Targeting MAT2A in CDKN2A/MTAP-deleted Cancers

American Association for Cancer Research
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Symposium on Exploiting Metabolic Vulnerabilities of Cancer
April 1st 2019
Targeting MAT2A in Cancers with Deletion of CDKN2A/MTAP

### MTAP deletion frequency

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>Mesothelioma</td>
<td>0% - 20%</td>
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<tr>
<td>Glioblastoma</td>
<td>20% - 40%</td>
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<tr>
<td>Bladder Carcinoma</td>
<td>40% - 60%</td>
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<tr>
<td>Esophageal cancer</td>
<td>60% - 80%</td>
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<tr>
<td>Melanoma</td>
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<tr>
<td>Pancreatic</td>
<td></td>
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<tr>
<td>Lung (squamous)</td>
<td></td>
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<tr>
<td>DLBCL</td>
<td></td>
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<tr>
<td>Head &amp; Neck</td>
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<tr>
<td>Lung (adenocarcinoma)</td>
<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td></td>
</tr>
<tr>
<td>Low-grade glioma</td>
<td></td>
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<tr>
<td>Cholangiocarcinoma</td>
<td></td>
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<tr>
<td>Gastric (adenocarcinoma)</td>
<td></td>
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<tr>
<td>Breast (TNBC)</td>
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### CDKN2A/MTAP Deleted Cancer Cell

- **Chromosome 9p21 deleted in 15% of cancer**
- MTAP enzyme is lost
- MTA accumulates
- MTA inhibits PRMT5
- AG-270

Marjon et al. Cell Reports 2016
Agios MAT2A Inhibitors Selectively Impact Proliferation of MTAP-null Cancers
MAT2A Inhibitor AG-270 Possesses Broad Activity in ‘Mouse Clinical Trial’ Using Patient Derived Xenograft Models

Anti-tumor activity observed in a variety of models, with examples of regressions / tumor stasis

N=3 per model; established tumors treated at 200 mpk AG-270 QD
MTAP-deletion Enriches for AG-270 Sensitivity in NSCLC PDX Models

MTAP-selective Tumor Growth Inhibition
In NSCLC PDX
(Unpaired t test p=0.02)

- MTAP-deleted and MTAP wt tumors have similar drug exposure

NSCLC PDX models validate MTAP-deletion as a biomarker
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MTAP-selective Tumor Growth Inhibition
In NSCLC PDX
(Unpaired t test p=0.02)

- MTAP-deleted and MTAP wt tumors have similar drug exposure and similar reductions in Tumor SAM but PRMT5 methyl marks only decrease in MTAP-deleted tumors

NSCLC PDX models validate MTAP-deletion as a biomarker
...and provide evidence supporting the MTAP→PRMT5/MAT2A mechanism
Methylation Proteomics Corroborates Role for PRMT5 as a Key Downstream Mediator of MAT2A Inhibition in MTAP-deleted Cells

HCT116 MTAP-/- and HCT116 MTAP wt cells

3-day treatment with MAT2Ai or DMSO

IP with PTM-specific antibodies
- Mono-Methyl-Arginine
- Asymmetrical di-methyl Arginine
- Symmetrical di-methyl Arginine
- Pan-methyl Lysine

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

<table>
<thead>
<tr>
<th>HCT116 MTAP+/+</th>
<th>HCT116 MTAP-/-</th>
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<tbody>
<tr>
<td>3</td>
<td>36</td>
</tr>
</tbody>
</table>

#SDMA peptides that decrease upon MAT2A inhibition in HCT116 MTAP-/-.

RNA Processing
MAT2A Inhibition Selectively Disrupts Splicing in MTAP-deleted Cells

- MAT2A Inhibition leads to substantial dysregulation of splicing, including large increase in transcripts containing Detained Introns (DIs)
- DI-containing transcripts fail to export into the cytosol and thus are not translated (Boutz et al G&D 2015)
- MAT2A Inhibitor treatment DIs include critical genes in DNA damage repair and cell cycle
AG-270 Treatment Induces Substantial Mitotic Defects in HCT116 MTAP\(^{-/-}\) cells

- Single Agent AG-270 treatment leads to DNA damage ($\gamma$H2AX) and micronuclei formation
- Effects are selectively observed in $MTAP^{-/-}$ cells and not in $MTAP$-wt cells
In Parallel, a Large-scale Synergy Screen in >30 Cell Lines Revealed Synergy between AG-270 and Antimitotics

**Large Scale Synergy Screen:**

- **20 candidate combo partners**
- **X**
- **36 MTAP-null cell lines**

- Full dose curve matrices
- Cell lines included (n):
  - Lung (17), Esophageal & Gastric (8), Pancreatic (7), Colorectal (3), Kidney (1)
AG-270 Synergizes with AuroraB Kinase Inhibitors and Other Anti-mitotic Agents \textit{in vitro}

- AG-270 synergizes with AuroraB Kinase Inhibitors and taxanes \textit{in vitro}
- Synergy is selectively observed in MTAP-/− cells and not in MTAP-wt cells
AG-270 Synergizes with Docetaxel in MTAP-null KP4 Pancreatic Ductal Adenocarcinoma Xenograft Model

**AG-270 + docetaxel combination in KP4**
- Single agent and combination treatments were well-tolerated (<5% BWL)
- AG-270 + docetaxel combo is synergistic

**Additional AG-270 + taxane combo studies**
- AG-270 + taxane combinations tested in a variety of PDX models
- Combination benefit seen in tumors of multiple indications, including lung, pancreatic, esophageal
- Combination benefit seen with both paclitaxel and docetaxel
- PK studies rule out a drug-drug PK interaction
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

5. DNA repair and cell cycle defects, leading to actionable combination partners including taxanes
Summary

• MAT2A inhibition selectively blocks the proliferation of MTAP-deficient cancers \textit{in vitro} and \textit{in vivo}

• Inhibition of MAT2A leads to MTAP-selective effects on PRMT5 methylation, leading to substantial increase in mis-spliced transcripts

• Mis-spliced transcripts include cell cycle regulators such as Aurora Kinase B; consistent with this, MAT2A inhibition leads to mitotic defects including micronuclei formation

• Mitotic defects downstream of MAT2A create a synergistic vulnerability to antimitotics including clinically-applicable taxanes which are used as standard of care in several malignancies with frequent deletion of MTAP
Acknowledgements

Agios Team