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

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## Background

- Sphingosine-1-phosphate (S1P) – a membrane-derived lysophospholipid signaling molecule – is implicated in a vast array of physiological and pathophysiological processes, primarily via extracellular activation of S1P1–S1P5 receptors[1,2]
- Although targeting S1P modulators provides opportunities for managing inflammatory conditions, both non-selective and selective S1P modulators have been associated with potentially serious adverse events, including bradycardia[3-5]
- 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, $I_{KAch}$, which has negative effects on both the sinoatrial and atrioventricular nodes[6-8]
- Etrasimod, an oral, potent, next-generation S1P modulator in clinical development for the chronic treatment of ulcerative colitis, has been designed to selectively target S1P receptor subtypes 1, 4, and 5 in order to provide systemic and local immune cell modulation,[9] 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 effects on ion $I_{KAch}$ currents in human atrial cardiomyocytes and other CV parameters in preclinical evaluations (*in vitro* and *in vivo*)
- Published data from other S1P modulators have been provided for comparison

## Methods

- The potency of etrasimod at human and non-human (mouse, rat, dog, monkey) S1P receptors was assessed in intracellular β-arrestin recruitment and cAMP accumulation assays using S1P receptor-expressing cells (PathHunter293 cell line)
  - At least 10 different etrasimod concentrations (≤10 μM) were tested and each determined in triplicate.
  - Other S1P modulators were included for comparison: FTY720 (fingolimod) and RPC1063, and two potential metabolites
- The effects of etrasimod and S1P on $I_{KAch}$ were tested in human atrial myocytes using standard voltage patch-clamping techniques
  - Concentrations tested were 0.01, 0.1, 1, 10, 100, and 1000 nM in DMSO vehicle
- The effects of single doses of etrasimod on CV parameters were evaluated in telemeterized conscious beagle dogs and rats
  - Both species were administered single doses of etrasimod or control (L-arginine hydrochloride) in deionized water vehicle, 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 approximately 1 hour 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 humans: its S1P1 selectivity was 24-fold and 4-fold versus S1P4 and S1P5, respectively (**Table 1**)
- Etrasimod was not active for either human S1P2 or S1P3 receptors; it had a ≥1000-fold selectivity for S1P1 versus S1P2 and S1P3
- Similar results were found in all species tested; mean concentration of drug giving half-maximal response ($EC_{50}$) values for S1P1 were 3.65-8.70 nM

### Effect of etrasimod and S1P on $I_{KAch}$

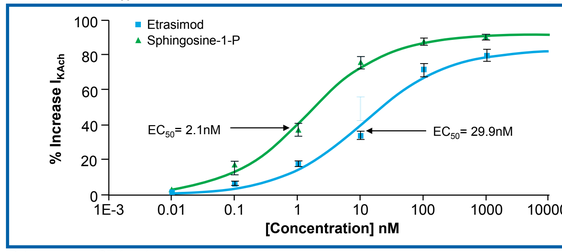
- Etrasimod activated $I_{KAch}$ in human atrial myocytes to a lesser extent than S1P, with mean $EC_{50}$ of 29.9 nM and 2.1 nM, respectively (**Figure 1**)
- As bradycardia is associated with an increase in $I_{KAch}$ of 50-75%, $I_{KAch}$ activation is likely to contribute to the mechanism underlying bradycardia in humans associated with S1P modulators
- Activation of GIRK channels in human atrial myocytes have 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 stable cell lines**

| Receptor subtype | Etrasimod | | Fingolimod phosphate | Ozanimod | | |
|---|---|---|---|---|---|---|
| | Mean $EC_{50}$, nM | Relative % S1P activity | [(S)-FTY720P] | RPC1063 | Met 1 | Met 2 |
| | | | Mean $EC_{50}$, nM | | | |
| **hS1P1** | 6.10 | 110 | 2.5 | 2.3 | 3.3 | 0.56 |
| **hS1P2** | No activity* | - | No activity* | No activity* | No activity* | No activity* |
| **hS1P3** | No activity* | - | 12.9 | No activity* | No activity* | No activity* |
| **hS1P4** | 147 | 63 | 2.32 | 482 | 252 | 225 |
| **hS1P5** | 24.4 | 73 | 0.95 | 3.96 | 3.8 | 3.9 |

Data presented here represent *in vitro* analysis of all analytes in a head-to-head profile under identical experimental conditions; *No activity, $EC_{50}$ >100 μM; Met 1 and 2, metabolites of RPC1063

**Figure 1. Etrasimod showed less activation of $I_{KAch}$, than S1P, with respective $EC_{50}$ values of 29.9 nM and 2.1 nM in human atrial myoctyes**




Data are mean ± SEM, n=4

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

| | Fingolimod phosphate* | Ozanimod* | | | MT-1303 phosphate*[b] |
|---|---|---|---|---|---|
| | | RPC1063 | Met 1 | Met 2 | |
| **Preclinical safety findings** | HR ↑ (monkey, rat) BP ↓ (dog)[a] | Preclinical HR and BP effects not reported[a] | | | No CV changes observed in any species |
| **Receptor specificity** | S1P 1, 3, 4 and 5[a] | S1P 1 and 5[a] | | | S1P 1, 4 and 5 |
| **S1P1 $EC_{50}$ (nM)** | 2.5 | 2.3 | 3.3 | 0.56 | 0.08 |
| **S1P2 $EC_{50}$ (nM)** | >10,000 | All >10,000 | | | >10,000 |
| **S1P3 $EC_{50}$ (nM)** | 12.9 | All >10,000 | | | >10,000 |
| **GIRK channel $EC_{50}$ (nM)** | 8.5[b] | Multiple active metabolites | | | 41.6 |
| **Heart: plasma ratio at $C_{max}$ (rats)** | ↑ relative to MT1303[b] | | | | ↓ relative to fingolimod |

*Not comparative head-to-head studies; [a]Preclinical safety findings and receptor selectivity data for fingolimod and ozanimod are from the current analysis; [b]Data previously reported by Sugahara et al. 2015.[10] BP, blood pressure; $C_{max}$ , maximum plasma concentration, CV, cardiovascular; HR, heart rate

### CV parameters in telemeterized rats and Beagle dogs

- There was no evidence of any meaningful changes in heart rate, electrocardiogram (ECG) parameters, pulse pressure, or body temperature with etrasimod administration in conscious telemeterized rats (**Figure 2**) or dogs (data not shown)
- In rats, no evidence of any meaningful changes in mean arterial pressure with etrasimod 10 or 30 mg/kg doses were found (**Figure 2**)
- In dogs, transient increases in systolic (11.7%), diastolic (14.2%), and mean arterial pressure (12.4%; **Figure 3**) were reported with etrasimod 40 mg/kg, compared with time-matched control values
- The no-observed-adverse-effect level (NOAEL) was therefore 40 mg/kg in this dog telemetry model
  - Mean plasma concentrations at NOAEL were 33.8-44.5 μg/mL 24 hours after administering etrasimod (40 mg/kg).
- Reductions in both heart rate and mean arterial pressure were shown with the non-selective S1P moderator (FTY720), compared with vehicle control (**Figure 2**)

**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**

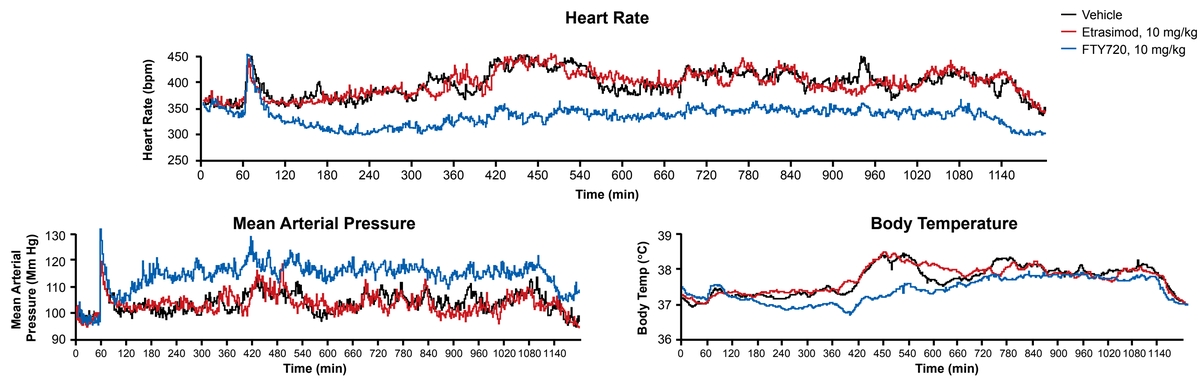
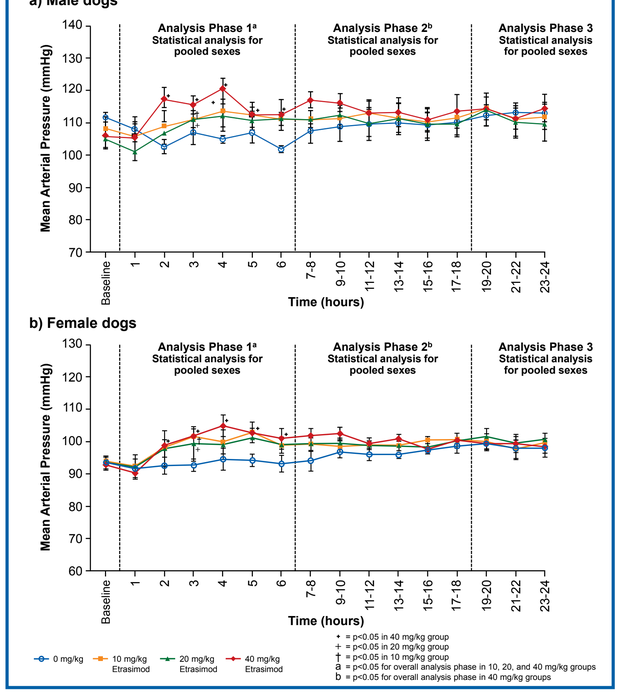



**Figure 3. Transient increases in mean arterial pressures were reported with etrasimod in conscious, telemeterized Beagle dogs**




Data are mean ± SEM, n=4; Each dosing period of the study was defined by a pre-dose and post-dose segment. For the purpose of statistical analysis, telemetry data were organized in the following phases: Phase 0: 1-hour pre-dose baseline; Phase 1: 6 sub-phases of 1 hour each for hours 1–6; Phase 2: 6 sub-phases of 2 hours each for hours 7–18; Phase 3: 3 sub-phases of 2 hours each for hours 19–24)

## 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 $I_{KAch}$ 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 transient increases in arterial blood pressure measures. No test article related differences were observed in heart rate, pulse pressure, body temperature, or any ECG parameter at any dose level tested (10, 20, or 40 mg/kg)

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## Author Disclosures

Ronald Christopher is an employee of Arena Pharmaceuticals, Inc. Joel Gatlin, Bruce Ennis, Kevin Whelan, Michael Morgan, Woo Hyun Yoon, Yong Tang, Hussien Al-Shamma, David Unett, and William Shanahan were employees of Arena Pharmaceuticals, Inc. during the conduct of the study.

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