Targeting MAT2A in MTAP-deleted Cancers
I have the following financial relationships to disclose:

Employee of: Agios Pharmaceuticals, Inc.
Stockholder in: Agios Pharmaceuticals, Inc.

- and -

I will not discuss off label use and/or investigational use in my presentation.
Learnings & Forward Strategy for Precision Medicine in Cancer Metabolism

- 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.
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

MTAP wt

MTAP null

Cycloleucine

AGI-24512
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

![Graph showing % inhibition of MAT2A activity vs log [inhibitor, μM] with AGI-24512 and Cycloleucine](image)

![Graphs showing tumor volume over days of treatment for HCT116 MTAP wt and HCT116 MTAP +/- with Vehicle and AGI-25696 300mg/kg](image)

![Bar graph showing tumor SAM (ng/g tumor tissue) for isogenic pair with Vehicle and AGI-25696](image)
MAT2A Inhibition Selectively Impacts PRMT5 Activity in MTAP-deleted Background

MAT2A Inhibition Selectively Modulates Splicing in MTAP-deleted Background

MAT2A Inhibition Selectively Modulates Splicing in MTAP-deleted Background
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

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

**Multiple Pharmacodynamic Biomarkers**

**Pathway Effects**
- MAT2A inhibition
- Tumor-selective inhibition of PRMT5
- Inhibition of PRMT5 targets including the spliceosome
- Antiproliferative & cytotoxic effects

**Clinically Applicable Biomarkers**
- Quantitative measurement of SAM in patient plasma
- IHC for PRMT5-mediated methyl marks in tumor biopsies
- Assess splicing using RNA seq & other assays
- Mechanistic studies on AG-270 suggest suitable response biomarkers

**Patient Selection Biomarkers**

1. **Next-gen sequencing for CDKN2A loss**
   - MTAP deficient PDAC
   - MTAP positive PDAC

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MTAP status</th>
<th>SDMA H-score</th>
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<td>AGI-25696 null</td>
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**IHC quantification: H-score**
- Histological score
- Combines % tumor stained cells with stain intensity (1-3)
- Score range: 0-300
MTAP status is predictive of basal SDMA levels and drug-induced reduction in SDMA

- 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

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

Penelope Kosinski, Hyeryun Kim, Victor Chubukov
Proteomics suggests most TIF proteins are from mouse blood

Typically observe depletion of Glutamine and enrichment for Lactate and Glutamate

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)
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

0% 20%
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 \textit{in vitro}, at concentrations that arise in MTAP-deficient cancers.

\textbf{Measure T cell proliferation in presence of MTA}

\textbf{In vitro MTA is immune suppressive}

\begin{itemize}
  \item \textbf{Proliferation Index (PI)}: \frac{\text{total number of divisions}}{\text{number of cells that went into division (peaks 1-5)}}
  \item \textbf{Division Index (DI)}: \frac{\text{total number of divisions}}{\text{number of cells in original population (peaks 0-5)}}
\end{itemize}

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

MTA (µM)
- 500
- 250
- 200
- 150
- 100
- 50
- 25
- DMSO

CTV

MTA IC50~10 µM

Proliferation Index (PI)
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
Agios discovered potent, cell and \textit{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 \textit{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