The Sphingosine-1-Phosphate Receptor (S1P) Modulator Etrasimod (APD334) Demonstrates Limited Effects on Heart Rate in Preclinical Testing

Ronald Christopher,1 Joel Gatlin,1 Bruce Ennis,1 Kevin Whelan,1 Michael Morgan,1 Woo Hyun Yoon,1 Yong Tang,1 Hussien Al-Shamma,1 David Unet,1 William Shannah1

1Arena Pharmaceuticals Inc., San Diego, CA, US

Background
- Sphingosine-1-phosphate (S1P) – a membrane-derived lysophospholipid signaling molecule – is involved in a vast array of physiological and pathophysiological processes, primarily via extracellular activation of S1P1–S1P5 receptors.
- Although targeting S1P modulators provides opportunities for managing inflammatory conditions, both native receptors and selectively S1P modulators have been associated with potentially serious adverse events, including bradycardia.
- These cardiovascular (CV) events are potentially due to S1P-mediated activation of GIRK (G protein coupled inwardly rectifying potassium) channels on atrial myocytes, leading to an inward potassium current, I_{K(ACh)}, which has negative effects on both the sinoatrial and atrioventricular nodes.
- Etrasimod, an oral, potent, next-generation S1P1 modulator in clinical development for the chronic treatment of inflammatory diseases, has been designed to selectively target only S1P receptor subtypes 1, 4, and 5 in order to provide systemic and local immune cell modulation, while avoiding potential serious adverse events associated with other S1P receptor activation.

The objective of this study was to evaluate the selectivity and potency of etrasimod at S1P receptors, and to assess its effect on I_{K(ACh)} currents in human atrial cardiomyocytes and other CV parameters in preclinical evaluations (in vivo and in vitro).

Published data from other S1P modulators have been provided for comparison.

Methods
- The potential of etrasimod at human and non-human (mouse, rat, dog, monkey) S1P receptors was assessed in in vitro baseline recruitment and cAMP accumulation assays using S1P receptor-expressing cells (PAH/Fh5025 cell line).
- At least 10 different etrasimod concentrations (nM) were tested and each determined in triplicate.
- Other S1P receptors were included for comparison: FTY720 (fingolimod) and RPC1063, and two potential metabolites.
- The effects of etrasimod and S1P on I_{K(ACh)} were tested in human atrial myocytes using standard voltage patch-clamping techniques.
- Concentrations tested were 0.1, 1, 10, 100, and 1000 nM in DMEM vehicle.
- The effects of single doses of etrasimod on CV parameters were evaluated in telemeterized conscious beagle dogs.
- Both species were administered single doses of etrasimod or control (L-arginine hydrochloride) intravenously, allowing approximately 7 days between dosing.
- CV parameters were recorded in both species, including (but not limited to): heart rate, arterial blood pressure, pulse pressure, and body temperature. These were collected continuously for an approximately 1-hour period prior to administration through approximately 24 hours post-dose.
- Similar assessments were conducted using the non-selective S1P modulator (FTY720, fingolimod, 10 mg/kg).

Results
Etrasimod S1P receptor selectivity and potency
- Etrasimod was shown to be a potent, full agonist at the S1P1 receptor and a partial agonist of both S1P4 and S1P5 receptors in human S1P1 selectivity was 24-fold for 4-fold versus S1P4 and S1P5, respectively (Table 1).
- Etrasimod was not active for either human S1P2 or S1P3 receptors; it had a 2100-fold selectivity for S1P1 versus S1P2 and S1P3.
- Similar results were found in all species tested; mean concentration of drug giving half-maximal response (EC50) values for S1P3 were 3.60 ± 0.47 nM.

Effect of etrasimod and S1P on I_{K(ACh)}
- Etrasimod activated I_{K(ACh)} in human atrial myocytes to a lesser extent than S1P, with mean EC50 of 22.9 nM and 2.1 nM, respectively (Figure 1).
- As bradycardia is associated with an increase in I_{K(ACh)} of 50–75%, I_{K(ACh)} activation is likely to contribute to the mechanism underlying bradycardia in humans associated with S1P modulators.
- Activation of GIRK channels in human atrial myocytes has also been reported with other S1P modulators (Table 2).

Table 1. Etrasimod is a potent agonist of the S1P1 receptor and partial agonist of S1P4 and S1P5 receptors in β-arrestin recruitment assay using human S1P receptor stably cell line

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Mean EC50 (nM)</th>
<th>Relative % activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1P1</td>
<td>22.9</td>
<td>100</td>
</tr>
<tr>
<td>S1P2</td>
<td>2250</td>
<td>25</td>
</tr>
<tr>
<td>S1P3</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>S1P4</td>
<td>29.9</td>
<td>50</td>
</tr>
<tr>
<td>S1P5</td>
<td>2.1</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 2. Other S1P modulators have shown activation of GIRK channels

<table>
<thead>
<tr>
<th>Receptor</th>
<th>EC50 (nM)</th>
<th>GIRK channel EC50 (nM)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1P1</td>
<td>3.65</td>
<td>2.9</td>
<td>GIRK1,2,3</td>
</tr>
<tr>
<td>S1P4</td>
<td>6.0</td>
<td>0.1</td>
<td>GIRK1,2,3</td>
</tr>
<tr>
<td>S1P5</td>
<td>8.0</td>
<td>0.1</td>
<td>GIRK1,2,3</td>
</tr>
</tbody>
</table>

Figure 1. Etrasimod showed less activation of I_{K(ACh)} than S1P, with respective EC50 values of 29.9 nM and 2.1 nM in human atrial myocytes

Figure 2. Etrasimod was associated with no meaningful changes in heart rate, mean arterial pressure, or body temperature in conscious, telemeterized rats, compared with control

Conclusions
- Etrasimod is a potent and selective S1P1 full agonist with greater than 1000-fold selectivity versus S1P2 and S1P3, and a partial agonist at S1P4 and S1P5 receptors.
- Etrasimod was an activator of I_{K(ACh)} channel, however with a lower potency than S1P.
- In conscious telemeterized rats, etrasimod showed no evidence of meaningful changes in heart rate or mean arterial pressure.
- In conscious telemeterized dogs, etrasimod administration (40 mg/kg dose level) resulted in no changes in arterial blood pressure measurements. No test article-related differences were observed in heart rate, pulse pressure, body temperature, or any EC5 parameter at any dose level tested (10, 20, 40, 100 mg/kg).

References

Author Disclosures
Ronald Christopher is employee of Arena Pharmaceuticals, Inc. Joel Gatlin, Bruce Ennis, Kevin Whelan, Michael Morgan, and William Shannah are employees of Arena Pharmaceuticals, Inc.

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