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Preclinical Safety Assessment of Etrasimod (APD334), an Oral Sphingosine-1-Phosphate Receptor (S1P) Modulator with a Favorable Profile

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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]
- 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,[6] while avoiding potential serious adverse events associated with other S1P receptor activation
- Here we present the favorable preclinical pharmacology and safety profile of etrasimod

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 (≤10μM) were tested and each determined in triplicate
- In a conventional panel of safety pharmacology studies, etrasimod was administered by oral gavage to rats (Sprague Dawley) and dogs (Beagle) and compared with vehicle control (L-arginine hydrochloride, USP in deionized water)
  - In rats, effects of single doses of etrasimod (25, 150, or 350 mg/kg) on the central nervous system (CNS; Irwin test: pre-dose and 30, 90, 150, and 200 minutes and 24 hours post-dose; 6 animals per group) and respiratory system (respiratory frequency and tidal volume assessed 60 minutes pre-dosing, and at least 5 hours and at 23 hours post-dose; 8 animals per group) were evaluated
  - Acute cardiovascular (CV) effects of etrasimod (10, 20, or 40 mg/kg) were assessed in conscious telemeterized dogs; assessments were conducted every hour for 24 hours included: heart rate, arterial blood pressure, pulse pressure and electrocardiogram (ECG), body temperature
  - Chronic safety profiles of etrasimod were evaluated by administering etrasimod once daily (QD) to rats (25, 75, 150, or 250 mg/kg/day) for 6 months, and to dogs (2, 5, 10, or 15 mg/kg/day) for 9 months; this was followed by a 1-month, post-dose observation period in both species (in the control and higher dose groups) to assess reversibility, progression and delayed appearance of any changes
- Comprehensive toxicokinetic assessments and evidence of reversibility were evaluated in each of the Good Laboratory Practice safety studies

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 and all other species tested (**Table 1**); it was not active for either S1P2 or S1P3 receptors
  - Mean concentration of drug giving half-maximal response (EC_{50}) values for S1P1 were 3.65-8.70nM (**Table 1**)
- Etrasimod S1P1 selectivity in humans was 24-fold and 4-fold versus S1P4 and S1P5, respectively, with ≥1000-fold selectivity for S1P1 versus S1P2 and S1P3
- Etrasimod was confirmed to be a potent, full agonist of the S1P1 receptor in the cAMP accumulation assay (**Table 1**)

Table 1. Similar EC_{50} values and efficacies were found in all species tested: mean EC_{50} values were 3.65-8.70nM and efficacy was >80% for S1P1 receptors in the β-arrestin recruitment assay

Receptor	Mean EC_{50}, nM	Mean efficacy, % S1P
β-arrestin recruitment assay		
hS1P1	6.10	110
mS1P1	3.65	82
dS1P1	4.19	101
mkS1P1	8.70	88
cAMP accumulation assay		
hS1P1	0.199	96
rS1P1	0.319	99

Etrasimod EC_{50} determinations in recombinant human (h), mouse (m), dog (d), monkey (mk) and rat (r) S1P1 receptors

Respiratory and CNS safety of etrasimod: acute administration in rats

- Single doses of etrasimod (≤350 mg/kg) in rats had no effect on respiratory frequency, tidal volume, minute volume, gross behavioral or neurological state (**Table 3**)
- No effects on physiological state, including body temperature, were found, except mild, transient exophthalmos with etrasimod 350 mg/kg

Table 3. Acute administration of etrasimod at ≤350 mg/kg had no effects on respiratory frequency, tidal volume or minute volume, or on gross behavioral or neurological state in rats

	Sprague Dawley rats Single acute dose, mg/kg		
Study evaluation	25	150	350
Respiratory frequency	No effect	No effect	No effect*
Tidal volume	No effect	No effect	No effect
Minute volume	No effect	No effect	No effect*
Gross behavioral state	No effect	No effect	No effect
Neurological state	No effect	No effect	No effect
Physiological state	No effect	No effect	Mild transient exophthalmos†

*Slight decreases reported for the overall analysis were considered to be incidental and not related to study drug; †Mild, transient exophthalmos with etrasimod 350 mg/kg: observed in four animals at 30 minutes and three animals at 90 minutes post-dose; resolving in all animals 150-minutes post dose

CV safety of etrasimod: acute administration in dogs

- Single doses of etrasimod (≤40 mg/kg) in dogs had no effect on heart rate, body temperature, or ECG parameters (**Table 4**)
- Transient increases in arterial blood pressure, 2–4 hours post-dosing, were found with etrasimod 40 mg/kg, compared with control values

Table 4. Acute administration of etrasimod at 10 and 20 mg/kg had no effect on any CV parameter or body temperature in dogs

	Beagle dogs Single acute dose, mg/kg		
Study evaluation	10	20	40
Heart rate	No effect	No effect	No effect
Body temperature	No effect	No effect	No effect
ECG parameters	No effect	No effect	No effect
Mean systolic pressure, vs. control values	No effect	No effect	Transient increase of 11.7% 2–4 hours post-dosing
Mean diastolic pressure, vs. control values	No effect	No effect	Transient increase of 14.2% 2–4 hours post-dosing
Mean arterial pressure, vs. control values	No effect	No effect	Transient increase of 12.4% 2–4 hours post-dosing
Pulse pressure	No effect	No effect	No effect

Chronic toxicology findings

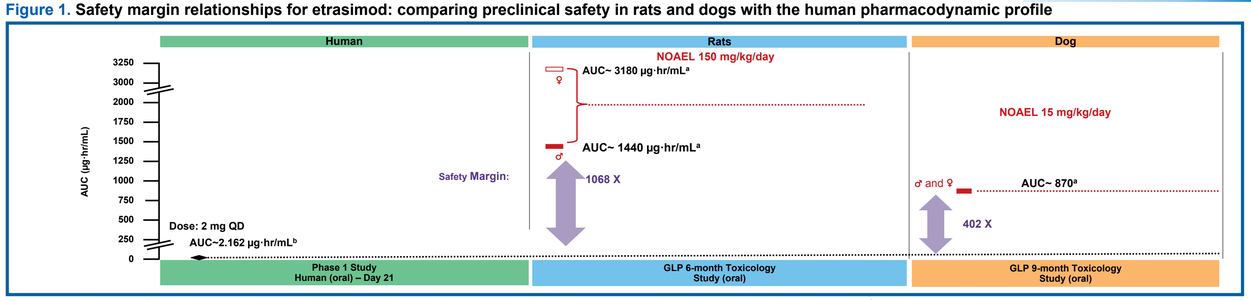
Chronic toxicology study in rats

- Etrasimod ≤150 mg/kg/day was generally well tolerated in rats, with expected pharmacological effects (**Table 5**)
- Evidence of reversibility was shown during the recovery period, including reversibility in body weight and food consumption decreases (less so with the highest dose)
- Mild reversible increases (+2.1X) in alanine aminotransferase (ALT) in males with etrasimod 250 mg/kg/day was related to hepatocellular necrosis (seen microscopically in some animals at this dose level) and resolved during a post-recovery phase

Chronic toxicology study in dogs

- Etrasimod ≤15 mg/kg/day for 9 months was generally well tolerated in dogs with no evidence of morbidity or mortality; all treatment-related effects were non-adverse (**Table 5**)

Figure 1. Safety margin relationships for etrasimod: comparing preclinical safety in rats and dogs with the human pharmacodynamic profile



[a]Mean values at steady state; [b]Mean plasma systemic exposure ($AUC_{0\text{-}24h}$) value from the multiple dose (21 day) study in healthy subjects with etrasimod. $AUC_{0\text{-}24h}$ at 2 mg dose (Day 21)[8]. AUC, area under the plasma concentration-time curve; NOAEL, no-observed adverse effects level; QD, once daily

Table 5. Etrasimod was generally well tolerated in rats, with expected and typical pharmacological effects with ≤150 mg/kg/day, and in dogs with doses ≤15 mg/kg/day

	Sprague Dawley rats Dose, mg/kg/day (n)				Beagle dogs Dose, mg/kg/day (n)			
Study evaluation	25 (25)	75 (25)	150 (20)	250 (20)	2 (8)	5 (8)	10 (8)	15 (8)
Death	None	1 – dosing injury	3 (2 dosing injury; 1 not established)	12 (9 not established; 2 liver inflammation/ necrosis; 1 liver inflammation/ obstruction caculi and urogenital inflammation)	None	None	None	None
Adverse clinical observations	None	None; few incidences of sporadic salivation	Salivation	Predominantly in animals that died	Fecal effects (mucoid, soft and/or watery)			
					None	Lacrimation		
					None	None	Thinness	
Body weight, change vs. control	No change	Non-adverse decrease:* males: 11.4%; females: 16.0%	Non-adverse decrease:* males: 18.9%; females: 16.0%	Decreased: males: 27.9%; females: 21.4%	No change	Decreased (males)		
Food consumption, vs. control	No change	Decreased†			No change	Decreased (males only)		Decreased
Clinical chemistry findings								
ALT	No change			Mild increase (males)	No change			
Bilirubin	Mild-to-moderate increase				No change			Minimal increase
Total protein	No change			Mild decrease	Mild decrease			
Albumin	No change			Mild decrease	Mild decrease			
Globulin	No change			Mild decrease	Mild decrease			
Triglyceride	Mild-to-moderate decrease				No change			
Ophthalmos copy	No change				No change	Conjunctivitis (≥5 mg/kg, males; ≥10 mg/kg, females); ocular lesions‡		
Urinalysis evaluation	No change				No change			
Macroscopic evaluation	No change				No change			Lungs: tan discolora-tion in 2/4 males
ECG	Not conducted				No change			
Post recovery phase	No relevant clinical findings persisted by end of the recovery phase				All clinical findings were reversible with the exception of body weight gain in high dose females and eye lacrimation in 1 male			

*Non-adverse based on the health status of the animals in these cohorts; †Peak magnitude of effect occurred at Week 1 with doses 75 and 50 mg/k/day, after which the decrease generally moderated; peak magnitude of effect occurred at Week 3 with the 250 mg/kg/day dose and remained significant throughout the study;‡Lacrimation or scleral injection; ALT, alanine aminotransferase; ECG, electrocardiogram

Histopathology findings

- Consistent with the pharmacological effect of etrasimod on lymphocyte trafficking, moderate-to-marked, partially reversible, decreases in circulating lymphocytes were found at all dose levels, versus control
- Microscopic findings associated with etrasimod with chronic dosing are mechanism-based and related to lymphoid depletion in the lymph nodes and spleen in rats, and increased thymus weights in dogs
- Evidence of reversibility of microscopic findings was noted at recovery in both rats and dogs after a four-week recovery period
- In dogs, clinical pathology changes were not adverse at any dose level, and ocular, pulmonary and mesenteric lymph node changes found were reversible

Human safety margin estimates: based on steady-state systemic exposures from the chronic toxicology studies in rats and dogs

- Human safety margin estimates for a clinically relevant dose of 2 mg etrasimod were 1,068-fold and 402-fold for rats and dogs, respectively (**Figure 1**)
- Etrasimod showed a substantially wider safety margin based on these chronic toxicology data than reported for RPC1063 (ozanimod) and its two metabolites (**Table 6**)[7]

Table 6. Etrasimod had a wide safety margin based on exposure date from the chronic toxicity studies in rat and dogs

Species	Drug	Study duration	NOAEL mg/kg/day	C_{max}[a] (µg/mL)	AUC[a] (µg·hr/mL)	Safety Margin[b]
Rats	Etrasimod	6 months	150	113 (males) 201 (females)	1440 (males) 3180 (females)	1,068
Dogs	Etrasimod	9 months	15	46	870	402
Monkey[c]	RPC1063	9 months	0.1	–	–	5
Monkey[c]	RP101988 (metabolite)	9 months	0.1	–	–	0.8
Monkey[c]	RP101075 (metabolite)	9 months	0.1	–	–	3.0

[a]Mean values from male and female animals at steady state.; [b]Safety margin at a 2 mg dose is derived from the ratio of $AUC_{0\text{-}24h}$ values determined at steady-state in the 6 and 9 month toxicology studies in rats and dogs, respectively, to the mean plasma $AUC_{0\text{-}24h}$ value from the multiple dose (21 day) study in healthy normal subjects with etrasimod. $AUC_{0\text{-}24h}$ at 2 mg dose (Day 21) is reported to be 2.162 µg·hr/mL;[8] [c]Based on published data from a 9-month toxicology study of ozanimod in cynomolgus monkeys.[7] $AUC_{0\text{-}24}$, area under the plasma concentration-time curve from time zero to 24 hours post-dosing; C_{max}, maximum plasma concentration

Conclusions

- Etrasimod is a potent, full agonist at the S1P1 receptor across a range of species, with selectivity at S1P1 versus the other S1P receptors
- Exceptional safety margins have been established for etrasimod, comparing steady-state systemic exposures in the chronic toxicology studies to clinically-relevant exposures in humans
- These safety findings support the continued development of etrasimod for ulcerative colitis and other potential autoimmune disorders

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Author Disclosures

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