Metabolic collateral vulnerabilities of MTAP-deleted cancers as therapeutic opportunities
The Challenge: Identifying Precision Medicine Approaches in Cancer Metabolism

Directly drugging ‘driver mutations’ has yielded transformative medicines but...DNA sequencing has identified only 1 classic, gain-of-function metabolic ‘driver’ mutation out of 2000+ metabolic genes

**EGFR WT NSCLC Patients**

- Gefitinib (n=25)
- Placebo (n=24)

Progression-free survival (%)

HR 0.86 (95% CI 0.48–1.51)

**EGFR Mutant NSCLC Patients**

- Gefitinib (n=15)
- Placebo (n=15)

Progression-free survival (%)

HR 0.17 (95% CI 0.07–0.42)


IDH1/2
History of targeting MTAP-null cancers: purine biosynthesis story

- MTAP-null cancer cell lines are not selectively sensitive to purine biosynthesis inhibition
- MTA selectively rescues MTAP wt cell lines from purine biosynthesis inhibition \textit{in vitro}

Astrid Ruefli-Brasse et al., J. of Cancer Therapy 2011
Hedy Lee Kindler et al., Invest. New Drugs 2009
shRNA Screening Identifies Candidate MTAP Synthetic Lethal Targets

**Diagram:**
- **MTAP** and **β-actin** expression levels in MTAP-WT and MTAP-/- cell lines.
- Barcoded shRNA library (50,468 shRNAs).
- HCT116 MTAP WT and HCT116 MTAP-/- subjected to 12 cell divisions.
- Measure shRNA depletion via RNA-seq of barcodes.

**Graph:**
- MTAP selectivity score (log2 depletion difference MTAP-/- vs MTAP-WT).
- Preferential depletion with MTAP loss.

**Graph genes:**
- MAT2A
- PRMT5

(ordered by MTAP selectivity)
Metabolomics Reveals Substantial Accumulation of MTAP Substrate MTA in MTAP-null Cells

From one-carbon pool (Serine→Tetrahydrofolate)

**Metabolomics in HCT116 MTAP−/− and HCT116 MTAP wt cells**

**Media MTA profiling in broad cell line panel (n=249)**

[Graph showing MTA and dcSAM levels in MTAP−/− and MTAP WT cells]
PRMT5 biochemical features make it sensitive to double hit of MTA accumulation and SAM reduction

PRMT5 is >20 fold more sensitive to MTA than any other methyltransferase tested in \textit{in vitro} assay

Biochemical profiling of methyltransferases indicates PRMT5 Km is poised near physiologic [SAM]
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
shRNA Screening Identifies Candidate MTAP Synthetic Lethal Targets

Barcoded shRNA library
(50,468 shRNAs)

HCT116
MTAP WT

MTAPwt

12 cell divisions

HCT116
MTAP-/

Measure shRNA depletion via RNA-seq of barcodes

MTAP-/

Preferential depletion with MTAP loss

MTAPwt

MTAP selectivity score

(log2 depletion difference MTAP-/- vs MTAPwt)

MAT2A

(ordered by MTAP selectivity)
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 \( \rightarrow \) regulate gene expression
  - sulfur
    - glutathione \( \rightarrow \) protect vs ROS
Genetic tools validate MAT2A as a Selective Vulnerability in MTAP-null Cancers

HCT116 MTAP isogenic pair

MTAP genotype:  WT  WT  -/-  -/-
shRNA:  NT  Mat2a  NT  Mat2a

MTAP genotype:  WT  WT  -/-  -/-
cDNA rescue:  Mat2a  MTAP  -  -

Growth (%)

-  +  -  +  -  +  -  +  -  +  -  +  -  +  -  +
shNT  shMat2a  shNT  shMat2a  shNT  shMat2a

p=0.009  p=0.015

SAM (nmol per 10^6 cells)

MTAP wt

MTAP +/-

DOX

Mat2a

MTAP

elF4e

MTAP +/-

MTAP wt

Tumor volume (mm^3)

Days post-dox

65% TGI

p=5e-8

+DOX

-DOX

-DOX

+DOX

0 0 0

3 3 3

6 6 6

9 9 9

12 12 12

15 15 15

0 0 0

500 500 500

1000 1000 1000

1500 1500 1500
In vitro and cellular activity of first-in-class MAT2A small molecule inhibitor

In vitro biochemical assay

SAM levels in HCT116 cells

SAM production rate in HCT116 cells
MAT2A Inhibitors Selectively Block Growth of MTAP-null cancer cells in vitro
MAT2A Inhibitors Selectively Block Growth of MTAP-null cancer cells in vitro

**4 day proliferation assay**

MAT2Ai I

MTAP wt

MTAP null

**IncuCyte Continuous growth assay**

HCT116 MTAP wt

HCT116 MTAP null

MTAP wt

MTAP null

**4 day proliferation assay**

MAT2Ai I with 5 µM MTA

MTAP wt

MTAP null

**IncuCyte Continuous growth assay with exogenously added MTA**

HCT116 MTAP wt + 5µM MTA

HCT116 MTAP null + 5µM MTA
MAT2A Inhibitors Selectively Block Growth of MTAP-null cancer cells \textit{in vitro}

MTAP predicts sensitivity in Cell Panel with MAT2Ai II

\[ p = 7.95 \times 10^{-14} \]
Genetic and Pharmacologic targeting of MAT2A Selectively Blocks Growth of MTAP-null cancer cells in vivo

**Impact in vivo with MAT2Ai**

- Similar SAM decrease is observed in HCT116-MTAP-/- and HCT116-MTAP+/+ tumors.
Treatment with MAT2Ai reduces growth of naturally MTAP-null KP4 pancreatic cancer cell line xenografts.

MAT2Ai had significant anti-tumor activity in the KP4 Sub-q model.
Strong reciprocal connection/synthetic lethality between MAT2A and MTAP

MAT2A is the top hit in an MTAP-selective shRNA screen

Meanwhile, MTAP deletion is the genetic feature that best predicts sensitivity to MAT2A inhibitor
PRMT5 is also a Selective Vulnerability in MTAP-null Cancers

HCT116 MTAP isogenic pair

**PRMT5 shRNA**

- **DOX**
  - **PRMT5**
  - **MTAP**
  - **β-actin**

**MTAP genotype:**
- WT
- +/-
- +/-
- WT
- +/-
- +/-

**cDNA rescue:**
- -
- -
- PRMT5
- R368A
- PRMT5

**Basal PRMT5 methyl marks**

**Cancer Cell Line Panel**

- **SDMA levels**
  - **MTAP wt**
  - **MTAP deleted**

**dox:**
- -
- +
- -
- +

**MTAP:**
- WT
- +/-
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:

<table>
<thead>
<tr>
<th>MTAP wt</th>
<th>MTAP −/−</th>
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<tbody>
<tr>
<td>0</td>
<td>0.1</td>
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<td>0.1</td>
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</table>

HCT116 MTAP+/+ HCT116 MTAP−/−

- 3
- 36

SDMA

β-actin
Methylation proteomics identifies loss of methylation of RNA processing machinery upon MAT2A inhibitor treatment
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)
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- SmD1, SmD3, SmB/B’ (Brahms, RNA 2001 and Friesen Mol Cell 2001)
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Splicing regulation is an essential step in lymphomagenesis:

- MYC directly upregulates the core snRNP assembly genes, including PRMT5
- PRMT5 is overexpressed in non-Hodgkin lymphoma (NHL) cell lines and clinical samples (Chang J Biol Chem 2013)
- PRMT5 is required for proliferation of B lymphoma cell lines (Chan-Penebre NCB 2015)
NHL B lymphoma MTAP-null models show reduced growth and downstream impact on PRMT5 SDMA marks upon treatment with MAT2Ai
MAT2Ai Treatment Perturbs Splicing Selectively in MTAP-null NHL B lymphoma Cell Lines

**Tophat2** (mapping reads to transcriptome)

**rMATS: alt splicing (FDR < 0.05)**

<table>
<thead>
<tr>
<th>MTAP Status</th>
<th>Cell Line</th>
<th>Treatment</th>
<th>Alternative 3' Splicing</th>
<th>Alternative 5' Splicing</th>
<th>Mutually Exclusive Exons</th>
<th>Retained Introns</th>
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Summary

• MTAP is frequently deleted in a variety of cancer indications (~15% of all human cancer)
  – MTAP deletion leads to its substrate MTA accumulation in MTAP null tumors
  – PRMT5 unique biochemical features make it sensitive to double hit of MTA accumulation and SAM reduction (downstream from MAT2A)

• Agios discovered first-in-class small molecule inhibitors of MAT2A

• MAT2A pharmacologic targeting substantially reduces SAM levels and SAM de novo synthesis in cells

• Pharmacologic inhibition of MAT2A recapitulates findings with MAT2A-targeting genetic tools and selectively attenuates growth of MTAP\(^{-/-}\) but not MTAP wt cancer cells in vitro and in vivo
  – Inhibition of MAT2A selectively attenuates growth of MTAP\(^{-/-}\) HCT116 cells and naturally MTAP-deleted cancer cell lines (n=330 cell lines)
  – Inhibition of MAT2A significantly attenuates growth of MTAP-deficient HCT116 and KP4 cells in vivo

• MAT2A inhibition and MTAP loss exhibit strong reciprocal connection mediated at least in part via impact on PRMT5 activity and downstream function (symmetric Arg di-methylation of RNA processing machinery and splicing)
Directly drugging ‘driver mutations’ has yielded transformative medicines but...DNA sequencing has only ID’d 1 classic, gain-of-fuction metabolic ‘driver’ mutation out of 2000+ metabolic genes.

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

Agios 2016 Founders Day Retreat

- Agios Pharmaceuticals team
- Cell Signaling Technology proteomics core