Low Risk of Drug-Drug Interactions for Ruzasvir Based Upon *In Vitro* Metabolism and Transporter Interaction Studies

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INTRODUCTION

- Approximately 50 million people globally are living with chronic HCV infection, with 1.0 million new infections occurring per year and 242,000 deaths per year[1]
- New HCV treatment regimens with direct-acting antivirals have become the standard of care, with sustained virological response (SVR) rates exceeding 95% and treatment duration reduced to 8–12 weeks depending on the regimen and patient population
- Despite high efficacy rates with existing therapies, better treatment options are needed for certain patient populations that include those with severe liver decompensation, active hepatocellular carcinoma, genotype 3 HCV infection, treatment failure due to resistance requiring at least 12 weeks of treatment, often with adjunctive ribavirin treatment, and those with comorbid conditions receiving concomitant medications leading to drug-drug interactions (DDI)
- Atea Pharmaceuticals, Inc. is developing bemnifosbuvir (BEM) in combination with ruzasvir (RZR, also known as AT-038, formerly known as MK-8408) for the treatment of HCV
- BEM is a novel, oral NS5B polymerase inhibitor; RZR is a novel, oral NS5A phosphoprotein inhibitor. RZR is a small molecule inhibitor of HCV nonstructural protein 5A (NS5A), an essential protein for HCV replication
- Both have individually demonstrated potent, pan-genotypic, antiviral activity against HCV[2,3]
- The combination of BEM-RZR, which has demonstrated a substantially greater inhibition of HCV replication *in vitro* than the sum of the activities of both agents alone, has the potential to offer a differentiated, short duration, pan-genotypic, protease inhibitor-sparing regimen for HCV-infected patients with or without cirrhosis
- For HCV patients with decompensated cirrhosis, the combination of BEM and RZR may have the additional potential for treatment without the co-administration of ribavirin, which can cause a wide range of serious side effects

METHODS

CYP450 inhibition using human liver microsomes (HLM)

- For direct CYP inhibition, RZR was pre-incubated in triplicate at 37ºC with HLM (reversible inhibition) and probe substrate in the absence of NADPH, followed with the addition of pre-warmed NADPH and incubation at 37ºC for 3–30 min depending on the individual CYP isoform
- For time-dependent inhibition, the test article was incubated at 37ºC with HLM in buffer for a duration ranging from 0 to 30 min in the absence of NADPH, followed by NADPH and probe substrate addition and incubation
- For UGT1A1 (uridine 5′-diphospho-glucuronosyltransferase enzyme 1A1) evaluation, the test article was incubated at 37°C with HLM for 20 min in a reaction mixture containing estradiol, UDPGA, and alamethicin
- Analyses were measured by LC-MS/MS

CYP450 induction in human hepatocytes

- Human cryopreserved hepatocytes from three donors were incubated in culture media spiked with RZR for 48 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, OCT1, and OCT2 transporters were conducted using inside-out membrane HEK293 vesicles and transporter-expressed MDCKII and HEK293 cells

RESULTS

Table 1. Recovery of radioactivity at 72 hours in urine, bile and feces following administration of [^{3}H]-RZR to BDC male Wistar Han rats and Beagle dogs

Species, (n)	Dose (mg/kg), route	Percent of dose recovered				Total percent
		Urine	Bile	Feces	Cage wash	
Rat, (n=3)	2, IV	0.24 ± 0.048	62 ± 12	20 ± 9.1	0.053 ± 0.023	83 ± 2.7
Dog, (n=3)	1, IV	0.25 ± 0.080	51 ± 16	35 ± 13	0.091 ± 0.081	86 ± 2.6
Dog, (n=3)	5, PO	0.21 ± 0.051	3.3 ± 1.9	78 ± 5.9	0.38 ± 0.28	82 ± 4.4

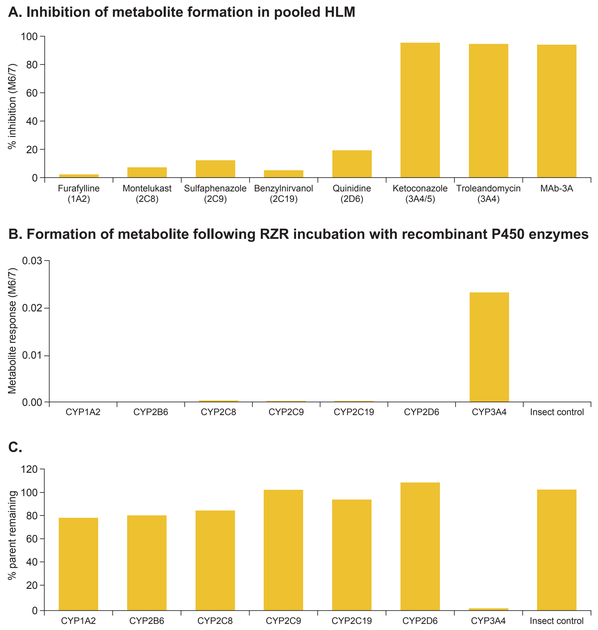
Excretion summary

- Small amounts of hydrolytic and oxidative metabolites were detected in bile and feces
- Unchanged [^{3}H]-RZR was the majority of drug-related radioactivity in circulation and in bile
- Since there was very little radioactivity observed in urine, substrate evaluation studies with kidney specific transporters were not conducted (OAT1, OAT3, OCT2, MATE1, MATE2-K)
- Labeled RZR was primarily recovered in bile and feces as unchanged drug in rats and dogs (**Table 1**)

CYP450 phenotyping

- *In vitro*, RZR was metabolized primarily by CYP3A4, resulting in the formation of mono-oxidative metabolites M6/7 (**Figure 1**)

Figure 1. RZR (1 µM) was incubated with HLM and known CYP inhibitors or inhibitory monoclonal antibody against CYP3A (A), and with recombinant human P450 enzymes (0.2 nmol/mL) and insect microsomes for 60 min at 37°C (B, C). The metabolites M6/7 (A, B) and the parent RZR (C) was then measured by LC-MS/MS in triplicate



Reversible CYP450 and UGT1A1 inhibition

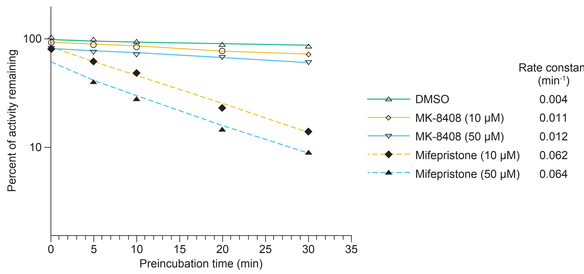
Table 2. CYP and UGT1A1 inhibitory potential of RZR in pooled HLM. Enzyme activities were compared to known inhibitors (control)

CYP	Reaction	IC^{50} (µM)	
		Control inhibitor	RZR[a]
CYP1A2	Phenacetin O-deethylation	0.013 (α-napthoflavone)	>10 (4.6 ± 0.77%)
CYP2B6	Bupropion hydroxylation	0.43 (ticlopidine)	>10 (4.8 ± 0.10%)
CYP2C8	Amodiaquine N-deethylation	0.32 (montelukast)	>10 (9.5 ± 1.2%)
CYP2C9	Diclofenac 4'-hydroxylation	0.82 (sulfaphenazole)	>10 (7.9 ± 1.0%)
CYP2C19	S-mephenytoin 4'-hydroxylation	0.22 (benzylnirvanol)	>10 (11 ± 0.46%)
CYP2D6	Dextromethorphan O-demethylation	0.18 (quinidine)	>10 (0%)[b]
CYP3A4M	Midazolam 1'-hydroxylation	0.029 (ketoconazole)	>10 (38 ± 7.3%)
CYP3A4T	Testosterone 6β-hydroxylation	0.039 (ketoconazole)	>10 (11 ± 1.2%)
UGT1A1	Estradiol-3-glucuronidation	2.69 ± 0.20 (nicardipine)	2.76 ± 0.70

[a]Value in parenthesis represents the percent inhibition (mean ± SD, n=3) at 10 µM RZR;
[b]Value in parenthesis represents the percent inhibition (mean, n=2) at 10 µM RZR.

- RZR was not a time-dependent inhibitor of CYP3A4 (**Figure 2**)

Figure 2. TDI Inhibition of CYP3A4 in HLM



- RZR (10 µM) inhibited CYP3A4 38% with midazolam as substrate but had no significant impact on the rest of CYPs tested
- When assessed in hepatocytes from 3 human donors, RZR did not induce mRNA expression or enzyme activity of CYP3A4
- Moreover, RZR did not induce mRNA expression of CYP2B6 and CYP1A2
- However, in one donor, a slight increase in CYP2B6 activity (15.2% of positive control) and a slight increase in CYP1A2 activity (12–23% of positive control) was observed

Transporter interaction

Table 3. Uptake and inhibition of RZR was evaluated in human hepatocytes and stably transfected cells expressing the transporter of interest *in vitro*, using known transporter inhibitors or substrates as controls

Transporter	Substrate potential	Inhibition IC$_{50}$ (µM)
P-gp	Yes	0.05 ± 0.03
BCRP	Inc	0.27 ± 0.02
BSEP	No	0.37 ± 0.02
OATP1B1	Inc	0.092 ± 0.004
OATP1B3	Inc	0.052 ± 0.002
OAT1	ND	No inhibition*
OAT3	ND	No inhibition*
OCT1	No	No inhibition*
OCT2	ND	No inhibition*
MATE1	ND	No inhibition*
MATE2-K	ND	No inhibition*

Inc, inconclusive; ND, substrate potential for urinary transporters were not determined because the urinary elimination of RZR was insignificant (<1%).
*No inhibition up to highest concentration tested (0.5 µM for OAT1/OAT3/OCT1/OCT2 and 2 µM for MATE1/MATE2-K).

CONCLUSIONS

- RZR is metabolized primarily by CYP3A4 *in vitro*, however the extent of *in vivo* metabolism was insignificant
- The main route of elimination of RZR in preclinical species was GI secretion and biliary excretion of the unchanged parent
- RZR exhibited weak direct inhibition of CYP3A4 *in vitro*, and no time-dependent inhibition of CYP3A4 was observed
- RZR was a substrate of P-gp and potentially of BCRP
- RZR inhibited P-gp, BCRP, BSEP, OATP1B1 and OATP1B3, however, because RZR was highly protein-bound (>99.9%), the risk of potential DDI is likely minimal

References

1. World Health Organization, Hepatitis C Fact Sheet. Available at: https://www.who.int/news-room/fact-sheets/detail/hepatitis-c (Accessed May 2024);
2. Good SS, et al. PLoS One 2020;15:e0227104;
3. Asante-Appiah E, et al. Antimicrob Agents Chemother 2018;62:e01280–18.

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Disclosures

The authors are employees of Atea Pharmaceuticals or Merck & Co.

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