Targeting MAT2A in MTAP-deleted Cancers

Disclosure Information

I have the following financial relationships to disclose:

Employee of: Agios Pharmaceuticals, Inc.
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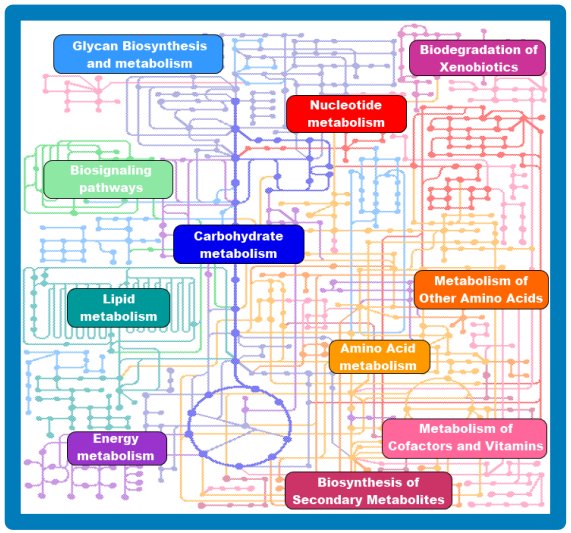
- and -

I will not discuss off label use and/or investigational use in my presentation.

Learnings & Forward Strategy for Precision Medicine in Cancer Metabolism



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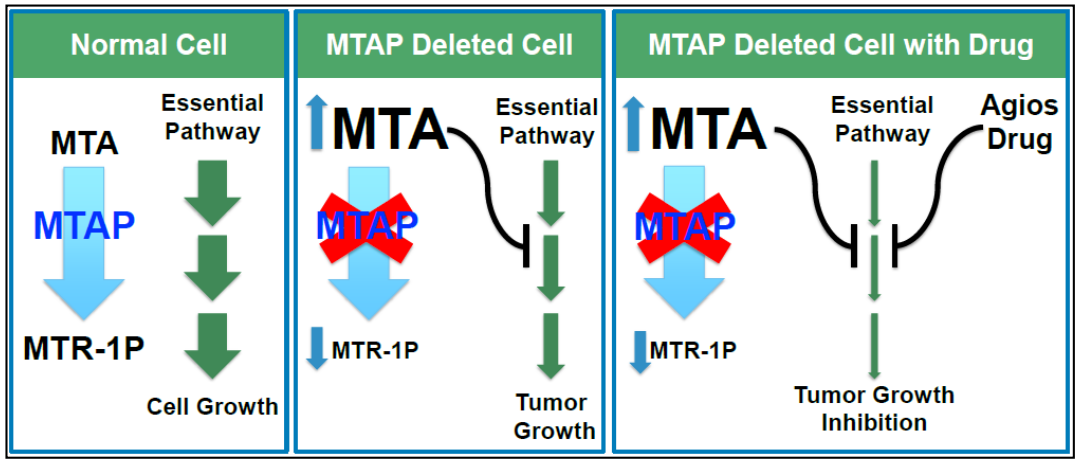
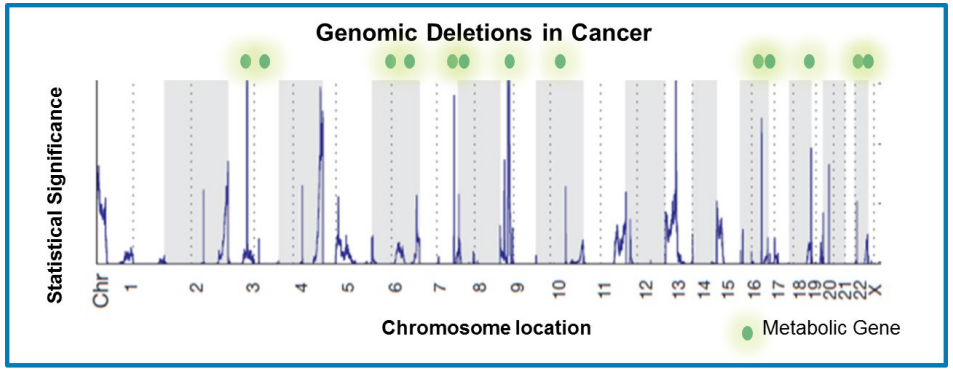


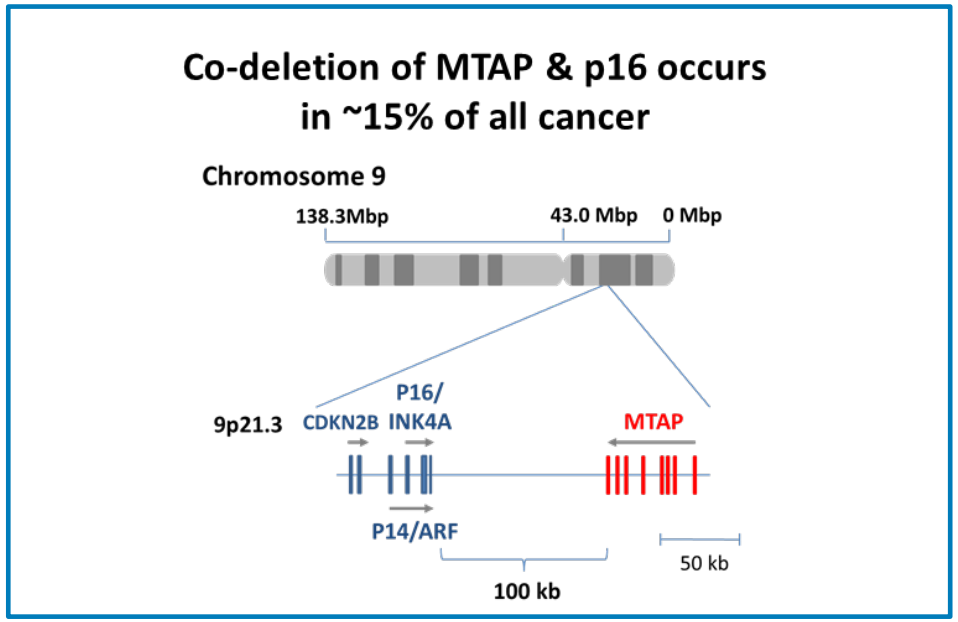
MTAP & others

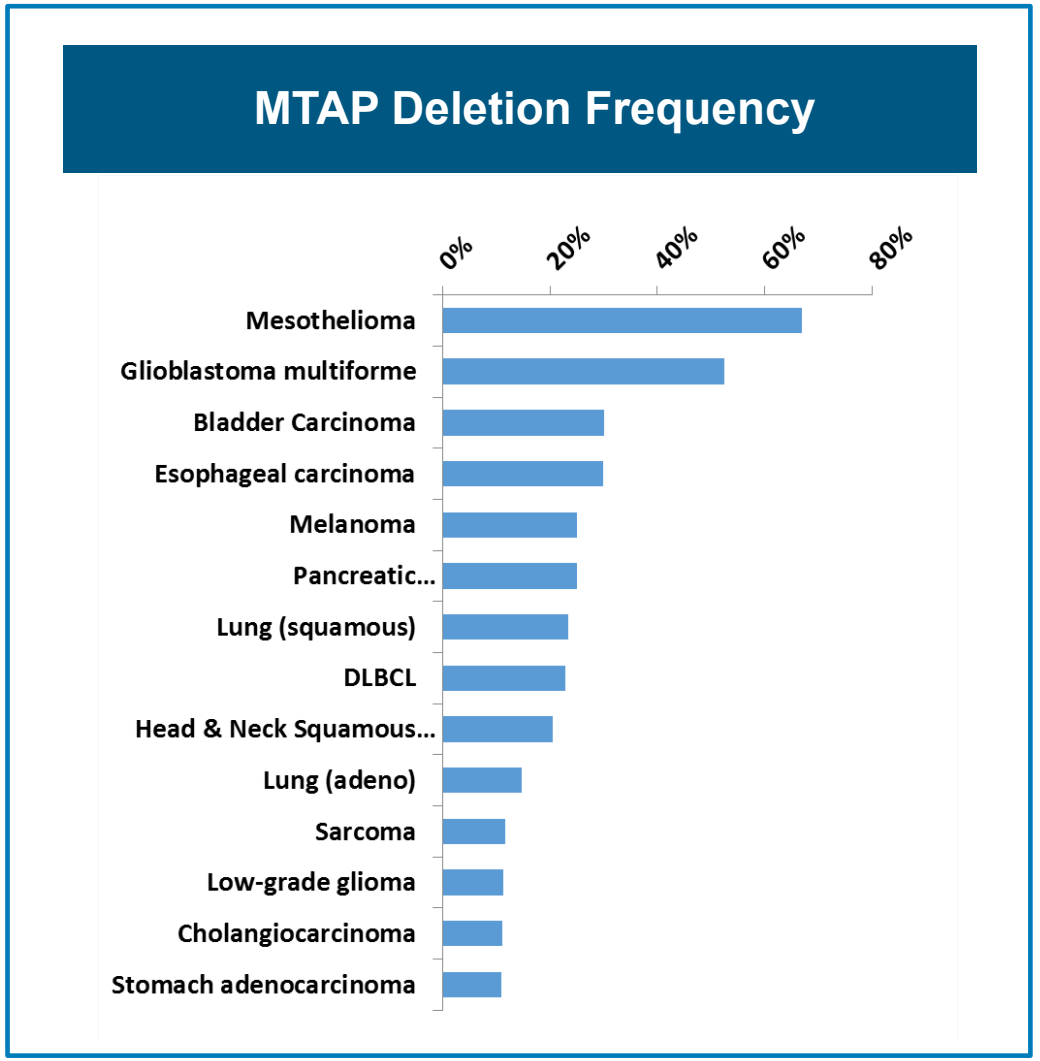
- Metabolites regulate signaling, epigenetics, and impact tumor microenvironment. ***Critical cancer vulnerabilities arise at the interfaces.***
- Synthetic lethality extends past tumor suppressors. ***Losses of adjacent genes create druggable vulnerabilities.***
- Synthetic lethality is often non-obvious. ***The best targets are found via integration of multiple approaches.***

While gain-of-function driver mutations are scarce, loss-of-function mutations are common



Beroukhim…Meyerson Nature 2010

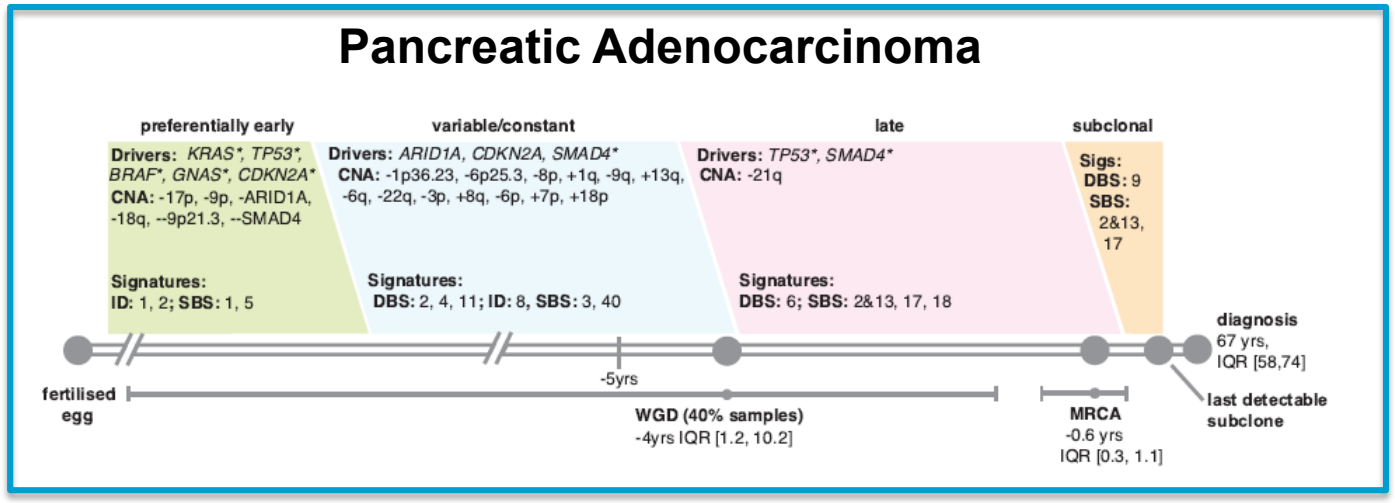


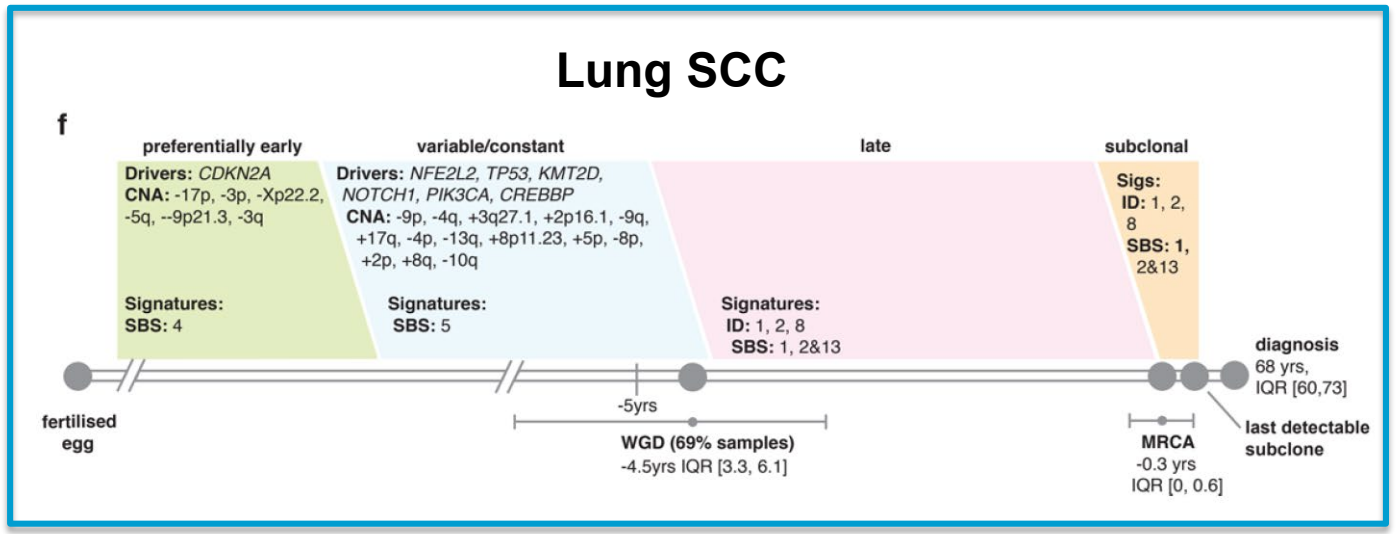


Marjon et al Cell Reports. 2016 Apr 19;15(3):574-587

Inc. & Illei et al Clinical Cancer Research. 2003 Jun; 9(6):2108-13.

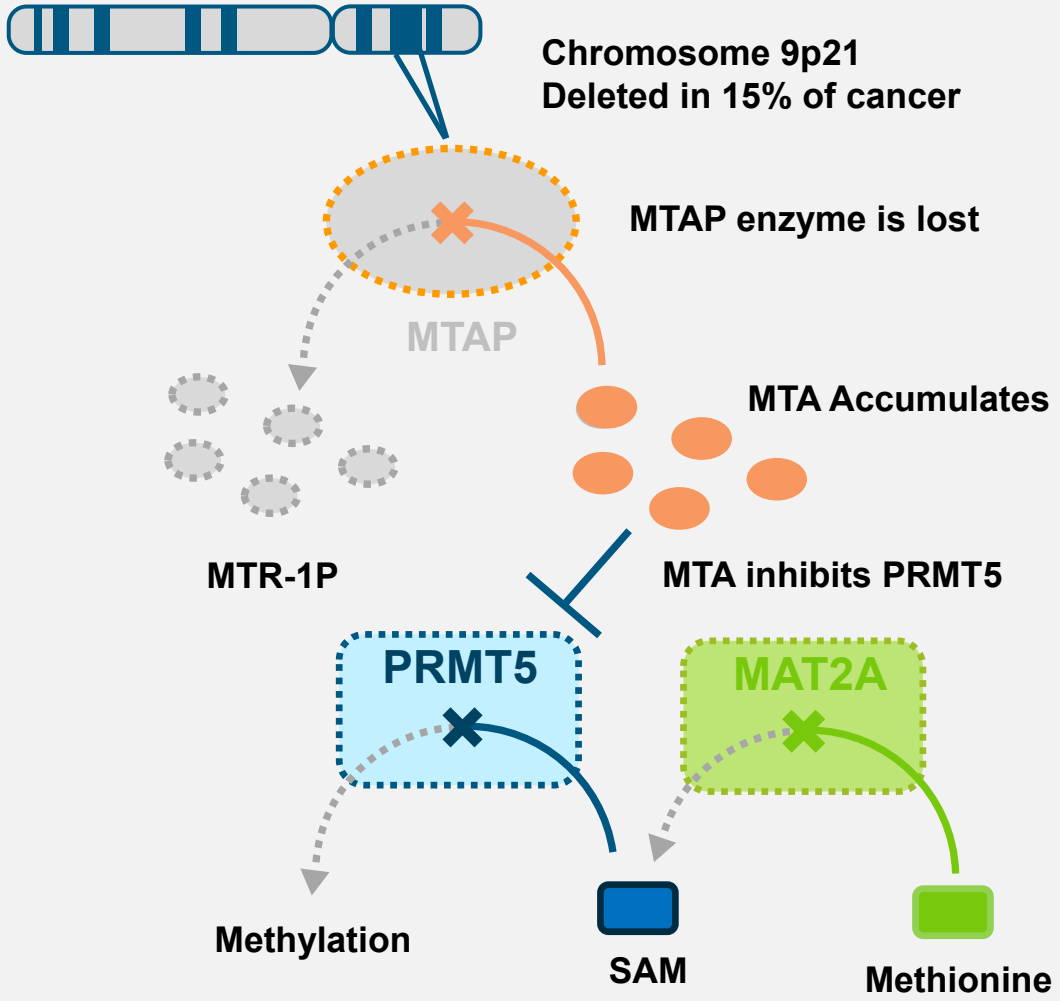
9p21 deletions are early/clonal events and therefore are homogenous throughout the tumor





A Key Insight: Deletion of MTAP Makes Cancers Vulnerable to Targeting of MAT2A

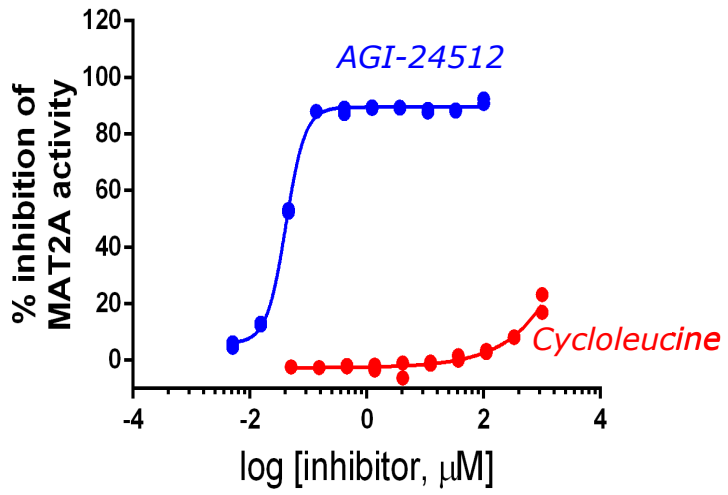
1. MTAP deletion
2. Substrate MTA accumulates
3. Partial Inhibition of PRMT5
4. Sensitivity to a 'second hit' : targeting **MAT2A** starves PRMT5 of its substrate



Discovery of First in Class MAT2A Inhibitors & Validation of the MAT2A/MTAP Hypothesis

Identification of MAT2A Inhibitors

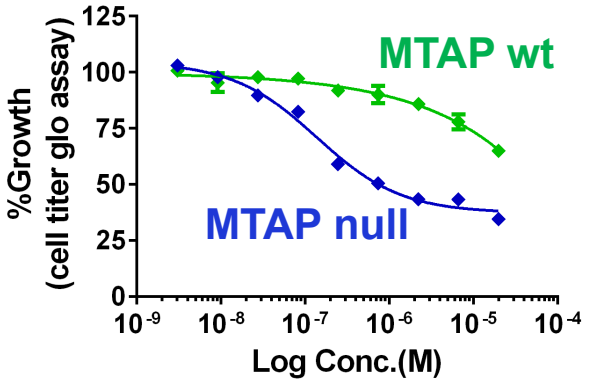
In vitro biochemical assay

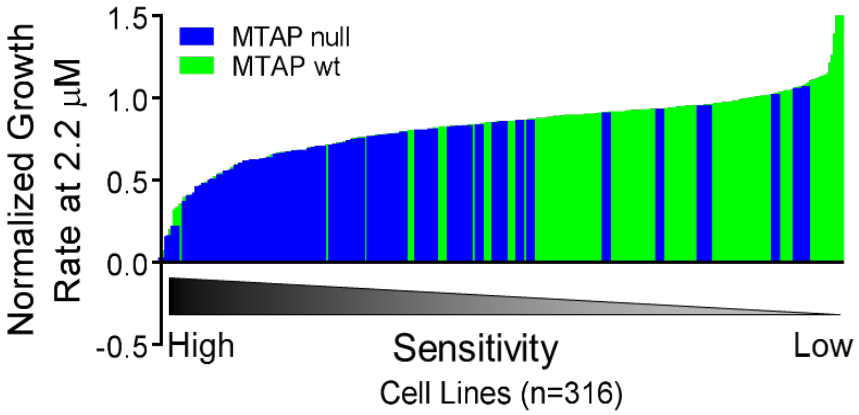


MTAP-selective growth inhibition

Growth Inhibition in Cancer Cells +/- MTAP

HCT116 isogenic pair





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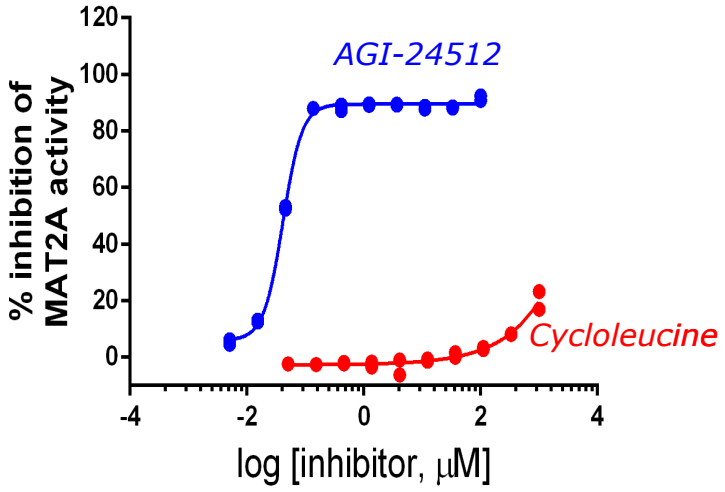
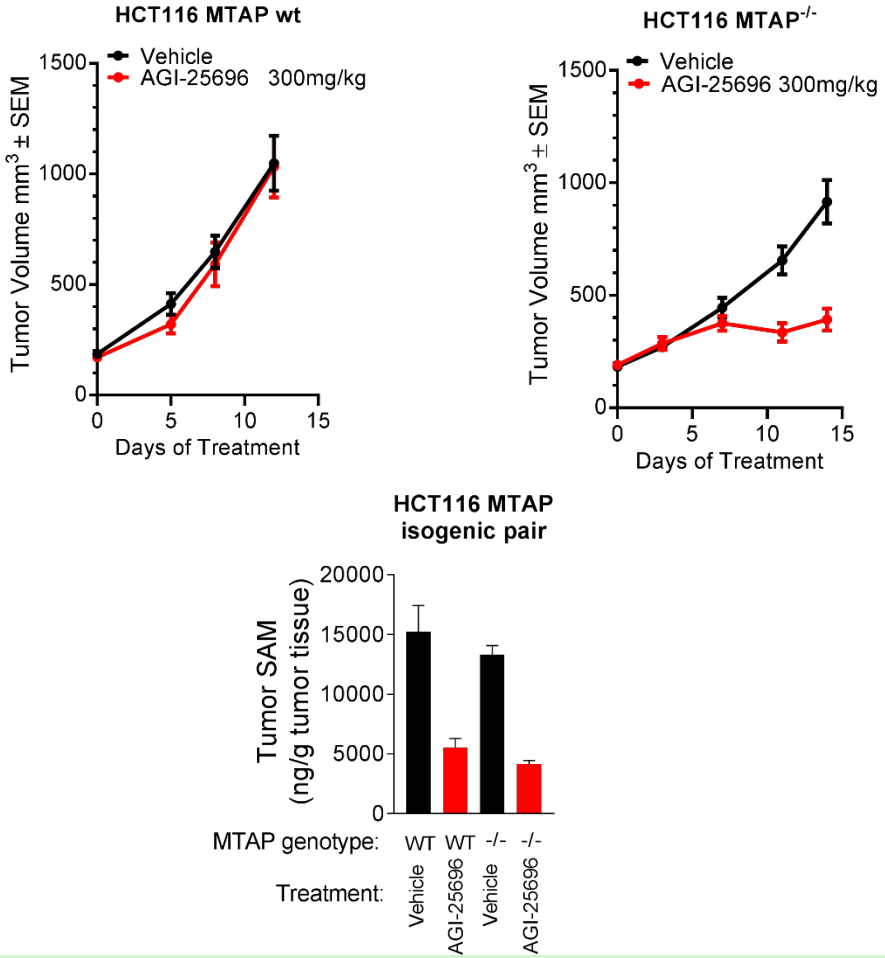
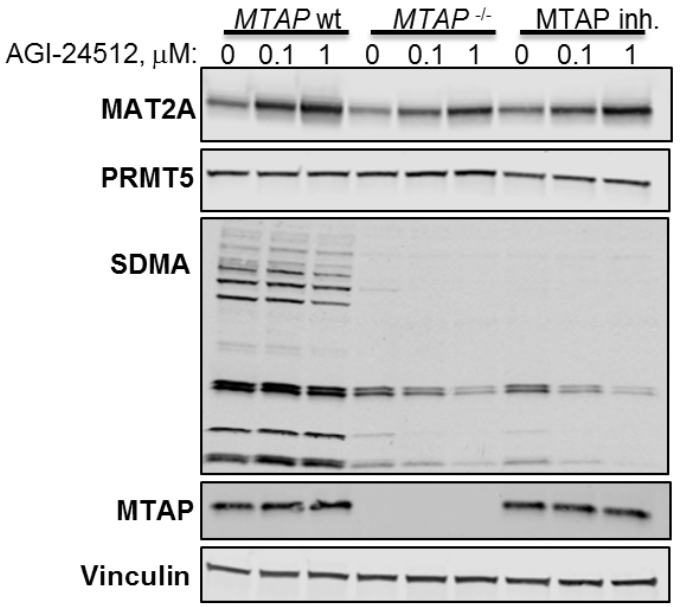
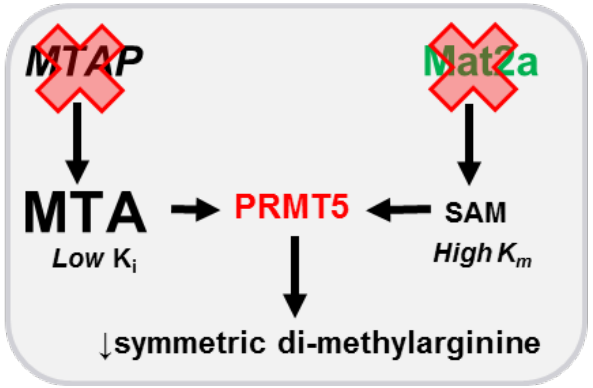


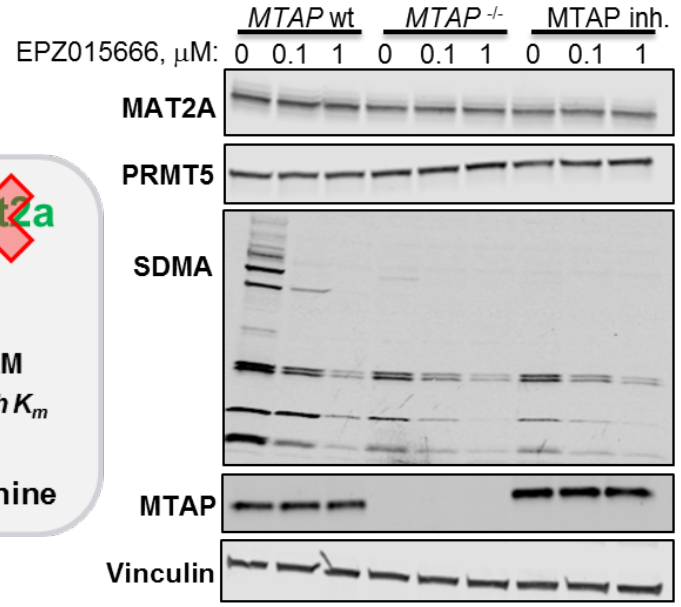
MTAP-selective growth inhibition

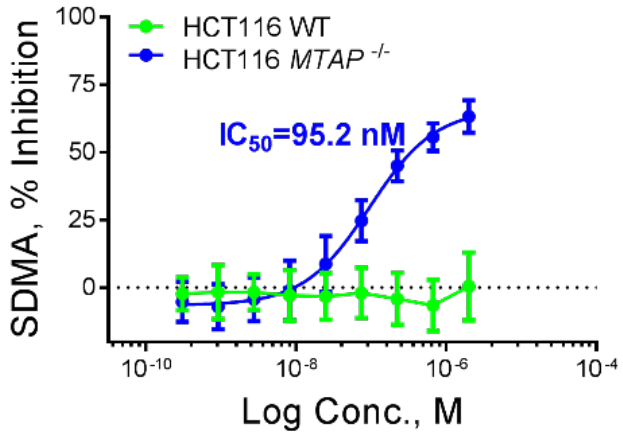


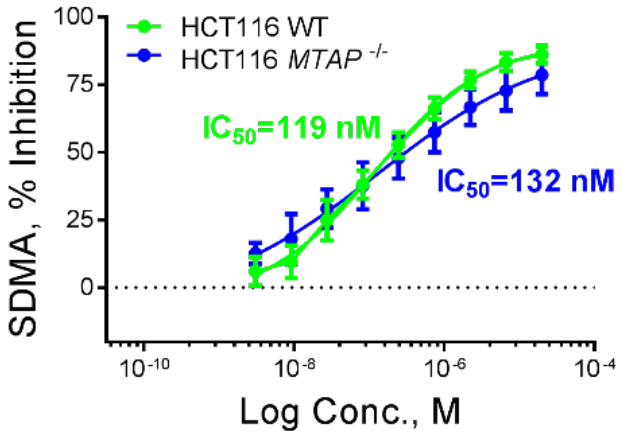
MAT2A Inhibition Selectively Impacts PRMT5 Activity in MTAP-deleted Background





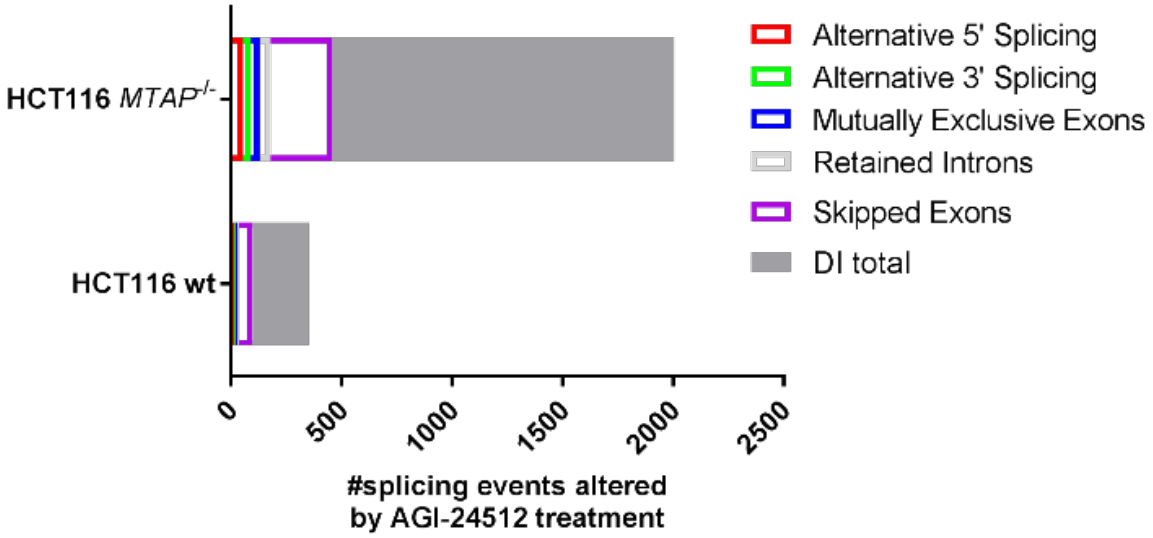


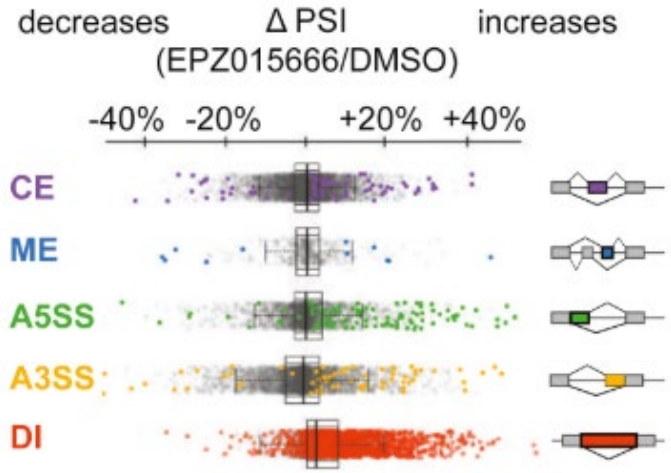


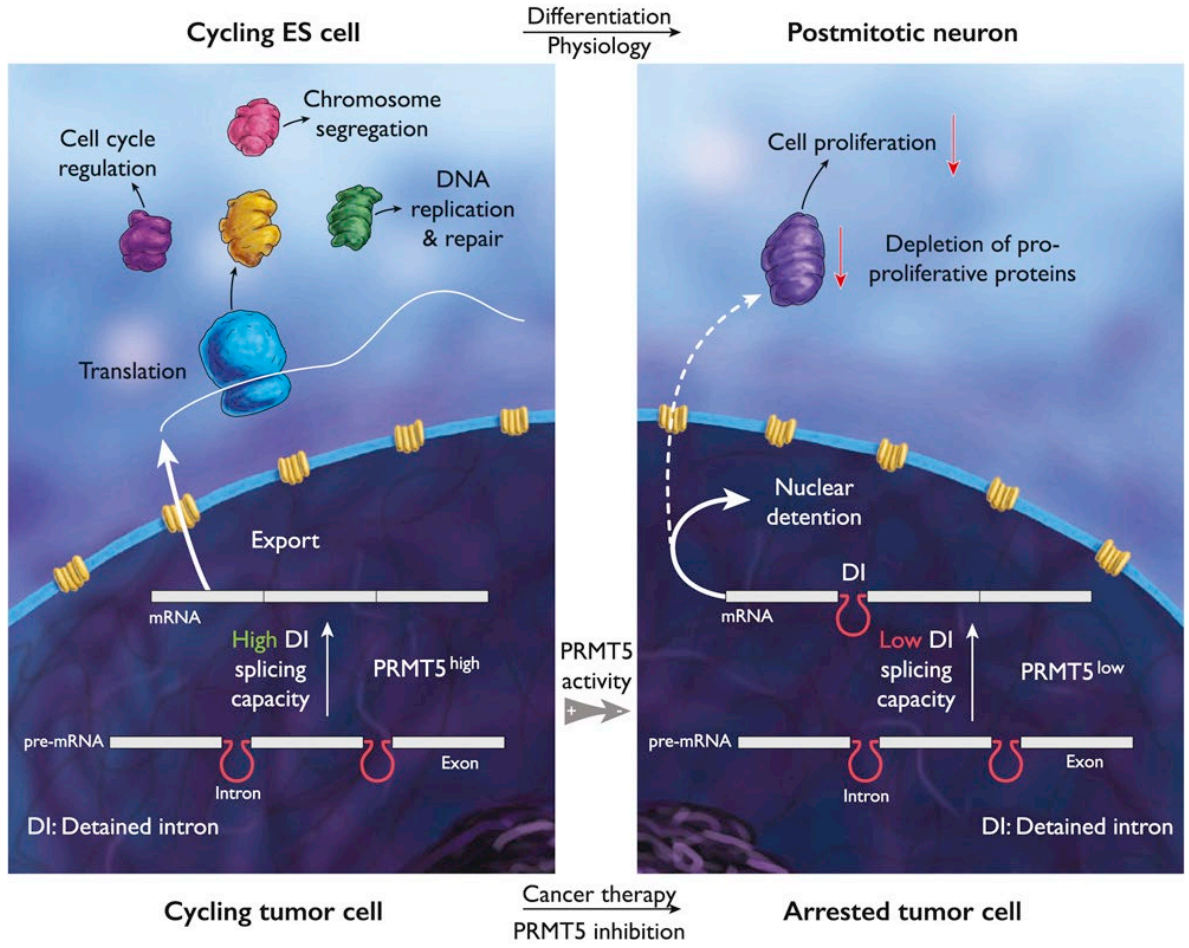


EPZ015666: Chan-Penebre E et al. *Nat Chem Biol* 2015;11(6):432-7.

MAT2A Inhibition Selectively Modulates Splicing in MTAP-deleted Background







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

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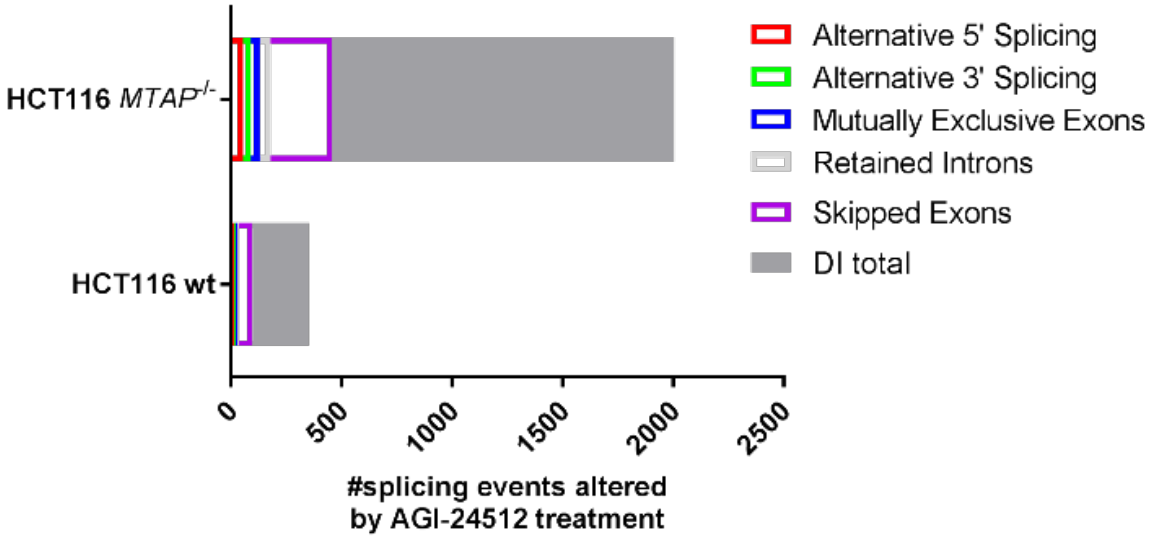
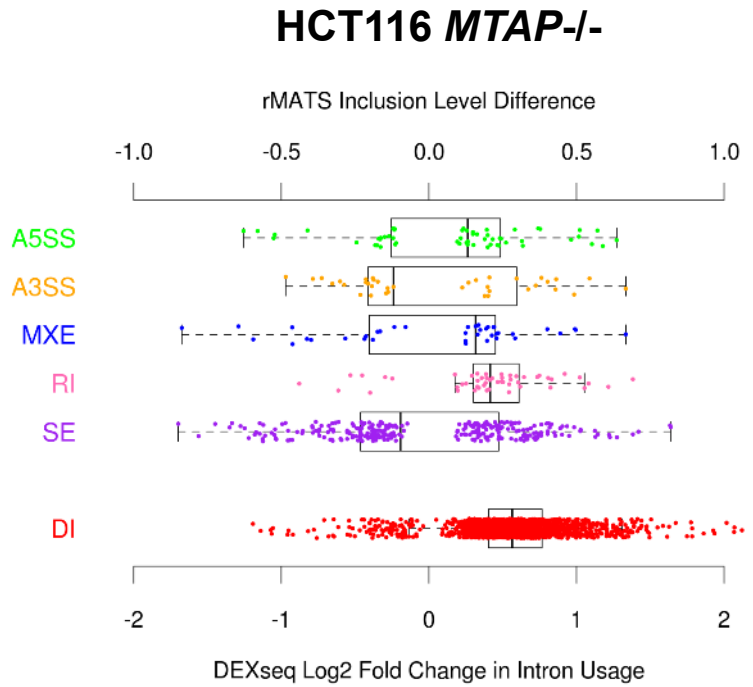
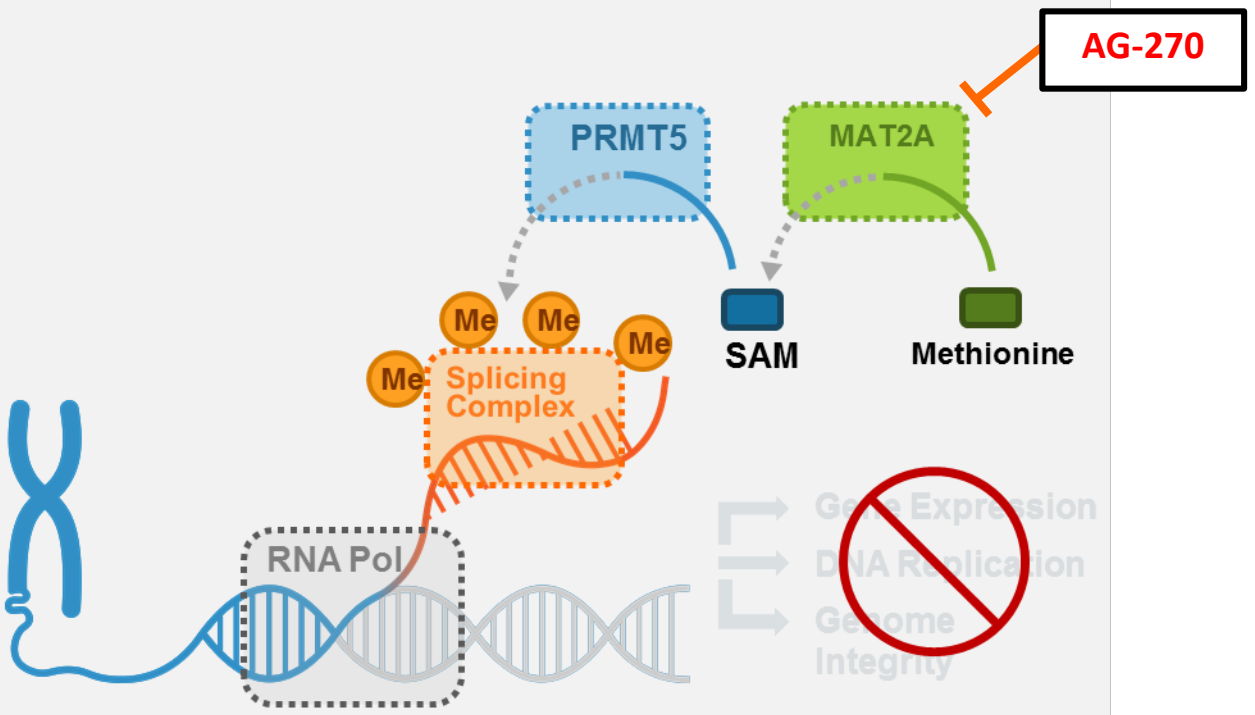




Emerging Mechanistic Understanding of the Pathway Downstream of MAT2A

1. RNA splicing concurrent with transcription
2. Splicing complex requires PRMT5
3. MAT2A inhibition blocks splicing
4. Defects in gene expression, DNA replication, genome integrity



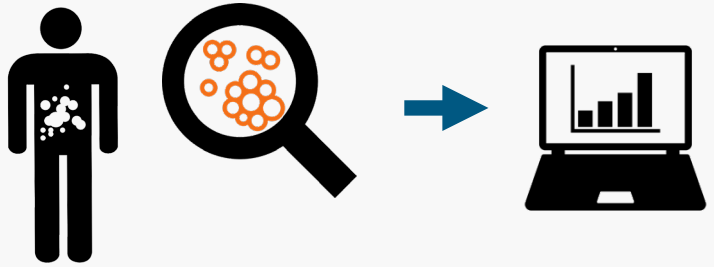
The AG-270 Program is Well-poised for Biomarker-driven Clinical Development

Multiple Pharmacodynamic Biomarkers

Pathway Effects	Clinically Applicable Biomarkers
MAT2A inhibition	
↓	Quantitative measurement of SAM in patient plasma
Tumor-selective inhibition of PRMT5	
↓	IHC for PRMT5-mediated methyl marks in tumor biopsies
Inhibition of PRMT5 targets including the spliceosome	
↓	Assess splicing using RNA seq & other assays
Antiproliferative & cytotoxic effects	Mechanistic studies on AG-270 suggest suitable response biomarkers

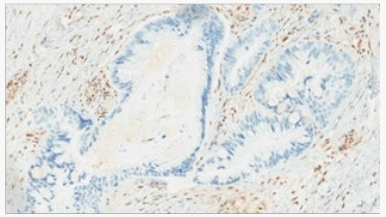
Patient Selection Biomarkers

1. **Next-gen sequencing for CDKN2A loss**

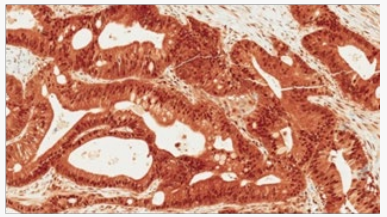


2. **Directly assess MTAP-status by IHC**

MTAP deficient PDAC

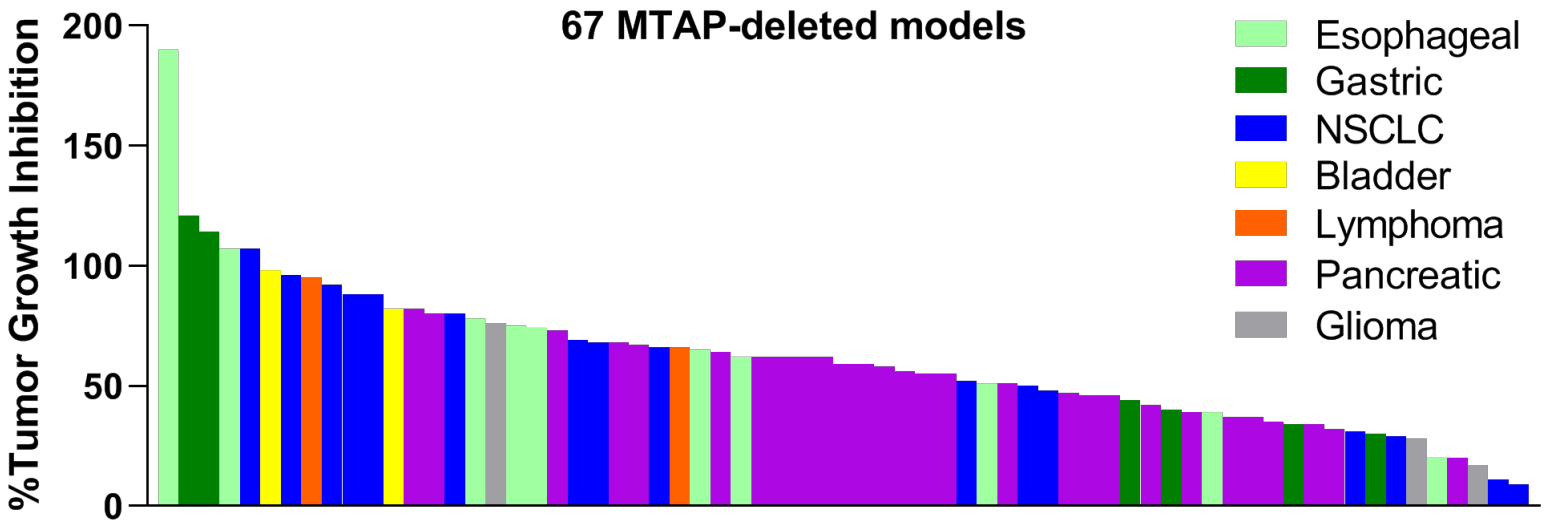


MTAP positive PDAC



Preclinical Studies Indicate Potential for use in Variety of MTAP-deleted Indications – PDX sensitivity by Tumor Type

Patient-derived Xenograft (PDX) 'clinical trial'

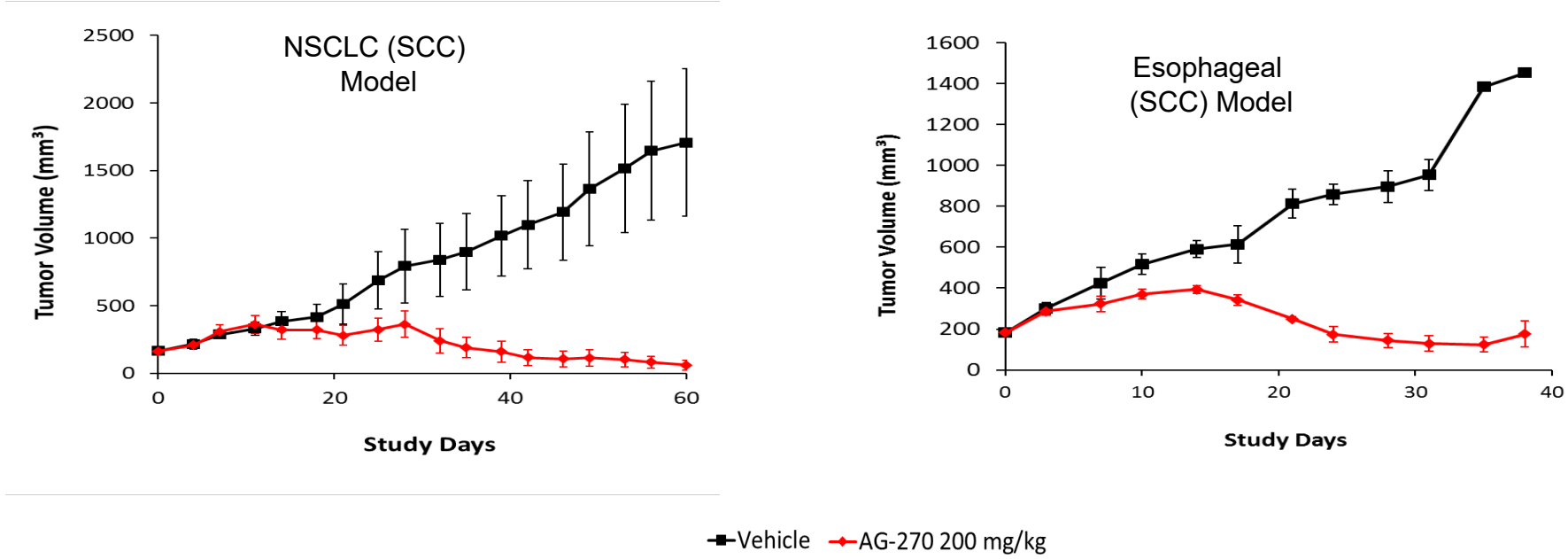


- N=3 PDX per model
- Samples were collected for LC-MS measurement of drug exposure and changes in levels of SAM
- Additional biomarkers included assessment of SDMA level by IHC

AG-270 is efficacious in MTAP-deleted PDX models from a variety of tissue origins including NSCLC, Pancreatic, Gastric & Esophageal

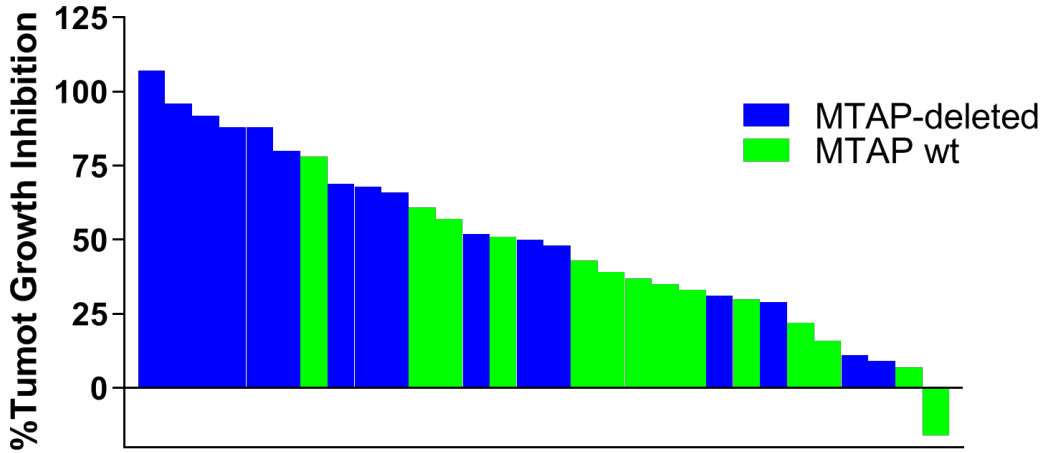
Preclinical Studies Indicate Potential for use in Variety of MTAP-deleted Indications

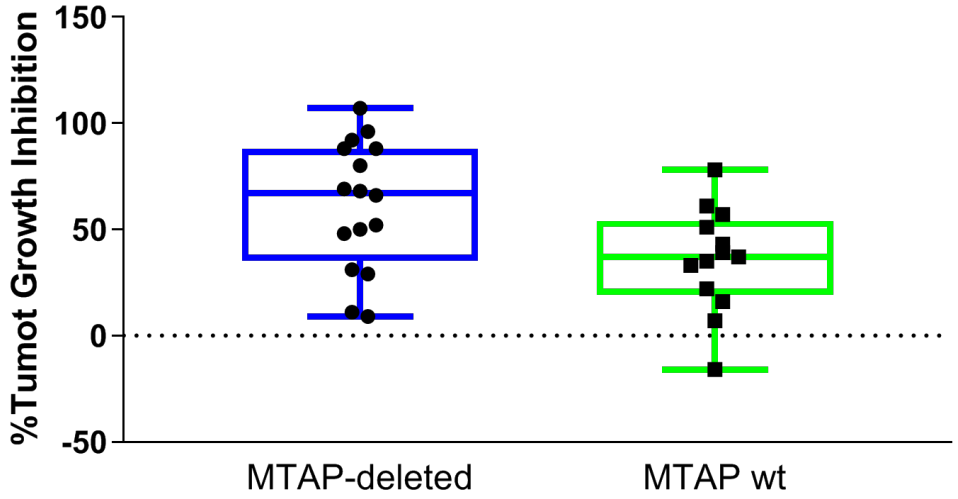
Regressions observed upon single agent AG-270 treatment in some models

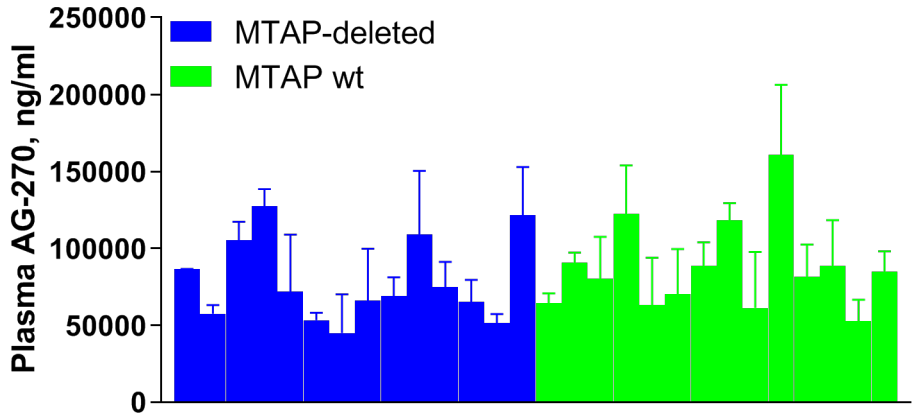


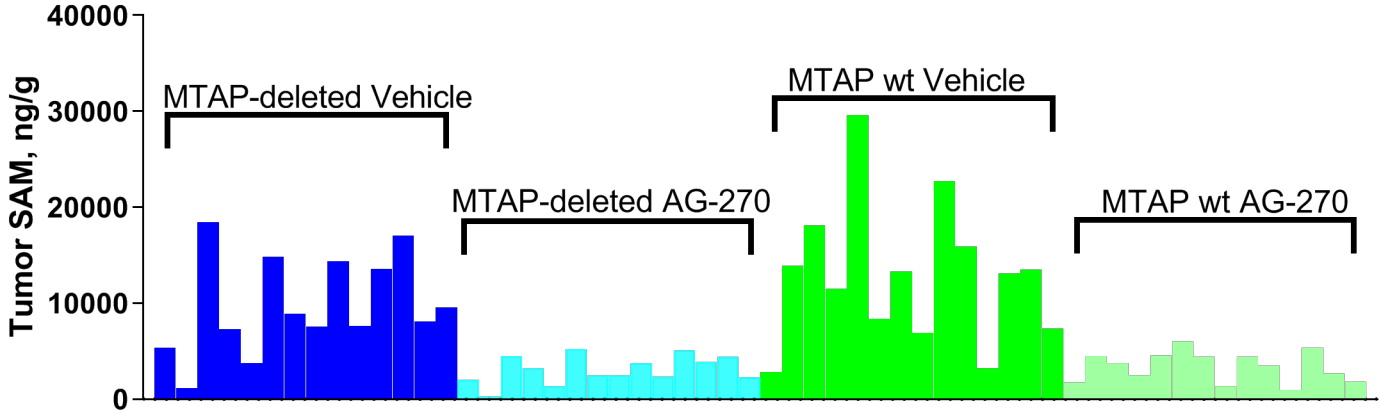
AG-270 is efficacious in MTAP-deleted PDX models from a variety of tissue origins including NSCLC, Pancreatic, Gastric & Esophageal

Preclinical Studies in NSCLC PDX models demonstrate segregation for sensitivity by MTAP status



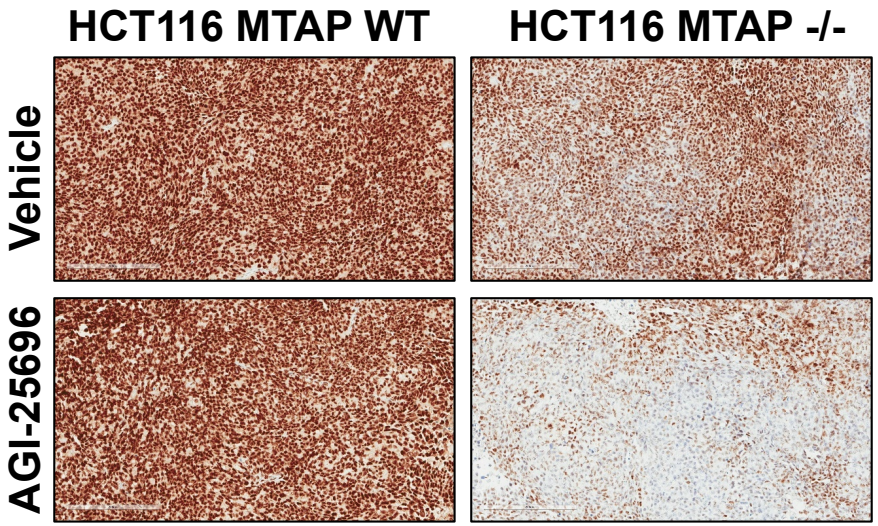




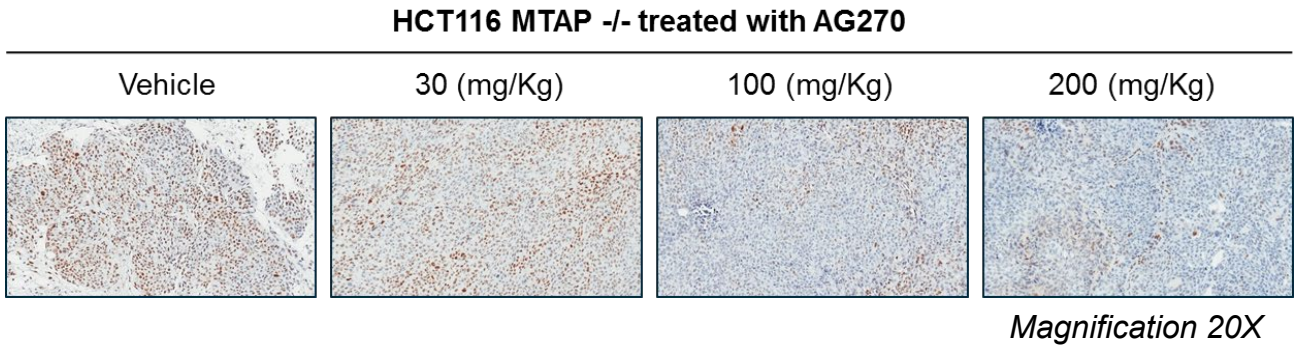


AG-270 is efficacious in MTAP-deleted NSCLC PDX more so than in MTAP wt NSCLC PDX models, while equivalent reduction in levels of SAM is observed

MTAP status is predictive of basal SDMA levels and drug-induced reduction in SDMA



Magnification 20X



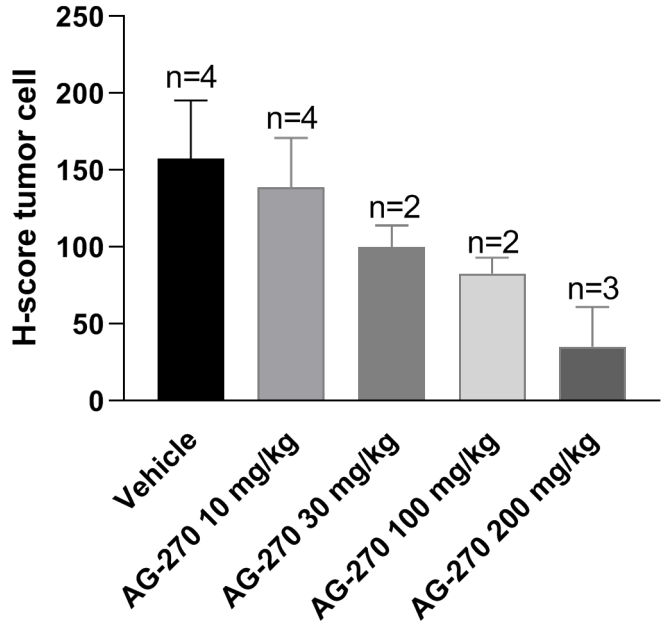
Magnification 20X

Treatment	MTAP status	SDMA H-score
Vehicle	WT	300
Vehicle	null	180
AGI-25696	WT	275
AGI-25696	null	100

IHC quantification: H-score

- Histological score
- Combines % **tumor** stained cells with stain intensity (1-3)
- Score range: 0-300

Tumor SDMA IHC staining (H-score)



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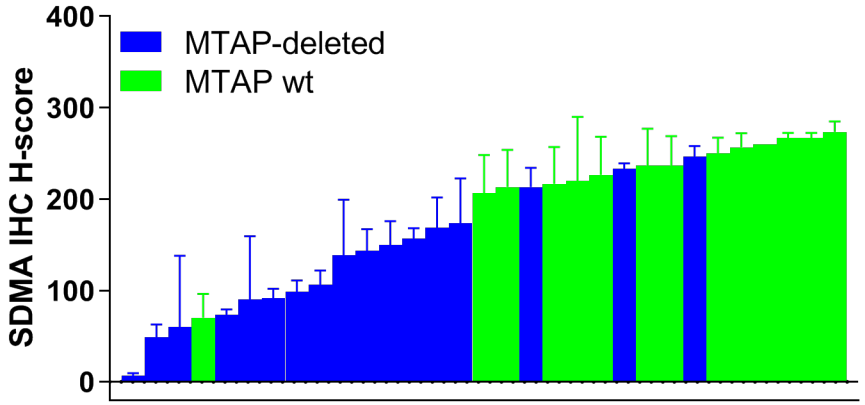
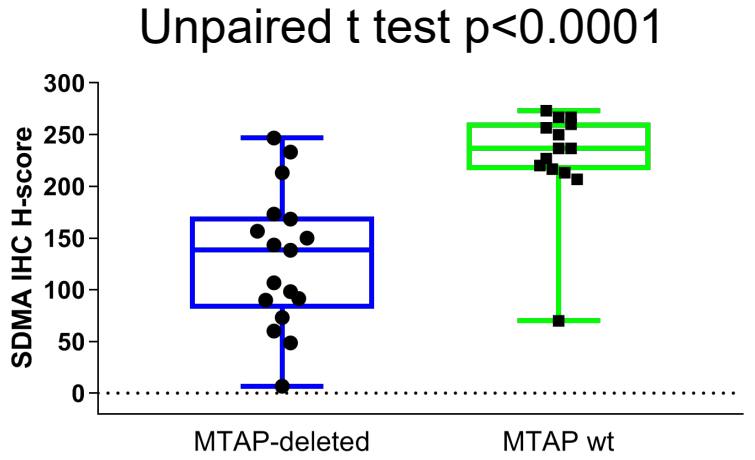
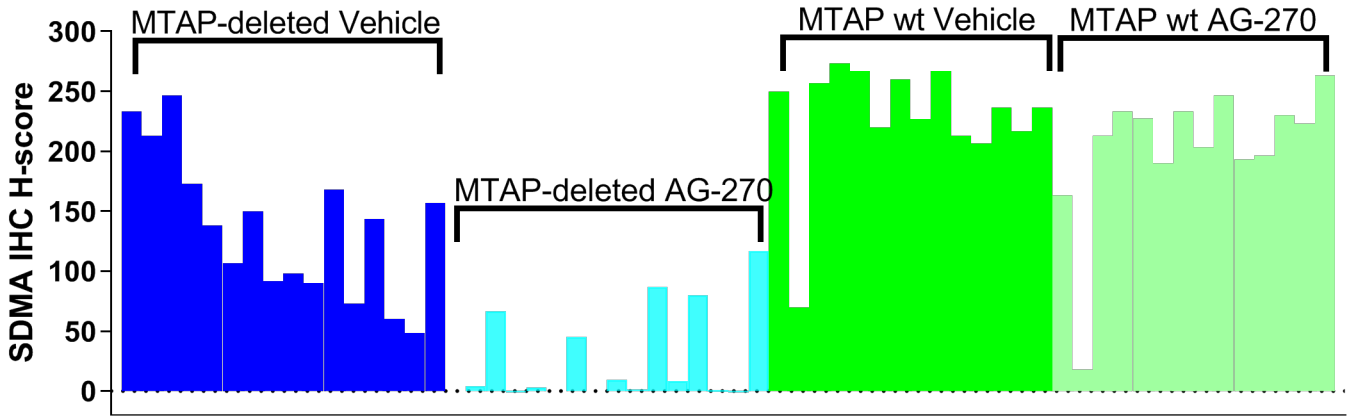


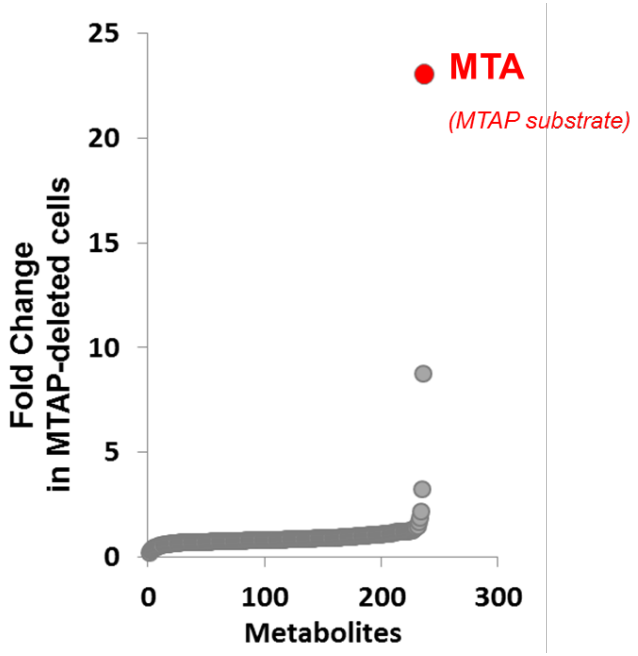




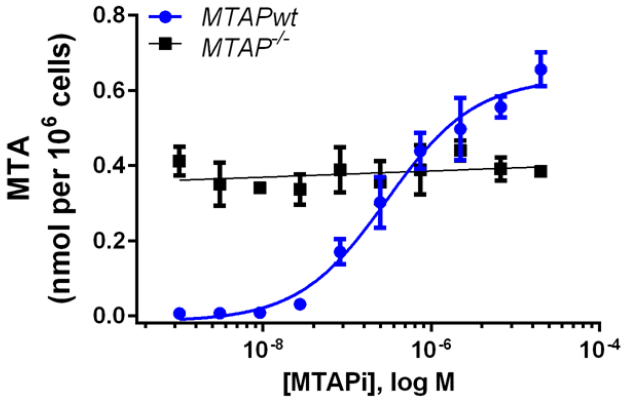
- **MTAP status correlates well with basal levels of SDMA marks by IHC in NSCLC PDX models**
- **AG-270 induces reduction in SDMA marks selectively in MTAP-deleted NSCLC PDX samples**

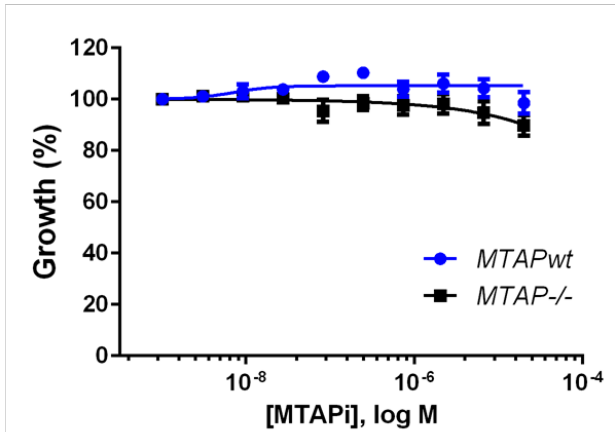
Genetic and pharmacologic modulation of MTAP controls MTA levels in cancer cells

MTAP deletion leads to increase in MTA levels

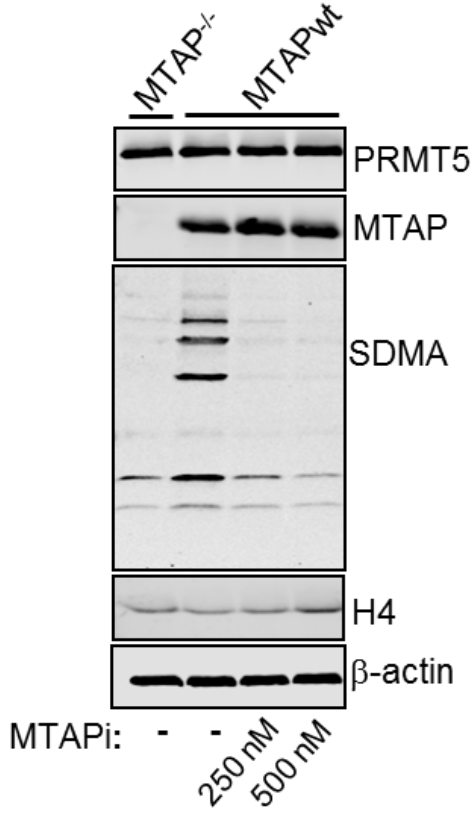


MTAP inhibitor leads to increase in MTA levels without impacting cell growth

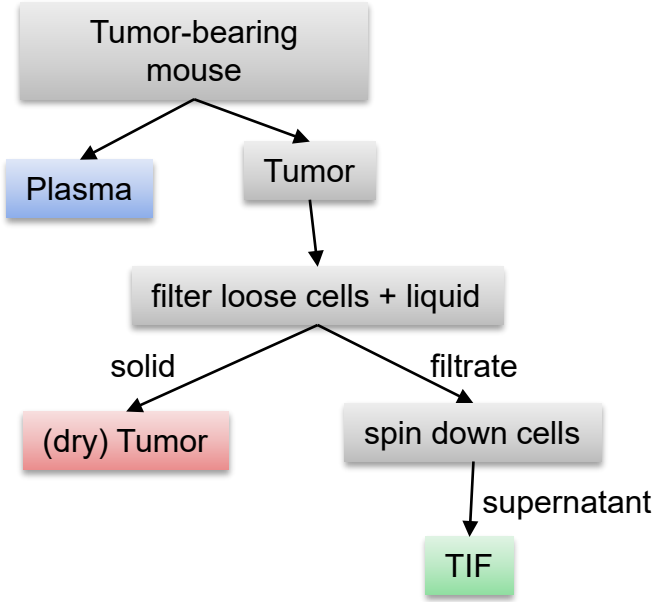


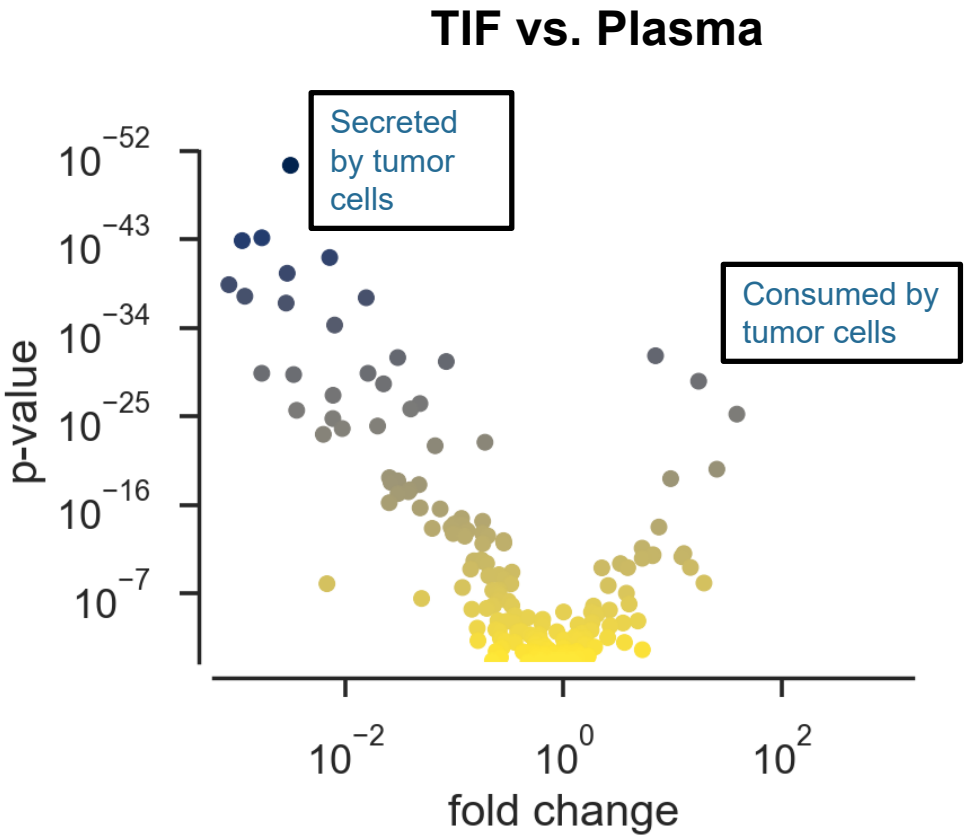


Pharmacologic elevation of MTA levels leads to reduction in PRMT5 methylation



Characterizing the metabolic composition of tumor microenvironment



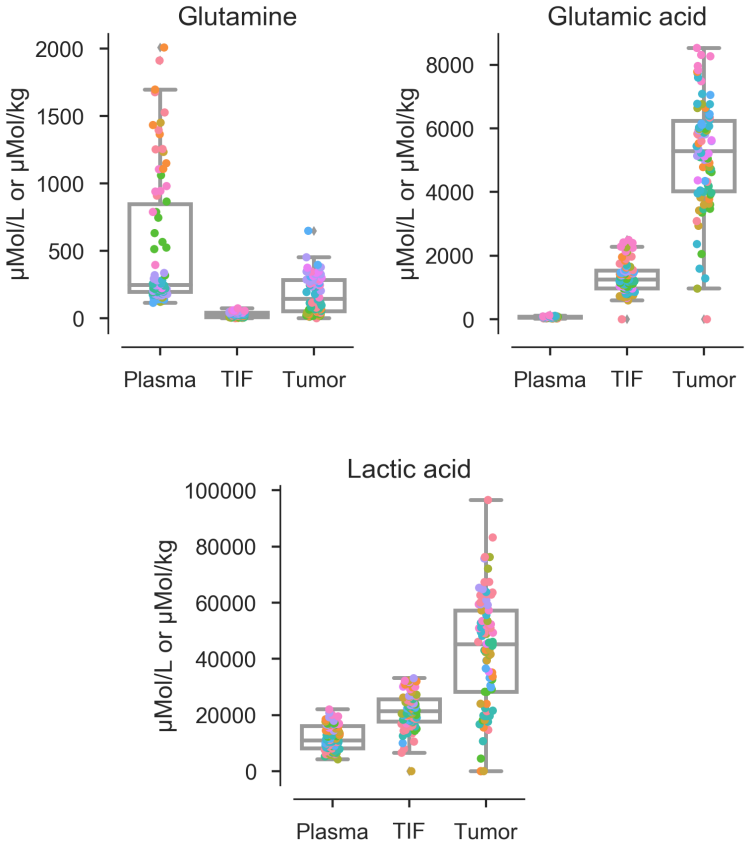


Hypothesis: **MTA in tumor microenvironment is impacted by neighboring normal cells**

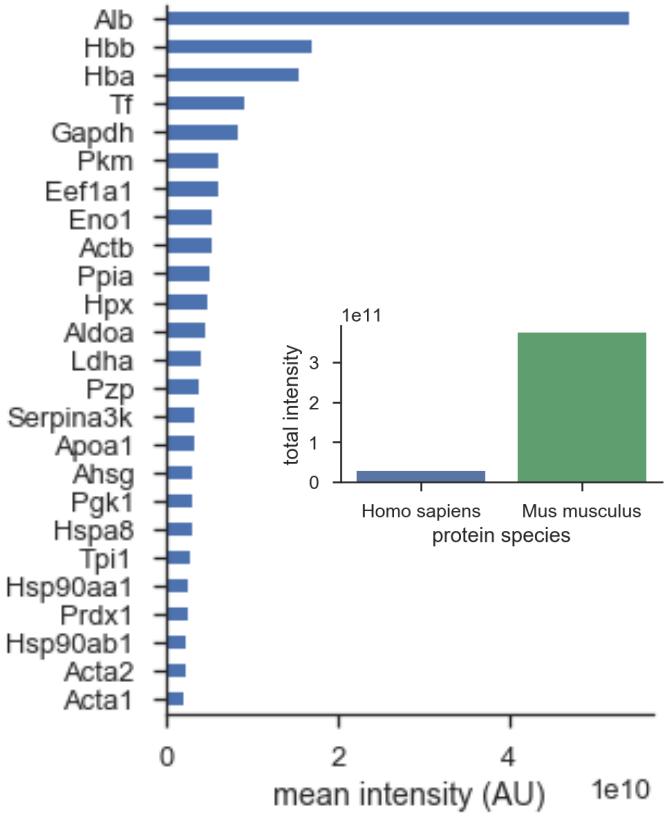
Goal: **differentiate intra-tumoral MTA from the MTA in tumor interstitial fluid (TIF)**

Characterizing the metabolic composition of tumor microenvironment

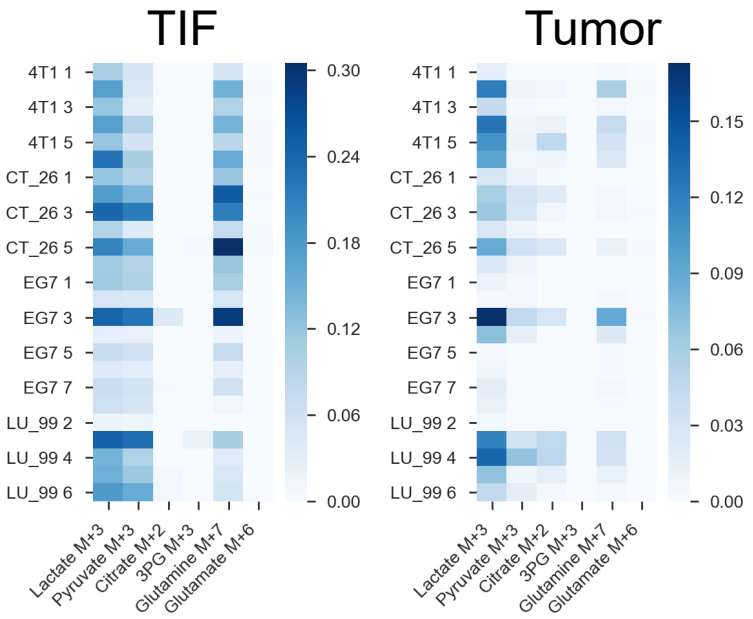
- Typically observe depletion of Glutamine and enrichment for Lactate and Glutamate



- Proteomics suggests most TIF proteins are from mouse blood

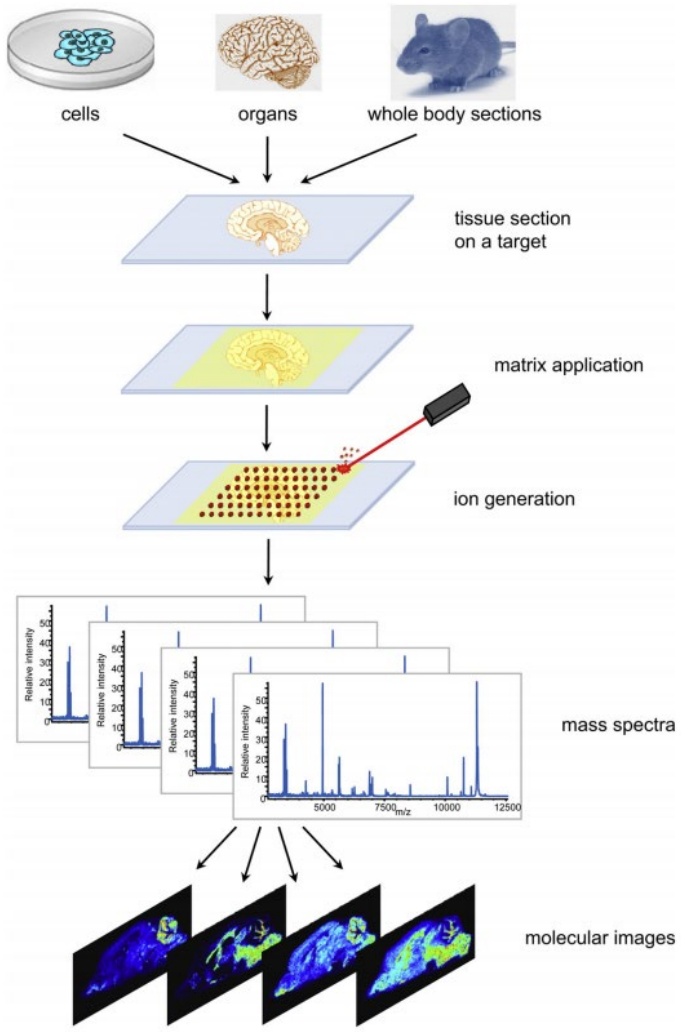


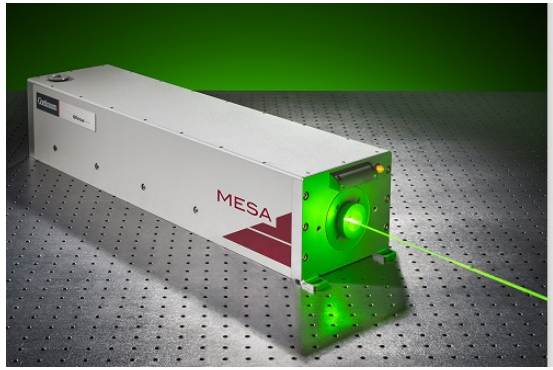
- Labeling studies show minimal metabolic activity during sample processing (exception: lactate/pyruvate equilibration by LDH)

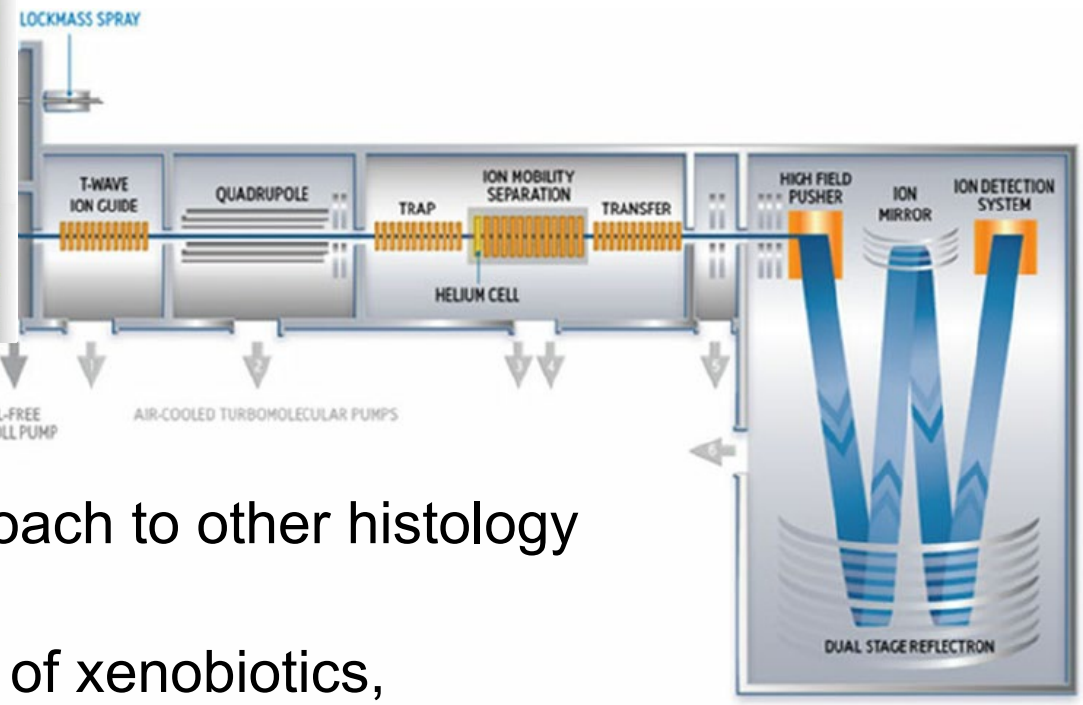


Penelope Kosinski, Hyeryun Kim, Victor Chubukov, Sebastian Hayes

Matrix-assisted laser desorption MS Imaging Analysis



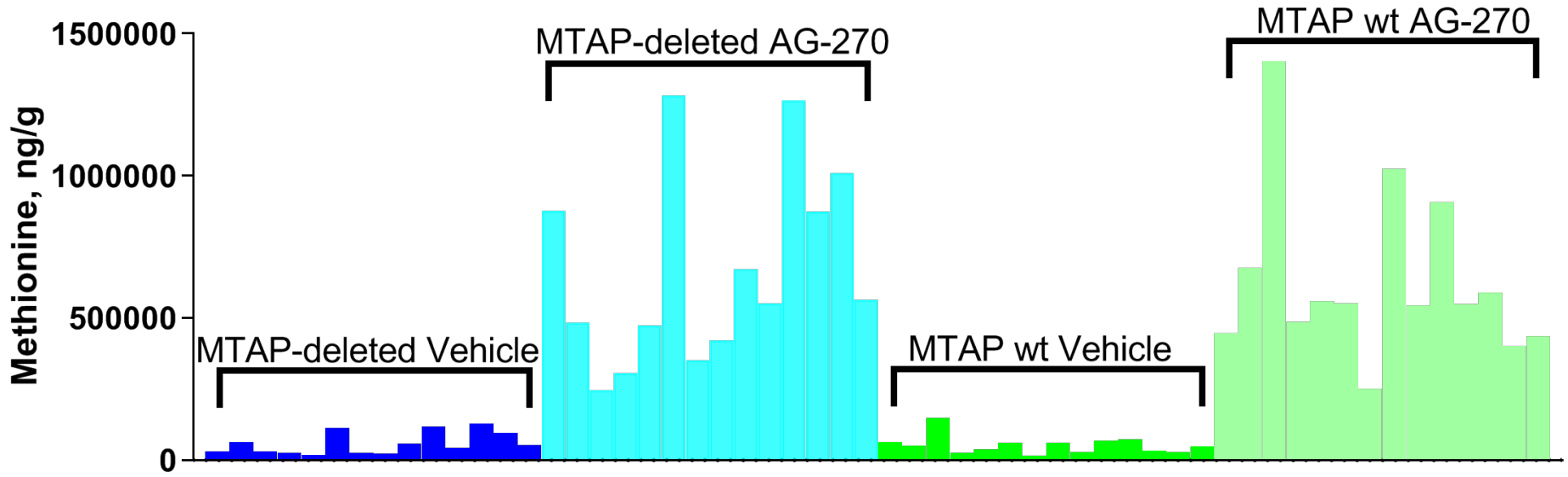




- Complementary approach to other histology techniques
- Molecular distribution of xenobiotics, metabolites, lipids and proteins
- Imaging System - Waters Synapt G2Si QTOF
- Mass resolution of ~30K
- MALDI image resolution 20-150 µm (adjustable)

David Pirman with Imabiotech

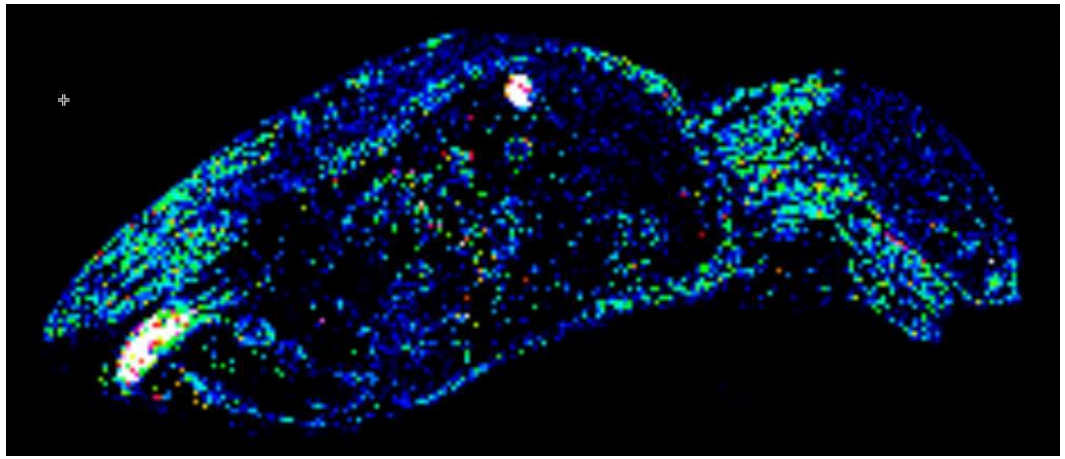
Methionine levels are upregulated upon AG-270 treatment *in vivo*



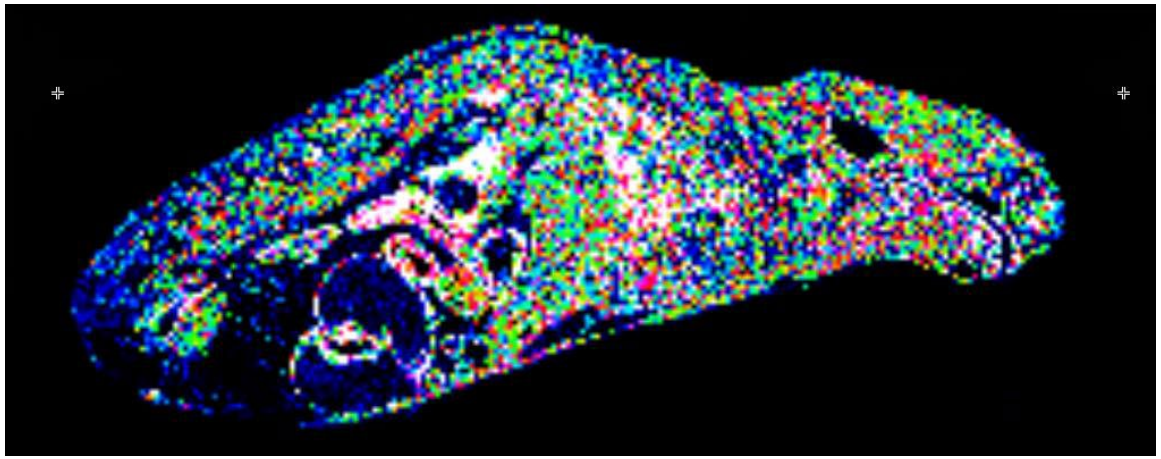
Data from NSCLC PDX samples is shown

Using MALDI MS to assess distribution of Methionine in control vs. AG-270 treated whole-body sections (non-tumor bearing)

Vehicle Control, Methionine by MALDI-MS



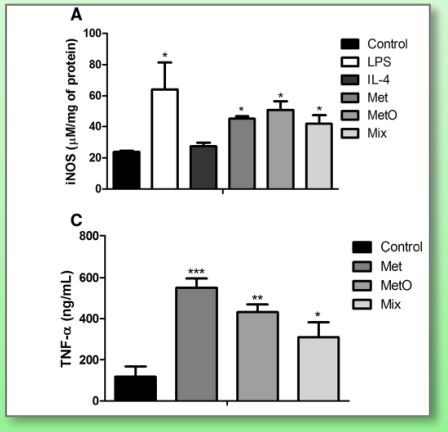
AG-270-treated, Methionine by MALDI-MS

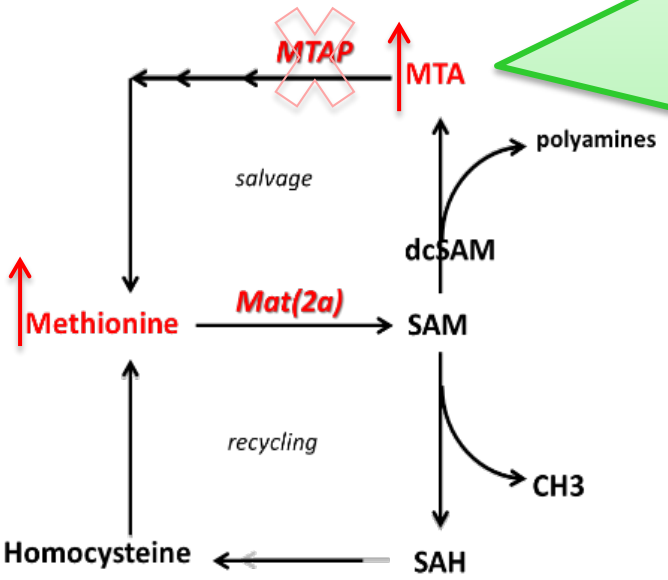


Recent literature suggests immunomodulatory effects of MTA and Methionine

Enhanced immune function

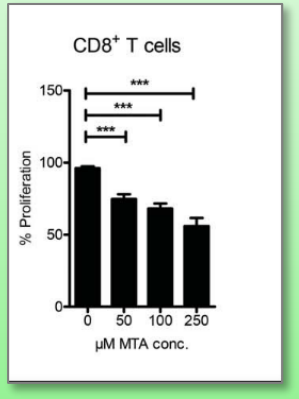
Impact on macrophages



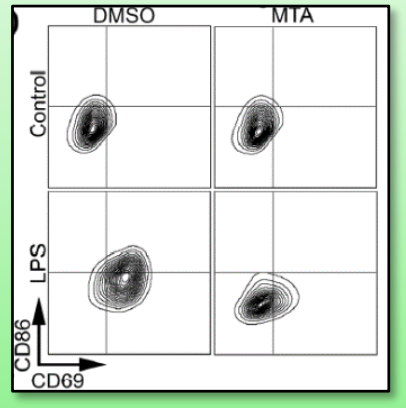


Immunosuppressive effects

Impact on T cells



Impact on macrophages

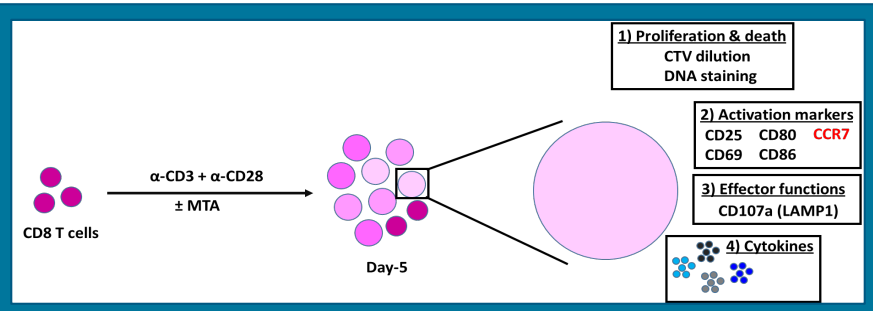


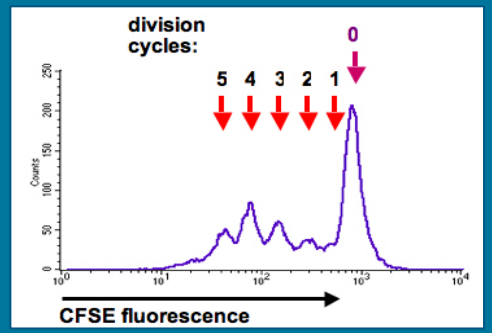
Dos Santos LM et al., Mol Cell Biochem 2016

Henrich et al., Oncoimmunology June 2016 Keyel P et al., Plos One 2014

MTA suppresses T-cell proliferation *in vitro*, at concentrations that arise in MTAP-deficient cancers

Measure T cell proliferation in presence of MTA



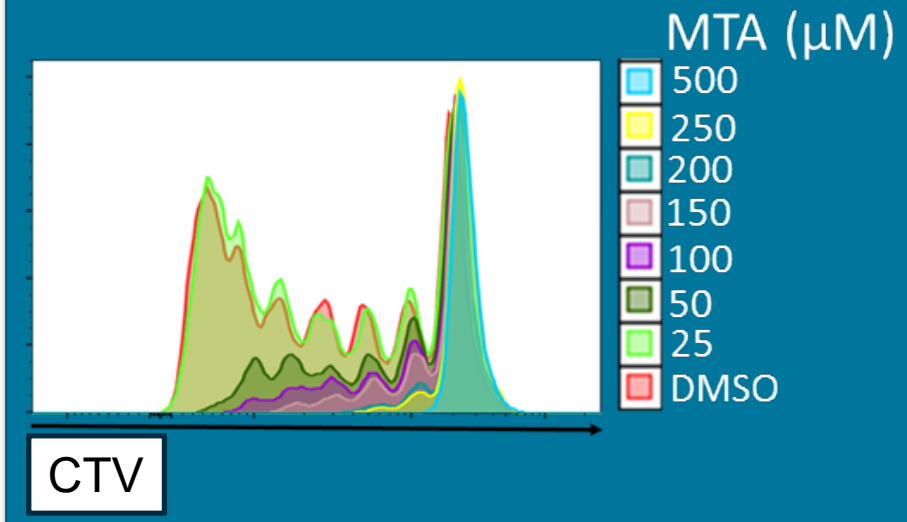


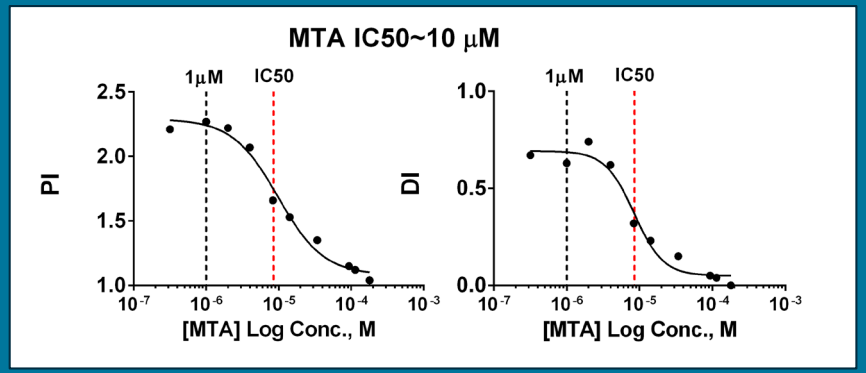
http://www.cyto.purdue.edu/cdroms/cyto10a/educationandresearch/flowanalysis.html

Proliferation Index (PI)= total number of divisions / number of cells that went into division (peaks 1-5)

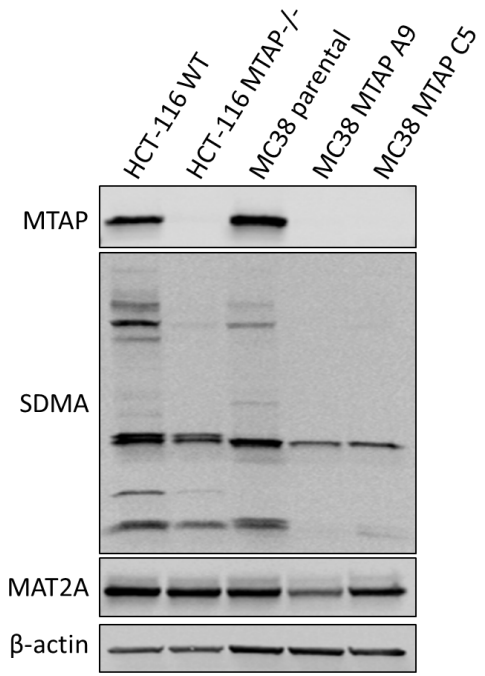
Division Index (DI)= total number of divisions / number of cells in original population (peaks 0-5)

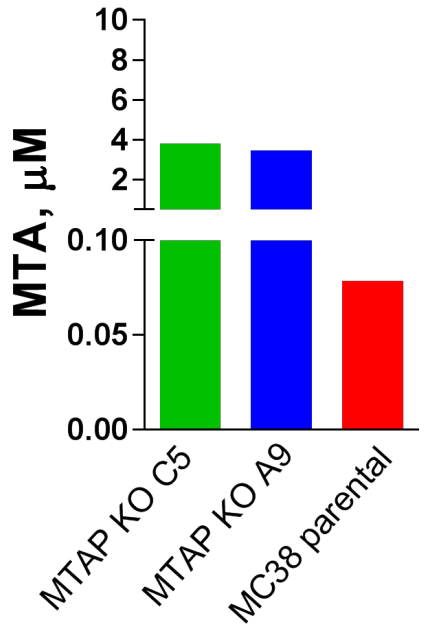
In vitro MTA is immune suppressive





Development of syngeneic mouse models to examine potential immunomodulatory impacts of MTAP loss and MAT2A Inhibition





Next Steps

- Immune phenotyping of MC38 isogenic pair
- Conduct anti-PD-1 efficacy studies in MC38 isogenic pair

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 with AG-270 was initiated in early 2018
- **Tumor intrinsic 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
- **Tumor extrinsic** impact of high **MTA** and other **Methionine** pathway metabolites is under investigation