#537 Low Risk of Drug-Drug Interactions (DDIs) for Bemnifosbuvir (BEM) Based Upon *In Vitro* Metabolism and Transporter Interaction Studies

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ABSTRACT

BEM, a prodrug of a guanosine nucleotide analog, is a potent inhibitor of SARS-CoV-2 under development for treatment of COVID-19. The activation of BEM to its active triphosphate involves CatA/CES1, HINT1, ADALP1, and the kinase GUK1 and NDPK enzymes (see pathway figure below). The drug-drug interaction (DDI) potential of BEM and its predominant metabolites was evaluated *in vitro*.

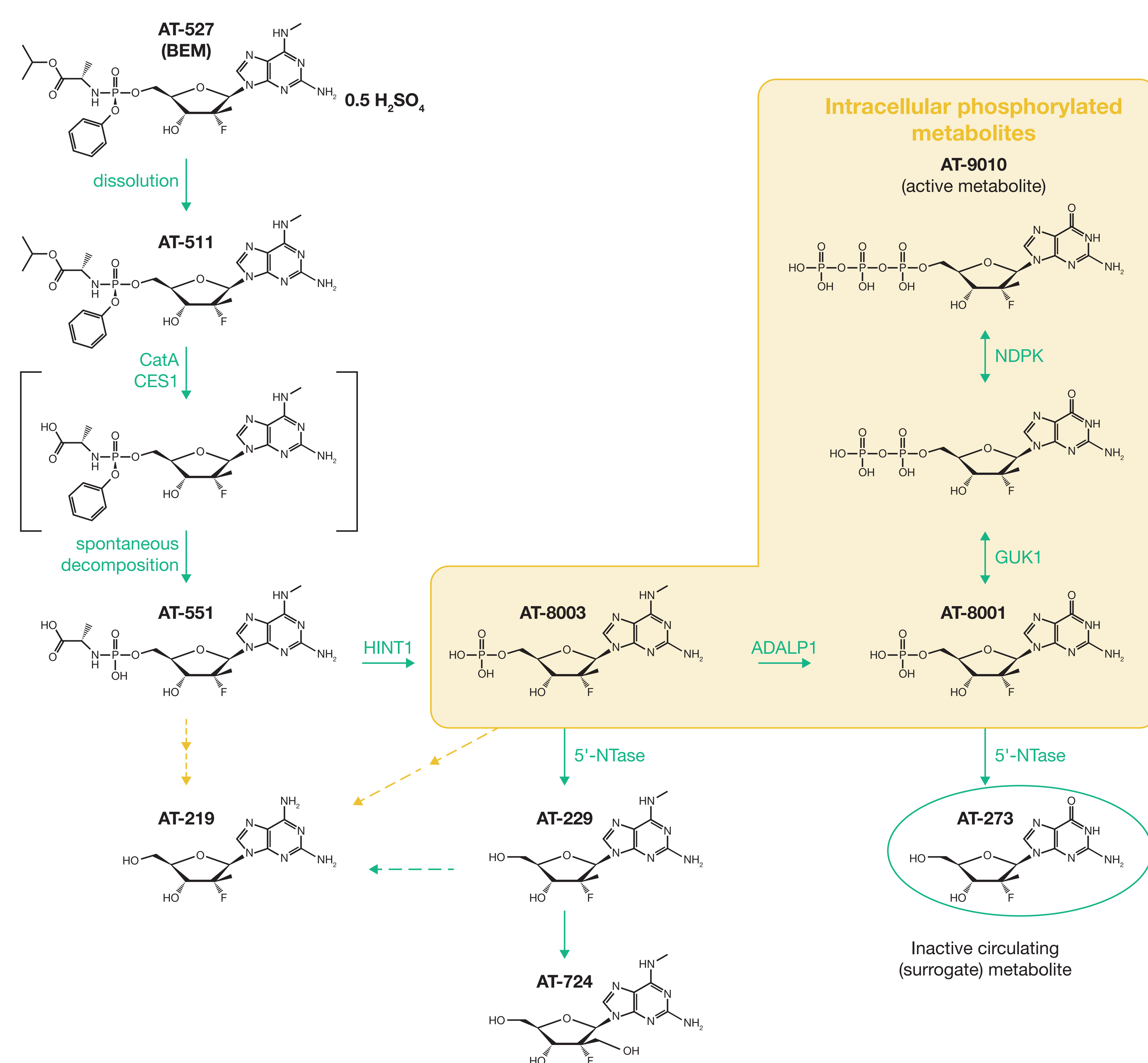
BEM directly inhibited CYP2C8, CYP2C19, CYP3A4m/t, and UGT1A1 with IC_{50} values of 51, 63, 19/83, and 44 µM, respectively. BEM was a time-dependent inhibitor of CYP3A4 with k_{inact} of 0.0135 min^{-1} and K_i of 4.551 µM using midazolam as a probe substrate; and k_{inact} of 0.0165 min^{-1} and K_i of 6.88 µM using testosterone as a probe substrate. None of the metabolites were direct or time-dependent inhibitors of CYP450 enzymes. BEM did not induce mRNA expression of CYP1A2 or 2B6 but was an inducer of CYP3A4 in a concentration-dependent manner. BEM was a substrate of P-gp and maybe a substrate of BCRP. BEM was not an inhibitor of OCT2 but was an inhibitor of P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, MATE1, and MATE2-K with IC_{50} values of 14.2, 183, 33.4, 54.6, 101, 34.6, 39.7, and 195 µM, respectively. None of BEM's metabolites inhibited any transporter.

Based on *in vitro* evaluations, there is low risk of clinically relevant DDIs when BEM is co-administered with other medications. The enzymes involved in the metabolic activation of BEM are of high capacity and not likely to be inhibited by commonly administered drugs. Since BEM metabolic activation does not involve CYP enzymes, there is low risk of drug interactions as a victim of CYP450 enzymes. The predicted low risk of CYP450- and transporter-mediated DDIs with BEM has been confirmed in clinical studies.

INTRODUCTION

- Atea Pharmaceuticals, Inc. is developing bemnifosbuvir (BEM; AT-527), an oral direct acting antiviral currently being evaluated in the global Phase 3 SUNRISE-3 clinical trial for the treatment of COVID-19
- BEM targets the SARS-CoV-2 RNA polymerase (nsp12), a highly conserved gene that is unlikely to change as the virus mutates and new variants continue to emerge. This gene is responsible for both replication and transcription of SARS-CoV-2
- BEM has a unique mechanism of action, with dual targets consisting of chain termination (RdRp) and nucleotidyltransferase (NiRAN) inhibition,[1] which has the potential to create a high barrier to resistance
- Here we show that BEM has low potential for DDIs *in vitro*

BEM metabolic and activation pathway



CatA, cathepsin A; CES1, carboxyesterase 1; HINT1: histidine triad nucleotide binding protein 1; ADALP1: adenosine deaminase like protein 1; GUK1: guanylate kinase 1; NDPK, nucleoside diphosphate kinase; 5'-Ntase, nucleotide phosphatase

METHODS

CYP450 inhibition using human liver microsomes (HLM)

- For direct CYP inhibition, BEM or individual metabolites was pre-incubated in triplicate at 37ºC with HLM for 3 minutes (reversible inhibition) and probe substrate in the absence of NADPH, followed with NADPH addition and incubation at 37ºC for 5–30 minutes depending on the individual CYP isoform
- For time-dependent inhibition, the test article was incubated at 37ºC with HLM in buffer for 30 minutes in the absence of NADPH, followed by NADPH and probe substrate addition and incubation. For UGT1A1 (uridine 5′-diphospho-glucuronosyltransferase enzyme 1A1) evaluation, similar procedures were conducted using HLM pre-treated with 10 µg/mL alamethicin
- Analytes were measured by LC-MS/MS

CYP450 induction in human hepatocytes

- Human cryopreserved hepatocytes from three donors were incubated in culture media spiked with BEM or individual metabolites for 48–72 hours in triplicate
- Hepatocyte cultures were also treated in parallel with vehicle control or control compounds
- Positive controls included omeprazole (50 µM) for CYP1A2, phenobarbital (1000 µM) for CYP2B6, and rifampicin (10 µM) for CYP3A4. Both mRNA expression and enzymatic activity were measured for each CYP

Transporter interaction studies

- For transcellular efflux assays, cell lines were cultured on semi-permeable inserts
- Transport measurements were performed at Day 3 or 4 after seeding to allow formation of confluent monolayers. Samples were quantified using LC-MS/MS. Transporter inhibition assays to investigate the interaction with the human BCRP, MDR1 (also known as P-gp), MATE1, MATE2-K, OATP1B1, OATP1B3, OAT1, OAT3, and OCT2 transporters were conducted using inside-out membrane HEK293 vesicles and transporter-expressed MDCKII and HEK293 cells

RESULTS

BEM has minimal inhibition potential on CYP450 enzymes and UGT1A1

Analyte	Inhibition IC_{50} (µM) UGT1A1	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4m	CYP3A4t	Time-dependent inhibition
BEM	44	>200	>200	51	>200	63	>200	19	83	Yes (CYP3A4)
AT-551	>200	>200	>200	>200	>200	>200	>200	>200	>200	No
AT-229	>200	>200	>200	>200	>200	>200	>200	>200	>200	No
AT-273	>200	>200	>200	>200	>200	>200	>200	>200	>200	No

CYP3A4 time-dependent inhibition kinetics

CYP450	Test article	k_{inact} (min^{-1})	K_i (µM)
3A4 (midazolam)	BEM	0.0135 ± 0.0007	4.55 ± 1.00
3A4 (testosterone)	BEM	0.0165 ± 0.0010	6.88 ± 1.52

- BEM was a time-dependent inhibitor of CYP3A4 with a relatively weak inhibitor constant K_i
  - BEM exposure is transient due to rapid and nearly complete activation *in vivo* by CES/CatA
  - CES/CatA are high capacity enzymes and are not part of the CYP450 metabolizing enzymes
  - Therefore, the risk of *in vivo* DDI with CYP3A4 caused by BEM is low
- None of the metabolites were direct or time-dependent inhibitors of CYP450 enzymes or UGT1A1

BEM has minimal induction potential on CYP3A4

BEM conc (µM)	CYP450 fold induction CYP1A2	CYP2B6	CYP3A4
1	1.27 ± 0.31	1.47 ± 0.21	1.47 ± 0.32
10	1.09 ± 0.28	1.20 ± 0.10	2.23 ± 0.51
100	0.93 ± 0.17	1.63 ± 0.31	7.57 ± 3.89

- BEM did not induce the mRNA expression of CYP1A2 or CYP2B6 but was an inducer of CYP3A4 in a concentration-dependent manner

Transporter substrate evaluation

Analyte	P-gp	BCRP	OATP1B1	OATP1B3	OAT1	OAT3	OCT2	MATE1	MATE2-K
AT-511	yes	maybe	nd	nd	nd	nd	nd	nd	nd
AT-551	no	no	nd	nd	nd	nd	nd	nd	nd
AT-229	no	yes	nd	nd	no	no	no	no	no
AT-273	no	no	nd	nd	no	no	no	no	no

nd, not determined

- BEM is an *in vitro* substrate of P-gp and a potential substrate of BCRP
- Metabolite AT-229 is a substrate of the BCRP transporter

BEM has minimal inhibition potential on ABC and SLC transporters

Analyte	Transporter inhibition IC_{50} (µM) P-gp	BCRP	OATP1B1	OATP1B3	OAT1	OAT3	OCT2	MATE1	MATE2-K
AT-511	14.2	183	33.4	54.6	101	34.6	>300	39.7	195
AT-551	>2000	>2000	>300	>300	>300	>300	>300	>300	>300
AT-229	>2000	870	>300	>300	>300	>300	>300	>300	>300
AT-273	>450	>450	>300	>300	>300	>300	>300	>300	>300

- BEM was a weak inhibitor of the ABC and SLC transporters above, however, the exposure is transient as the prodrug is quickly activated by CES/CatA, therefore the risk of clinically relevant DDIs is low
- The metabolites appeared to have very low, or no inhibition of the transporters evaluated

Preliminary clinical DDI data

- Emerging data from clinical DDI studies in healthy subjects suggest that BEM may be administered with P-gp inhibitors or inducers without dose adjustment
- BEM was a weak inhibitor (ratio <2) of CYP3A4 (midazolam probe substrate); *in vitro* induction effect was mitigated by inhibitory effect after chronic dosing[2]
- BEM was a weak inhibitor of BCRP/OATP1B1 (rosuvastatin as probe substrate)[3]
- BEM was a weak and transient inhibitor of P-gp (digoxin as probe substrate)[3]

CONCLUSIONS

- *In vitro* evaluation indicates BEM has low risk of DDIs with CYP450 enzymes, UGT1A1 or ABC/SLC transporters
- While BEM induced the mRNA expression of CYP3A4, at clinically relevant concentrations (clinical C_{max} ~5 µM), this induction is considered weak
- The *in vitro* observations are corroborated with preliminary clinical DDI studies

References

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Acknowledgments

This study was funded by Atea Pharmaceuticals. We thank Dr. Kerry-Ann da Costa for her excellent assistance in preparing this poster presentation.

Disclosures

All the authors are employees of Atea Pharmaceuticals.

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Poster presented at the 36th International Conference on Antiviral Research (ICAR) 2023 Conference, 13–17 March, Lyon, France.