. Whittington, Steven A. Lombardo, Binzhang Shen, Hannah Stowe, Samuel R. Meier, Hongxiang Zhang, Satoshi Yoda, Shangtao Liu, Brian Doyon, Isabella Ribeiro, Wenhai Zhang, Minjie Zhang, Kevin M. Cottrell, Heidi Rego, Jennifer Morawiak, Ellen Hooper, Yi Yu, Heather DiBenedetto, Adam S. Crystal, Teng Teng, and Kimberly J. Briggs

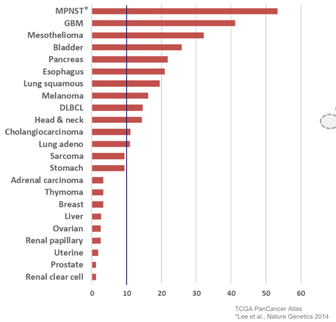
Introduction

Homozygous deletion of the MTAP gene occurs in 10-15% of all human cancers. To benefit this large and diverse patient population, MTA-cooperative PRMT5 inhibitors, including TNG908 and TNG462, have been developed to leverage the synthetic lethal relationship between MTAP deletion and PRMT5 inhibition. MTA-cooperative PRMT5 inhibitors selectively bind the PRMT5·MTA complex driving selective inhibition of PRMT5 in MTAP-deleted cancers while sparing normal, MTAP-proficient cells. Our PRMT5 inhibitors are currently in Phase I/II clinical trials (NCT05275478 and NCT05732831), and eligibility is restricted to patients with tumors with confirmed MTAP loss either detected by next-generation sequencing (NGS) or immunohistochemistry.

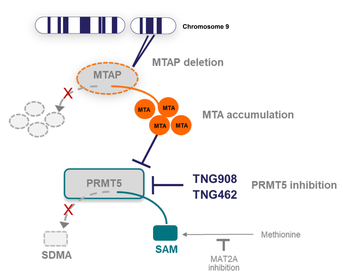
MTAP gene loss occurs in cancers because of its chromosomal proximity to one of the most common genetically altered tumor suppressor genes, CDKN2A, but the chromosomal 9p breakpoints for the co-deletion are not uniform. Indeed, while clinical NGS testing and preclinical data confirm that homozygous intragenic MTAP breakpoints occur, the functional consequence of any given breakpoint on MTAP enzymatic activity and protein function remains unknown. Given the potential implications for homozygous intragenic MTAP deletions to impact the clinical response to MTA-cooperative PRMT5 inhibitors, we have started to evaluate the loss-of-function phenotype of various MTAP truncations to determine whether they retain MTAP activity. Here, we present our initial functional genomics analysis of this important diagnostic biomarker using in vitro cDNA reconstitution approaches for MTAP activity combined with analysis of PRMT5 inhibitor sensitivity. Ultimately, these data may help refine patient enrollment on clinical trials to drive the maximum benefit for patients with MTAP-deleted cancers.

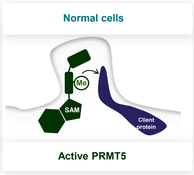
MTA-cooperative PRMT5 inhibitors are synthetic lethal with MTAP deletion

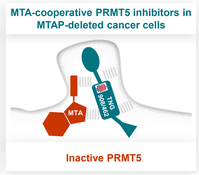
Homozygous MTAP deletion frequency



TCGA PanCancer Atlas
*Lee et al., Nature Genetics 2014

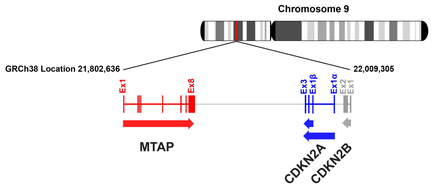






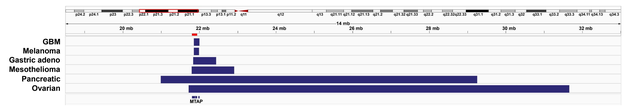
MTA-cooperative PRMT5i in clinical development	Clinical stage
TNG908	Ph1/2
TNG462	Ph1/2
AMG 193	Ph1/2
MRTX1719	Ph1/2
AZD3470	Ph1/2

"Tail-to-tail" orientation of MTAP and CDKN2A genes on chr9p

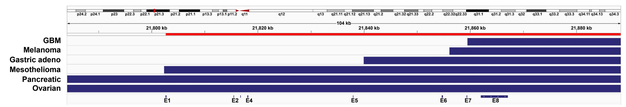


Chr9p breakpoints may cause intragenic MTAP deletions

Chr9p breakpoints are variable in tumors representing multiple histologies

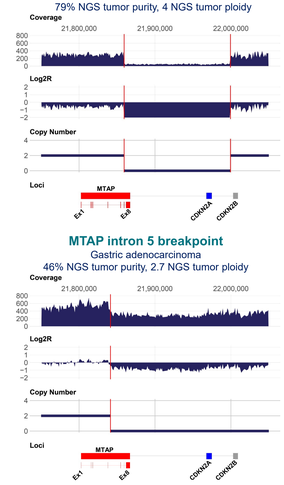


Intragenic MTAP breakpoints identified



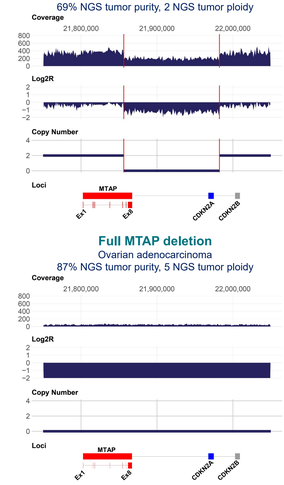
MTAP intron 7 breakpoint

Glioblastoma
79% NGS tumor purity, 4 NGS tumor ploidy



MTAP intron 6 breakpoint

Cutaneous melanoma
69% NGS tumor purity, 2 NGS tumor ploidy



MTAP intron 5 breakpoint

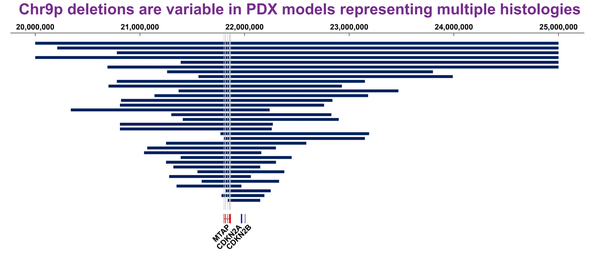
Gastric adenocarcinoma
46% NGS tumor purity, 2.7 NGS tumor ploidy

Full MTAP deletion

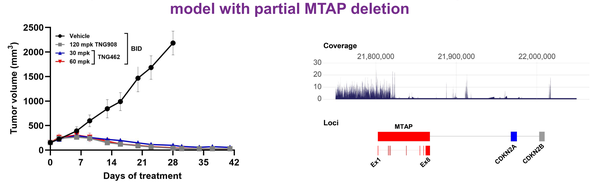
Ovarian adenocarcinoma
87% NGS tumor purity, 5 NGS tumor ploidy

PDX model with partial MTAP loss responds to MTA-cooperative PRMT5 inhibitors

Chr9p deletions are variable in PDX models representing multiple histologies



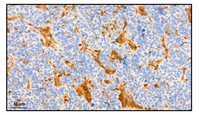
MTA-cooperative PRMT5 inhibitors drive near complete response in PDX model with partial MTAP deletion



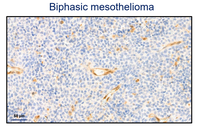
Loss of exon 8 may not be sufficient for loss of MTAP protein

MTAP immunohistochemistry of tumors

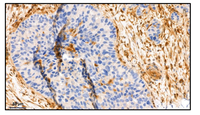
Full MTAP loss
Ovarian adenocarcinoma



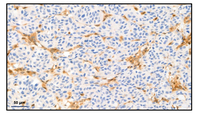
Full MTAP loss
Biphasic mesothelioma



MTAP loss exons 6-8
Gastric adenocarcinoma

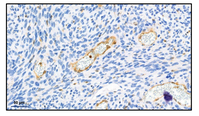


MTAP loss exons 7-8
Cutaneous melanoma

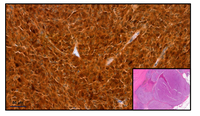


MTAP loss exon 8

Glioblastoma

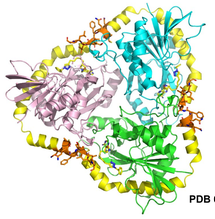


Cutaneous melanoma



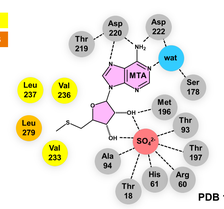
Loss of exons 7 and 8 is predicted to significantly impact MTAP activity

Loss of exons 7-8 likely impacts MTAP active site structure



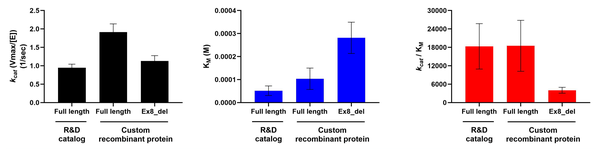
exon 7: Val231-Lys271
exon 8: Asn272-His283

PDB 6DZ2



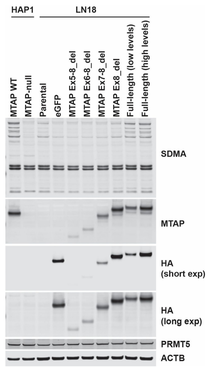
PDB 1CG6

Loss of exon 8 reduces MTAP catalytic efficiency



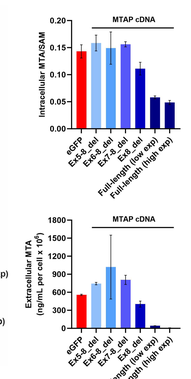
Loss of exon 8 significantly impacts MTAP activity in cells

Immunoblots



MTA levels

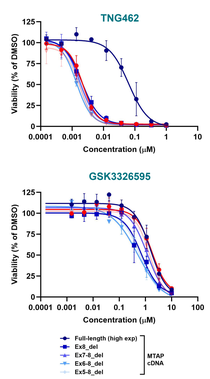
LN18 cell lines



7-day viability assays

LN18 cell lines

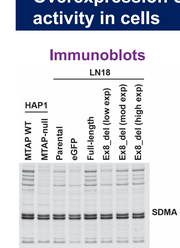
TNG462



GSK3326595

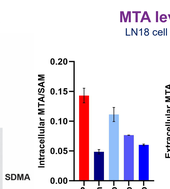
Overexpression of exon 8-deleted MTAP protein partially rescues activity in cells

Immunoblots



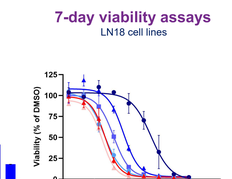
MTA levels

LN18 cell lines



7-day viability assays

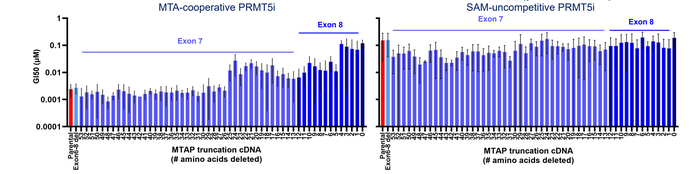
LN18 cell lines



Cellular screen suggests p.I258* is sufficient to ablate MTAP activity

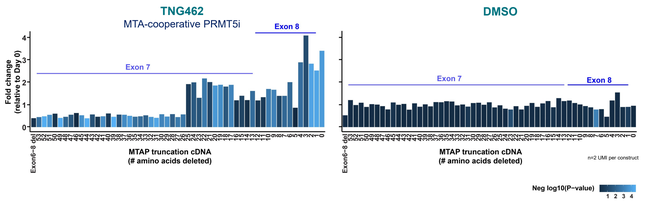
7-day viability assay (CellTiter-Glo)

LN18 cell lines



14-day enrichment assay (UMI barcodes)

LN18 cell lines



Summary

- Loss of CDKN2A on chr9p is caused by deletions of variable size that frequently include MTAP
- Chr9p breakpoints can be located within the MTAP gene causing partial gene loss
- Homozygous loss of exon 8 impairs but does not ablate MTAP activity in preclinical studies
- Preliminary results suggest that partial loss of exons 7 and 8, with or without loss of additional exons, is sufficient to ablate MTAP activity
- Data suggest that clinical NGS providers should report exon-level detail when calling homozygous MTAP deletions to improve patient outcomes on MTA-cooperative PRMT5 inhibitor clinical trials

Acknowledgements

The authors gratefully acknowledge the generous contributions from their colleagues at Tango Therapeutics and from the scientific teams at BostonGene, CellCarta, FMI, Biortus, ChemPartner, Champions Oncology, Crown Biosciences, Enamine, Pharmaron, WuXi AppTec, and XenoSTART

References

Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401-4.

Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):pl1.

Lee W, Teckie S, Wiesner T, et al. PRC2 is recurrently inactivated through EED or SUZ12 loss in malignant peripheral nerve sheath tumors. Nat Genet. 2014; 46(11):1227-32.