Mitotic defects induced by MAT2A inhibitors guide translational drug combination strategies with AG-270 and taxanes

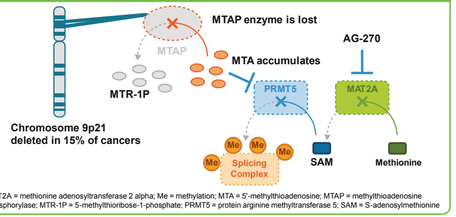
Peter Kalev, Marc L Hyer, Mark Fletcher, Peili Zhang*, Elia Aguado-Fraile, Everton Mandley, Sheila Newhouse, Yesim Tuncay, Zenon Konteatis, Jeremy Travins†, Kevin M Marks, Katya Marjon

Agios Pharmaceuticals, Inc., Cambridge, MA, USA
Current affiliations: *Forward Informatics, LLC, Winchester, MA, USA; †Takeda, Cambridge, MA, USA

BACKGROUND

- The *MTAP* gene is adjacent to the *CDKN2A* gene on chromosome 9p21, both of which are often co-deleted in approximately 15% of all human cancers.[1,2]
- Biallelic loss of the *MTAP* gene sensitizes cancer cells to genetic depletion of PRMT5 and the upstream metabolic enzyme MAT2A (**Figure 1**).[3-5]
  - The encoded enzyme, MTAP, metabolizes MTA, a byproduct of polyamine biosynthesis.
  - MTAP loss results in accumulation of MTA, which partially inhibits the function of PRMT5.[3]
  - PRMT5 catalyzes the formation of symmetrically di-methylated arginine residues (SDMAs) in target proteins using SAM, which is produced by MAT2A.
- MAT2A inhibition in *MTAP*-null cells, which have high levels of MTA, selectively reduces PRMT5 activity[3] and leads to mitotic defects, thus forming a basis for combinations with antimitotic agents such as taxanes.
- We have developed a first-in-class, highly potent, orally bioavailable MAT2A inhibitor, AG-270, which is currently under investigation in a phase 1 clinical trial (ClinicalTrials.gov NCT03435250).

Figure 1. Targeting MAT2A in cancers with *MTAP* deletion



MAT2A = methionine adenosyltransferase 2 alpha; Me = methylation; MTA = 5'-methylthioadenosine; MTAP = methylthioadenosine phosphorylase; MTR-1P = 5-methylthioribose-1-phosphate; PRMT5 = protein arginine methyltransferase 5; SAM = S-adenosylmethionine

OBJECTIVES

- To evaluate the growth inhibitory activity of AG-270 in both *MTAP* wild type (wt) and *MTAP*-null cells.
- To elucidate the mechanisms underlying the selective growth inhibition of *MTAP*-null cells by AG-270, using an isogenic cell line pair (HCT116).
- To investigate the combined activity of AG-270 and the antimitotic taxane docetaxel, in both *in vitro* and *in vivo* cancer models.

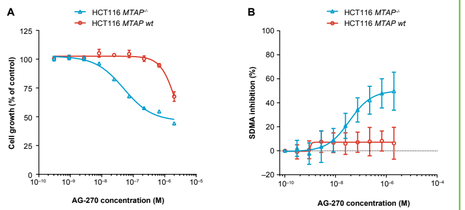
METHODS

- HCT116 human colon carcinoma isogenic pair cell lines were purchased from Horizon Discovery:
  - HCT116 parental, with wt *MTAP* (*MTAP wt*)
  - HCT116 *MTAP* knock-out clone (*MTAP*$^{-/-}$).
- DNA damage was measured *in vitro* using indirect immunofluorescence visualization of γH2AX foci.
  - γH2AX was detected using anti-phospho-histone H2A.X (Ser139) antibody, clone JBW301 (Millipore #05-636). Cells were classified as positive for DNA damage when ≥10 foci per nucleus were counted. More than 400 cells were analyzed for each sample.
- γH2AX immunohistochemistry on formalin-fixed paraffin-embedded tumor tissues was performed by Mosaic Laboratories in accordance with validated procedures. Six mice were used per treatment arm. All tissues were collected 12–24 hr after the last dose.
- SDMA immunohistochemistry was designed and validated as a laboratory-developed test and was performed in accordance with Mosaic Laboratories' standard operating procedures.
- Splicing changes were determined from RNAseq data using rMats version 3.2.5; changes in usage of detained introns (DIs)[6,7] were determined using DEXseq.
  - The splicing changes were selected using the criterion false discovery rate–adjusted p-value <0.05.
- Drug combination studies were assessed using the Chou-Talalay model.[8]
  - Combination Index (CI) score was used to measure drug-drug interactions.

RESULTS

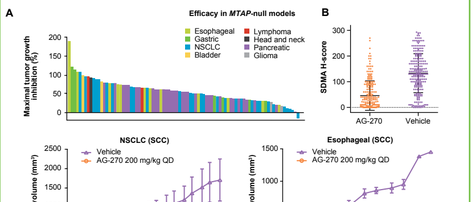
- MAT2A inhibition by AG-270 inhibited growth in HCT116 *MTAP*-null cells *in vitro* (**Figure 2A**).
- Treatment with AG-270 selectively inhibited SDMA formation in HCT116 *MTAP*-null cells but not in HCT116 *MTAP*-wt cells (**Figure 2B**).
- Treatment with AG-270 reduced SDMAs and inhibited the growth of *MTAP*-null patient-derived xenografts (PDX; **Figure 3**).
  - Tumor regression and tumor stasis were observed in selected models of non–small-cell lung cancer (NSCLC) and esophageal cancer (**Figure 3**).
- Proteomic analysis was undertaken to investigate the mechanisms responsible for tumor growth inhibition, and demonstrated that the methylation changes following MAT2A inhibition included peptides with SDMAs that depend on the activity of PRMT5 (**Figure 4**).
- SDMA peptides that were reduced >4-fold after treatment with a MAT2A inhibitor were enriched for peptides corresponding to proteins that regulate RNA processing.

Figure 2. AG-270 inhibited cell growth in an *MTAP*-selective manner *in vitro*



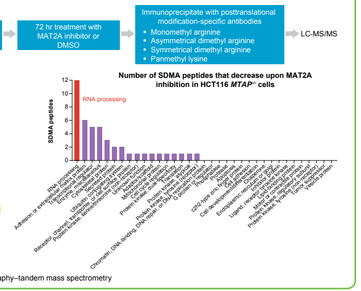
A. Mean (±SD) percentage growth of HCT116 *MTAP*-null and *MTAP*-wt cells as measured by CellTiter Glo assay upon treatment with various doses of AG-270 for 96 hr, normalized to DMSO control. **B.** Mean (±SD) percentage inhibition of SDMA in HCT116 *MTAP*-null and *MTAP*-wt cells after 72 hr of treatment with DMSO control or various doses of AG-270, measured by in-cell western
DMSO = dimethyl sulfoxide

Figure 3. AG-270 reduced SDMAs and demonstrated anti-tumor activity in a range of *MTAP*-null patient-derived xenograft models



A, bottom panels. Mean ± SEM tumor volume following oral vehicle or AG-270, n=3 per group. **B.** SDMAs in a range of MTAP-null models (horizontal bar denotes mean ± SD)
H-score = semiquantitative immunohistochemistry measure ranging from 0 to 300; SCC = squamous cell carcinoma; QD = once daily

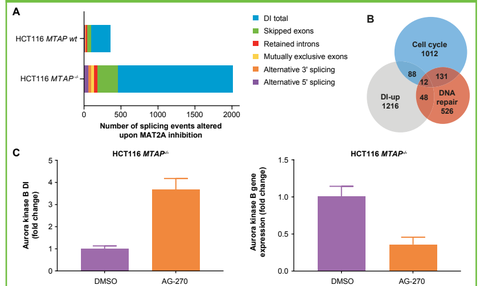
Figure 4. Methylation proteomics corroborated the role of PRMT5 as a key downstream mediator of MAT2A inhibition in *MTAP*-null cells



LC-MS/MS = liquid chromatography–tandem mass spectrometry

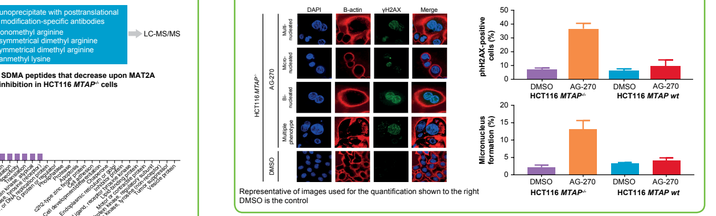
- MAT2A inhibition led to substantial dysregulation of splicing, including an increased number of transcripts containing DIs, in *MTAP*-null cells (**Figure 5A**).
- DI-containing transcripts fail to export into the cytosol and thus are not translated.[6]
- MAT2A inhibition–induced DIs included genes involved in the DNA damage response and cell cycle regulation, such as Aurora kinase B (**Figure 5B**).
- Quantitative RT-PCR analysis demonstrated that treatment with AG-270 led to increased expression of DI-containing transcripts and decreased levels of total (exon-exon) Aurora B expression (**Figure 5C**).
- Treatment with AG-270 led to increased DNA damage and micronucleus formation in HCT116 *MTAP*-null cells, estimated by γH2AX immunofluorescence and DAPI staining analysis (**Figure 6**).
- After treatment with AG-270, a substantial increase in binucleated and multinucleated cells was seen in HCT116 *MTAP*-null cells but not in *MTAP*-wt cells.

Figure 5. MAT2A inhibition disrupted splicing and altered gene expression in *MTAP*-null cells



A. Number of splicing changes on MAT2A inhibition in HCT116 *MTAP*-null and -wt cells, determined by DEXseq (DIs) and rMATS (others). **B.** The Venn diagram shows the following three sets: genes with DIs upregulated on MAT2A inhibition in HCT116 *MTAP*-null cells, genes in pathway GO:6281 DNA repair, and genes in pathway GO:0278 cell cycle. **C.** Quantitative RT-PCR demonstrates Aurora B expression upon AG-270 treatment; DMSO is the control

Figure 6. Treatment with AG-270 induced DNA damage and mitotic defects in *MTAP*-null cells



Representative of images used for the quantification shown to the right
DMSO is the control

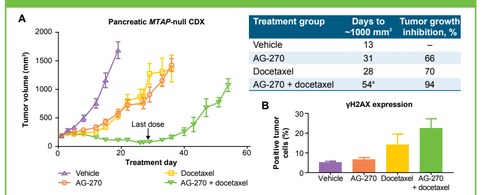
- AG-270 demonstrated an additive to synergistic effect, as defined by Chou and Talalay,[8] when administered in combination with docetaxel in lung and colorectal cell lines *in vitro* (**Table 1**).
  - AG-270 demonstrated synergism when combined with docetaxel (CI=0.5) in HCT116 *MTAP*-null cells, and a nearly additive effect (CI=0.99) in HCT116 *MTAP*-wt cells.[8]
  - AG-270 demonstrated synergism with docetaxel (CI<0.6) in H2122 cells where MTAP was pharmacologically inhibited, and a nearly additive effect (CI<1) in H2122 *MTAP*-wt cells.[8]
- The combination of AG-270 and docetaxel resulted in increased tumor growth inhibition compared with either agent alone in an *MTAP*-null pancreatic (KP4) cell–derived xenograft (CDX) model *in vivo* (**Figure 7A**).
  - AG-270 treatment in combination with docetaxel was well tolerated, with a mean body weight loss of <5%.
- In an additional KP4 model, AG-270 in combination with docetaxel led to increased γH2AX-positive tumor cells (**Figure 7B**).
- AG-270 demonstrated increased anti-tumor activity when combined with docetaxel in a lung PDX mouse model (**Figure 8**).

Table 1. AG 270 demonstrated synergism when combined with docetaxel in an *MTAP*-dependent manner *in vitro*[8]

Cell line	CI at 72 hr	Drug-drug interaction AG-270/docetaxel
HCT116 *MTAP* wt	0.99	No synergy
HCT116 *MTAP* null	0.53	Synergistic
H2122 *MTAP* wt	1.0	No synergy
H2122 *MTAP* wt + MTAP inhibitor	0.62	Synergistic

CI 0.1–0.3 = strong synergism, 0.3–0.7 = synergism, 0.7–0.85 = moderate synergism, 0.85–0.9 = slight synergism, 0.9–1.1 = nearly additive, >1.1 = antagonism[8]

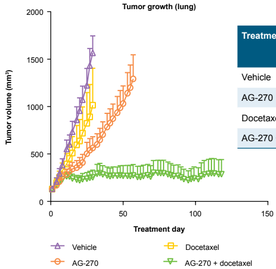
Figure 7. Tumor growth inhibition in a KP4 *MTAP*-null xenograft mouse model upon treatment with AG-270 and/or docetaxel



Treatment group	Days to ~1000 mm³	Tumor growth inhibition, %
Vehicle	13	–
AG-270	31	66
Docetaxel	28	70
AG-270 + docetaxel	54*	94

A. KP4 tumor–bearing CB-17 SCID mice were treated for 29 days with vehicle, AG-270 (oral 100 mg/kg once daily), docetaxel (intravenous 5 mg/kg once weekly), or the drug combination, n=12 per group, mean ± SEM shown in graph. **B.** Tumor tissues were collected on Day 21 from a repeat study with similar efficacy results. n=6 per group, mean + SEM
*25 days after stopping dosing
SCID = severe combined immunodeficiency

Figure 8. AG-270 enhanced docetaxel treatment in an NSCLC (SCC) *MTAP*-null mouse model



Treatment group	Days to ~1000 mm³	Tumor growth inhibition, %
Vehicle	21	–
AG-270	48	70
Docetaxel	28	38
AG-270 + docetaxel	–	91

Four animals in the combination arm with tumors were removed on Day 120. The remaining four animals were tumor free when the last dose was delivered on Day 114 and these animals remained tumor free until the arm was terminated on Day 141

Treatment with vehicle, AG-270 (oral 100 mg/kg once daily), docetaxel (intravenous 2.5 mg/kg Q7D × 18 doses), or AG-270 + docetaxel in a lung (LUX001) PDX model. Body weight loss (BWL) was observed in several animals from multiple groups; all groups were given AG-270 dosing holidays on Days 16–21. In the AG-270 + docetaxel group, one animal reached 20% BWL and was given AG-270 holidays on Days 54–59, 65–73, and 77–83; one animal reached >20% BWL and was given an AG-270 dosing holiday on Days 38–46 and a docetaxel dosing holiday on Day 42. Body weight recovered in all animals and maximum mean BWL for the combination group was 6%. N = 8 animals per group, mean + SEM
Q7D = every 7 days

CONCLUSIONS

- The MAT2A inhibitor AG-270 selectively inhibited PRMT5 activity and cell growth in *MTAP*-null cells.
- Inhibition of PRMT5 activity altered splicing and gene expression, leading to impaired DNA replication and genome integrity in *MTAP*-null cells.
- The DNA repair and cell cycle defects triggered by AG-270 treatment in *MTAP*-null cells suggested the potential for favorable combinations with standard-of-care agents such as taxanes (e.g. docetaxel).
- Combinations of AG-270 and docetaxel demonstrated additive to synergistic activity in *MTAP*-null *in vitro* and xenograft models.
- Taxane and AG-270 combinations led to tumor regression and complete responses in several models of *MTAP*-null solid tumors, including NSCLC (SCC) and pancreatic ductal adenocarcinoma.

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