The MAT2A inhibitor AG-270 combines with both taxanes and gemcitabine to yield enhanced antitumor activity in patient-derived xenograft models

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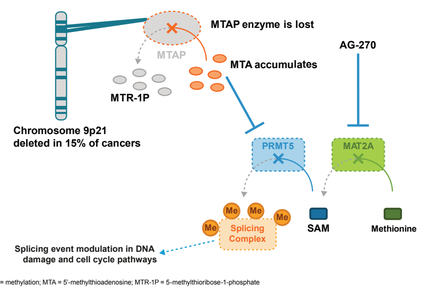
BACKGROUND

- AG-270 is a first-in-class, oral, potent, reversible inhibitor of methionine adenosyltransferase 2A (MAT2A), currently being evaluated in a phase 1 trial in patients with advanced solid tumors and lymphomas with *MTAP* (S-methyl-5'-thioadenosine phosphorylase) deletion (ClinicalTrials.gov NCT03435250)
- MAT2A is a key enzyme in the methionine salvage pathway, responsible for generating the universal methyl donor, S-adenosylmethionine (SAM)[1,2]
- *MTAP* is deleted in ~15–25% of non–small cell lung cancers (NSCLC), ~25% of pancreatic, and ~30% of esophageal cancers[3]
- Biallelic loss of the *MTAP* gene sensitizes cancer cells to genetic depletion of protein arginine methyltransferase 5 (PRMT5) and the upstream metabolic enzyme, MAT2A (**Figure 1**)[4]
- To prioritize candidate combination partners for AG-270, a cell-based *in vitro* screening approach was employed using *MTAP*-null cell lines, in which AG-270 was combined with standard-of-care (SOC) agents as well as agents targeting pathways with hypothesized mechanistic links to MAT2A[5]

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



Me = methylation; MTA = 5'-methylthioadenosine; MTR-1P = 5-methylthioribose-1-phosphate

OBJECTIVES

- To identify combination partners that potentiate the cell growth inhibition effects of MAT2A blockade
- To assess the tolerability and antitumor activity of AG-270 combined with SOC agents in clinically relevant *in vivo* patient-derived xenograft (PDX) and cell-derived xenograft (CDX) models
- To investigate the mechanism of action associated with AG-270 combined with taxane therapy

METHODS

- An *in vitro* synergy screen tested 20 candidate combination partners in 37 *MTAP*-null cell lines, with full-dose curve matrices performed at Horizon using a 96-hr CellTiter-Glo readout
  - Cell lines included kidney (n = 1), colorectal (n = 4), esophageal and gastric (n = 7), pancreatic (n = 8), and lung (n = 17)
  - The strength of potential synergistic interactions between AG-270 and enhancer compounds *in vitro* in excess of Loewe additivity was measured using the Horizon-developed Synergy Score
- Splicing changes were determined from RNA sequencing data using rMATS 3.2.5; changes in usage of detained introns[6] were determined using DEXSeq
  - The splicing changes were selected using the criterion false discovery rate–adjusted p-value < 0.05
- *In vivo* xenograft studies were performed using institutional animal care and use committee guidelines; the combination analysis used belongs to the 'response additivity' class of synergy analysis,[7,8] with area under the tumor growth curves (AUC) compared between single-agent and combination treatments
- Hematoxylin and eosin (H&E) staining on formalin-fixed paraffin-embedded tumor tissues was performed by Mosaic Laboratories in accordance with validated procedures, with the percentage of multinucleated tumor cells and cytomegaly assessed by a pathologist
  - Six mice were evaluated per treatment arm; all tissues were collected 12–24 hr after the last dose of AG-270

RESULTS

- A large-scale synergy screen revealed potential synergistic combinations (**Figure 2**)
- MAT2A inhibition led to substantial dysregulation of splicing in *MTAP*-null cells, including an increased number of transcripts containing detained introns (**Figure 3A**)
- Detained intron–containing transcripts fail to export into the cytosol and thus are not translated[6]
- MAT2A inhibitor–induced detained introns included genes involved in the DNA damage response and cell-cycle regulation, such as Aurora kinase B (**Figure 3B**)
- Quantitative reverse transcriptase (RT)-PCR analysis demonstrated that treatment with a MAT2A inhibitor led to increased expression of detained intron–containing transcripts and decreased levels of total (exon-exon) Aurora B expression (**Figure 3C**)

Figure 2. AG-270 synergized with antimitotic taxanes and gemcitabine in a cell-based assay

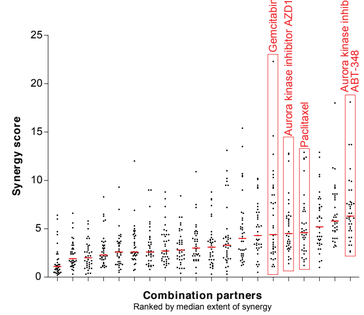
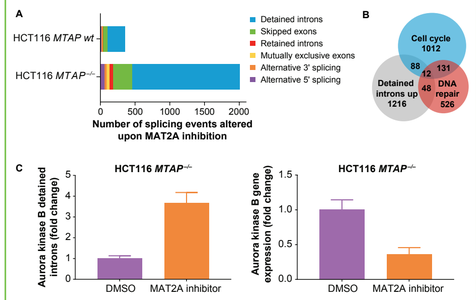


Figure 3. MAT2A inhibition disrupted splicing and altered gene expression in *MTAP*-null cells



A MAT2A inhibitor tool compound (AGI-24512) was used for all experiments shown in Figure 3
A. Splicing changes associated with MAT2A inhibition in HCT116 *MTAP*-null and *MTAP*-wt cells, determined by DEXSeq (detained introns) and rMATS (others)
B. The Venn diagram shows the following three sets: genes with detained introns 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 analysis demonstrated that treatment with a MAT2A inhibitor affected expression of detained intron–containing transcripts and total (exon-exon) Aurora B expression. Data shown are from a representative experiment performed in triplicate
DMSO = dimethyl sulfoxide; wt = wild type

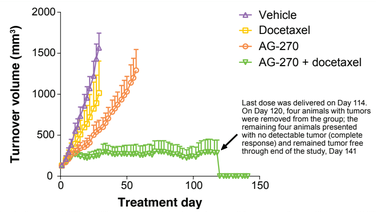
- A summary of the efficacy of AG-270 and taxanes as single agents and in combination in xenograft models is shown in **Table 1**
- AG-270 demonstrated increased antitumor activity when combined with docetaxel in a lung PDX mouse model (**Figure 4**)
- AG-270 demonstrated increased antitumor activity when combined with docetaxel in an esophageal PDX mouse model (**Figure 5**)
- The combination of AG-270 with taxanes induced cytomegaly (abnormal cell enlargement) and multinucleation in xenograft tumor cells (**Figure 6**)
- A summary of the efficacy of AG-270 and gemcitabine as single agents and in combination in xenograft models is shown in **Table 2**
- AG-270 demonstrated increased antitumor activity when combined with gemcitabine in a pancreatic CDX mouse model (**Figure 7**)

Table 1. Efficacy data summary of AG-270 and taxanes, alone and combined

Tumor model (indication, subtype)	Taxane partner	AG-270 tumor growth inhibition, %	Taxane tumor growth inhibition, %	Combination tumor growth inhibition, %	Tolerability: Maximum mean BWL, %	Combination analysis[7,8]
KP4 (pancreatic)	Docetaxel	66	70	94	< 5	Synergy
ESX030 (esophageal, SCC)	Docetaxel	68	44	90	< 2.5	Synergy
LUX001 (NSCLC, SCC)	Docetaxel	70	45	91	6	Synergy
PA0372 (pancreatic)	Paclitaxel	59	29	84	< 2.5	Additive
ESX2263[a] (esophageal, SCC)	Docetaxel	27	12	57	< 5.0	Additive
LUX034 (NSCLC, SCC)	Docetaxel	41	27	60	< 6	Additive
LU6412[a] (NSCLC, Ad)	Docetaxel	52	57	92	13.3	Additive
PAX001 (pancreatic)	Paclitaxel	94	22	94	7	Weakly additive
CTG-1194 (NSCLC, Ad)	Docetaxel	15	42	52	0	Additive
PAX041 (pancreatic)	Paclitaxel	55	12	50	< 2.5	Weakly additive

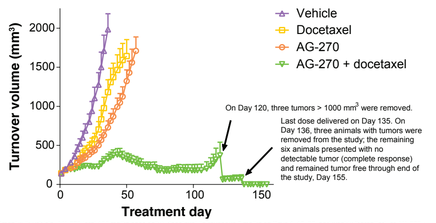
Tumor growth inhibition % = [1–(TV$_T$–TV$_0$)/(TV$_V$–TV$_0$)] ×100%; where TV$_T$ is the average tumor volume of the test group on a specific day and TV$_0$ is the average tumor volume of the same group on day 0, and TV$_V$ is the average tumor volume of the vehicle group on a specific day and TV$_{V_0}$ is the average tumor volume of the vehicle group on day 0
Paclitaxel doses used in PAX001 and PAX041 were 7.5 mg/kg; in PA0372, 15 mg/kg. Docetaxel doses used in KP4, ESX030, ESX2263, CTG-1194, LU6412, LUX034 were 5 mg/kg; in LUX001, 2.5 mg/kg. Number of animals used per arm per model was 8–12
[a]Dosing holidays were provided
[b]1 of 12 animals was found dead in the combination group
Ad = adenocarcinoma; BWL = body weight loss; SCC = squamous cell carcinoma

Figure 4. AG-270 enhanced docetaxel therapy in an NSCLC (SCC) *MTAP*-null PDX mouse model



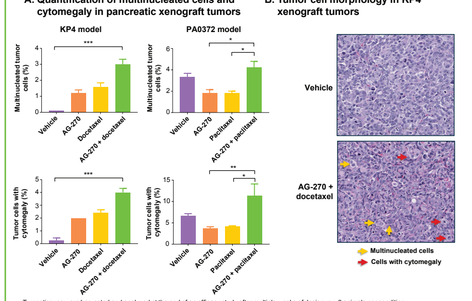
Treatment with vehicle, AG-270 (oral 100 mg/kg once daily), docetaxel (intravenous 2.5 mg/kg every 7 days × 18 doses), or AG-270 + docetaxel in a lung (LUX001) PDX model. BWL was observed in two animals in the AG-270 group (20% and 16%) and all groups were given AG-270 dosing holidays on Days 16–21. In the AG-270 + docetaxel group, one animal reached 20% BWL and was given AG-270 dosing 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%. Y-axis shows mean + SE; n = 8 animals per group

Figure 5. AG-270 enhanced docetaxel therapy in an esophageal (SCC) *MTAP*-null PDX mouse model



Treatment with vehicle, AG-270 (oral 100 mg/kg once daily), docetaxel (intravenous 5 mg/kg on Days 1, 15, 29, and weekly thereafter), or AG-270 + docetaxel in an esophageal (ESX030) PDX model. One animal in the AG-270 + docetaxel group experienced BWL > 20% and received an AG-270 dosing holiday from Days 55 to 60; BWL recovered. Maximum mean BWL for the combination group was < 3%. Y-axis shows mean + SE; n = 12 animals per group

Figure 6. Treatment with AG-270 combined with taxanes enhanced mitotic defects in xenograft tumors



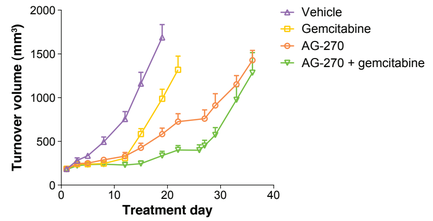
Tumor tissues were harvested and analyzed at the end of an efficacy study after multiple weeks of dosing; n = 6 animals per condition
A. Quantification of the percentage of multinucleated cells and cells with cytomegaly by H&E staining and pathologist review in pancreatic xenograft models (KP4 and PA0372) treated with AG-270 as a single agent or in combination with taxanes.
A nonparametric Kruskal-Wallis test with multiple comparison correction was used; * p < 0.05, ** p < 0.01, *** p < 0.001
B. Representative images of abnormal tumor cell morphology in KP4 xenografts treated with AG-270 in combination with docetaxel or vehicle control. Yellow arrows indicate multinucleation examples and red arrows indicate cells with cytomegaly

Table 2. Efficacy data summary of AG-270 and gemcitabine, alone and combined

Tumor model (indication, subtype)	AG-270 tumor growth inhibition, %	Gemcitabine tumor growth inhibition, %	Combination tumor growth inhibition, %	Tolerability: Maximum mean BWL, %	Combination analysis[7,8]
KP4 (pancreatic)	66	43	82	< 5	Additive
PAX041 (pancreatic)	55	16	69	< 5	Additive
LU6431 (NSCLC, SCC)	45	43	69	< 5	Additive
PAX001 (pancreatic)	83	56	89	< 5	Weakly additive
LU1513[a] (NSCLC, SCC)	74	90	101	< 11	Weakly additive

Tumor growth inhibition calculated as described for Table 1
Number of animals used per arm per model was 8–12
[a]In the LU1513 study, one of 12 animals in the combination arm lost > 20% BWL on Day 11 and was removed from the study; maximum mean BWL in this group was 10%

Figure 7. AG-270 enhanced gemcitabine therapy in an *MTAP*-null pancreatic CDX (KP4) mouse model



Treatment with vehicle, AG-270 (oral 100 mg/kg once daily), gemcitabine (intraperitoneal 20 mg/kg every 3 days), or AG-270 + gemcitabine in a pancreatic CDX model (KP4). Maximum mean BWL in the combination group was < 5%. Y-axis shows mean + SE; n = 12 animals per group

CONCLUSIONS

- **A cell-based screen identified taxanes and gemcitabine as therapeutic agents that could yield combination benefits with AG-270**
- **MAT2A inhibition led to substantial dysregulation of splicing, including an increased number of transcripts containing detained introns, in *MTAP*-null cells, including in pathways that regulate the DNA damage response and cell cycle, which we suggest are mechanistically relevant to efficacy**
- **These data form the basis for combining AG-270 with taxanes (antimitotic agents) and gemcitabine (DNA-damaging agent)**
- **Combining AG-270 with taxanes and gemcitabine yielded additive-to-synergistic antitumor activity, with the docetaxel combination yielding 50% complete tumor regressions in select models; combination benefits were observed in PDX models derived from esophageal, NSCLC, and pancreatic cancers**
- **The combination of AG-270 with taxanes induced mitotic defects in tumor cells, as demonstrated by increased cytomegaly and multinucleation**
- **These preclinical findings have inspired an ongoing phase 1 study exploring AG-270 combined with docetaxel or gemcitabine/nab-paclitaxel (ClinicalTrials.gov NCT03435250)**

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