**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.
- 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 groups 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, and those with comorbid conditions receiving concomitant medications leading to drug-drug interactions (DDIs).
- Asa Pharma, Inc., is developing bemnifosbuvir (ISE) in combination with ritonavir-based PIs. Our goal was to evaluate the drug-drug interaction potential of RZV for inhibition of drug-metabolizing enzymes and transporters.

**METHODS**

### CYP450 inhibition using human liver microsomes (HLM)

- For direct CYP inhibition, RZV was pre-incubated in tris buffers (37°C) with HLM (reversible inhibition) and probe substrate in the absence of NADPH.
- For time-dependent inhibition, the test article was incubated at 37°C 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 30 min.
- For UGT inhibition, RZV was incubated with a panel of recombinant human UGT enzymes in the presence of UDPGA and all methionine.

### CYP450 induction in human hepatocytes

- Human cryopreserved hepatocytes from three donors were incubated in media spiked with RZV for 48 hours in triplicate.
- Hepatocyte cultures were also treated in parallel with vehicle control or with recombinant human P450 enzymes (0.2 nmol/mL) and insect control.
- Metabolism
  - The main route of elimination of RZV in preclinical species was unchanged drug in rats and dogs.
  - The extent of GI secretion and biliary excretion of the unchanged parent remained similar in all species.

### Transporter interaction

- Transporter assays were conducted using known transporter inhibitors or substrates as controls.

**RESULTS**

### Excretion summary

- Small amounts of hydrophilic and oxidative metabolites were detected in bile and feces.
- Unchanged [14C]-RZV was the majority of drug-related radioactivity in circulation and in bile.

### Table 1. Recovery of radioactivity at 72 hours in urine, bile and feces following administration of [14C]-RZV to BCD male Wistar Han rats and Beagle dogs

<table>
<thead>
<tr>
<th>Species/Route</th>
<th>Urine %</th>
<th>Feces</th>
<th>Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, (n=3)</td>
<td>2, IV</td>
<td>0.24 ± 0.048</td>
<td>62 ± 1.2</td>
</tr>
<tr>
<td>Dog, (n=3)</td>
<td></td>
<td>0.03 ± 0.023</td>
<td>83 ± 2.7</td>
</tr>
</tbody>
</table>

### CYP450 phenotyping

- In vitro, RZV was metabolized primarily by CYP3A4, resulting in the formation of mono-conjugate metabolites M6/7 (Figure 1).

### Table 2. CYP and UGT1A1 inhibitory potential of RZV in pooled HLM.

<table>
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<th>CYP</th>
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<th>M2(%)</th>
<th>IC50 (µM)</th>
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<tr>
<td>CYP1A2</td>
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<td>(nanoflavone)</td>
<td>4.6 ± 0.77%</td>
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### CONCLUSIONS

- RZV is metabolized primarily by CYP3A4 in vitro, however the extent of in vivo metabolism is insignificant.
- The main route of elimination of RZV in preclinical species was GI secretory and biliary excretion of the unchanged parent.
- RZV exhibited weak direct inhibition of CYP3A4 in vitro, and no significant independent inhibition of CYP3A4 was observed.
- RZV was a substrate of P-gp and potentially of BCRP.
- RZV inhibited P-gp, BCRP, BSEP, OATP1B1 and OATP1B3, however, because RZV was highly protein-bound (>99%), the risk of potential DDI is likely minimal.

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**Disclosures**

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