Targeting MAT2A in MTAP-deleted Cancers

Presented at the American Association for Cancer Research (AACR) Annual Meeting, April 14-18, 2018, Chicago, IL, USA

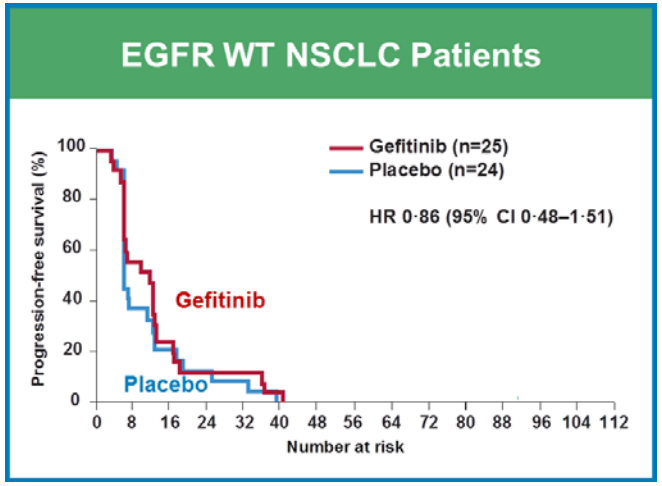
Acknowledgements

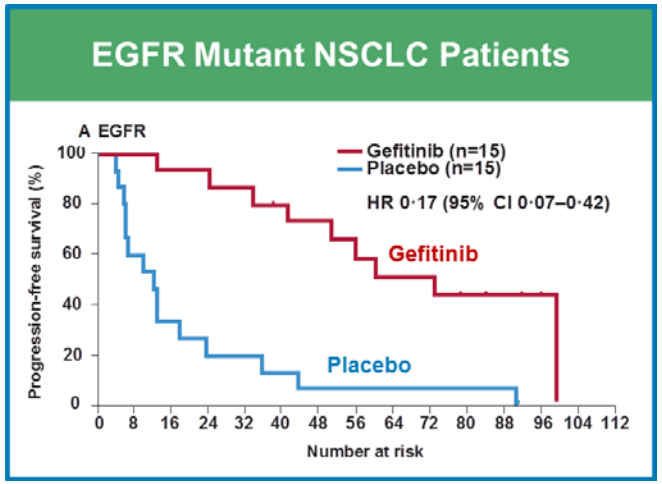
Agios 2017 Founders Day Retreat

"Hire great people, think big, have fun, follow the science and do what's right for patients"

The Challenge: Identifying Precision Medicine Approaches in Cancer Metabolism

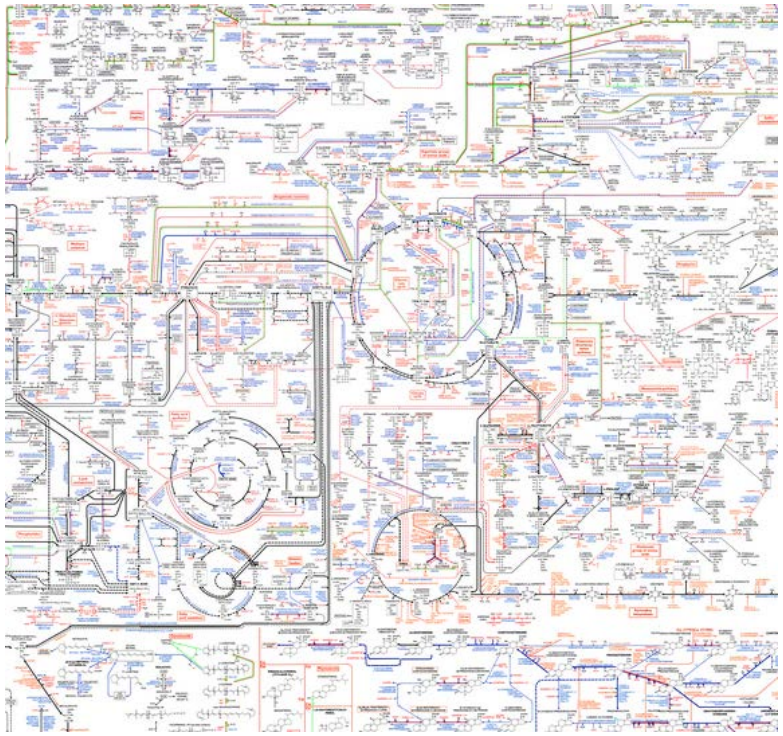
Directly drugging 'driver mutations' has yielded transformative medicines





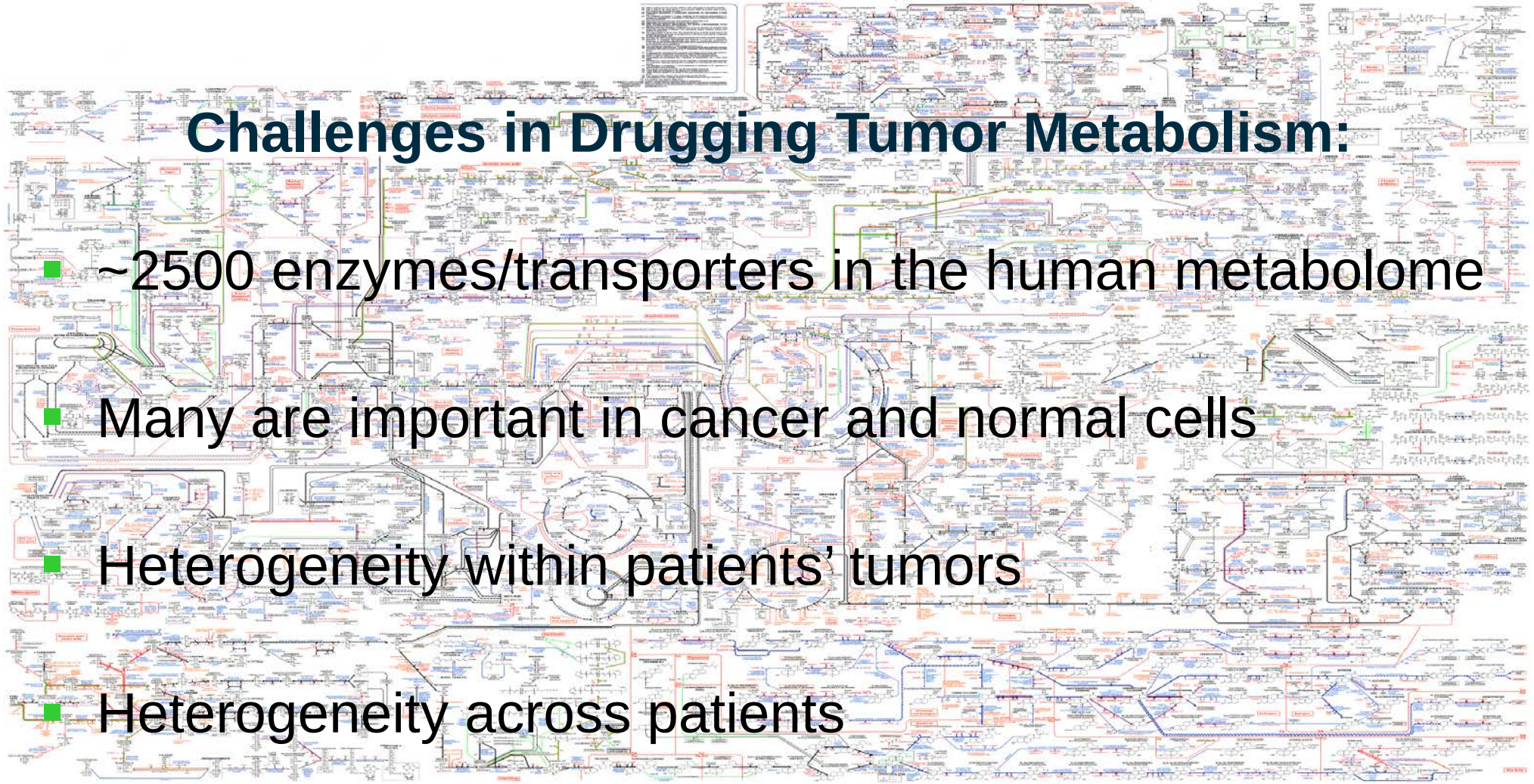
Zhang L et al. *Lancet Oncol* 2012;13(5):466-75.

but…DNA sequencing has identified only 2 gain-of-function metabolic 'driver' mutations out of 2000+ metabolic genes



IDH1/2

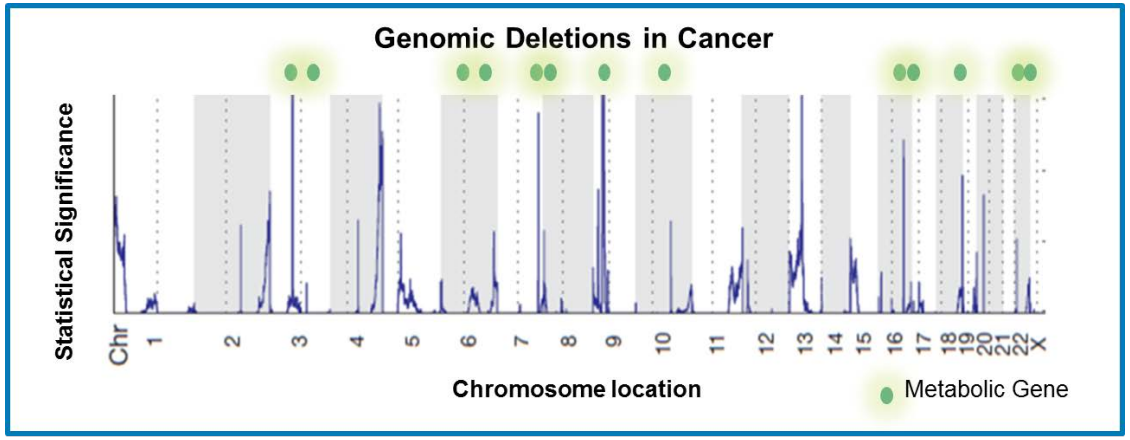
Which Pathways and Targets to Drug?



Challenges in Drugging Tumor Metabolism:

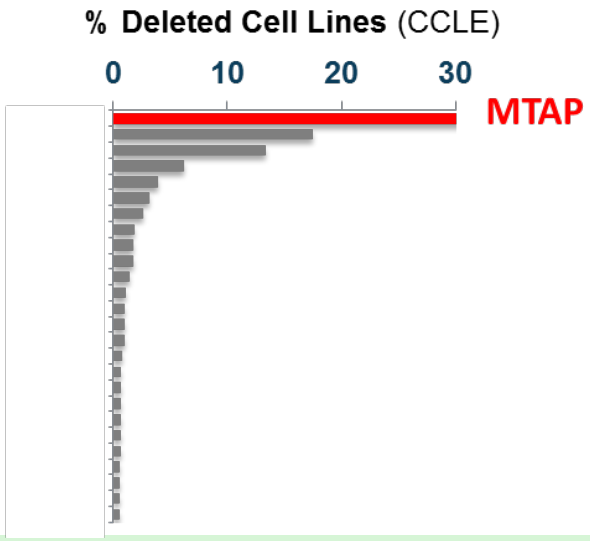
- ~2500 enzymes/transporters in the human metabolome
- Many are important in cancer and normal cells
- Heterogeneity within patients' tumors
- Heterogeneity across patients

Homozygous Deletions of Metabolic Genes in Cancer

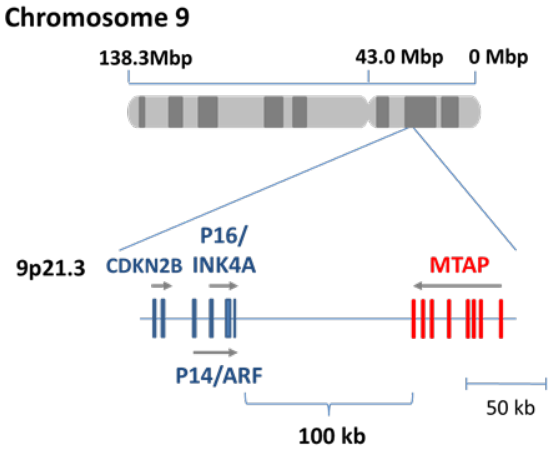


Beroukhim R et al. *Nature* 2010;463(7283):899-905.

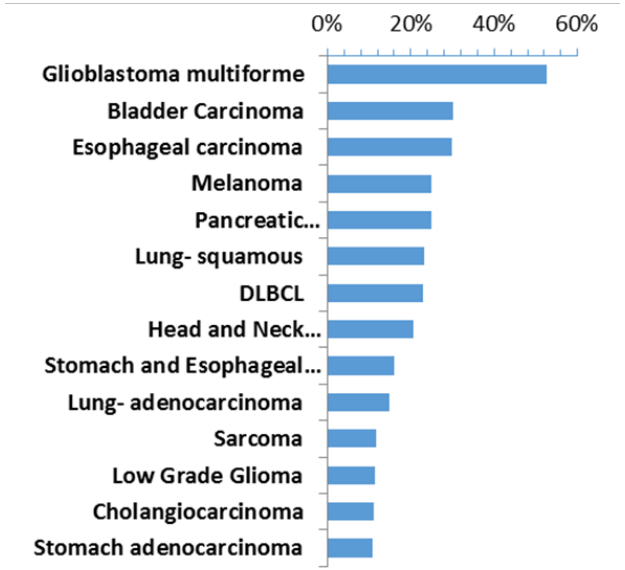
MTAP is the metabolic gene most frequently mutated in cancer (deletion)



Co-deletion of MTAP & p16 occurs in ~15% of all cancer



MTAP deletion frequency in tumors



Marjon K et al. *Cell Rep*. 2016;15(3):574-87.

A Key Insight: Deletion of MTAP Makes Cancers Vulnerable to an Unexpected Target

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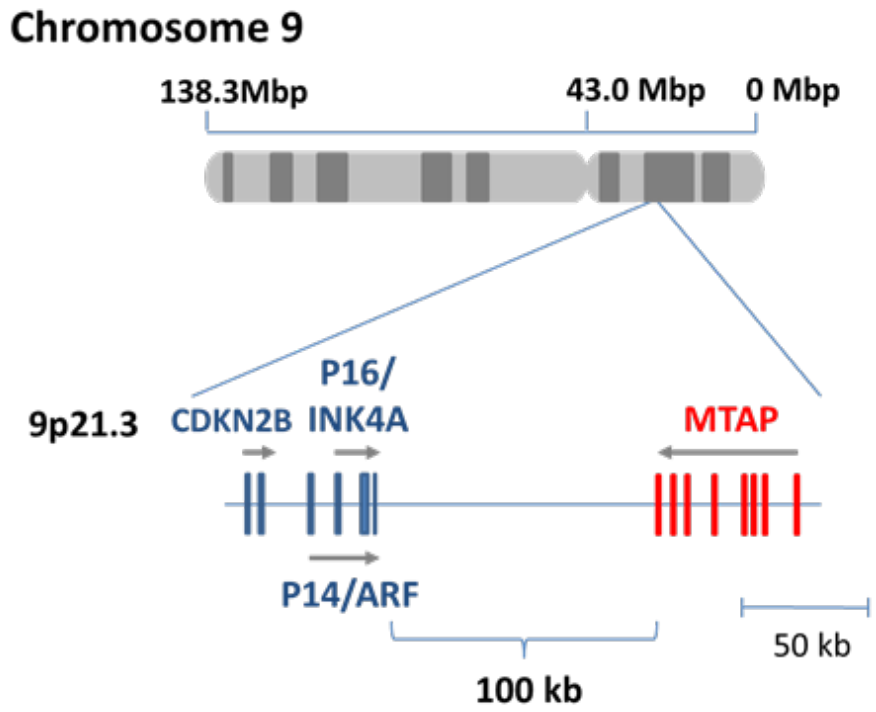
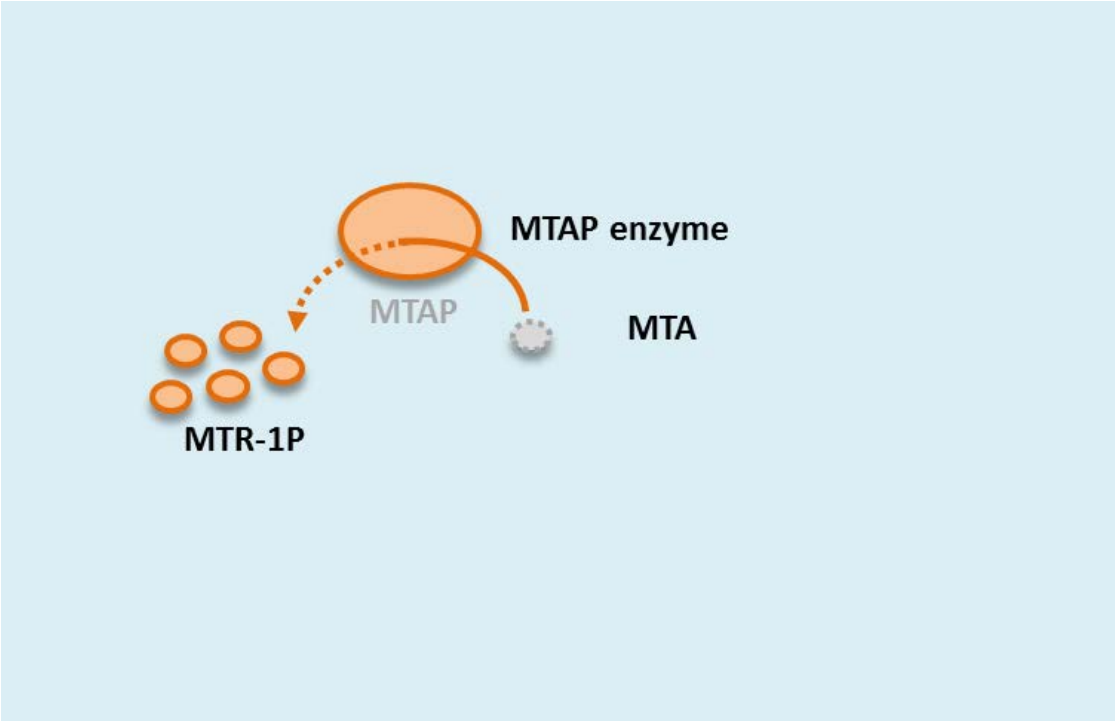


MTAP deletion



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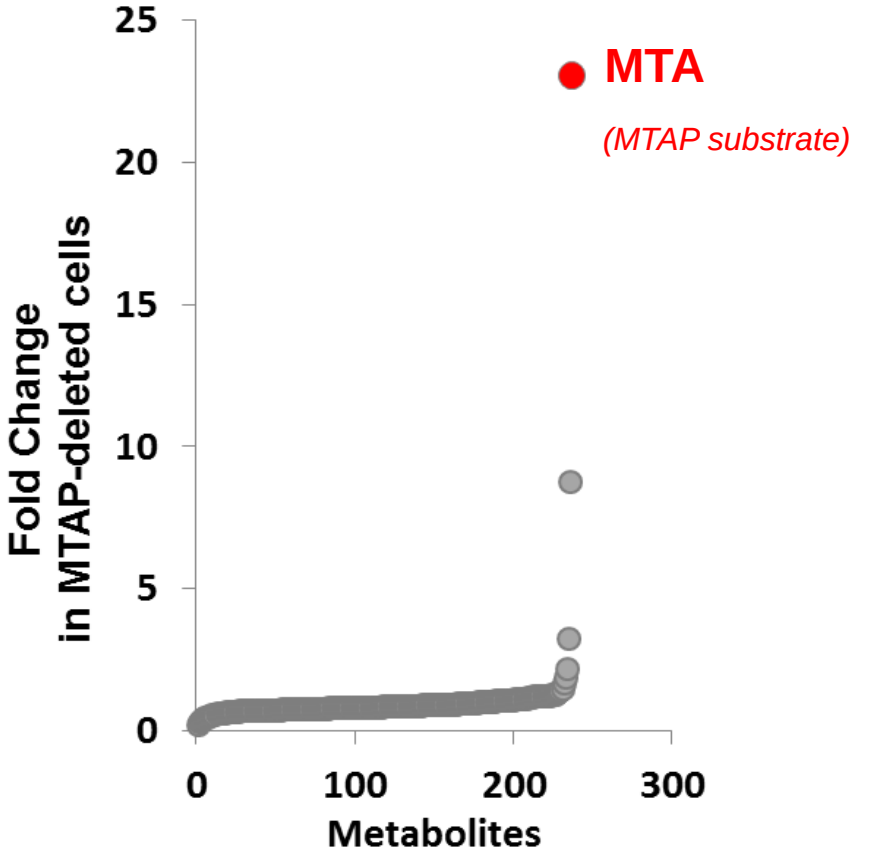
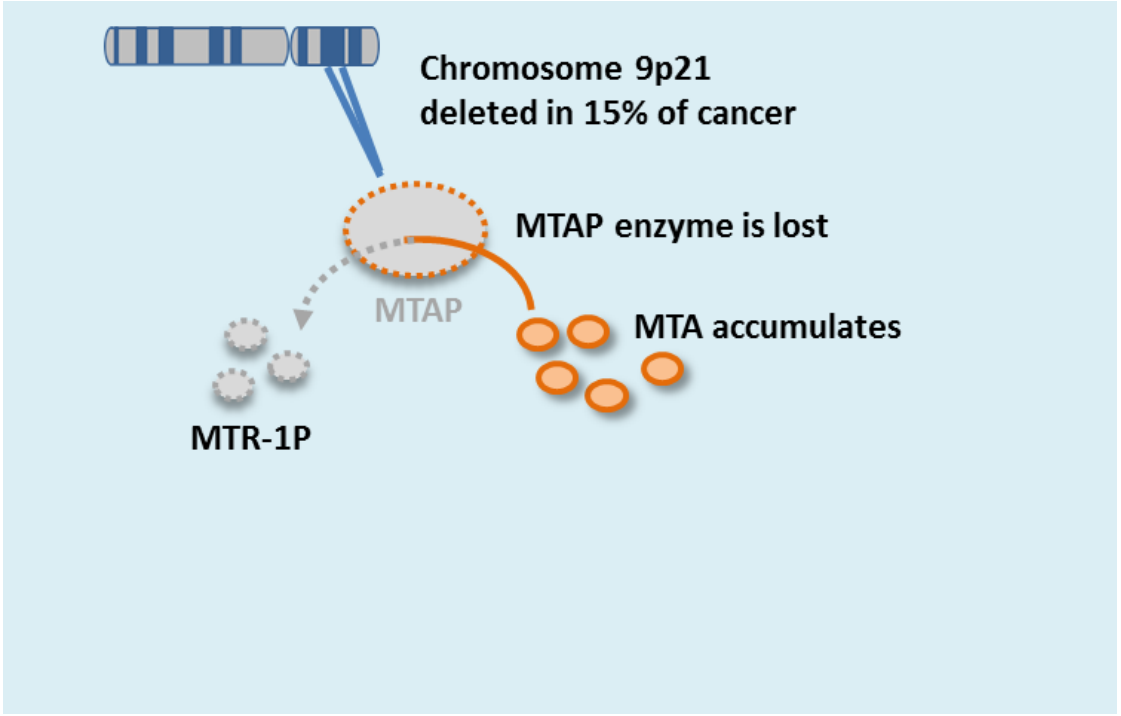
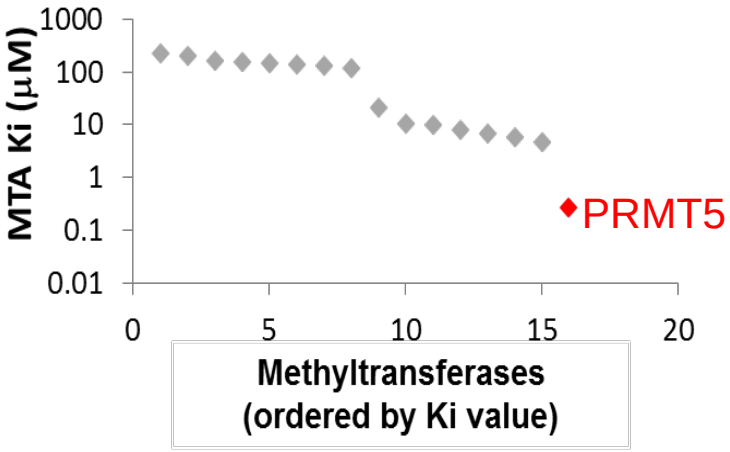
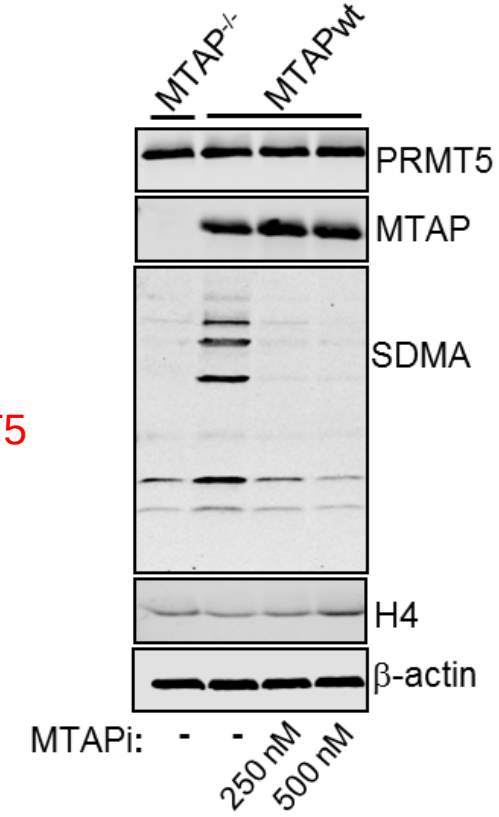


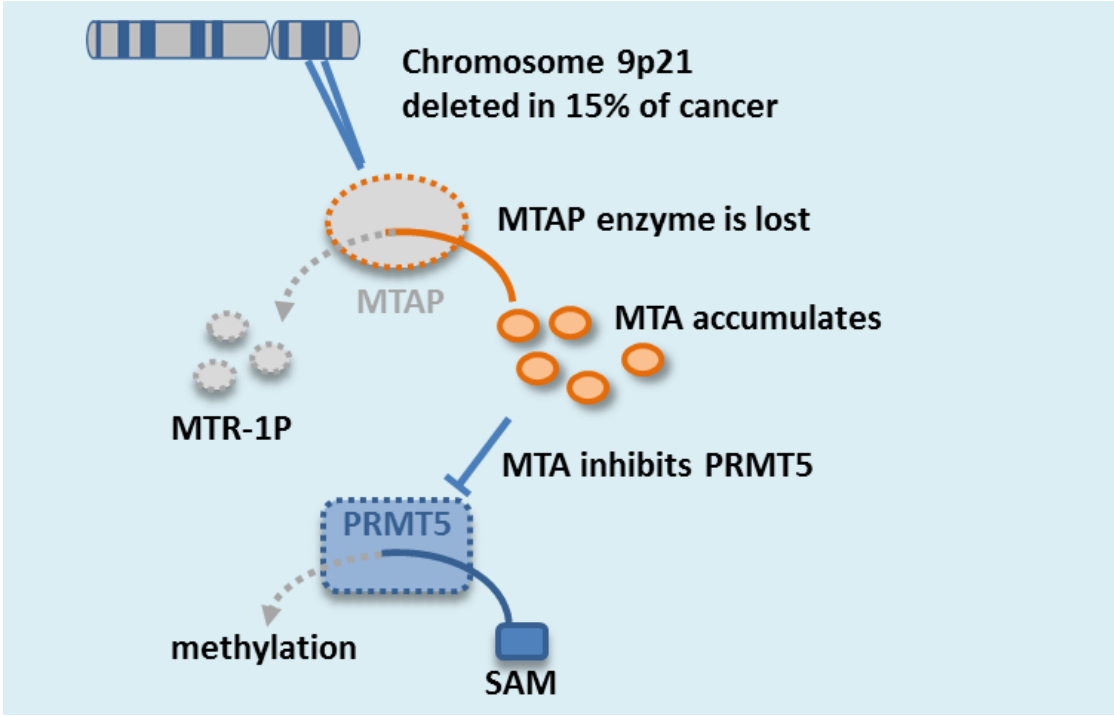


MTAP deletion → **Substrate MTA accumulates** →

A Key Insight: Deletion of MTAP Makes Cancers Vulnerable to an Unexpected Target



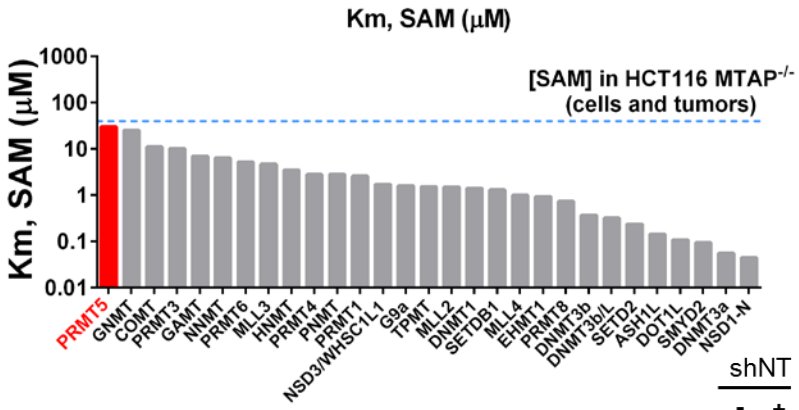


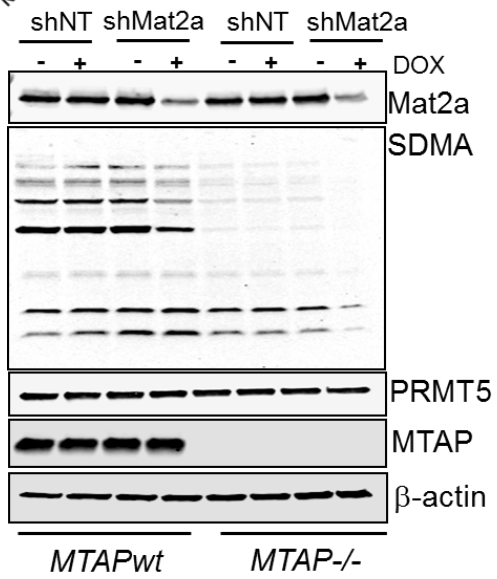


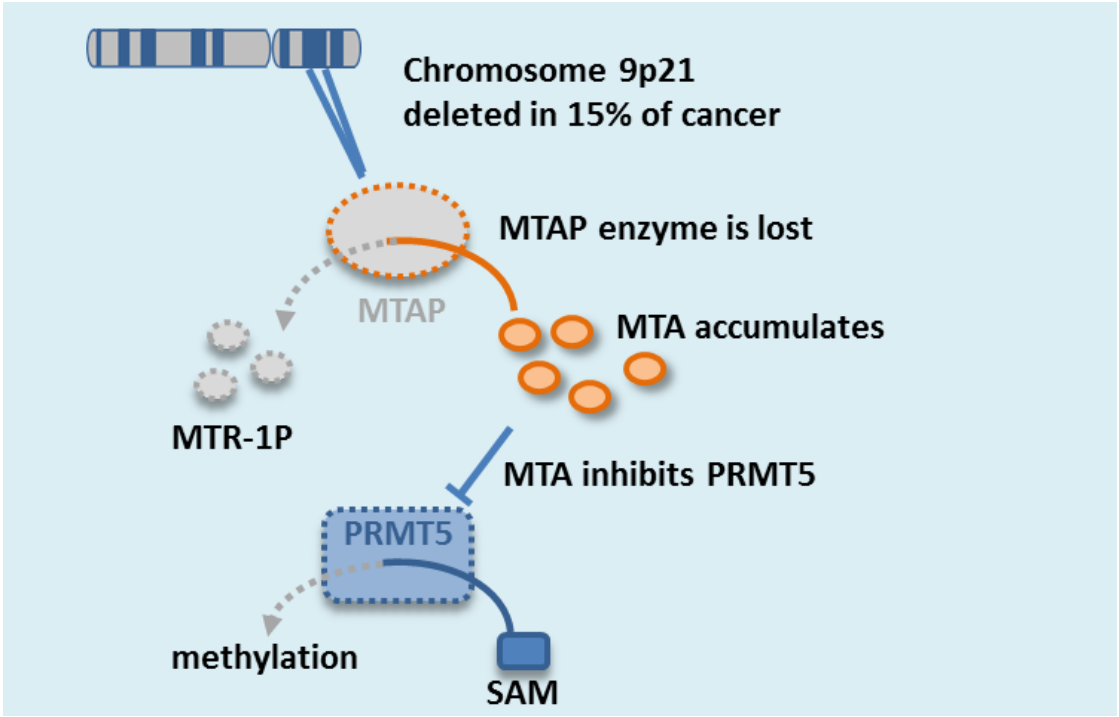
MTAP deletion → **Substrate MTA accumulates** → **Partial Inhibition of PRMT5** →

Marjon K et al. *Cell Rep*. 2016;15(3):574-87.

A Key Insight: Deletion of MTAP Makes Cancers Vulnerable to an Unexpected Target



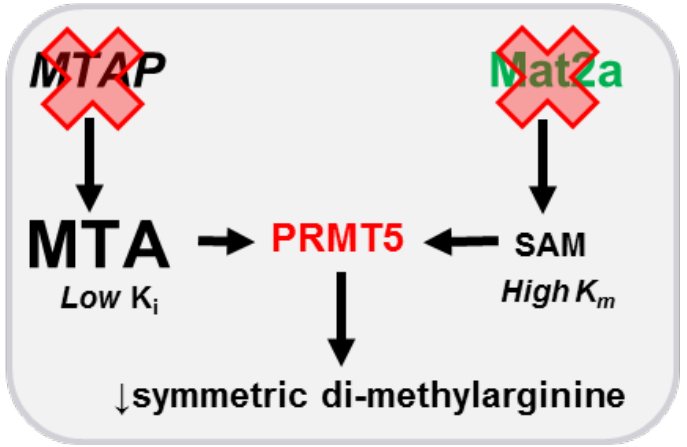


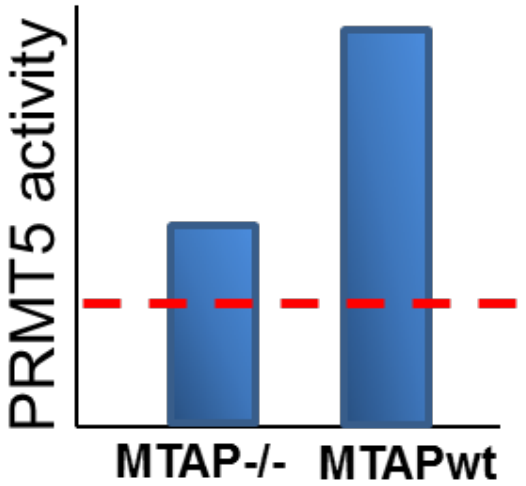


MTAP deletion → **Substrate MTA accumulates** → **Partial inhibition of PRMT5** → **Sensitivity to a 'second hit' : targeting MAT2A starves PRMT5 of its substrate**

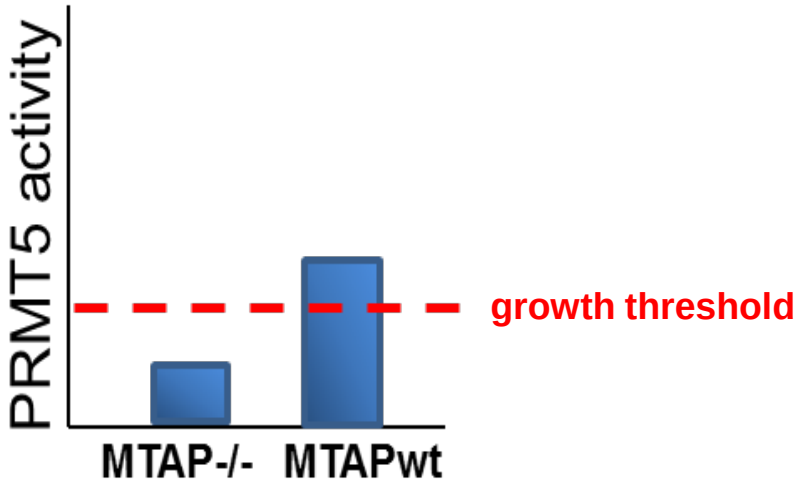
Marjon K et al. *Cell Rep*. 2016;15(3):574-87.

Fortuitous Biochemical Features of PRMT5 Can Explain the Vulnerability of PRMT5 and MAT2A in MTAP-deleted Cancers





PRMT5 is inhibited by MTA at concentrations that arise in MTAP-null cancers



Low affinity of PRMT5 for SAM leads to further reduction in activity upon MAT2A ablation

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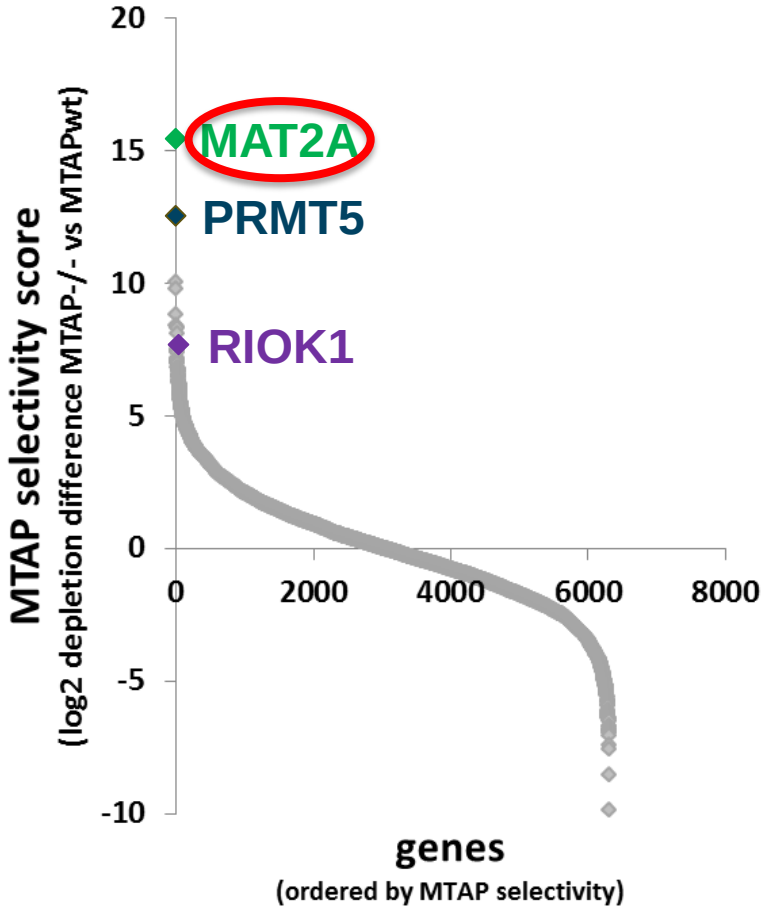
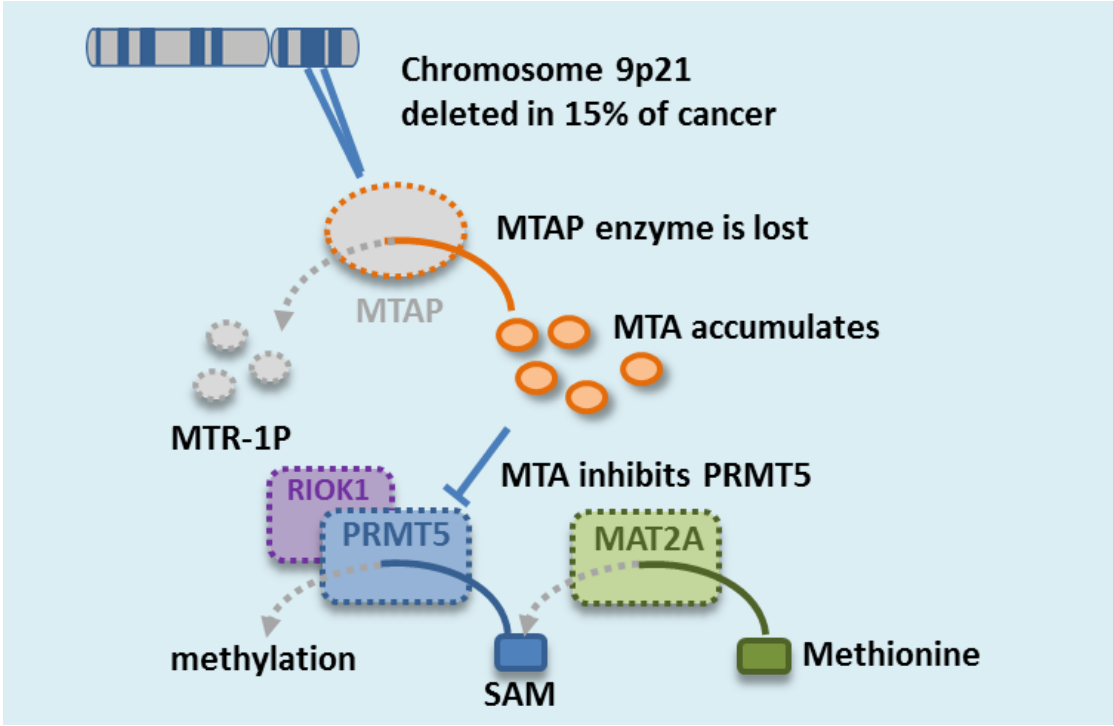
A Key Insight: Deletion of MTAP Makes Cancers Vulnerable to an Unexpected Target





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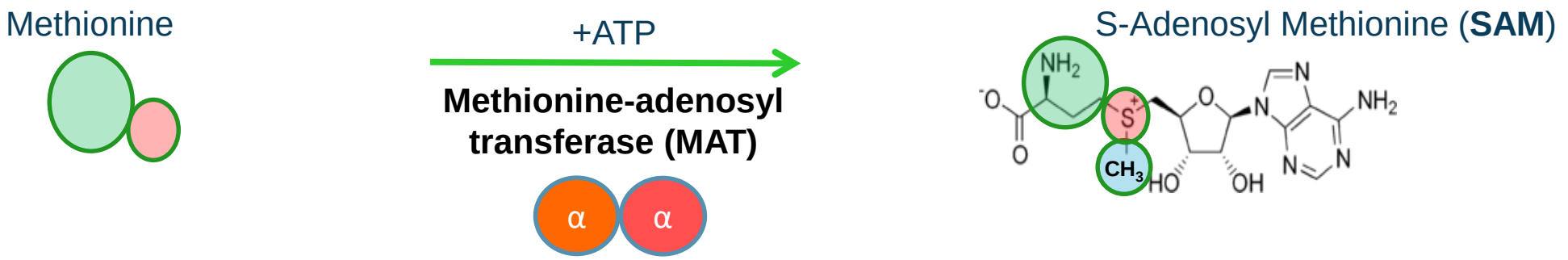
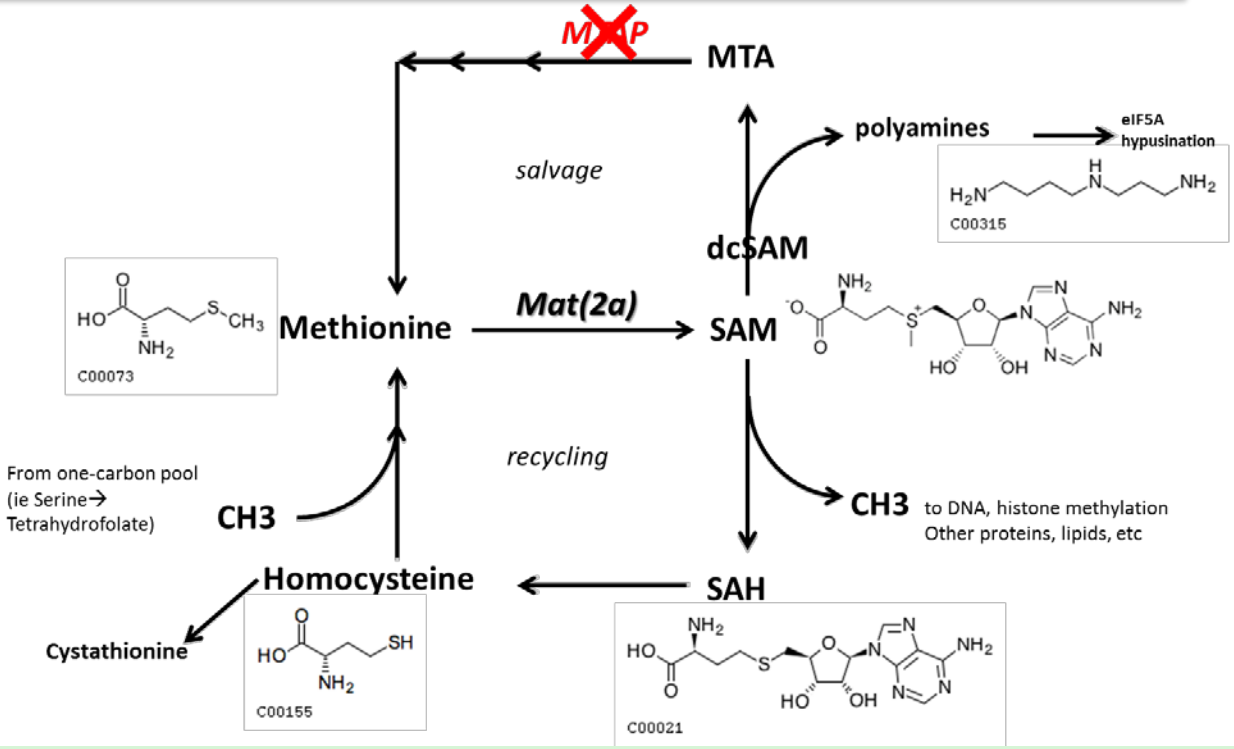
MAT2A: Methionine Adenosyltransferase 2A



- MAT2A is the key enzyme that produces SAM in cancer & normal cells
- SAM (S-adenosyl methionine) is a 'hub' metabolite utilized in a number of pathways. Fates include:
  - **methyl group**
    - methylation of histone, DNA, protein, lipid
  - **aminopropyl group**
    - polyamines → regulate gene expression
  - **sulfur**
    - glutathione → protect vs ROS



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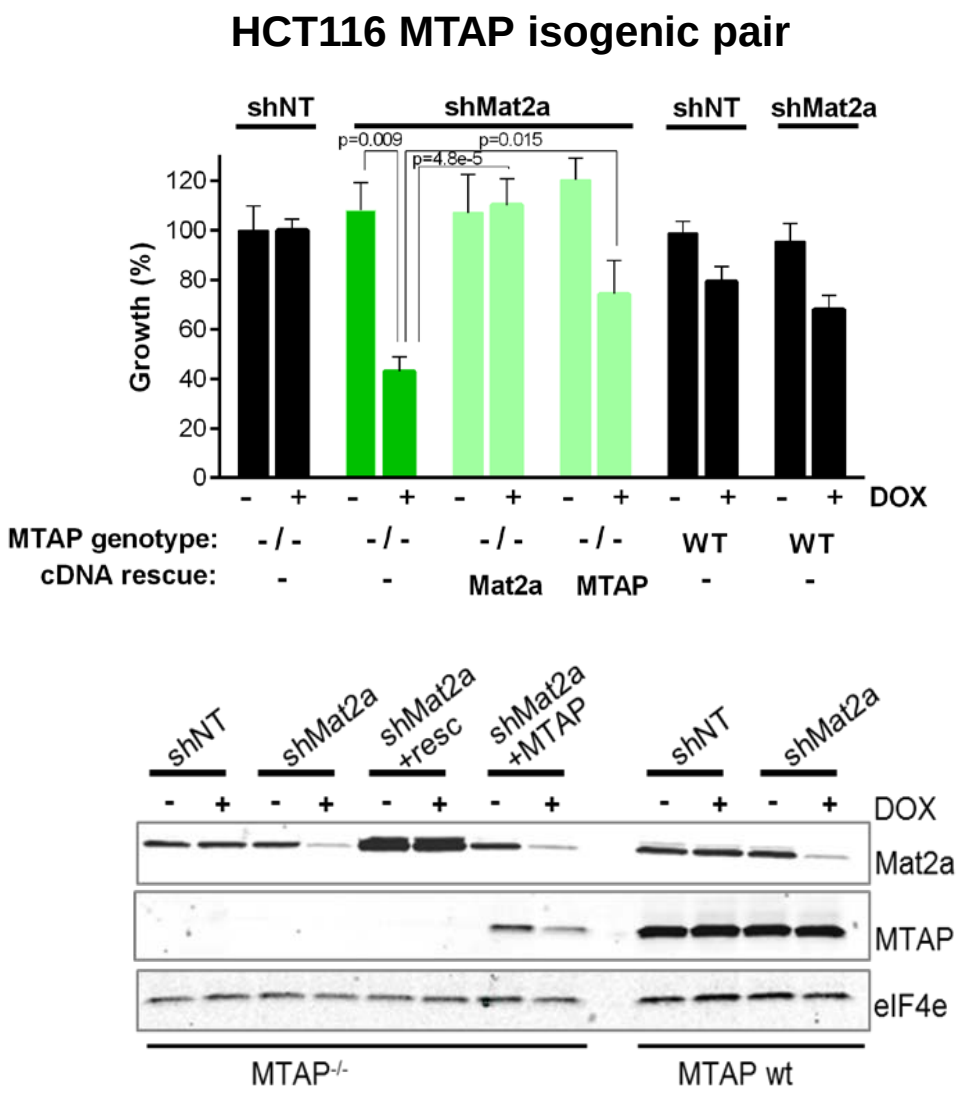
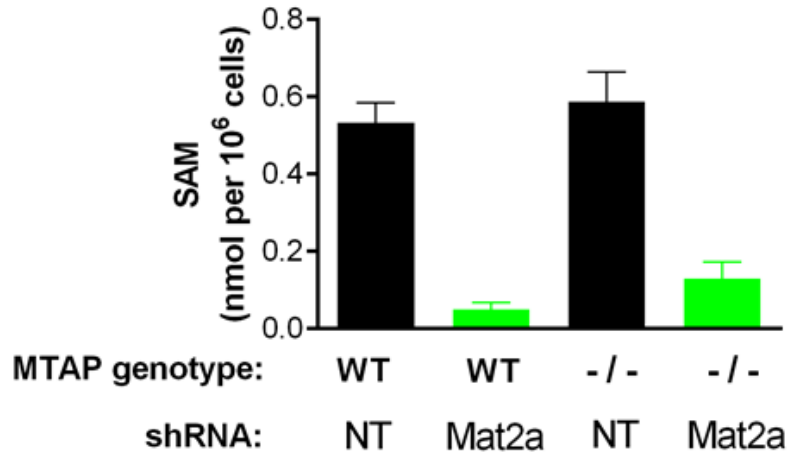
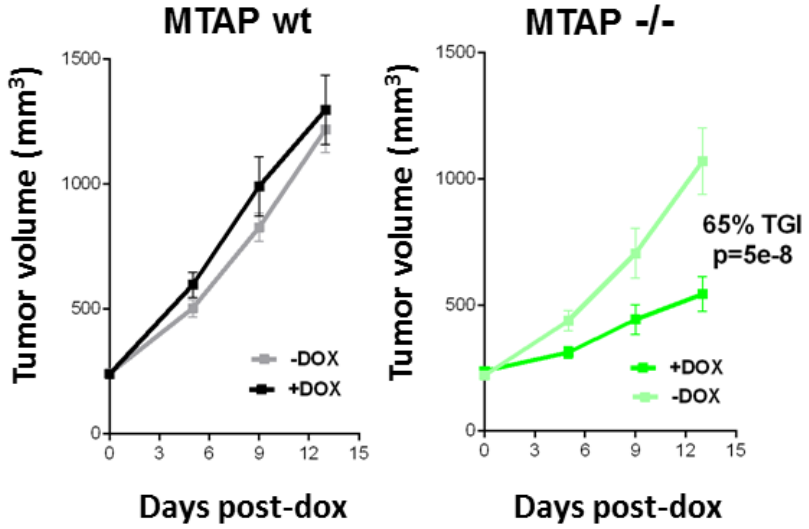
Genetic Tools Validate MAT2A as a Selective Vulnerability in MTAP-deleted Cancers







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Fragment-Based Screening for Discovery of MAT2A Inhibitors

Fragment Library (~3K fragments)

MAT2A

fragment

fragment

fragment

Loose Filter (Ultrafiltration/LCMS)

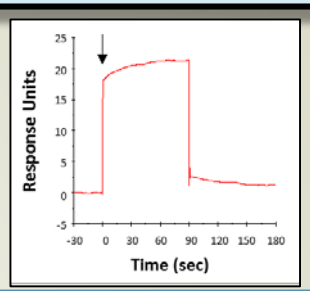
binders

binder

binder

binder

Tight Filter (SPR)



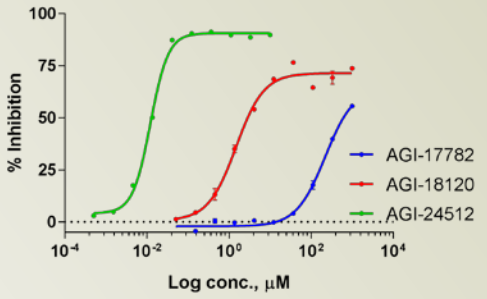
Validated fragments

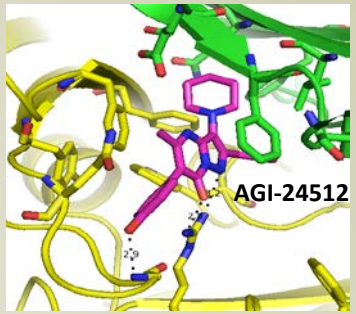
Validated Fragments

Crystallography Co-complex

Structure Guided Design

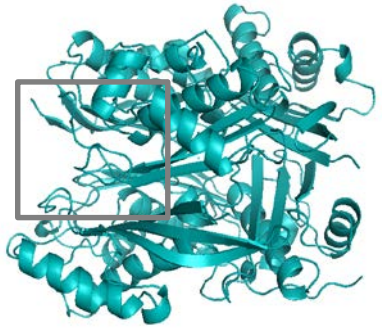
MAT2A enzyme assay

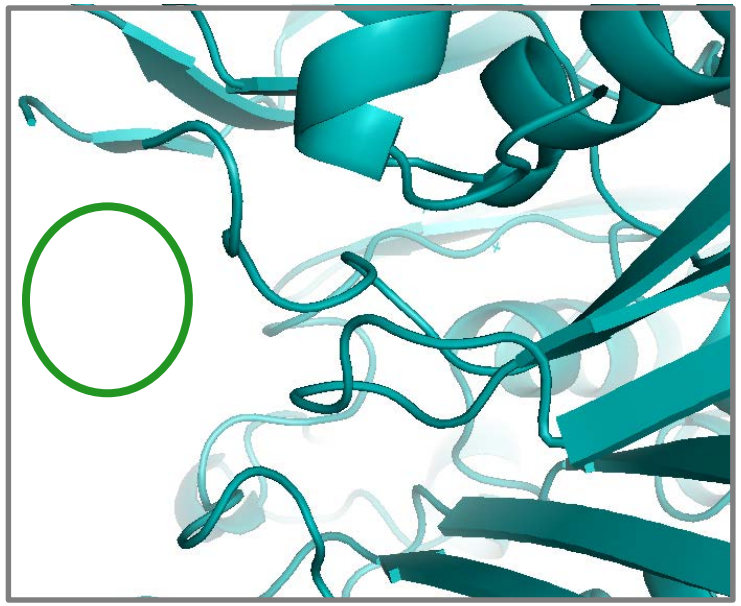




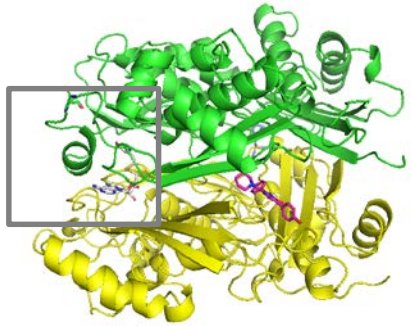
MAT2A Inhibitor Binding in Allosteric Site Impacts Conformation of the Active Site Gate Loop

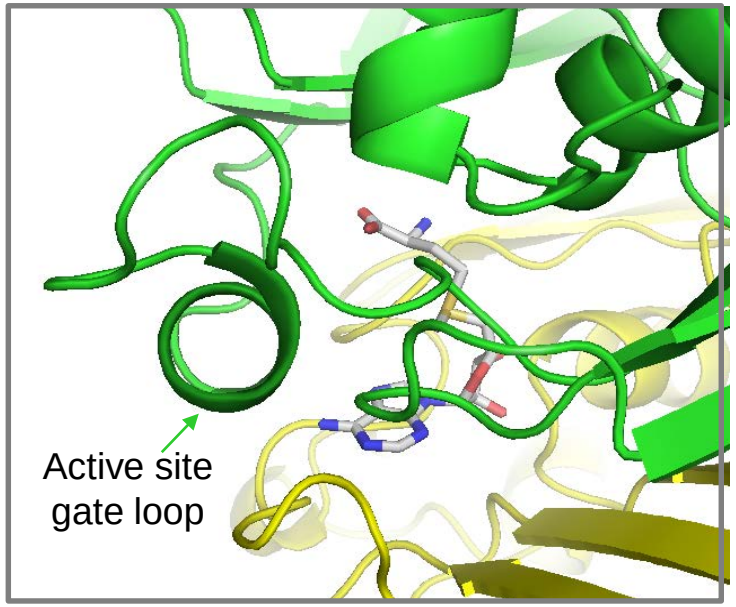
MAT2A apo form
(PDB Code: 5a19)





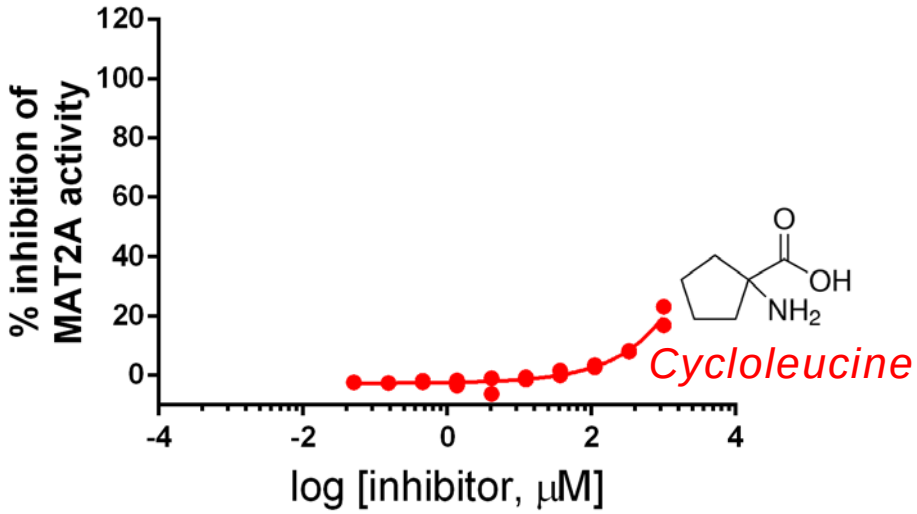
AGI-24512:MAT2A cocrystal





In vitro and Cellular Activity of MAT2A Small Molecule Inhibitor

In vitro biochemical assay



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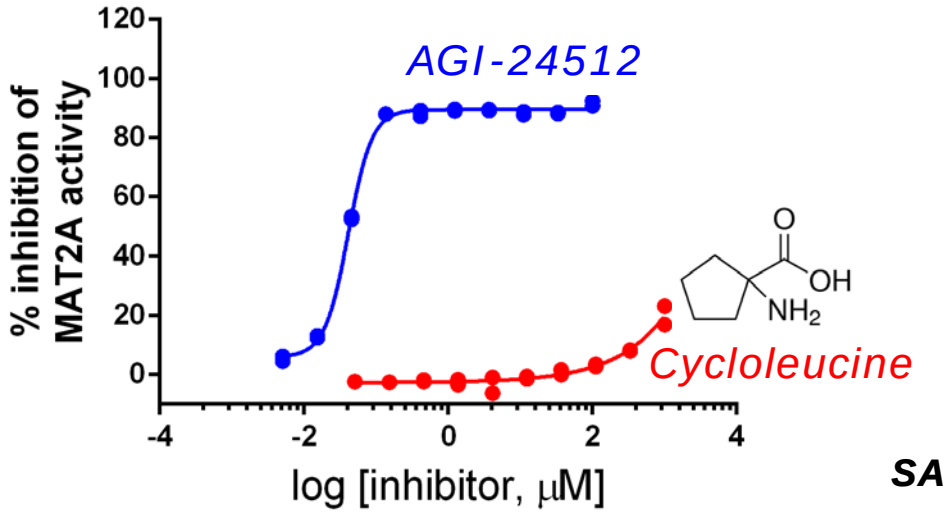
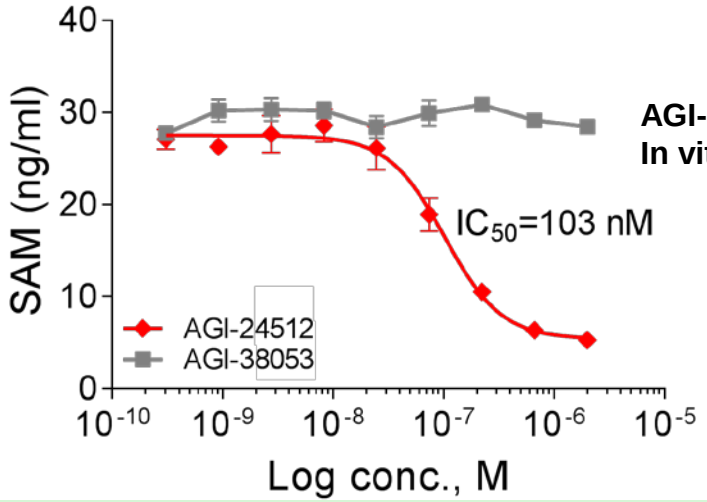
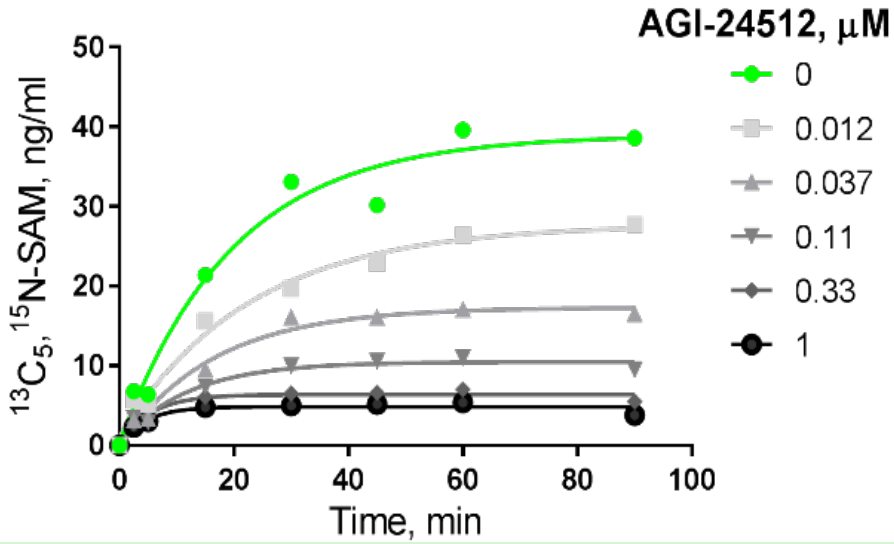


SAM levels in HCT116 cells

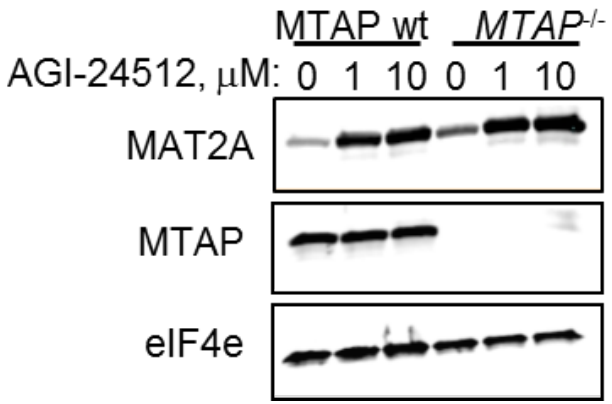


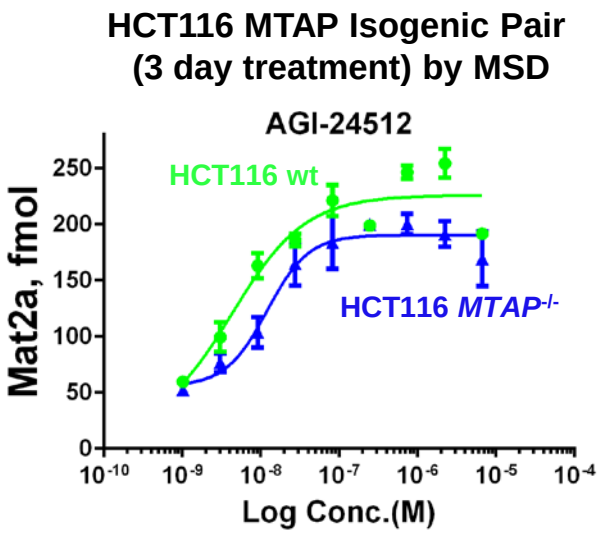
AGI-38053
In vitro IC_{50}>20 μM

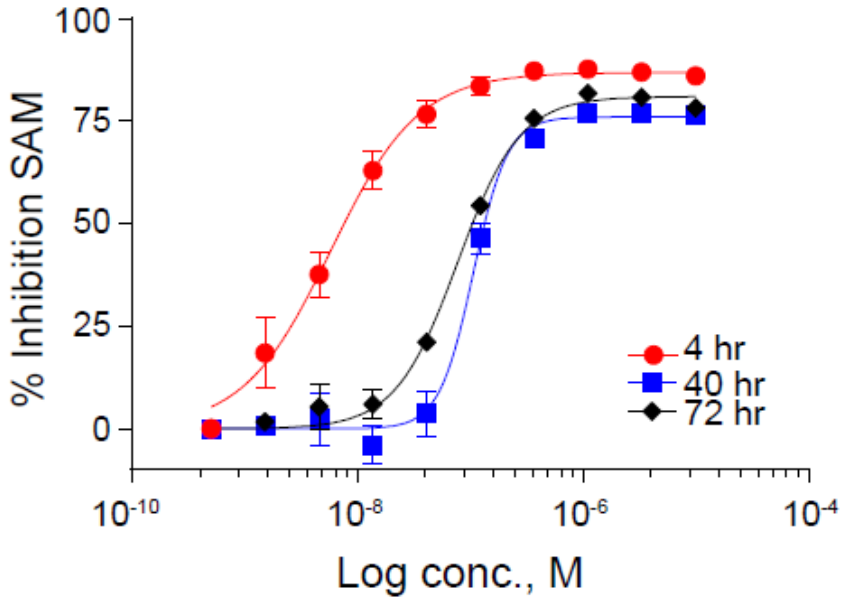
SAM production rate in HCT116 cells



MAT2A Inhibition Causes MAT2A Protein Upregulation and Potency Shift That is Limited and Surmountable

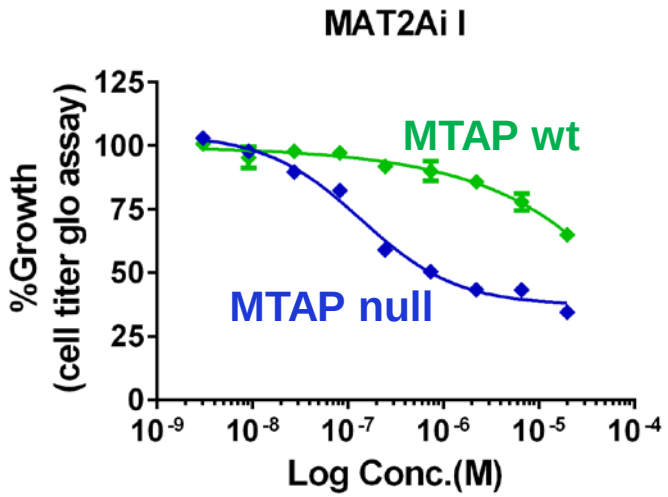




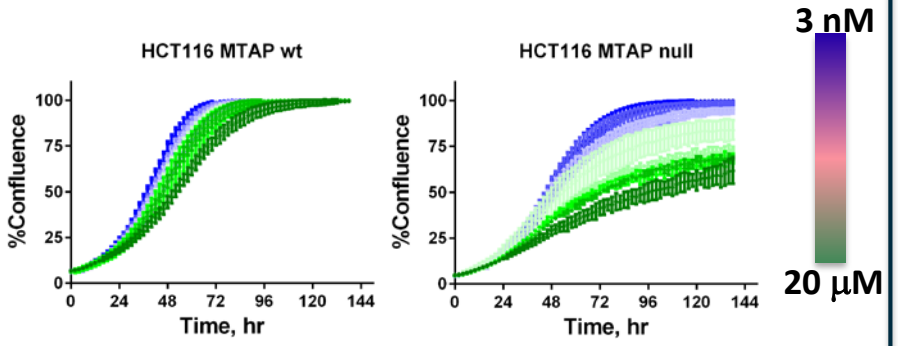


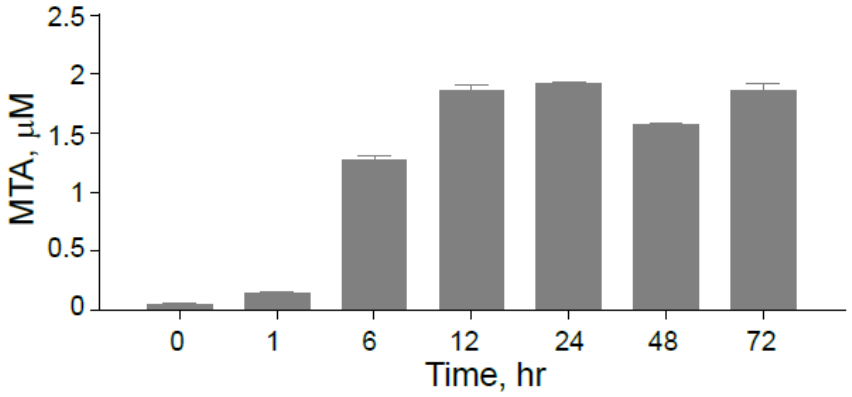
MAT2A Inhibitors Selectively Block Growth of MTAP-deleted Cancer Cells *in vitro*

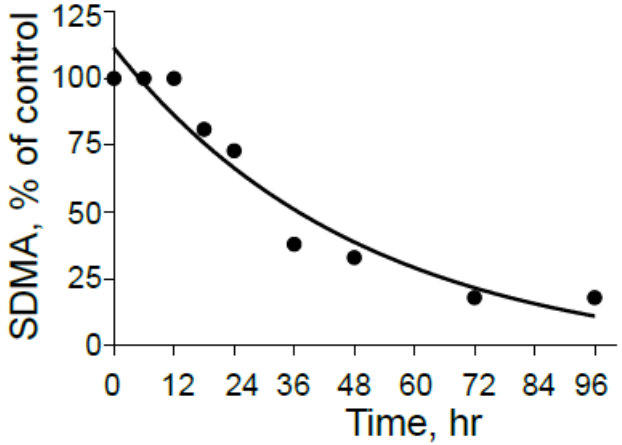
4 day proliferation assay



IncuCyte continuous growth assay

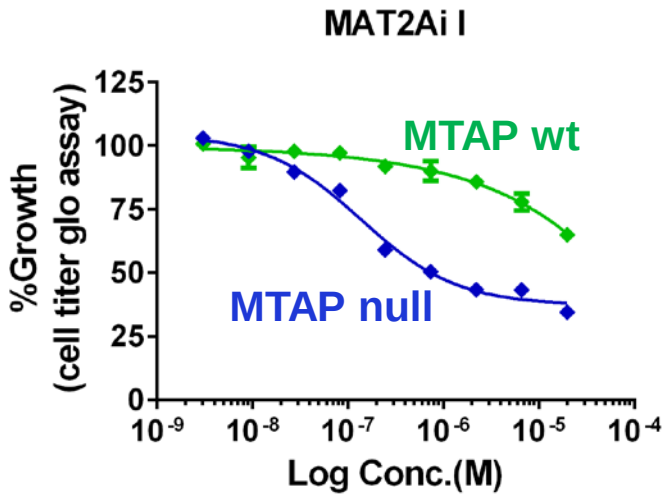






MAT2A Inhibitors Selectively Block Growth of MTAP-deleted Cancer Cells *in vitro*

4 day proliferation assay



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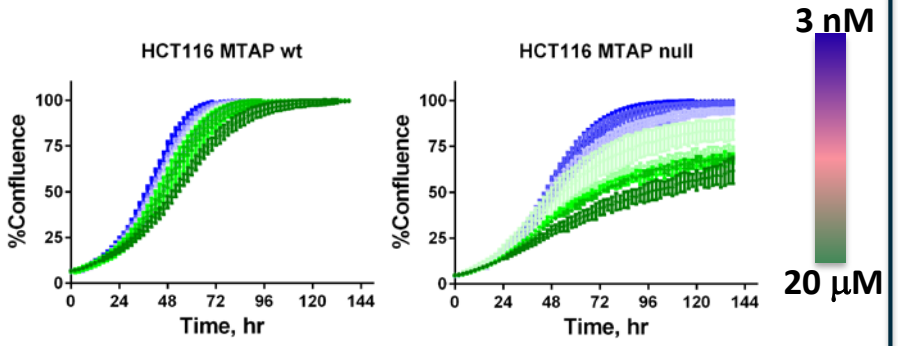
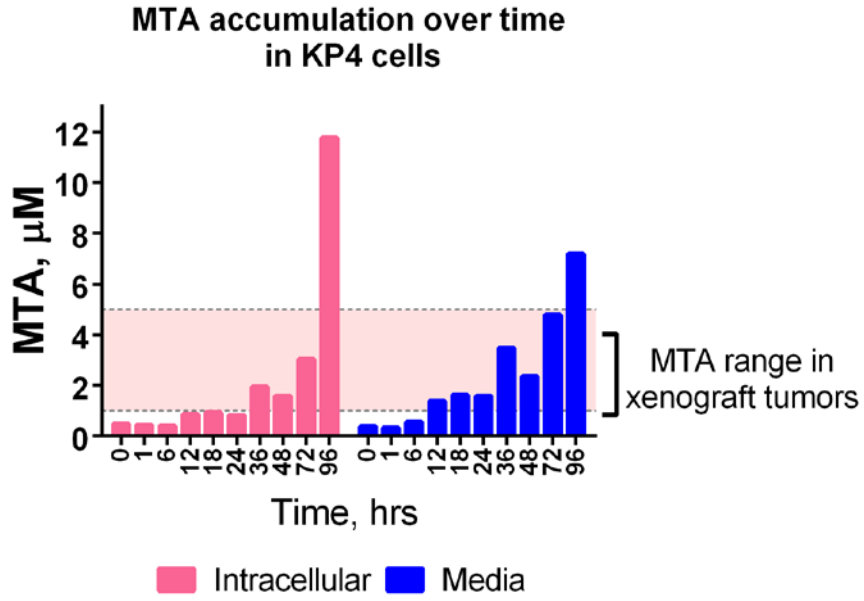
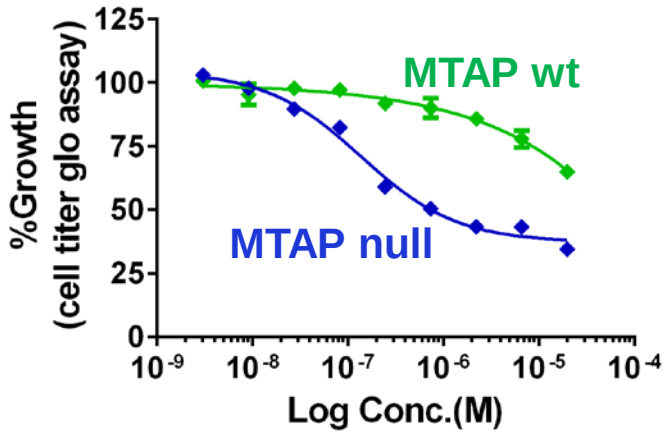




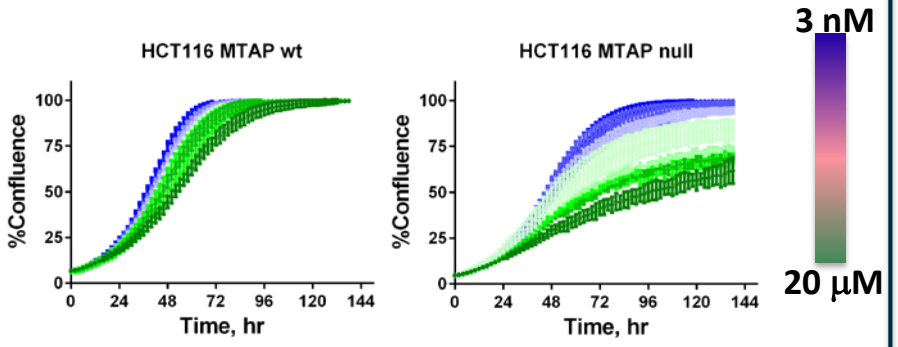
MAT2A Inhibitors Selectively Block Growth of MTAP-deleted Cancer Cells *In Vitro*

4 day proliferation assay

MAT2Ai I

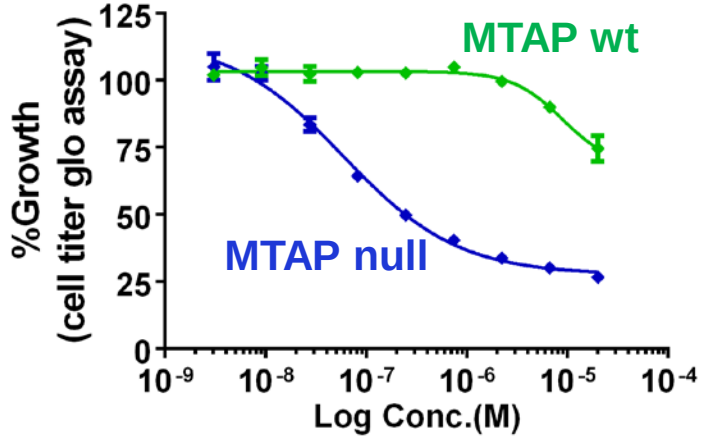


IncuCyte continuous growth assay

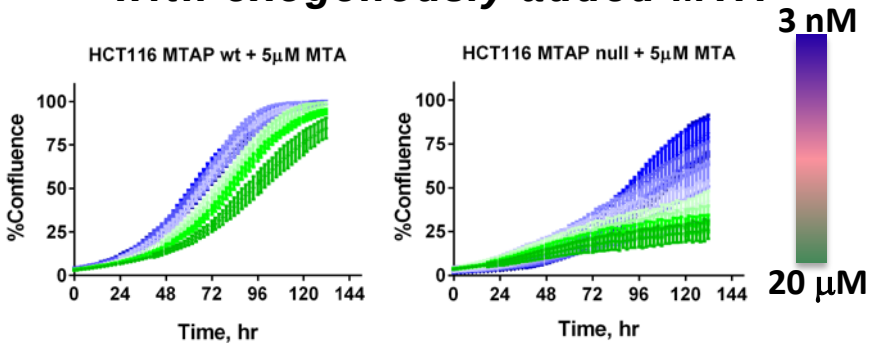


4 day proliferation assay

MAT2Ai I with 5 µM MTA



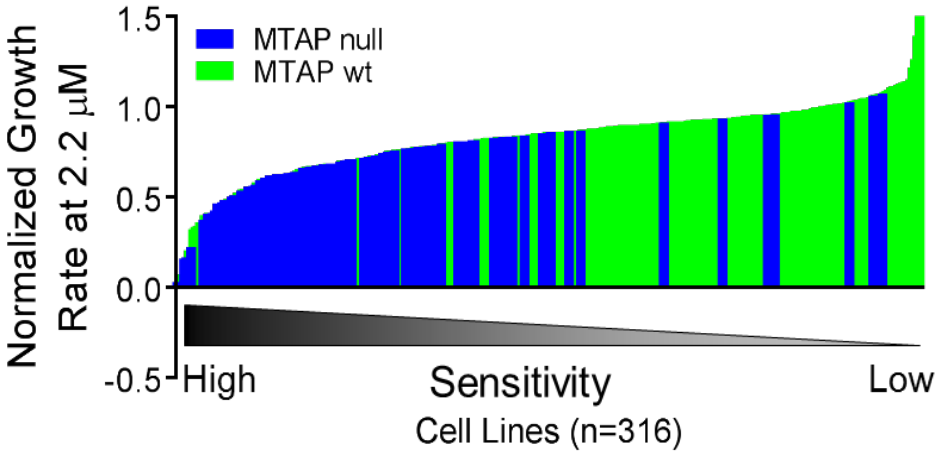
IncuCyte continuous growth assay with exogenously added MTA

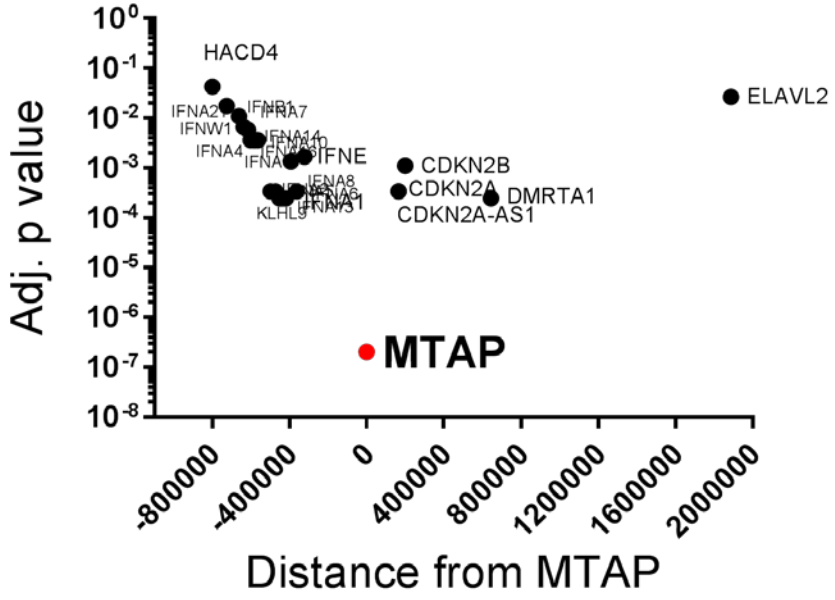


MAT2A Inhibitors Selectively Block Growth of MTAP-deleted Cancer Cells *In Vitro*

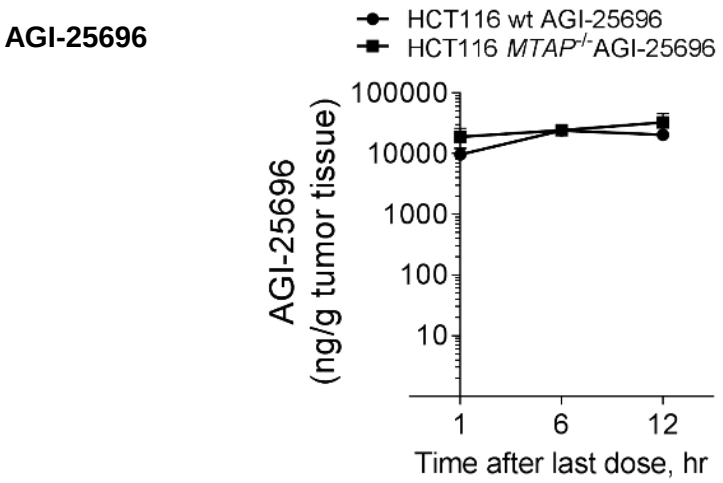
MTAP predicts sensitivity in Cell Panel with AGI-24512

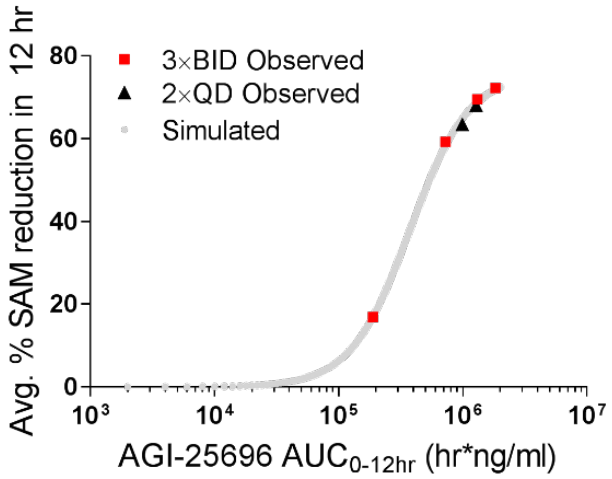
(p=1.29e-15)

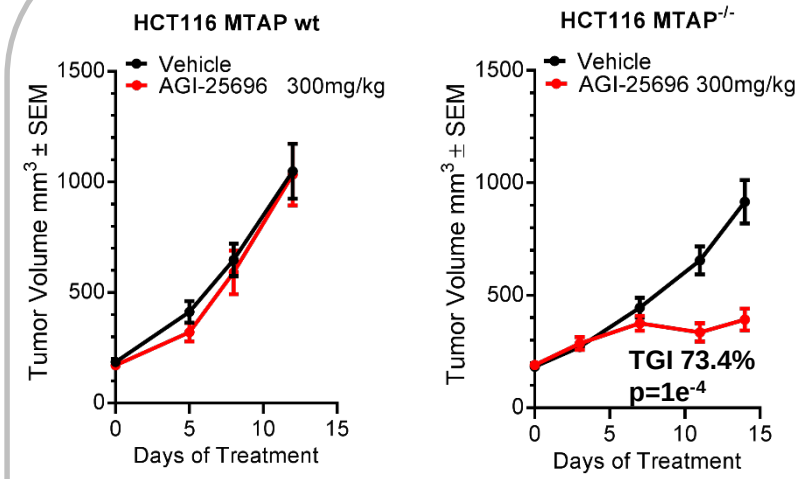


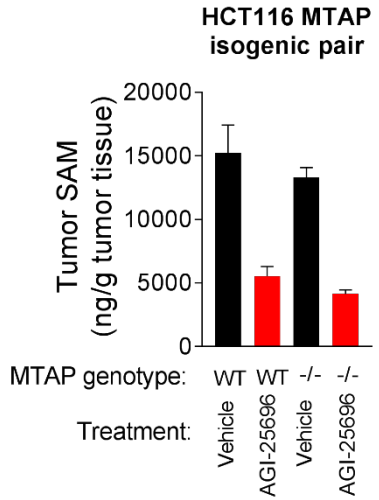


Pharmacologic Targeting of MAT2A with *In Vivo* Tool Molecule Selectively Blocks Growth of MTAP-deleted Tumors *in vivo*

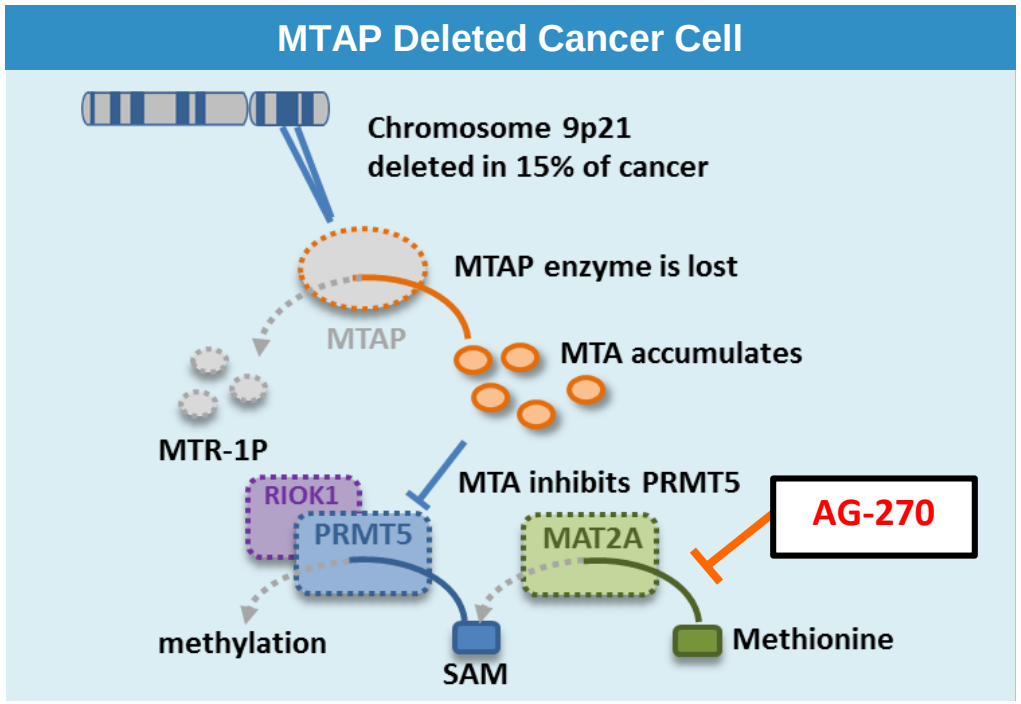




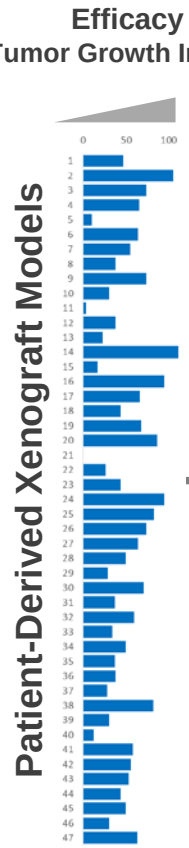


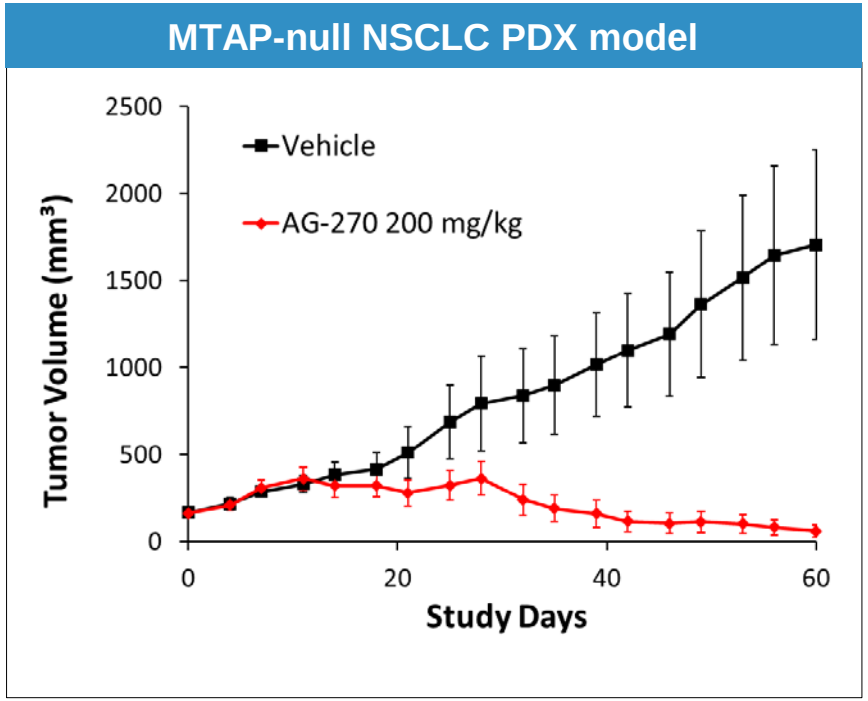


AG-270 Active in Wide Variety of MTAP-deleted Cancer Models



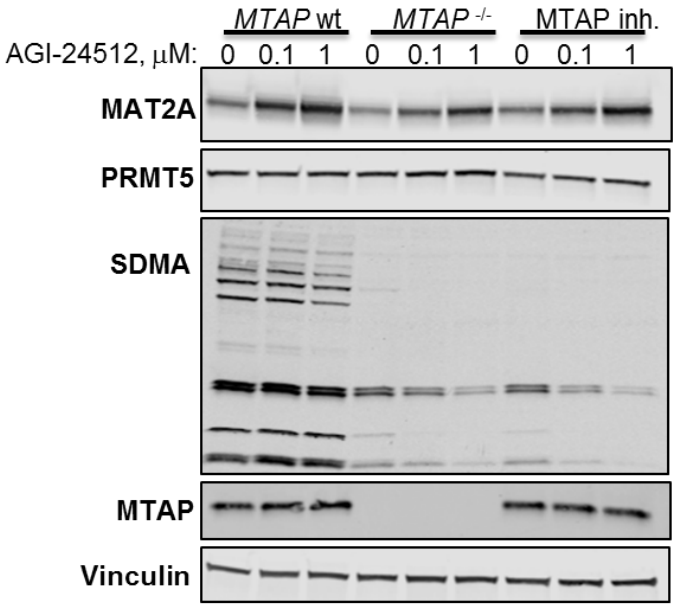
Agios publication: Marjon et al. Cell Reports 2016

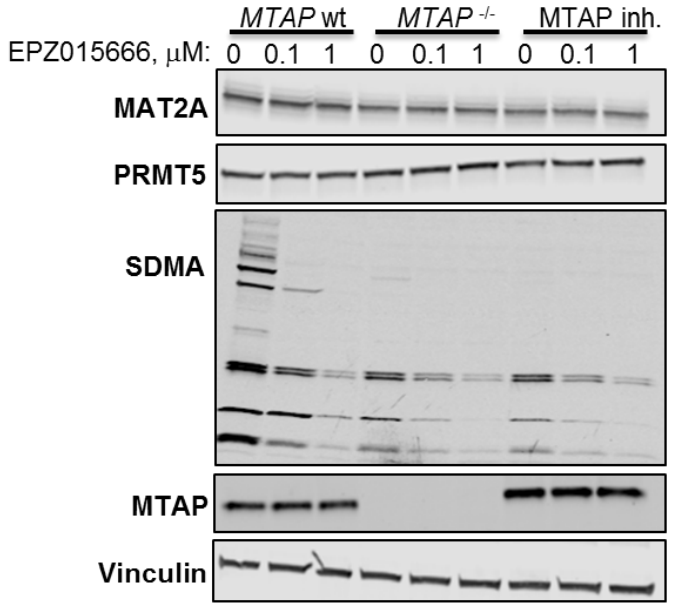


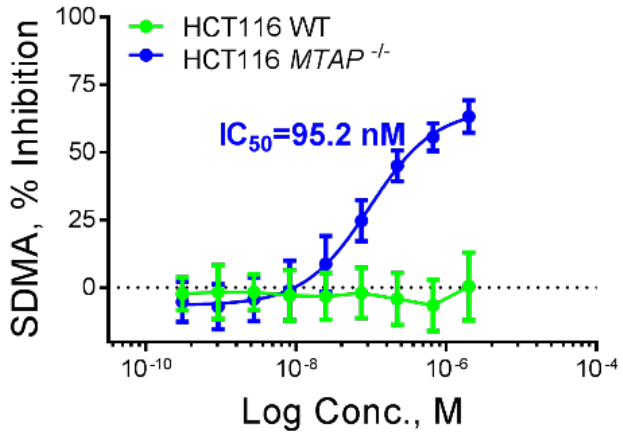


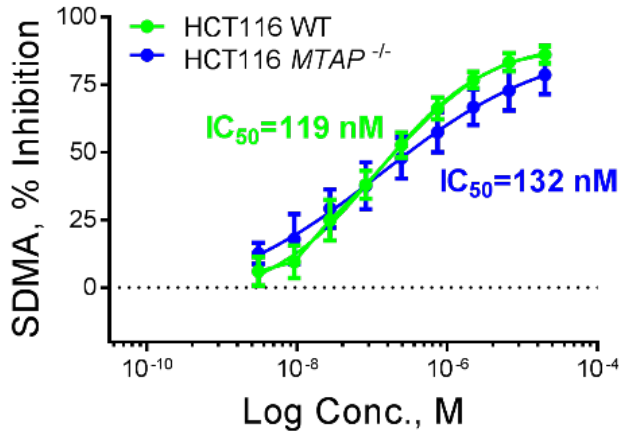
First-in-human Phase 1 dose-escalation clinical trial started Q1 2018

MAT2A Inhibition Selectively Impacts PRMT5 Activity in MTAP-deleted Background









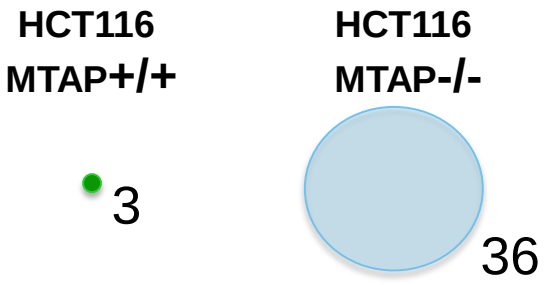
EPZ015666: Chan-Penebre E et al. *Nat Chem Biol* 2015;11(6):432-7.

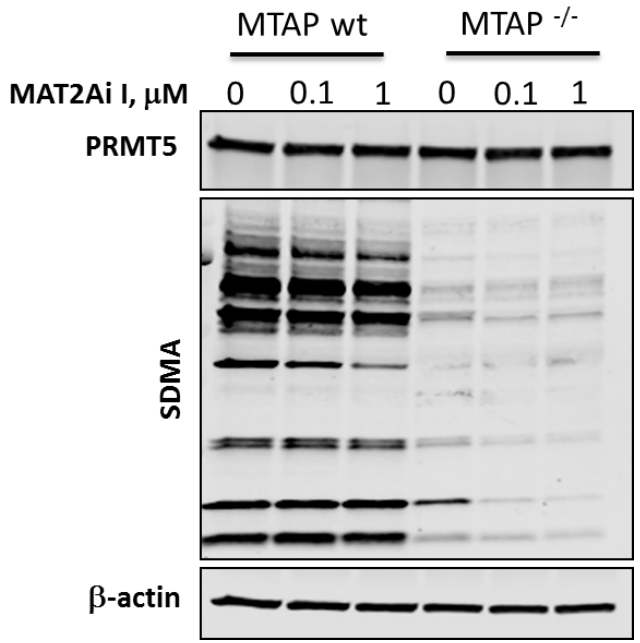
Methylation Proteomics Corroborates Role for PRMT5 as a Key Downstream Mediator of MAT2Ai in MTAP-deleted Cells

HCT116 MTAP$^{-/-}$ and HCT116 MTAP wt cells → **3-day treatment with MAT2Ai or DMSO** → **IP with PTM-specific antibodies** → **LC-MS/MS**

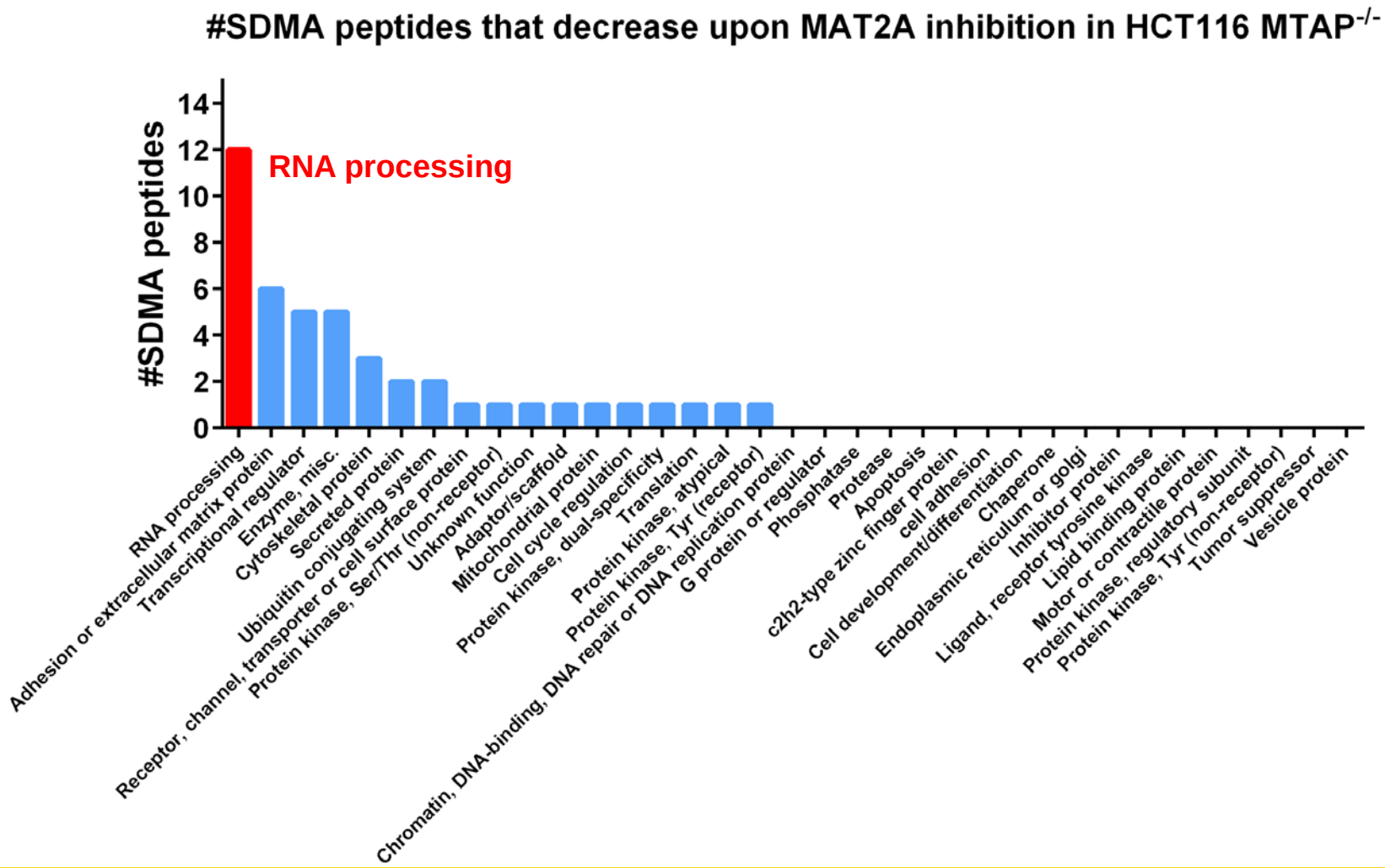
- Mono-Methyl-Arginine
- Asymmetrical di-methyl Arginine
- Symmetrical di-methyl Arginine
- Pan-methyl Lysine

 # PRMT5 SDMA peptides reduced >4-fold upon MAT2Ai II treatment:





Methylation Proteomics Indicates MAT2A Inhibition Reduces Methylation of RNA Processing Machinery in MTAP-deleted Cells

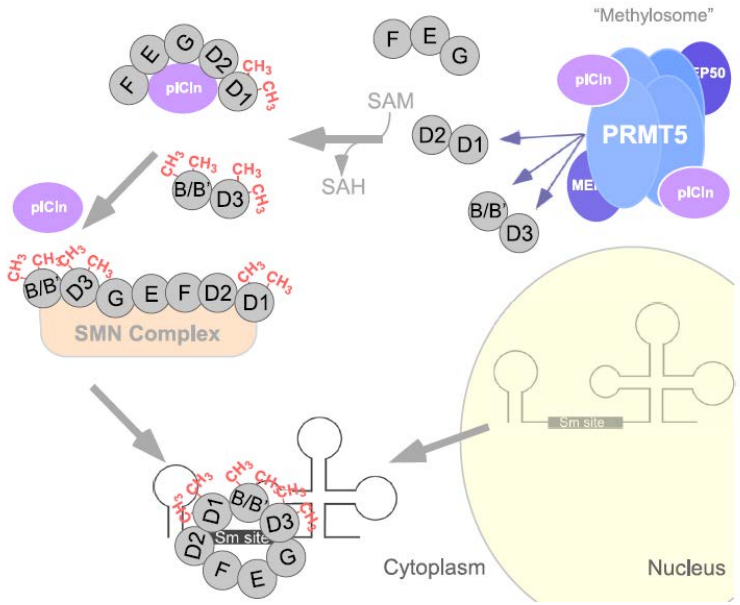


Methylation proteomics identifies loss of methylation of RNA processing machinery upon MAT2A inhibitor treatment

Symmetric Arginine Methylation of Spliceosome Components by PRMT5 is Important for Spliceosome Maturation

Published substrates include:

- SmD1,SmD3,SmB/B’
  (*Brahms, RNA 2001 and Friesen Mol Cell 2001)*
  - Methylation is required for interaction w/ SMN
- PRMT5 KO mouse NPCs have splicing defects
  (Bezzi, Genes Dev 2013)



PRMT5 Modulation Impacts Various Splicing Modalities

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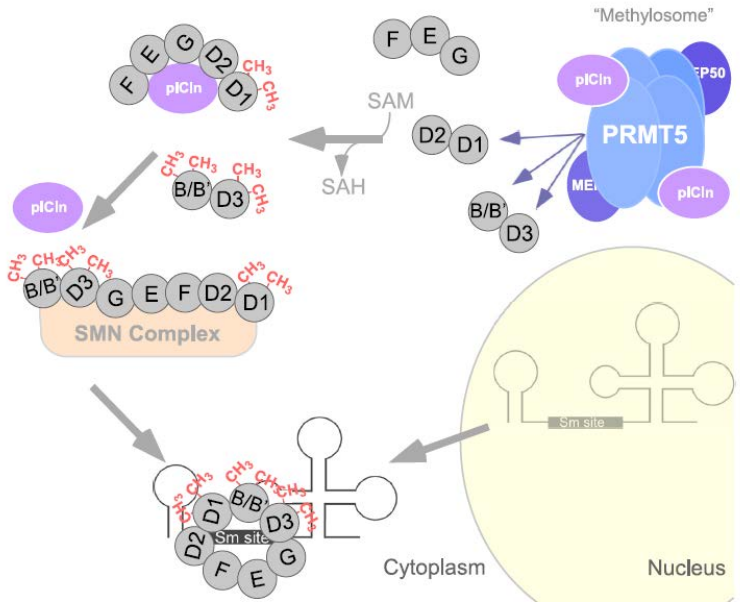
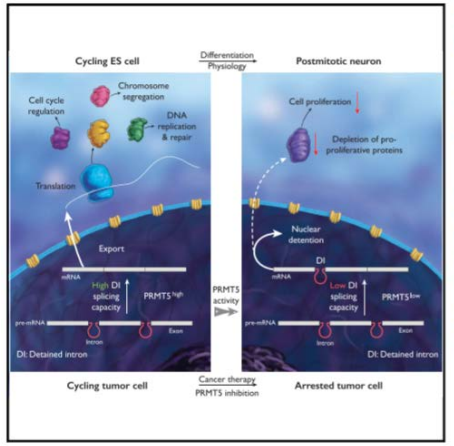


Article

Cancer Cell

Coordinated Splicing of Regulatory Detained Introns within Oncogenic Transcripts Creates an Exploitable Vulnerability in Malignant Glioma

Graphical Abstract



Authors

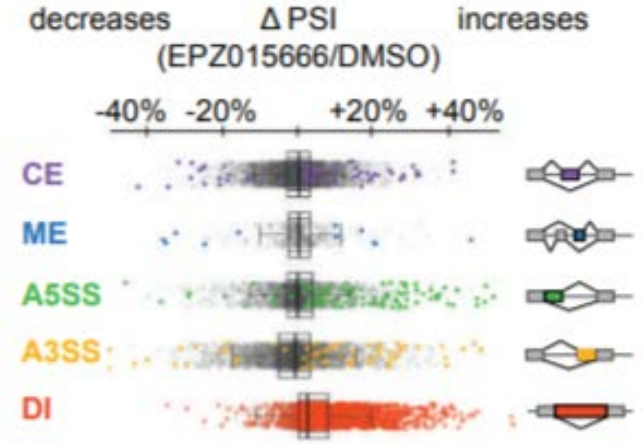
Christian J. Braun, Monica Stanciu, Paul L. Boutz, ..., Phillip A. Sharp, Michael T. Hemann, Jacqueline A. Lees

Correspondence

hemann@mit.edu (M.T.H.), jalees@mit.edu (J.A.L.)

In Brief

Braun et al. show that glioblastoma is selectively sensitive to the inhibition of PRMT5 and identify a predictive biomarker for this sensitivity. PRMT5 inhibition primarily disrupts the removal of detained introns, which results in the reduction of functional transcripts of mainly proliferation-associated genes.



Braun CJ et al. *Cancer Cell* 2017;32(4):411-26.

MAT2A Inhibition Selectively Modulates Splicing in MTAP-deleted Background

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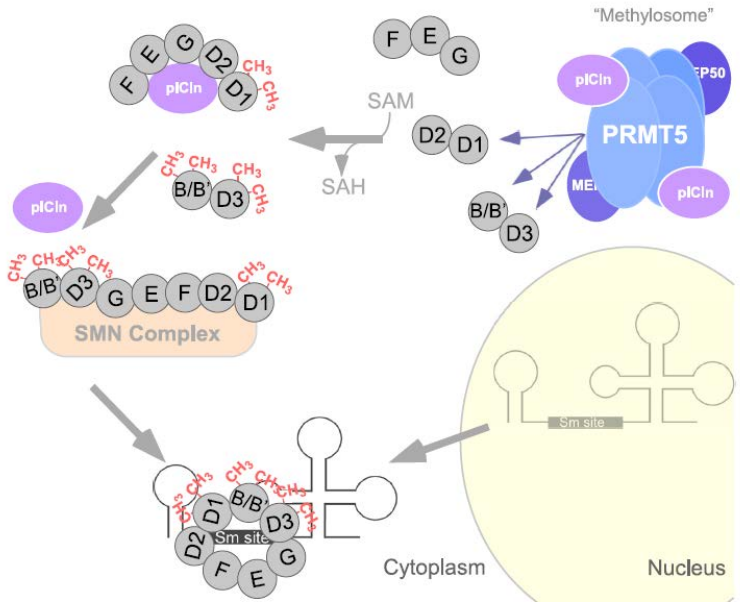
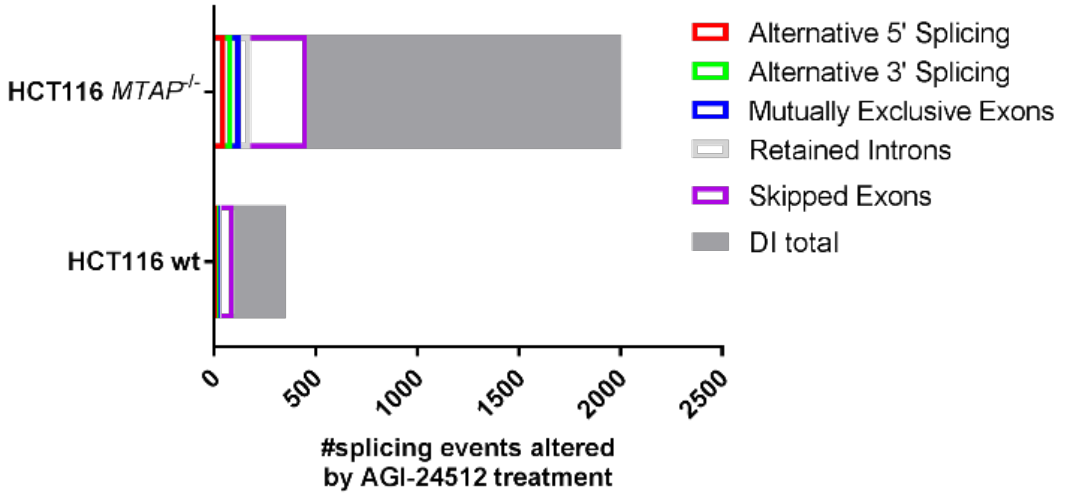
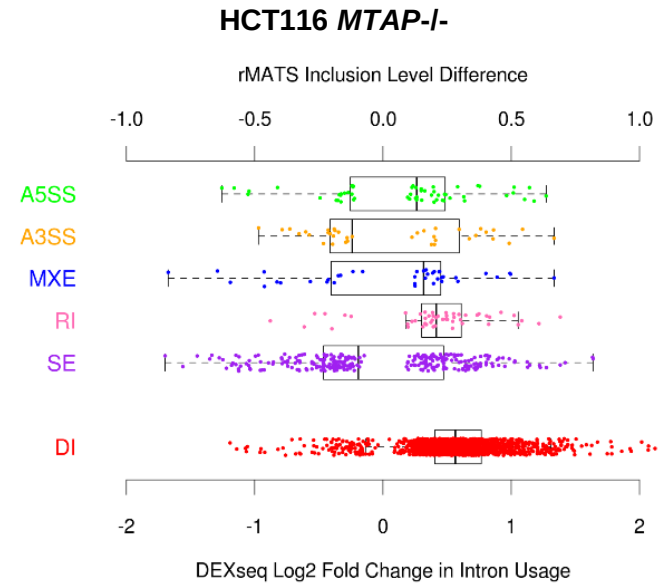
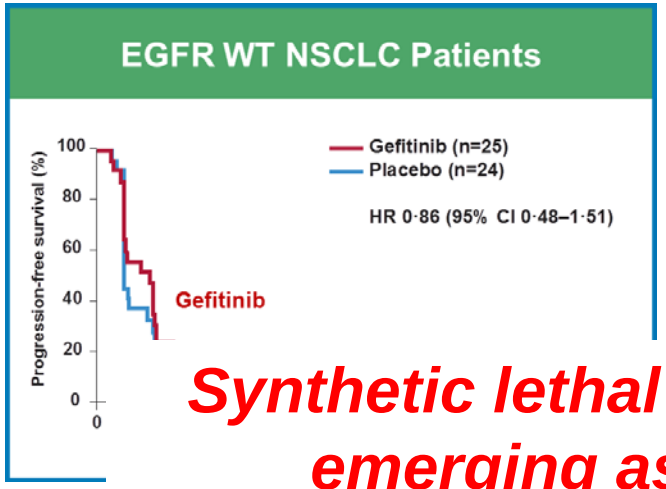


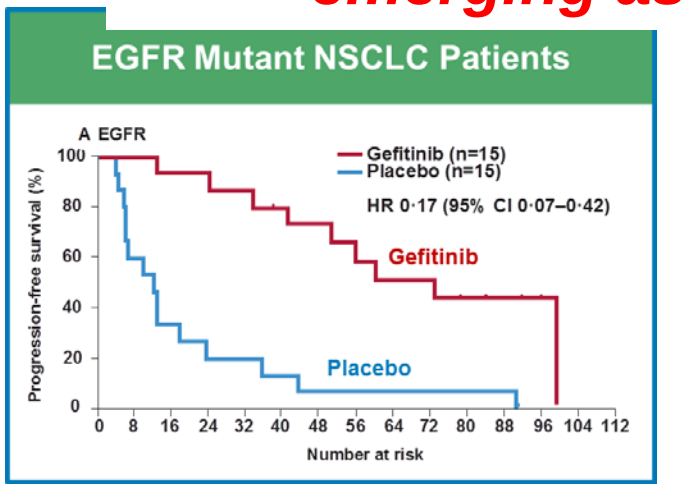




The Challenge: Identifying Precision Medicine Approaches in Cancer Metabolism

Directly drugging 'driver mutations' has yielded transformative medicines





Zhang L et al. *Lancet Oncol* 2012;13(5):466-75.

but…DNA sequencing has identified only 2 gain-of-function metabolic 'driver' mutations out of 2000+ metabolic genes

Synthetic lethal targeting of collateral vulnerabilities emerging as a key solution to this challenge

IDH1/2

Summary

- **Agios discovered potent, cell and *in vivo* active small molecule inhibitors of MAT2A**
  - MAT2A inhibitors selectively block growth of MTAP-deleted cancer cells and tumors
  - Phase I clinical trial was recently initiated for AG-270

- **Surprising specificity of cellular effects following reduction of universal donor of methyl groups SAM** is mediated at least in part via impact on PRMT5 activity and downstream splicing biology