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RESEARCH**

APPLICATION NUMBER:

216956Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review

Clinical Review

Non-Clinical Review

Statistical Review

Clinical Pharmacology Review

NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	216956
Priority or Standard	Standard
Submit Date(s)	October 14, 2022
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PDUFA Goal Date	October 14, 2023
Division/Office	Division of Gastroenterology
Review Completion Date	October 12, 2023
Established/Proper Name	Etrasimod
(Proposed) Trade Name	Velsipity
Pharmacologic Class	Sphingosine 1-phosphate receptor modulator
Applicant	Pfizer Inc.
Dosage form	Tablet (2mg)
Applicant proposed Dosing Regimen	2 mg taken orally once daily
Applicant Proposed Indication(s)/Population(s)	Treatment of moderately to severely active ulcerative colitis in adults
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	64766004 Ulcerative colitis (disorder)
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	Treatment of moderately to severely active ulcerative colitis in adults
Recommended SNOMED CT Indication Disease Term for each Indication (if applicable)	78712000 Moderate chronic ulcerative colitis (disorder)
Recommended Dosing Regimen	2 mg taken orally once daily

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OPQ=Office of Pharmaceutical Quality
 OPDP=Office of Prescription Drug Promotion
 OSI=Office of Scientific Investigations
 OSE= Office of Surveillance and Epidemiology
 DEPI= Division of Epidemiology
 DMEPA=Division of Medication Error Prevention and Analysis
 DRM=Division of Risk Management
 DTPM= Division of Translational and Precision Medicine

Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AR	adverse reaction
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DHOT	Division of Hematology Oncology Toxicology
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Conference on Harmonisation
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
OCS	Office of Computational Science

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OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert (also known as Patient Information)
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event

1 Executive Summary

1.1. Product Introduction

Etrasimod is an orally bioavailable sphingosine 1-phosphate (S1P) receptor modulator that binds with high affinity to S1P receptors 1, 4, and 5. S1P receptor modulation partially and reversibly sequesters specific lymphocytes in lymph nodes, thus reducing activated lymphocytes in the tissue. The proposed indication is for the treatment of moderately to severely active ulcerative colitis (UC) in adults.

S1P signaling is involved in multiple immune functions. The mechanism by which etrasimod exerts therapeutic effects involves lymphocyte retention in lymphoid tissues and the reduction of lymphocyte migration to sites of inflammation including the intestine. As UC is an immune-mediated inflammatory disease, the retention of lymphocytes in the lymphoid tissue has the potential to prevent recruitment of inflammatory cells, local release of proinflammatory cytokines, and ongoing damage to the colonic mucosa. Reduction of this inflammatory response may allow a decrease in disease activity and subsequent healing of the mucosa.

Etrasimod is a new molecular entity (NME) for UC. Other S1P modulators have been approved for UC (e.g., Zeposia [ozanimod]), multiple sclerosis, psoriasis, and graft-versus-host disease. The proposed dosing for etrasimod in UC is 2 mg orally once daily.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The multidisciplinary review team recommends approval of this original NDA for Velsipity (etrasimod) for the treatment of moderately to severely active UC in adults.

The Applicant submitted the results of two adequate and well-controlled phase 3 trials (APD334-301 and APD334-302), which formed the basis of the substantial evidence of effectiveness. A clinically meaningful and statistically significant benefit was demonstrated on the coprimary endpoints for APD334-301 and primary endpoint for APD334-302. Multiple secondary endpoints supported the primary efficacy results in each trial. Thus, the Applicant has met the evidentiary standard for efficacy to support approval of etrasimod for the proposed indication.

The safety profile was acceptable and generally consistent with known adverse reactions for drugs in the same class. Identified risks will be mitigated through labeling.

The overall benefit-risk assessment supports approval as described in the Benefit-Risk Assessment below.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Moderately to severely active ulcerative colitis (UC) is a serious chronic disease associated with morbidity and mortality when inadequately treated. Multiple therapies are approved for the treatment of moderately to severely active UC; however, there remains a need for novel therapies as not all patients will respond or have continued response to any given treatment.

Etrasimod demonstrated efficacy in inducing clinical remission after 12 weeks of treatment with 2 mg daily, and in maintaining clinical remission when continued over 40 weeks of treatment. Efficacy was demonstrated in two adequate and well-controlled investigations:

- APD334-301: A 52-week, randomized, double-blinded, placebo-controlled “treat-through” trial
- APD334-302: A 12-week, randomized, double-blinded, placebo-controlled induction trial

In the 52-week, “treat-through” trial, etrasimod demonstrated efficacy in achieving the coprimary endpoints of clinical remission at Week 12 and Week 52, compared to placebo. In the 12-week induction trial, etrasimod demonstrated efficacy in achieving the primary endpoint of clinical remission at Week 12, compared to placebo. In both trials, efficacy was demonstrated on multiple clinically relevant secondary endpoints at Week 12 (APD334-301 and APD334-302) and Week 52 (APD334-301), compared to placebo. Overall, the results provided substantial evidence of effectiveness for etrasimod in the treatment of moderately to severely active UC in adults. Therefore, the Applicant has met the evidentiary standard for efficacy to support approval of etrasimod for the proposed indication.

The safety profile of etrasimod 2 mg once daily for up to 52 weeks was acceptable and supports approval of the dosing regimen. The safety database included data from the 52-week phase 3 trial APD334-301, the 12-week phase 3 trial APD334-302, and 12-week phase 2 trial APD334-003. The size of the safety database was adequate to support approval of the proposed dosing regimen. However, the size of the controlled 52-week trial does not allow for a definitive characterization of the potential for rare, serious safety concerns (e.g., mortality, malignancy, progressive multifocal leukoencephalopathy [PML], cryptococcal meningitis) that have been observed in S1P receptor modulators, including ozanimod (ZEPOSIA) that is approved for the treatment of moderately to severely active UC. There are several known potential risks related to the S1P receptor modulator class which were observed in the etrasimod development program (i.e., infections, bradyarrhythmia and atrioventricular conduction delays, liver injury, macular edema, increased blood pressure, and respiratory effects), which were consistent with other S1P receptor modulators. The most common adverse reactions observed in UC subjects treated with etrasimod during the 52-week trial

APD334-301 were headache (9%), elevated liver tests (6%), and dizziness (5%). Additionally, a greater proportion of subjects who received etrasimod had an increase of at least 40 µm in macular thickness compared to placebo (16/95, 17% vs 2/26, 8%), which is concerning for macular edema. The interpretation of this finding is challenging because a limited number of subjects had optical coherence tomography (OCT) performed at baseline and throughout the trial. The most common adverse reactions during the 12-week trials (APD334-302 and APD334-003) were generally similar to the 52-week trial. The labeling is considered adequate to communicate these risks and provide appropriate risk mitigation.

Based on the above considerations, the overall benefit-risk assessment is favorable to support approval of etrasimod for the treatment of moderately to severely active ulcerative colitis (UC) in adults.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Ulcerative colitis (UC), an inflammatory bowel disease (IBD), is a chronic, relapsing disease of the colonic mucosa. The peak age of occurrence is between the ages of 30 and 40. UC may affect as many as 907,000 Americans; the annual incidence rates of UC in the United States range from 1.55 to 15.0 cases per 100,000 person-years, and the prevalence ranges from 117 to 238 cases per 100,000 persons. • Patients with UC most commonly present with bloody diarrhea, rectal bleeding, tenesmus, urgency, abdominal pain, and passage of mucus in the stool. Disease of moderate to severe activity may be associated with systemic symptoms, including fatigue, fever, anorexia, nausea, weight loss, and dehydration. Patients may also experience symptoms secondary to anemia and hypoalbuminemia, including dyspnea and peripheral edema. • UC is associated with many extraintestinal manifestations, which have been reported to affect a wide variety of organ systems, most commonly joints, skin, eyes, kidneys, and hepatobiliary tract.	• UC is a chronic, relapsing inflammatory disorder of the colonic mucosa. • Patients with UC commonly present with bloody diarrhea, rectal bleeding, tenesmus, urgency, abdominal pain, and passage of mucus in the stool. Left untreated, patients may experience significant morbidity and/or mortality.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current Treatment Options	- The overall goal in the treatment and management of UC is to reduce signs and symptoms of active disease, decrease the underlying mucosal inflammation, and prevent short-term (e.g., severe bleeding, bowel perforation) and long-term complications (e.g., colon cancer). The choice of therapy is guided by the disease severity, extent of disease, and presence of other manifestations (i.e., extraintestinal complications, malabsorption). - Therapeutic options for treatment include 5-ASA products (e.g., mesalamine), corticosteroids, antibiotics, immunomodulators (e.g., AZA, 6-MP, MTX), biologic therapies (e.g., TNFα blockers, anti-integrin receptor blockers), S1P receptor modulators, and JAK inhibitors. Corticosteroids are not recommended for long-term use given the toxicities associated with chronic steroid use. While these medications are widely used in clinical practice, not all are FDA approved for the treatment of UC. There remains a need for novel therapies as not all patients will respond or have continued response to any given treatment.	- Etrasimod is a S1P receptor modulator with high affinity to S1P receptors 1, 4, and 5, proposed for the treatment of moderately to severely active UC. Another S1P receptor modulator (that binds with high affinity to S1P receptors 1 and 5), ozanimod (ZEPOSIA), is approved for the same indication. - Additional therapies are needed to treat moderately to severely active UC, as many patients do not respond or lose response over time to the currently available therapies. Etrasimod represents another oral, daily treatment option for patients.
Benefit	- Efficacy of etrasimod was demonstrated in one 52-week, randomized, double-blinded, placebo-controlled “treat-through” trial (APD334-301) and one 12-week, randomized, double-blinded, placebo-controlled induction trial (APD334-302). - In both trials, subjects with moderately to severely active UC were enrolled. A total of 408 adult subjects with a baseline modified Mayo Score (mMS) of 5 to 9, including an endoscopic subscore (ES) of at least 2, were enrolled in APD334-301, of which 121 had prior biologic/JAK inhibitor exposure. A total of 333 adult subjects with the same definition of moderately to severely active UC were enrolled in APD334-302, of which 112 had prior biologic/JAK inhibitor exposure.	- The two trials, APD334-301 and APD334-302, provided evidence for the effectiveness of etrasimod compared to placebo for achieving clinical remission in subjects with moderately to severely active UC. - Subgroup analyses of subjects with prior biologic/JAK inhibitor exposure support that these subjects, who are typically more refractory to treatment than biologic-naïve subjects, also derived benefit from

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	● In APD334-301, efficacy was demonstrated on the coprimary endpoints of clinical remission (defined as a mMS score of 0 to 2 with a stool frequency subscore [SFS] of 0 or 1, rectal bleeding subscore [RBS] of 0, and ES of 0 or 1 [excluding friability]) at Week 12 and Week 52. The treatment difference in the proportion of subjects achieving clinical remission between etrasimod and placebo was 20.1% (p <0.0001) and 25.7% (p<0.0001) for Week 12 and Week 52, respectively. ○ The coprimary endpoint results were further investigated in subgroup analyses for subjects with prior biologic/JAK inhibitor exposure. Efficacy results were consistent across both subgroups. The treatment difference for clinical remission between etrasimod and placebo was 15.1% and 16.4% for Week 12 and Week 52, respectively, in the prior biologic/JAK inhibitor exposure subgroup. ○ The majority of the secondary endpoints were statistically significant, including endoscopic improvement (Week 12 and 52), histologic-endoscopic mucosal improvement (Week 12 and 52), corticosteroid-free clinical remission (Week 52), and maintenance of clinical remission (Week 52). ● In APD334-302, efficacy was demonstrated on the primary endpoint of clinical remission at Week 12. The treatment difference in the proportion of subjects achieving clinical remission between etrasimod and placebo was 11.2% (p=0.010). Although the treatment difference observed for APD334-302 was smaller than that observed for APD334-301, baseline demographic and disease characteristics were similar between APD334-301 and APD334-302. The difference observed was attributed to the intrinsic variability of the placebo	treatment with etrasimod.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	response rate. ○ The primary endpoint results were further investigated in subgroup analyses for subjects with prior biologic/JAK inhibitor exposure. The treatment difference for clinical remission between etrasimod and placebo was 5.8% at Week 12 in the prior biologic/JAK inhibitor exposure subgroup. ○ Several secondary endpoints were statistically significant at Week 12, including endoscopic improvement and histologic-endoscopic mucosal improvement.	
Risk and Risk Management	● The safety of etrasimod was evaluated in two adequate and well-controlled trials, APD334-301 (52-week duration) and APD334-302 (12-week duration). Additionally, data from APD334-003, a phase 2, 12-week, randomized, double-blind, placebo-controlled induction trial was pooled with data from APD334-302 because the two trial designs and populations were sufficiently similar, to better assess the adverse event profile for the 12-week trials. ● The safety profile of etrasimod for the treatment of moderately to severely active UC appears to be consistent with the labeled safety profile of other S1P receptor modulators, including ozanimod, which is approved for the same indication. ● The label contains warnings and precautions for the following, which are consistent with other S1P receptor modulators: ○ Infections ○ Bradycardia and Atrioventricular Conduction Delays ○ Liver Injury ○ Macular Edema ○ Increased Blood Pressure	● The demonstrated and anticipated risks will be managed through labeling, including the following: ○ Infections – Obtain a complete blood count (CBC) before initiation of treatment and monitor for infection during treatment and for (b) (4) weeks after discontinuation. Consider interruption of treatment with etrasimod if a serious infection develops. ○ Bradycardia and AV conduction delays – Obtain an electrocardiogram (ECG) to assess for preexisting cardiac conduction abnormalities before starting

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	○ Fetal Risk ○ Malignancies ○ Posterior Reversible Encephalopathy Syndrome ○ Respiratory Effects ○ Unintended Additive Immune System Effects from Prior Treatment with Immunosuppressive or Immune-Modulating Drugs ○ Immune System Effects After Stopping Velsipity ● Adverse reactions (>2%) reported during APD334-301 include headache (9%), elevated liver tests (6%), dizziness (5%), arthralgia (4%), hypertension (3%), urinary tract infection (3%), nausea (3%), and herpes viral infection (2%). ● Adverse reactions (>2%) reported during APD334-302 and APD334-003 include headache (6%), elevated liver tests (5%), nausea (4%), bradycardia (3%), and urinary tract infection (3%). ● The size of the safety database is adequate to support a benefit-risk assessment; however, the size of the controlled 52-week trial does not allow for a definitive characterization of the potential for rare, serious safety concerns (e.g., mortality, malignancy, progressive multifocal leukoencephalopathy [PML], cryptococcal meningitis) that have been observed with the use of S1P receptor modulators.	etrasimod. ○ Liver injury – Obtain transaminase and bilirubin level before initiating etrasimod and discontinue if significant liver injury is confirmed. ○ Macular edema – Obtain a baseline evaluation of the macula and monitor during treatment. Consider discontinuing etrasimod if macular edema develops. ○ Increased blood pressure – Monitor blood pressure during treatment. ○ Malignancies – Perform a skin examination at the start of treatment and monitor during treatment. Promptly evaluate suspicious skin lesions. ○ Respiratory effects – Assess pulmonary function if clinically indicated. ● The labeling is considered adequate to communicate the demonstrated and anticipated risks and provide appropriate risk mitigation.

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1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

X	The patient experience data that were submitted as part of the application include:	Section of review where discussed, if applicable
☒	Clinical outcome assessment (COA) data, such as	
☒	Patient reported outcome (PRO)	Section 8.1 – The modified Mayo Score was used to derive the primary endpoint and incorporates patient reported outcomes. Additional endpoints were assessed for abdominal pain using the Abdominal Pain Numeric Rating Scale (NRS) and bowel urgency using the Urgency NRS.
☐	Observer reported outcome (ObsRO)	
☐	Clinician reported outcome (ClinRO)	
☐	Performance outcome (PerfO)	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)	
☐	Patient-focused drug development or other stakeholder meeting summary reports	
☐	Observational survey studies designed to capture patient experience data	
☐	Natural history studies	
☐	Patient preference studies (e.g., submitted studies or scientific publications)	
☐	Other: (Please specify):	
X	Patient experience data that were not submitted in the application, but were considered in this review:	
☐	Input informed from participation in meetings with patient stakeholders	
☐	Patient-focused drug development or other stakeholder meeting summary reports	

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	☐ Observational survey studies designed to capture patient experience data	
☒	Other: (Please specify):	The Division of Gastroenterology regularly participates in public meetings, workshops, and scientific conferences that engage the IBD community.
☐ Patient experience data was not submitted as part of this application.		

2 Therapeutic Context

2.1. Analysis of Condition

Ulcerative colitis (UC) is a chronic gastrointestinal inflammatory disorder that involves the colonic surface mucosa, including the infiltration of neutrophils and other inflammatory cells into the epithelium and submucosa of the colon. The etiology of UC is multifactorial, but likely includes a dysregulated mucosal immune response against commensal non-pathogenic bacteria of the colon, resulting in bowel inflammation. Onset of disease most commonly occurs between 15 and 40 years of age. Prevalence estimates per 100,000 persons in North America range from 139.8 (Quebec, Canada) to 286.3 (Minnesota, USA), while in Europe they range from 2.42 (Romania, 2003) to 505 (Norway, 1991-94), including 412 in Germany (2010), 340 in Hungary (2013), and 90.8 in the UK (1989). Globally, the prevalence of UC continues to rise, particularly in North America and Europe, and is expected to increase in newly industrialized countries in Africa, Asia, and South America (Ng et al. 2017).

The clinical course of UC is characterized by a lifelong course of remissions and exacerbations. Patients with UC suffer from recurrent episodes of diarrhea, rectal bleeding, weight loss, abdominal pain, fever, and are at an increased risk of perforated bowel, toxic megacolon, and colorectal cancer. The estimated risk of colorectal cancer is approximately 2% after 10 years, 5% to 10% after 20 years, and 12% to 30% after 30 to 35 years of UC (Bernstein et al. 2001; Eaden, Abrams, and Mayberry 2001; Feuerstein and Cheifetz 2014).

Patients have a 10% cumulative risk of colectomy 5 years after diagnosis, and 15% at 10 years after diagnosis (Fumery et al. 2018) However, with colectomy, there is a 50% risk of continued inflammation in the residual intestinal pouch (pouchitis); after 10 years, approximately 12% of patients experience pouch failure and require conversion to a permanent ileostomy (Zesos and Saibil 2015; Mark-Christensen et al. 2018). Surgical complications of proctocolectomy with ileostomy include stenosis, prolapse, and other abdominal/pelvic sequelae including small bowel obstruction, fistula, infection, persistent pain, unhealed perineal wound, sexual and bladder dysfunction, and infertility.

Patients with UC may also experience extra-intestinal manifestations including primary sclerosing cholangitis or eye, joint, or skin manifestations. Improved intestinal disease activity in UC is associated with an improvement in extra-intestinal manifestations.

2.2. Analysis of Current Treatment Options

The goals of UC treatment include decreasing mucosal inflammation, reducing signs and symptoms, reducing long-term corticosteroid use, reducing colon cancer risk, and improving patient quality of life.

Treatment of UC is challenging due to its multifactorial nature and incompletely understood pathogenesis. UC is also a chronic disease, and in general, most drugs that are initiated for induction of remission are continued as maintenance therapy, if they are effective (Feuerstein et al. 2020).

Therapeutic options for the treatment of moderately to severely active UC include a variety of pharmacologic therapies such as tumor necrosis factor (TNF)- α antagonists (infliximab, adalimumab, golimumab), anti-integrin agents (vedolizumab), Janus kinase (JAK) inhibitors (tofacitinib, upadacitinib), interleukin (IL) 12/23 antagonist (ustekinumab), sphingosine 1-phosphate (S1P) receptor modulator (ozanimod), and other immunomodulators (thiopurines, methotrexate). However, a proportion of patients who have initially responded to these therapies may subsequently lose response and experience recurrence of UC symptoms. Recent systematic reviews and meta-analyses have reported that the estimated incidence rates of loss of response (LOR) to vedolizumab was 39.8 (per 100 person-years of follow-up) in patients with UC (Peyrin-Biroulet et al. 2019) and the loss of response to anti-TNF agents was approximately 25% in one year and in 33% in 3 years in patients with IBD (Guberna et al. 2021).

Although multiple approved therapies are available, there remains a need for novel therapies for patients who have had an inadequate response, intolerance, or loss of response to available therapies and would benefit from the availability of additional therapeutic options.

The currently approved systemic therapies for the treatment of moderately to severely active UC are summarized in Table 1.

Table 1. Summary of Systemic Therapies for The Treatment of Moderately to Severely Active Ulcerative Colitis

Product(s) Name	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues
Tumor Necrosis Factor (TNF) Blocker				
Infliximab (Remicade®) BLA 103772 Approval: 1998	Reducing signs and symptoms, inducing and maintaining clinical remission and mucosal healing, and eliminating corticosteroid use	IV: 5 mg/kg at 0, 2 and 6 weeks, then every 8 weeks	69% and 62% of patients taking infliximab 5 mg/kg and 10 mg/kg respectively achieved clinical response at week 8.	Boxed Warning: Increased risk of serious infections. leading to hospitalization or death Perform test for latent TB; if positive, start treatment for TB prior to starting Infliximab. Lymphoma and other malignancies, some fatal, have been reported in children and adolescent patients treated with tumor necrosis factor (TNF) blockers, including Infliximab. Postmarketing cases of fatal hepatosplenic T-cell lymphoma (HSTCL) have been reported in patients
Infliximab biosimilars: Avsola® (Infliximab-AXXQ) BLA 761086 Inflectra® (Infliximab-DYYB) BLA 125544			39% and 32% of patients taking infliximab 5 mg/kg and 10 mg/kg, respectively, achieved clinical remission at week 8.	

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Product(s) Name	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues
Ixifi® (Infliximab- QBTX) BLA 761072			35% and 34% of patients taking infliximab 5 mg/kg and 10 mg/kg respectively achieved clinical remission at week 54.	treated with TNF blockers including Infliximab.
Renflexis® (Infliximab- ABDA) BLA 761054			62% and 59% of patients taking infliximab 5 mg/kg and 10 mg/kg respectively achieved mucosal healing at week 8.	Warnings and precautions: Serious infections including TB, invasive fungal infections, hepatitis B reactivation, malignancies (including lymphoma, melanoma, and Merkel cell carcinoma), congestive heart failure, demyelinating disorders, hematologic cytopenias, Lupus-like syndrome, hypersensitivity reactions.
			45% and 47% of patients taking infliximab 5 mg/kg and 10 mg/kg respectively achieved mucosal healing at week 54.	Most common adverse reactions (>10%) infections (e.g., upper respiratory, sinusitis, pharyngitis), infusion-related reactions, headache, and abdominal pain.

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Product(s) Name	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues
Adalimumab (Humira®) BLA 125057 Approval: 2002	Inducing and sustaining clinical remission in adult patients with moderately to severely active UC who have had an inadequate response to immunosuppressants such as corticosteroids, azathioprine or 6-mercaptopurine (6- MP). The effectiveness of HUMIRA has not been established in patients who have lost response to or were intolerant to TNF blocker.	SQ: Initial dose: (Day 1): 160 mg Second dose: two weeks later (Day 15): 80 mg Two weeks later (Day 29): Begin a maintenance dose of 40 mg every other week.	Study I: 18.5% of patients receiving adalimumab 160/80 mg achieved clinical remission at week 8. Study II: 16.5% of patients receiving adalimumab 160/80 mg achieved clinical remission at week 8. 8.5% of patients receiving adalimumab 160/80 mg achieved sustained clinical remission (clinical remission at both weeks 8 and 52).	Boxed Warning Same as infliximab. Warnings and precautions: Same as infliximab. Most Common AEs (>10%): Infections (upper respiratory, sinusitis), injection site reactions, headache and rash.
Adalimumab biosimilars:				
Abrilada® (Adalimumab-AFZB) BLA 761118				
Amjevita® (Adalimumab-ATTO) BLA 761024				
Cyltezo® (Adalimumab-ADBM) BLA 761058				
Hadlima® (Adalimumab-BWWD) BLA 761059				
Hulio® (Adalimumab-FKJP) BLA 761154				
Hyrimoz® (Adalimumab-ADAZ) BLA 761071				
Idacio (Adalimumab-AACF) BLA 761255				
Yusimry (Adalimumab-AQVH) BLA 761216				

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Product(s) Name	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues
Golimumab (Simponi®) BLA 125289 Approval: 2009	Moderate to severe UC with an inadequate response or intolerant to prior treatment or requiring continuous steroid therapy for: • inducing and maintaining clinical response • improving endoscopic appearance of the mucosa during induction • inducing clinical remission • achieving and sustaining clinical remission in induction responders	SQ: Initial dose 200 mg at week 0, followed by 100 mg at week 2 and then 100 mg every 4 weeks	Study I: 51% of patients receiving golimumab 200/100 mg achieved clinical response at week 6 18% of patients achieved clinical remission at week 6 42% of patients achieved improvement in endoscopic appearance of the mucosa at week 6 Study II: 50% of patients receiving golimumab 100 mg achieved clinical response through week 54 28% of patients achieved clinical remission at both week 30 and week 54	Boxed Warning: Same as infliximab. Warnings and Precautions: Same as infliximab. Most Common AEs (>10%): Upper respiratory tract infection, nasopharyngitis, and injection site reactions.
Integrin Receptor Antagonist (inhibits $\alpha 4\beta 7$ integrin)				
Vedolizumab (Entyvio®) BLA 125476 Approval: 2014	Adult patients with moderately to severely active UC	IV: 300 mg infused over 30 minutes at 0, 2 and 6 weeks, then every 8 weeks thereafter	Study I: 47% of patients achieved clinical response at week 6 17% of patients achieved clinical remission at week 6 Study II: 42% of patients achieved clinical remission at week 52	Warnings and Precautions: infusion related hypersensitivity reactions, Infections, Progressive Multifocal Leukoencephalopathy (PML). Most Common AEs: nasopharyngitis, headache, arthralgia, nausea, pyrexia, upper respiratory tract infection, fatigue, cough, bronchitis, influenza, back pain, rash, pruritus, sinusitis, oropharyngeal pain, and pain in extremities.

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Product(s) Name	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues
			57% of patients achieved clinical response at both weeks 6 and 52	
			52% of patients achieved improvement of endoscopic appearance of the mucosa at week 52	
Janus Kinase (JAK) Inhibitors				
Tofacitinib (Xeljanz®/ Xeljanz XR™) NDA 203214/ NDA 208246	Adult patients with moderately to severely active UC, who have had an inadequate response or who are intolerant to TNF blockers. Use in combination with biological therapies for UC or with potent immunosuppressants such as azathioprine and cyclosporine is not recommended.	Xeljanz: 10 mg twice daily for at least 8 weeks for a maximum of 16 weeks; then 5 or 10 mg twice daily. Xeljanz XR: 22 mg once daily for at least 8 weeks for a maximum of 16 weeks, then 11 mg once daily.	Study I: 60% of patients achieved clinical response at week 8 7% of patients achieved normalization of endoscopic appearance of the mucosa at week 8 Study II: 55% of patients achieved clinical response at week 8 7% of patients achieved normalization of endoscopic appearance of the mucosa at week 8 Study III: 52% and 62% taking 5 mg twice daily and 10 mg twice daily respectively achieved maintenance of clinical response at both baseline and week 52 46% and 56% taking	Boxed Warning: avoid use during an active serious infection (e.g., TB, viral reactivation), including localized infections. Most Common AEs: thrombosis (including pulmonary, deep venous and arterial), gastrointestinal perforations, lymphoma and other malignancies, lymphopenia, neutropenia, anemia, abnormal liver enzymes, dyslipidemia, and hypersensitivity reactions.

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Product(s) Name	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues
			5 mg twice daily and 10 mg twice daily respectively achieved maintenance of clinical remission at week 52	
			15% and 17% taking 5 mg twice daily and 10 mg twice daily respectively achieved normalization of endoscopic appearance at week 52	
Upadacitinib (Rinvoq®) NDA 211675 Approval: 2021	Adult patients with moderately to severely active ulcerative colitis, who have had an inadequate response or intolerance to one or more TNF blockers LOU: Rinvoq is no recommended for use in combination with other JAK inhibitors, biological therapies for ulcerative colitis, or with other potent immunosuppressants such as azathioprine and cyclosporine	Extended-Release Tablets Induction: 45 mg once a day for 8 weeks Maintenance: 30 mg or 15 mg once daily (30 mg QD for patients with refractory, severe, or extensive disease)	Induction studies: Study I: 26.1 % of patients achieved clinical remission at 8 weeks Study II: 33.1 % of patients achieved clinical remission at 8 weeks Maintenance Study: Study II: 52%, and 42% of subjects treated with 30 mg, and 15 mg, respectively achieved maintenance of clinical remission at 52 weeks	Boxed Warning: serious infections, mortality, malignancy, major adverse cardiovascular events (MACE), and thrombosis. Warnings and Precautions: Avoid use during an active serious infection, including localized infection, hypersensitivity, GI perforation, laboratory abnormalities in lymphocytes, neutrophils, hemoglobin, liver enzymes and lipids, Embryo-Fetal Toxicity, avoid use with live vaccines. Adverse reactions (≥ 5%): upper respiratory tract infections, increased blood creatine phosphokinase, acne, neutropenia, elevated liver enzymes, and rash

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Product(s) Name	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues
Human Interleukin (IL 12 and 23) Antagonist				
Ustekinumab (Stelara®) BLA 761044 Approval: 2009	Adult patients with moderately to severely active UC	SQ or IV: A single IV infusion using weight-based dosing. Induction: Up to 55 kg: 260 mg (2 vials), greater than 55 kg to 85 kg: 390 mg (3 vials), greater than 85 kg: 520 mg (4 vials) Maintenance (8 weeks after initial dose): 90 mg SQ injection every 8 weeks	Study I: 19% of patients achieved clinical remission at 8 weeks (7% plc). 58% of patients achieved clinical response at 8 weeks (31% plc). 25% of patients achieved endoscopic improvement at 8 weeks (13% plc). 17% of patients achieved histologic endoscopic mucosal improvement at 8 weeks (8% plc) Study II: 66% of patients who achieved clinical remission 8 weeks after induction achieved maintenance of clinical remission at 44 weeks (36% plc). 74% of patients achieved maintenance of clinical response at 44 weeks (48% plc) 47% of patients achieved endoscopic improvement at 44 weeks (27% plc)	Warnings and Precautions: serious bacterial, mycobacterial, fungal and viral infections such as gastroenteritis, ophthalmic herpes zoster, pneumonia, and listeriosis. Tuberculosis, malignancies, hypersensitivity reactions, reversible posterior leukoencephalopathy syndrome, and noninfectious pneumonia. Most Common AEs: nasopharyngitis, headache, abdominal pain, influenza, fever, diarrhea, sinusitis, fatigue, and nausea.

Product(s) Name	Relevant Indication	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues
Sphingosine 1-Phosphate (S1P) Receptor Modulator				
Ozanimod (Zeposia®) NDA 209899 Approval: 2020	Adult patients with moderately to severely active ulcerative	Capsule Titration is required for treatment initiation Days 1-4: 0.23 mg once daily Days 5-7: 0.46 mg once daily Maintenance dosage Day 8 and thereafter: 0.92 mg once daily	Induction study: Study I: 18 % of patients achieved clinical remission at 10 weeks (6% plc) Maintenance Study: Study II: 37% achieved maintenance of clinical remission at 52 weeks (19% plc)	Warnings and Precautions: infections, bradyarrhythmia and atrioventricular conduction delays, liver injury, fetal risk, increased blood pressure, respiratory effects (may cause a decline in pulmonary function), macular edema. Most Common AEs (incidence ≥4%): liver enzymes increased, upper respiratory infection, and headache

Source: Reviewer's table.

Abbreviations: AE, adverse event; IV, intravenous; plc, placebo; SQ, subcutaneous

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

At the time of this review, etrasimod has not been authorized for marketing in any country outside of the U.S.

On October 14, 2023, Pfizer, Inc. submitted a new drug application (NDA) for new molecular entity (NME), etrasimod. Etrasimod (also referred to as APD334, APD334 L-arginine or AR401959) is a S1P receptor modulator. The proposed indication for etrasimod is the treatment moderately to severely active UC in adults at a recommended dose of 2 mg orally once daily.

3.2. Summary of Presubmission/Submission Regulatory Activity

A Summary of the Presubmission and Submission Regulatory activity is described in Table 2.

Table 2. Presubmission/Submission Regulatory Activity

Date	Activity	Description
October 30, 2018	Type B (End of Phase 2) meeting	Discussion centered around the proposed phase 3 program, including the following recommendations: • Assess exposure of metabolite M3 • Conduct DDI studies • A “treat-through” design appeared reasonable • Update the definition of moderately to severely active UC to a modified Mayo score of 5 to 9 with an endoscopic subscore of at least 2. • Include a plan to assess for benefit of treatment at an early timepoint. • Consider a co-primary endpoint approach of clinical remission at Week 52 and Week 12. • Provide justification for the use of the Geboes index for histologic endpoints • Perform optical coherence tomography (OCT) in all patients, regardless of changes in visual acuity • Include discontinuation criteria for pulmonary adverse events • Perform first-dose cardiac monitoring in subjects that experience an interruption of treatment
February 20, 2019	Advice/Information request	FDA recommended hourly vital signs (HR, BP) and ECG for 4 hours after drug administration with discontinuation criteria for subjects who experience cardiac adverse events
September 16, 2020	Type C WRO request	FDA did not agree with the proposed modifications of the cardiac monitoring plan, which included change from inpatient monitoring to outpatient Holter monitoring. As a result, the Applicant did not change the cardiac monitoring plan.
June 15, 2021	Type C WRO request	FDA agreed with the Applicant’s proposed definitions for adverse events of special interest (AESI). FDA requested narratives and case report forms (CRFs) for adverse events leading to discontinuation and pregnancy.
June 15, 2021	Type C WRO request	The following recommendations and comments were provided to the Applicant regarding their SAPs for APD334-301 and APD334-302: • The primary analysis population should include subjects with an mMS of 5 to 9 with an ES of at least 2 to support an indication of moderately to severely active UC. • The primary missing data approach should include a multiple imputation approach for missing at random (e.g., due to COVID-19). • The proposal to modify visit windows due to the COVID-19 pandemic appeared reasonable.

Date	Activity	Description
		FDA raised concerns that the proposed minimum number of diary records (i.e., two consecutive days in a seven-day window) for the calculation of the RBS and SFS could under-represent the weekly RBS and SFS. FDA recommended an exploratory analysis of the co-primary endpoint with a minimum of 3 consecutive days or 4 nonconsecutive days of RBS and SFS within the proposed 7-day window.
October 29, 2021	Type C WRO request	The following recommendations and comments were provided to the Applicant regarding their integrated summary of efficacy (ISE) and integrated summary of safety (ISS): - FDA stated that the ISE would be considered supportive, and that the primary determination of efficacy would be based on the results of the individual studies APD334-301 and APD334-302. - FDA agreed with the Applicant's proposed pooling strategy and analysis plan for the ISS.
Preliminary Comments: May 31, 2022 Sponsor response: June 2, 2022 Meeting Request Cancelled letter: June 7, 2022	Type B Pre-NDA Meeting	The following recommendations and comments were conveyed to the Applicant regarding their plan to submit an NDA for etrasimod for the treatment of moderately to severely active UC: - FDA agreed that the nonclinical, clinical pharmacology, and biopharmaceutics data packages appeared adequate to support an NDA but that the final determination of the adequacy of the data would be assessed during the NDA review. - FDA recommended clinical investigators update their financial disclosure information as it pertains to Pfizer, which acquired Arena Pharmaceuticals within one year of completion of the study. - FDA recommended clinical remission be defined as an mMS of 0 to 2, including an SFS of 1 or 1, RBS of 0, and ES of 0 or 1 (score of 1 modified to exclude friability).

3.3. Data Quality and Integrity

Compliance With Good Clinical Practices

The trial was performed in accordance with consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki, and the International Conference on Harmonization guidelines for Good Clinical Practice, all applicable laws, rules, and regulations.

Data Quality and Integrity

The data were of sufficient quality to permit a substantive review.

Financial Disclosure

Etrasimod was originally developed by Arena Pharmaceuticals Inc. (Arena), which was acquired by Pfizer Inc. (Pfizer) on March 11, 2022. The two covered clinical studies (APD334-301 and APD334-302) were conducted, completed, and achieved database lock before the completion of this acquisition. Per guidance received from FDA in Pre-NDA meeting minutes (Reference ID 4992019) dated May 31, 2022, since the acquisition occurred within 1 year of conclusion of the studies, both Arena and Pfizer are considered sponsors for the covered clinical studies. As such, the financial disclosures were provided pertaining to both entities (collectively “Applicant”).

Financial disclosure information was self-disclosed by investigators. All investigators were assessed for equity interest, significant payments of other sorts, other compensation by the Sponsor, and propriety interest. Self-disclosures pertaining to Arena have been collected from 97% of investigators. Per FDA’s request communicated in the same Pre-NDA meeting minutes, the Applicant has made efforts to collect updated financial disclosure information from investigators identifying both Arena and Pfizer as sponsors. As of the disclosure cutoff date of September 6, 2022, for this NDA, 33% of investigators have provided the requested updates; due diligence to obtain these updates will continue for 1 year after study completion for each covered clinical study.

Seven investigators had reportable financial interests of any significant payments from the Applicant for the covered study, APD334-301, such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation, or honoraria. The seven investigators represent 1% (7/793) of the investigators for APD334-301. Given the total number of investigators, the potential impact of this financial interest was not expected to influence the trial outcome.

Five investigators had reportable financial interests of any significant payments from the Applicant for the covered study, APD334-302, such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation, or honoraria. The five investigators represent 0.5% (5/933) of the investigators for APD334-302. Given the total number of investigators, the potential impact of this financial interest was not expected to influence the trial outcome.

A detailed summary of financial disclosures is included in the Appendix Section 16.2.

4 Significant Issues From Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

Site inspections were conducted to assess the quality and integrity of the data submitted in this

marketing application. Clinical investigators (CI), George Aaron DuVall, MD (Site 10004, Tyler, Texas), Timothy E. Ritter, MD (Site 10074, Southlake, Texas), and Rafal Drozda, MD (Site 43034, Lodz, Poland) were selected for inspections based on large subject enrollment, high treatment response, and no recent inspection history. The non-US CI, Dr. Drozda in Latvia, was selected due to the large amount of data from sites outside the US in the application.

No significant good clinical practice (GCP) violations were observed. The two audited studies appear to have been conducted in compliance with GCP principles and regulations. The audited data for the three CIs appear acceptable in support of the NDA.

Refer to the Clinical Inspection Summary by Dr. Glenn Mannheim for full details of the review (DARRTS ID: 5204110).

4.2. Product Quality

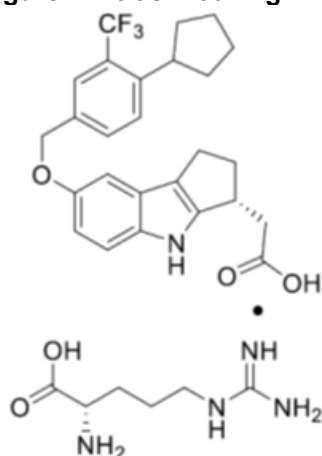
4.2.1. Drug Substance

Etrasimod arginine is the arginine salt of the active moiety etrasimod and is a synthetic small molecule sphingosine 1-phosphate receptor modulator. Etrasimod has not been previously approved or marketed in the United States and therefore, it is classified as a New Molecular Entity (NME).

Etrasimod arginine is a white, off-white, to light brown crystalline solid. It has a melting point of 203.5C -208.1C, a pKa of 4.5, and a log P of 6.8 – 9.0. It is non-hygroscopic with a solubility of 1.38mg/mL in water at pH of 8.9. To date, one thermodynamically stable crystalline form has been identified for etrasimod arginine. The drug substance as free acid has one chiral center, and the R-configuration has been confirmed by single crystal X-ray technique.

Etrasimod arginine is the L-arginine salt of (R)-2-(7-(4-cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl) acetic acid. It has a molecular formula of C₃₂H₄₀F₃N₅O₅ (C₂₆H₂₆F₃N₃O₃ as free acid), a molecular weight of 631.69 g/mol (457.48 g/mol as free acid), and the chemical structure as shown in Figure 1.

Figure 1. Etrasimod Arginine Structural Formula



Source: General Information, 2.3.S Drug Substance, Page 2 of 3.

The drug substance, etrasimod arginine, for this application is manufactured in accordance with current Good Manufacturing Practice (cGMP) requirements by AMPAC™ Fine Chemicals, LLC., Rancho Cordova, CA. It is tested and released against a specification that assures identity, strength, purity, and quality of the drug substance at release and throughout its proposed re-test period of (b) (4) months. The proposed re-test date of (b) (4) months is supported by the stability data provided in the application and is granted. Based on the results from stability testing of this drug substance, it should be stored at (b) (4)

4.2.2. Drug Product

The drug product, Velsipity (etrasimod) Tablets, 2 mg is indicated for the treatment of moderately to severely active ulcerative colitis in adults. The recommended dosage is one tablet taken orally once daily. The tablet should be swallowed as a whole with or without food.

Velsipity (etrasimod) tablets are green, round, film-coated immediate release tablets debossed with “ETR” on one side and “2” on the other side. Each tablet contains 2.762 mg of etrasimod arginine equivalent to 2 mg of etrasimod as the active ingredient and magnesium stearate, mannitol, microcrystalline cellulose, and sodium starch glycolate, with a film coating containing FD&C blue #1/brilliant blue FCF aluminum lake, FD&C blue #2/indigo carmine aluminum lake, FD&C yellow #5/tartrazine aluminum lake, macrogol 4000 JP/PEG 3350, polyvinyl alcohol (partially hydrolyzed), talc, and titanium dioxide as inactive ingredients.

Velsipity (etrasimod) tablets are supplied as 30 tablets in a child resistant 100 mL high density polyethylene (HDPE) white, round bottle with 4 g silica gel as desiccant integrated directly into the 45 mm white polypropylene (PP) closure.

Velsipity (etrasimod) tablets are manufactured in accordance with cGMP requirements by Penn Pharmaceutical Services Limited, United Kingdom and it is tested and released against a specification that assures the identity, strength, purity, and quality of the drug product at release and throughout its proposed expiration dating period of 24 months. The proposed

expiration dating period of 24 months is supported by the stability data provided in the application and is granted. This drug product should be stored at 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C to 30°C (59°F to 86°F) [see USP Controlled Room Temperature].

4.2.3. Office of Pharmaceutical Quality (OPQ) Recommendation

- The Applicant of this 505(b)(1) new drug application has provided sufficient CMC information to assure the identity, strength, purity, and quality of the drug substance, etrasimod arginine, and the drug product, Velsipity (etrasimod) Tablets, 2 mg.
- The Office of Pharmaceutical Manufacturing Assessment has made the overall recommendation of adequate for the facilities involved in this application.
- The CMC PI labeling recommended changes as well as the recommended revisions for the container and carton labels have been appropriately addressed.
- The Applicant's request for categorical exclusion from preparation of environmental assessment has been found to be adequate and is granted.

Therefore, from the OPQ perspective this application is recommended for approval with an expiration dating period of 24 months.

4.3. Clinical Microbiology

Not applicable

4.4. Devices and Companion Diagnostic Issues

Not applicable

5 Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

From a nonclinical perspective, no approvability issues have been identified for etrasimod at the proposed clinical dose of 2 mg/day.

Nonclinical studies conducted with etrasimod include pharmacology studies, safety pharmacology studies, pharmacokinetics, repeat-dose toxicology studies of up to six months duration in rats and nine months duration in dogs, a standard genotoxicity battery, two-year carcinogenicity studies in mice and rats, a standard battery of developmental and reproductive toxicity studies in rats and rabbits, and an in vitro phototoxicity study.

Etrasimod is a sphingosine-1-phosphate receptor subtype 1, 4, and 5 (S1P_{1,4,5}) agonist with no significant pharmacological activity at S1P₂ or S1P₃ receptors. Etrasimod agonism of S1P₁ reduces lymphocyte egress from lymph nodes, resulting in decreased circulating lymphocytes.

In a standard battery of nonclinical safety pharmacology studies, etrasimod had no significant effects on hERG channel current in vitro, cardiovascular function in dogs, CNS parameters in rats, or respiratory function in rats. In an in vitro assay using human atrial cardiomyocytes, etrasimod activated I_KAch channels at concentrations less than the predicted steady state C_{max} and C_{trough} values at the clinical etrasimod dose of 2 mg/day; this finding may provide an underlying mechanism for the transient etrasimod-induced decrease in heart rate observed in clinical trials.

In repeat-dose toxicity studies, mortalities, decreased body weight gain, and histopathological findings in the liver and thymus were observed in rats at exposures > 1000-fold the exposure at the maximum recommended human dose (MRHD) of 2 mg/day (based on AUC comparison). No adverse effects were observed in rats or dogs at exposures up to 738- and 446-fold, respectively, the exposure at the MRHD (based on AUC comparison). Dose-dependent decreases in circulating lymphocytes were observed in both rats and dogs and correlated with lymphoid depletion in the lymph nodes and spleen of rats only; these findings were attributed to the pharmacological activity of etrasimod and not considered adverse.

Etrasimod did not demonstrate genotoxic potential in a standard battery of in vitro and in vivo studies. Process-related impurity (b) (4) was considered positive for mutagenicity in the bacterial reverse mutation assay and is controlled in the (b) (4) manufacturing product at < (b) (4) µg/day in accordance with ICH M7(R1), option 2.

In a two-year carcinogenicity study in mice, a statistically significant positive dose-response in the incidences of hemangiosarcoma malignant tumor (multicentric neoplasm) and combined

hemangioma/hemangiosarcoma were observed at exposures $\geq$ 46-fold the exposure at the MRHD (based on AUC comparison). In a two-year carcinogenicity study in rats, no evidence of carcinogenic potential was observed at exposures up to 88- and 198-fold the exposure at the MRHD in males and females, respectively (based on AUC comparison). The Executive Carcinogenicity Assessment Committee concurred with these conclusions. Notably, these findings are consistent with those observed in studies with other approved S1P receptor modulators.

No adverse effects of etrasimod were observed on male or female fertility parameters at doses up to 42 and 121 times, respectively, the exposure at the MRHD (based on AUC comparison). In an embryo-fetal development study in rats, no maternal toxicity was observed at up to 23-fold the exposure at the MRHD, but a developmental NOAEL could not be determined based on the observed dose-dependent increase in the incidence of post-implantation loss (primarily early resorptions), decreased number and proportion of viable fetuses, and dose-dependent incidence of fetal malformations (aortic vessel abnormalities and septal defects) at exposures $\geq$ 6 times the exposure at the MRHD. In an embryo-fetal development study in rabbits, no maternal toxicity was observed at up to 12 times the exposure at MRHD. Based on the findings of increased post-implantation loss, decreased litter proportions of viable fetuses, and increased fetal malformations (aortic arch defects and fused sternbrae) at exposures 7 and 11 times the exposure at the MRHD, the NOAEL for developmental toxicity in rabbits was considered the low dose of 2 mg/kg/day (approximately the same exposure as MRHD). In a pre- and post-natal development study in rats, maternal toxicity including the premature termination of one female during delivery, increased post-implantation loss, increased number of females with stillborn pups, increased number of stillborn pups per litter, decreased viability index, and decreased lactation index was observed at exposures 10 and 24 times the exposure at the MRHD; thus, the NOAEL for maternal toxicity was considered the low dose of 0.4 mg/kg/day (approximately 2 times the exposure at the MRHD). Based on findings of increased perinatal mortality, adverse clinical signs and findings of decreased body weight/body weight gain during the preweaning period, as well as reduced number of implantations and increased rate of pre-implantation loss correlated with a decrease in the number of live embryos per litter, a developmental NOAEL could not be determined (with effects occurring at exposures less than that predicted at the MRHD).

No novel excipients are used in the formulation of the etrasimod drug product, and all excipients are used at acceptable levels that are consistent with those used in other FDA approved, orally administered products. No leachables are expected given the solid tablet formulation. Elemental impurities in the etrasimod drug substance meet the allowable limits established by ICH Q3D, option 1, for oral administration. All impurities, including those with genotoxic potential, are controlled in accordance with ICH Q3A and/or ICH M7(R1) as appropriate. Thus, there are no safety concerns regarding excipients, impurities, or extractables and leachables from the drug product or container closure system.

5.2. Referenced NDAs, BLAs, DMFs

None.

5.3. Pharmacology

Etrasimod is a sphingosine-1-phosphate receptor subtype 1, 4, and 5 (S1P_{1,4,5}) agonist with no significant pharmacological activity at S1P₂ or S1P₃ receptors. At clinically relevant concentrations, etrasimod had no significant binding or activity when tested against panels of other receptors, ion channels, transporters, and enzymes. Etrasimod agonism of S1P₁ reduces lymphocyte egress from lymph nodes, resulting in decreased circulating lymphocytes. In nonclinical species, etrasimod produced a dose-dependent decrease in blood lymphocytes, with EC₅₀ values of 25 ng/mL in rats and dogs and 45 ng/mL in mice and monkeys (approximately 4.5 and 2.5 times lower, respectively, than the steady state C_{max} at the clinical etrasimod dose of 2 mg/day; 113 ng/mL).

A standard battery of nonclinical safety pharmacology studies did not demonstrate significant safety concerns regarding acute etrasimod administration. Etrasimod inhibited hERG signaling in vitro, with a maximal inhibitory effect of 59% at the highest tested dose of 3 μM; a specific IC₅₀ value could not be determined based on the study design but is likely between 1000-3000 nM (460-1370 ng/mL; 4-12 times the steady state C_{max} at the MRHD). In an in vitro assay using human atrial cardiomyocytes, etrasimod activated IK_{ACh} channels (EC₅₀: 29.9 nM or 18.9 ng/mL; 6 times lower than the steady state C_{max} at the MRHD). This finding may suggest an underlying mechanism for the transient drug-induced decrease in heart rate observed in clinical trials with etrasimod in various indications. A single oral dose of etrasimod did not produce acute CNS or clinically relevant respiratory effects in rats (NOELs: 350 mg/kg/day) or cardiovascular effects in dogs (NOEL: 350 mg/kg/day).

5.4. ADME/PK

Available nonclinical data regarding the pharmacokinetics of etrasimod are described in

NDA/BLA Multi-Disciplinary Review and Evaluation NDA 216956
Velsipity (etrasimod)

Table 3 and Table 4 below.

Table 3. Pharmacokinetic Studies with Etrasimod

Type of Study	Major Findings
Absorption	BALB/C mice (1 mg/kg, single dose) ¹ - Oral bioavailability = 100% - Oral half-life: 12.3 hours
	Sprague-Dawley rats (1 or 10 mg/kg, single dose) ^{2,3} - Oral bioavailability = 54% - Oral half-life = 9.6-21.1 hours - Detectable through 120 hours post-dose - Blood and plasma concentrations were ≈ 50% higher in females compared to males, though drug-related exposures were only 7-14% higher
	Dogs (2 mg/kg, single dose) ^{4,5} - Oral bioavailability = 73% - Oral half-life = 29-41 hours - Detectable through 168 hours post-dose
Distribution	- Etrasimod is highly bound (> 95%) to plasma proteins in multiple species⁷ - No selective association of etrasimod with the cellular component of blood in rats or dogs^{3,5} - Etrasimod is primarily portioned into the extracellular component of blood in multiple species⁶ - In rats, early drug distribution was widespread (blood, plasma, and all analyzed tissues except the lens of the eye), with maximum concentrations observed at 4-8 hours post dose³ - Tissues with the highest exposure include the GI tract and organs of elimination (kidney and liver)³
Metabolism	In vitro - The disappearance half-life of etrasimod was >60 minutes in liver microsomes from mouse, rat, dog, monkey, and human, and approximately 30 minutes in microsomes from rabbit⁸ - Metabolites identified using human liver microsomes included M1 (glucuronide conjugate), M2 and M3 (mono-hydroxyl metabolites), as well as M4 and M5 (glucuronide conjugates of M2 and M3)⁹ - Metabolites identified using hepatocytes from various species were 29 in total, with the most abundant including M1, M3, and M6, as well as hydroxyl glucuronides M14, M15, and M17¹⁰ - M3 and M6 were both observed in hepatocytes from rat, dog, and monkey¹⁰ - No unique human metabolites identified in vitro^{9,10} - Glucuronidation of etrasimod is primarily catalyzed by UGT1A7 in liver microsomes from humans as well as mouse, rat, dog, and monkey¹¹
	In vivo - Primary human metabolites M3 and M6 were detected in the plasma and feces of rats³ and feces of dogs⁵ - Metabolism of etrasimod in dogs involved oxidation, dehydrogenation, and conjugation via sulfate or glucuronidation⁵
Excretion	Rats Elimination primarily via the bile/feces (> 80% within 48 hours, > 95% within 168 hours post-dose)³
	Dogs Elimination primarily via the feces, with 68% recovered by 96 hours post-dose and > 81% through 168 hours post-dose⁵

Type of Study	Major Findings
1. Study MPR-11-014: Pharmacokinetics in Male BALB/c Mice after Intravenous and Oral Administration	
2. Study MPR-11-013: Pharmacokinetics in Male Sprague-Dawley Rats after Intravenous and Oral Administration	
3. Study APD334.DMPK.002: Pharmacokinetics, Absorption, Metabolism, and Excretion of [14C] APD334 Following Oral Administration to Rats	
4. Study MPR-11-015: Pharmacokinetics in Male Beagle Dogs after Intravenous and Oral Administration	
5. Study APD334.DMPK.001: Pharmacokinetics, Absorption, Metabolism, and Excretion of [14C] APD334 Following Oral Administration to Dogs	
6. Study APD334.DMPK.003: In vitro Blood-to-Plasma Protein Binding in Rat, Dog, Monkey and Human	
7. Study MPR-12-001: Determination of APD334 Plasma Protein Binding in Mouse, Rat, Rabbit, Dog, Monkey and Human Plasma Using an Ultracentrifugation Method	
8. Study MPR-11-003: Species Comparison of the Metabolic Stability of APD334 in Mouse, Rat, Rabbit, Dog, Monkey, and Human Liver Microsomes in the Presence of NADPH	
9. Study MPR-11-005: Identification of the In Vitro Metabolites of APD334 in Liver Microsomes from Mouse, Rat, Rabbit, Dog, Monkey, and Human	
10. Study MPR-16-003: In Vitro Identification of 14C-APD334 Metabolites in Hepatocytes of Various Species	
11. Study MPR-16-004: Species Comparison APD334-glucuronide formation and Identification of Human UGT Enzymes Involved in the Glucuronidation of APD334	

Table 4. Pharmacokinetics of Etrasimod in Other Toxicology Studies

Type of Study	Major Findings
General Toxicology Studies	
Study TX14009: 6-month toxicology study in rats NOAEL = 150 mg/kg/day based on mortalities, decreased body weight gain, and histopathological findings in the liver at 250 mg/kg/day	Sprague-Dawley rats (25, 75, 150, 250 mg/kg/day) C _{max} at NOAEL: - M: 113 µg/mL - F: 201 µg/mL AUC at NOAEL: - M: 1440 µg·h/mL - F: 3180 µg·h/mL Accumulation: Yes 1.4- to 1.8-fold in males, 2.4- to 3.0-fold in females Dose proportionality: Yes Sex differences: Yes 1.4-fold higher AUC in females compared to males on day 1; 2- to 3-fold higher AUC in females compared to males on day 182
Study TX14008: 9-month toxicology study in dogs NOAEL = 15 mg/kg/day based on the lack of adverse effects at all doses	Beagle dogs (2, 5, 10, 15 mg/kg/day) C _{max} at NOAEL: 43.6 µg/mL AUC at NOAEL: 870 µg·h/mL Accumulation: No Dose proportionality: Yes Sex differences: No

Type of Study	Major Findings
Reproductive and Developmental Toxicology Studies	
Study TX14017: APD334 L-Arginine: An Oral Embryo-Fetal Developmental Toxicity Study in Rats - Maternal NOAEL = 4 mg/kg/day based on the lack of adverse effects at all doses - Developmental NOAEL could not be determined due to increased post-implantation loss and fetal malformations at all doses	Sprague-Dawley rats (1, 2, 4 mg/kg/day) GD 6 AUCs: 1 mg/kg/day = 7.0 µg·h/mL 4 mg/kg/day = 34.2 µg·h/mL GD 17 AUCs: 1 mg/kg/day = 11.0 µg·h/mL 4 mg/kg/day = 44.5 µg·h/mL
Study TX14019: APD334 L-Arginine: An Oral Embryo-Fetal Developmental Toxicity Study in Rabbits - Maternal NOAEL = 20 mg/kg/day based on the lack of adverse effects at all doses - Developmental NOAEL = 2 mg/kg/day based on increased post-implantation loss, lower number of viable fetuses, and increased fetal malformations at 4 mg/kg/day	New Zealand White rabbits (2, 10, 20 mg/kg/day) GD 7 AUCs: 2 mg/kg/day = 2.21 µg·h/mL 20 mg/kg/day = 35.0 µg·h/mL GD 20 AUCs: 2 mg/kg/day = 1.70 µg·h/mL 20 mg/kg/day = 24.2 µg·h/mL
Study APD334.TOX.011: An Oral Pre- and Postnatal Development Study of APD334 in Rats, Including Toxicokinetics and a Postnatal Neurobehavioral and Reproductive Functional Evaluation - Maternal NOAEL = 0.4 mg/kg/day based on perinatal mortality, increased post-implantation loss, and/or increased number of females with stillborn pups at higher doses in the absence of overt maternal toxicity - Developmental NOAEL could not be determined based on observations of increased perinatal mortality, adverse clinical signs and findings of decreased body weight/body weight gain during the preweaning period, as well as reduced number of implantations and increased rate of pre-implantation loss correlating with a decrease in the number of live embryos per litter	Sprague-Dawley rats (0.4, 2, 4 mg/kg/day) GD 6 AUC: 0.4 mg/kg/day = 3.73 µg·h/mL GD 17 AUC: 0.4 mg/kg/day = 4.32 µg·h/mL PND 20 AUC: 0.4 mg/kg/day = 2.39 µg·h/mL
Carcinogenicity Studies	
Study APD334.TOX.003: APD334 L-Arginine: A 104-Week Oral Oncogenicity Study in Mice A dose-dependent effect on mortality and dose-dependent increase in the incidence of combined hemangioma and hemangiosarcomas (multicentric neoplasm) was observed in both male and female mice at ≥ 6 mg/kg/day	CD1(ICR) mice (2, 6, 20 mg/kg/day): AUCs (day 364): 2 mg/kg/day = 42.1 µg·h/mL 6 mg/kg/day = 90.6 µg·h/mL 20 mg/kg/day = 261 µg·h/mL

Type of Study	Major Findings
Study APD334.TOX.002: APD334 L-Arginine: A 104-Week Oral Oncogenicity Study in Rats Etrasimod was negative for carcinogenicity in rats at all tested doses	Sprague-Dawley rats (2, 6, 20 mg/kg/day): AUCs (day 364): 20 mg/kg/day: M = 172 µg·h/mL F = 387 µg·h/mL

5.5. Toxicology

5.5.1. General Toxicology

Repeat-dose toxicity studies of up to six months duration in rats and nine months duration in dogs were conducted to support the chronic use of etrasimod in humans. Mortalities, decreased body weight gain, and histopathological findings in the liver and thymus were observed in rats at exposures > 1000-fold the exposure at the MRHD, based on AUC comparison. No adverse effects were observed in rats or dogs at exposures up to 738- and 446-fold, respectively, the exposure at the MRHD.

Study TX14009: APD334 L-Arginine: A 6-Month Oral Toxicity Study in Rats with a 4-Week Recovery Period

Conducting laboratory and location:



GLP compliance:

Yes

Dose and frequency of dosing

0, 25, 75, 150, 250 mg/kg

Route of administration

Oral gavage

Formulation/vehicle

L-arginine hydrochloride in deionized water

Species/strain

Sprague Dawley rat

Number/sex/group

15-20/sex/group

Age

6 weeks

Satellite groups/ unique design

Toxicokinetic groups: 4/sex for vehicle control, 11/sex/group treatment; 1-2 animals were included as replacement animals in each group because of mortalities

Deviation from study protocol affecting interpretation of results

No

Key Study Findings

- Etrasimod-related mortalities occurred at 150 mg/kg/day (n = 1) and 250 mg/kg/day (n = 12).

- Decreased body weight gain, correlating with decreased food consumption, occurred at all doses, but most severely at 250 mg/kg/day. Findings in the liver (single-cell necrosis, hypertrophy) and thymus (hypertrophy) were considered adverse at 250 mg/kg/day due to increased severity and/or correlating changes in clinical chemistry values.
- Based on the observed mortalities, decreased body weight gain, and histopathological findings in the liver and thymus at 250 mg/kg/day, the NOAEL was considered the mid dose of 150 mg/kg/day.

Etrasimod-related findings are summarized in Table 5.

Table 5. Observations and Results in the 6-Month Study in Rats (TX14009)

Parameters	Major Findings (Changes from Control)
Mortality	• Two males administered 250 mg/kg/day died due to liver inflammation/necrosis, while one male administered 250 mg/kg/day male died due to urogenital inflammation and calculi. • One male in the 150 mg/kg/day group, three males in the 250 mg/kg/day group, and five females in the 250 mg/kg/day group were either found dead or terminated in extremis for undetermined reasons that were considered related to etrasimod treatment. • In the 250 mg/kg/day TK satellite groups, four males and three females were found dead or terminated in extremis with the cause of death undetermined.
Clinical Signs	• Thinness, hunched posture, few or absent feces, colored material around the nose and eyes, and salivation were noted in surviving animals at 250 mg/kg/day.
Body Weights	• Dose-dependent decreases in body weight gain were observed. In males, body weight gain was decreased by 11.4%, 18.9%, and 27.9% at 75, 150, and 250 mg/kg/day, respectively. In females, body weight gain was decreased by 16%, 16%, and 21.4%, respectively. • Decreases in body weight gain correlated with decreased food consumption at all doses and treatment-related adverse clinical signs at 250 mg/kg/day. • Decreased body weight gain was reversible following the 4-week recovery period in both sexes.
Ophthalmoscopy	• None.

Parameters	Major Findings (Changes from Control)
Hematology	• Dose-dependent decreases in circulating lymphocytes (up to -78%) and total leukocytes (up to -70%) were observed and correlated with histopathological findings of lymphoid depletion in the lymph nodes and spleen. These are expected pharmacological effects. • Increased platelets (up to 37%), prolonged APTT (up to 2.3x), and prolonged prothrombin times (up to 2.1x) were observed at ≥ 150 mg/kg/day.
Clinical Chemistry	• Increased total bilirubin (up to 4.7x), creatinine (up to 93%), and urea nitrogen (up to 34%) were observed at all doses. • Increased ALT (2.1x) was observed in males administered 250 mg/kg/day and correlated with histopathological findings of hepatocyte necrosis.
Urinalysis	• None.
Gross Pathology	• None.
Organ Weights	• Relative (to body weight) liver weight increased in males at ≥ 75 mg/kg/day and females at ≥ 150 mg/kg/day and correlated with histopathological findings of hepatocyte hypertrophy. These findings were reversible following the recovery period. • Relative spleen weight irreversibly decreased in all treated animals and correlated with histopathological findings of decreased lymphocytes. • Relative thyroid/parathyroid weight irreversibly increased at ≥ 150 mg/kg/day and correlated with histopathological findings of thyroid follicular cell hypertrophy.
Histopathology Adequate battery: Yes	• In the liver, minimal to mild single cell necrosis and minimal to moderate centrilobular hepatocellular hypertrophy were observed at ≥ 150 mg/kg/day. • Partially reversible, minimal to mild hyperplasia (atypical, epithelial cell, medullar lymphoid) of the thymus was observed at all doses, with moderate severity in males administered 250 mg/kg/day.

Study TX14008: APD334 L-Arginine: 9-Month Oral Toxicity Study in Dogs with a 4-Week Recovery Period

Conducting laboratory and location:



GLP compliance:	Yes
Dose and frequency of dosing	0, 2, 5, 10, 15 mg/kg
Route of administration	Oral gavage
Formulation/vehicle	L-arginine hydrochloride in deionized water
Species/strain	Beagle dog
Number/sex/group	4-6/sex/group
Age	5-6 months
Satellite groups/ unique design	2 animals/sex from the vehicle, 10, and 15 mg/kg/day groups were maintained for a 4-week recovery period after 273 consecutive days of dosing.
Deviation from study protocol affecting interpretation of results	None

Key Study Findings

- Noteworthy effects on the eye (lacrimation, conjunctivitis, injected sclera) were observed with etrasimod at ≥ 10 mg/kg/day. These effects were fully reversible following the recovery period and were considered non-adverse due to lack of histopathological correlates or effects on the overall health of the animals.
- Based on a lack of adverse effects at all doses, the NOAEL was considered the highest tested dose of 15 mg/kg/day.

Etrasimod-related findings are summarized in Table 6.

Table 6. Observations and Results in the 9-Month Study in Dogs (TX14008)

Parameters	Major Findings (Changes from Control)
Mortality	• None.
Clinical signs	• Etrasimod-related clinical signs included increased incidences of fecal alterations (mucoid, soft, and/or watery) in all groups, red discolored gums in all groups, brown discolored teeth in males administered 15 mg/kg/day, skin warm to touch in all females, lacrimation in all groups, sclera injected in all males, and interdigital cysts in females at ≥ 5 mg/kg/day.
Body weights	• Dose-dependently decreased body weight gain was observed in treated males (up to -50.3%) and correlated with decreased feed consumption at all doses. Decreased body weight gain in males persisted following the recovery period. • In females, body weight gain was decreased (up to -21.8%), but the decrease was independent of dose. Decreased body weight gain correlated with decreased food consumption at 15 mg/kg/day. These findings were fully reversible following the recovery period.

Parameters	Major Findings (Changes from Control)
Ophthalmoscopy	A drug-related trend for conjunctivitis and ocular discharge (clear or purulent) was observed primarily at ≥ 10 mg/kg/day. These observations resolved following the recovery period.
ECG	None.
Hematology	- Decreased lymphocytes (up to -86%, partially reversible) and decreased total leukocytes (up to -62%, not reversible) were observed in all treated groups. Mild decreases in monocytes, eosinophils, basophils, and/or large unclassified cells were also observed in all treated groups. These findings are expected pharmacological effects of etrasimod, and thus were not considered adverse. - Reversible APTT prolongation was observed (up to 12%) in all treated groups with no dose-dependence.
Clinical chemistry	- Reversibly increased total bilirubin (up to 92%) was observed in both sexes at 15 mg/kg/day without histopathological correlates. - Decreased total protein (up to -18%), albumin (up to -19%), globulin (up to -21%), and cholesterol (up to -41%) were observed in all treated groups. Following recovery, these changes were resolved in males but remained decreased in females.
Urinalysis	None.
Gross pathology	Lung discoloration was observed in two of four males in the 15 mg/kg/day group and correlated with non-adverse histopathological findings of alveolar histiocytosis and pleural fibrosis (lack of dose dependence).
Organ weights	- Absolute and relative (to body weight) lung weights were irreversibly increased in all treated groups and correlated with histopathological findings of fibrosis of the pleural surface of the lung. - Absolute and relative thymus weights were irreversibly increased in all treated groups. These findings correlated with hematologic evidence of lymphopenia, suggesting sequestration of lymphocytes in the thymus, but no histopathological changes were reported. - Absolute and relative prostate weight were irreversibly decreased in males at all doses with no histopathological correlates.
Histopathology Adequate battery: Yes	None.

5.5.2. Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study TX10011: APD334 L-Arginine Bacterial Reverse Mutation Test

Key Study Findings:

- Etrasimod was not mutagenic under the tested conditions.

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GLP compliance: Yes

Test system: *S. typhimurium* TA98, TA100, TA1535, and TA1537; *E. coli* WP2uvrA

Study is valid: Yes

Study APD334.TOX.006: (b) (4) Bacterial Reverse Mutation Assay

Key Study Findings:

- Positive mutagenic responses were observed with (b) (4) in strains TA98 and WP2 uvrA in the presence and absence of S9 activation and with TA1537 in the presence of S9 activation. Equivocal responses were observed with TA100 in the presence of S9 activation and TA1537 in the absence of S9 activation.
- Based on these findings, (b) (4) (a process-related impurity) was considered positive for mutagenicity.

GLP compliance: Yes

Test system: *S. typhimurium* TA98, TA100, TA1535, and TA1537; *E. coli* WP2uvrA

Study is valid: Yes

Study APD334.TOX.006: (b) (4) Bacterial Reverse Mutation Assay

Key Study Findings:

- (b) (4) (a process-related impurity) was considered negative for mutagenicity under the conditions of the assay.

GLP compliance: Yes

Test system: *S. typhimurium* TA98, TA100, TA1535, and TA1537; *E. coli* WP2uvrA

Study is valid: Yes

In Vitro Assays in Mammalian Cells

Study TX10012: L-Arginine: In Vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes

Key Study Findings:

- Etrasimod did not induce structural aberrations in human peripheral blood lymphocytes under the conditions of the study, with or without metabolic activation.

GLP compliance: Yes

Test system: Human peripheral blood lymphocytes

Study is valid: Yes

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study TX10013: APD334 L-Arginine: Oral Bone Marrow Micronucleus Test in Rats

Key Study Findings:

- Etrasimod was positive for inducing bone marrow micronuclei in female rats at the highest tested dose of 300 mg/kg/day, a systemically toxic dose; however, this increase was within the historical control range, substantially lower than the positive control, and not observed in males.
- Overall, etrasimod was considered negative for genotoxicity in this assay under the tested conditions.

GLP compliance: Yes

Test system: Sprague-Dawley rats

Study is valid: Yes

5.5.3. Carcinogenicity

Etrasimod was positive for carcinogenicity in mice but negative for carcinogenicity in rats.

In a two-year carcinogenicity study in mice, a statistically significant positive dose-response in the incidences of hemangiosarcoma malignant tumor (multicentric neoplasm) and combined hemangioma/hemangiosarcoma were observed in males and females at 6 and 20 mg/kg/day compared to controls. These doses correspond to 46- and 134-fold, respectively, the exposure at the MRHD of 2 mg/day (based on AUC comparison). The Executive Carcinogenicity Assessment Committee concurred with these findings and considered the study to be adequately conducted.

In a two-year carcinogenicity study in rats, no carcinogenic findings were observed in males or females administered up to 20 mg/kg/day for 86 to 89 weeks, after which mortalities precluded continued conduct of the study. This dose corresponds to 88- and 198-fold the exposure at the MRHD in males and females, respectively (based on AUC comparison). The Executive Carcinogenicity Assessment Committee concurred with these findings and considered the study to be adequately conducted.

5.5.4. Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study TX15007: APD334 L-Arginine: An Oral Fertility and Early Embryonic Development Toxicity Study in Rats

Conducting laboratory and location:



GLP compliance:

Yes

Dose and frequency of dosing

Females: 0, 1, 2, 4 mg/kg/day
Males: 0, 25, 100, 200 mg/kg/day
Once daily

Route of administration
Formulation/vehicle

Oral gavage
L-arginine hydrochloride in deionized water (same concentration as the maximum dose of the test article: 9.2 mg/mL for males, 1.8 mg/mL for females)

Species/strain
Number/sex/group
Satellite groups
Study design

Sprague Dawley rats
25/sex/group
None
Females were dosed once daily for at least 14 days prior to cohabitation, during cohabitation, and through gestation day 7 (total: 22-35 doses).
Males were dosed once daily for 28 days prior to mating and up to the day before euthanasia (total: 63-64 doses).
Toxicokinetic measurements were not conducted.

Deviation from study protocol affecting interpretation of results

No

Key Study Findings

- Based on a lack of adverse effects, the NOAELs for female and male reproductive toxicity were considered the highest tested doses of 4 and 200 mg/kg/day, respectively.
- Based on the observed adverse effects on body weight and food consumption at ≥ 100 mg/kg/day, the NOAEL for systemic toxicity in males was considered the low dose of 25 mg/kg/day.

Etrasimod-related findings are summarized in Table 7.

Table 7. Observations and Results in the Fertility and Early Embryonic Development Study in Sprague Dawley Rats (TX15007)

Parameters	Major Findings (Changes from Control)
Mortality	• None.
Clinical Signs	• In males, a slight increase in incidence of dried red material around the mouth and/or nose was observed 5 hours post-dose in all groups; this finding was neither persistent nor consistently observed (single occurrences in each dose group). Thus, this finding was considered non-adverse.
Body Weights	• In males, decreased body weights were observed at 100 mg/kg/day on days 4-17 and at 200 mg/kg/day on day 4-63, respectively. Weight gain was decreased in all male groups prior to mating. • In females, transient but drug-related and dose-dependent decreases in weight gain corresponding to decreased feed consumption were observed. Transient, rebound weight gain was observed following scheduled cessation of treatment in these animals on GD 7.
Necropsy findings	• None.

Embryo-Fetal Development

Dose-Range Finding Studies in Rats and Rabbits

Two dose-range finding studies were conducted with etrasimod to determine the doses for the definitive embryo-fetal development (EFD) study in rats. In the first study using doses of 25, 75, 150, and 250 mg/kg/day (Study TX14016), severe maternal toxicity and decreased intrauterine survival were observed such that, among all treated groups, there was only a single viable fetus from the 25 mg/kg/day group and no others. The single fetus in the 25 mg/kg/day group (121 times the clinical dose, based on HED) was observed with a meningocele.

In the second study using doses of 2, 5, 10, and 20 mg/kg/day (Study TX14025), increased post-implantation loss with corresponding lower numbers/proportions of viable fetuses was observed at ≥ 5 mg/kg/day. A meningocele was observed in a single fetus each in the 5 and 10 mg/kg/day groups, and spina bifida was observed in one fetus at 10 mg/kg/day. These findings occurred at > 21 times the exposure at the clinical dose, based on AUC (based on the available AUC data at 4 mg/kg/day) or 24 times the clinical dose, based on HED.

While these findings suggest the potential of etrasimod exposure during organogenesis to result in neural defects, the toxicological significance of these findings is unclear due to the sporadic nature of the findings, lack of clear dose effect, and overall developmental failure at higher doses. However, the presence of these findings is unsurprising, as S1P receptors play an essential role in embryogenesis. Based on the totality of findings in these studies, the doses in the definitive EFD study in rats were set to 1, 2, and 4 mg/kg/day.

In the dose-range finding study in rabbits using doses of 2, 25, 75, and 150 mg/kg/day (Study TX14018), moribundity or abortion were observed in 5/6 females at 75 mg/kg/day and all

females at 150 mg/kg/day (726 and 1452 times the clinical dose, respectively, based on HED). The single surviving female at 75 mg/kg/day was observed to have increased post-implantation loss. At 25 mg/kg/day (242 times the clinical dose, based on HED), increased post-implantation loss and a lower number of viable fetuses were observed. No adverse effects were observed at 2 mg/kg/day (19 times the clinical dose, based on HED, or less than the clinical dose, based on AUC). Based on these findings, the doses in the definitive EFD study in rabbits were set to 2, 10, and 20 mg/kg/day.

Study TX14017: APD334 L-Arginine: An Oral Embryo-Fetal Developmental Toxicity Study in Rats

Conducting laboratory and location:



GLP compliance:

Yes

Dose and frequency of dosing

0, 1, 2, 4 mg/kg/day
Once daily

Route of administration

Oral gavage

Formulation/vehicle

L-arginine hydrochloride in deionized water (same concentration as the maximum dose of the test article: 1.8 mg/mL)

Species/strain

Sprague-Dawley rat

Number/sex/group

25/group (females only)

Satellite groups

TK: 8/group

Study design

Pregnant dams were dosed from GD 6-17.

Deviation from study protocol affecting interpretation of results

No

Key Study Findings

- Based on a lack of adverse effects, the NOAEL for maternal toxicity was considered the highest tested dose of 4 mg/kg/day.
- Based on the findings of a dose-dependent increase in the incidence of post-implantation loss (primarily early resorptions), decreased number and proportion of viable fetuses, and a dose-dependent increase in the incidence of fetal malformations (aortic vessel abnormalities and septal defects) at all tested doses, a NOAEL for developmental toxicity could not be determined.

Etrasimod-related findings are summarized in Table 8.

Table 8. Observations and Results in the Embryo-Fetal Development Study in Rats (TX14017)

Parameters	Major Findings (Changes from Control)
Mortality	None.
Clinical signs	None.
Body weights	- Body weight gain was transiently decreased at ≥ 2 mg/kg/day following the first two days of dosing (GD 7-8), but no significant effects were observed for the remainder of the dosing period. - Following the cessation of treatment (GD 18-20), slightly decreased body weight gain, final body weight, and gravid uterine weight were observed at 4 mg/kg/day. These findings were considered secondary to the increased incidence of post-implantation loss and decrease in viable fetuses in this group.
Necropsy findings Cesarean section data	- Increased post-implantation loss (primarily early resorptions) and decreased number and proportion of viable fetuses per litter were observed at 4 mg/kg/day. - A small but dose-dependent increase in the percentage of late resorptions was also observed (0.2%, 0.7%, and 3.1% at 1, 2, and 4 mg/kg/day, respectively).
Necropsy findings Offspring	- Increased fetal malformations (primarily visceral malformations in cardiovascular tissues, including aortic vessel abnormalities and septal defects) were observed at all tested doses. - Increased incidence of individual fetuses with multiple drug-related malformations was observed at 2 and 4 mg/kg/day.

Study TX14019: APD334 L-Arginine: An Oral Embryo-Fetal Developmental Toxicity Study in Rabbits

Conducting laboratory and location:



GLP compliance:	Yes
Dose and frequency of dosing	0, 2, 10, 20 mg/kg/day Once daily
Route of administration	Oral gavage
Formulation/vehicle	L-arginine hydrochloride in deionized water (same concentration as the maximum dose of the test article: 9.2 mg/mL)
Species/strain	NZW Rabbits
Number/sex/group	22/group (females only)
Satellite groups	TK: 4/group
Study design	Pregnant dams were dosed from GD 7-20.
Deviation from study protocol affecting interpretation of results	No

Key Study Findings

- Based on a lack of adverse effects, the NOAEL for maternal toxicity was considered the highest tested dose of 20 mg/kg/day.
- Based on the findings of increased post-implantation loss, decreased litter proportions of viable fetuses, and increased fetal malformations (aortic arch defects and fused sternebrae) at 10 and 20 mg/kg/day, the NOAEL for developmental toxicity was considered the low dose of 2 mg/kg/day.

Etrasimod-related findings are summarized in Table 9.

Table 9. Observations and Results in the Embryo-Fetal Development Study in Rabbits (TX14019)

Parameters	Major Findings (Changes from Control)
Mortality	• None.
Clinical signs	• None.
Body weights	• None.
Necropsy findings Cesarean section data	• Increased early resorptions were observed at 10 and 20 mg/kg/day. This corresponded with a decreased number and litter proportion of viable fetuses at 20 mg/kg/day.
Necropsy findings Offspring	• Drug-related fetal malformations including aortic arch defects (10 and 20 mg/kg/day) and fused sternebrae (20 mg/kg/day) were observed.

Prenatal and Postnatal Development

Study APD334.TOX.011: An Oral Pre- and Postnatal Development Study of APD334 in Rats, Including Toxicokinetics and a Postnatal Neurobehavioral and Reproductive Functional Evaluation

Conducting laboratory and location:



GLP compliance:

Yes

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Dose and frequency of dosing	0, 0.4, 2, 4 mg/kg/day Once daily
Route of administration	Oral gavage
Formulation/vehicle	L-arginine monohydrochloride at 0.189 mg/mL in 0.5% (v/v) Tween 20, 10 mM Tris, pH 8.5-9.0
Species/strain	Sprague-Dawley rat
Number/sex/group	22/group (females only)
Satellite groups	TK groups: control = 4, treated = 8/group
Study design	Pregnant females were dosed from GD 6 through lactation day (LD) 20. Offspring were not directly dosed and were maintained until approximately PND 127 for males and PND 92 for females. Male and female offspring were cohabitated starting at PND 92-95 for evaluation of reproductive capacity.
Deviation from study protocol affecting interpretation of results	No

Key Study Findings

- Based on the premature termination of one female during delivery (4 mg/kg/day), increased post-implantation loss (4 mg/kg/day), increased number of females with stillborn pups (2 and 4 mg/kg/day), increased number of stillborn pups per litter (2 mg/kg/day), decreased viability index (2 and 4 mg/kg/day), and decreased lactation index (4 mg/kg/day), the NOAEL for maternal toxicity was considered the low dose of 0.4 mg/kg/day.
- Based on increased perinatal mortalities at ≥ 2 mg/kg/day, adverse clinical signs and findings of decreased body weight/body weight gain during the preweaning period at all doses, as well as the observations of reduced number of implantations and increased rate of pre-implantation loss correlating with a decrease in the number of live embryos per litter at 4 mg/kg/day, a developmental NOAEL could not be established (with effects occurring at exposures less than that expected at the clinical dose).

Etrasimod-related findings are summarized in Table 10.

Table 10. Observations and Results in the Pre- and Postnatal Development Study in Rats (APD334.TOX.011)

Generation	Major Findings (Changes from Control)
F0 Dams	• One female at 4 mg/kg/day was prematurely terminated on GD 22 due to severe, adverse clinical signs during delivery. • A significant increase in post-implantation loss was observed at 4 mg/kg/day. • A significant increase in the number of females with stillborn pups was observed at 2 and 4 mg/kg/day as well as an increased number of stillborn pups per litter at 2 mg/kg/day. • Viability index was decreased at 2 and 4 mg/kg/day, while lactation index was decreased at 4 mg/kg/day.
F1 Generation	Preweaning • An increased number of pups were found dead or found missing and presumed to be cannibalized, predominantly between PND 0-4, at 2 and 4 mg/kg/day. • Clinical observations from birth through PND 4 included cold to touch, suspected dehydration (based on skin turgor), discolored skin, skin pallor, and swelling at 4 mg/kg/day and no milk band in the stomach at 2 and 4 mg/kg/day. • After culling (PND 4-21), clinical signs included suspected dehydration (based on skin turgor) and thin appearance at 4 mg/kg/day. • Decreased maternal feed consumption during lactation corresponded to decreased pup body weights and body weight gain at 2 and 4 mg/kg/day. Pup body weights at the end of the preweaning period (PND 21) were 10%, 12%, and 20% lower than controls at 0.4, 2, and 4 mg/kg/day, respectively, while total body weight gains were 11%, 14%, and 23% lower than controls, respectively. • Macroscopic findings in prematurely terminated or stillborn pups included full body edema at 4 mg/kg/day and no milk in the stomach at 2 and 4 mg/kg/day. Postweaning • A reduced number of implantations and increased rate of pre-implantation loss were observed at 4 mg/kg/day and correlated with a decrease in the number of live embryos per litter in this group.

5.5.5. Other Toxicology Studies

Phototoxicity

Etrasimod did not demonstrate phototoxic potential in an in vitro neutral red uptake phototoxicity assay using BALB/c mouse fibroblasts.

Excipients and Leachables

Etrasimod is orally administered at a dose of 2 mg/day. The etrasimod drug product is a round, immediate release, film-coated tablet available in a 2 mg form only. There are no novel excipients or excipients of human or animal origin used in the manufacturing of the etrasimod

drug product. Levels of excipients used in this drug product are similar to or lower than those used in other FDA approved, orally administered products. (b) (4)

(b) (4) all ingredients used in the manufacture of the film coating are at levels below those used in other FDA approved, orally administered products.

Due to the formulation of the etrasimod drug product as a solid tablet, there are no safety concerns with regard to leachables.

Impurities Assessment

Elemental impurities in etrasimod L-arginine drug substance meet the allowable limits established by ICH Q3D, option 1, for oral administration.

A genotoxic/mutagenic hazard assessment was conducted on etrasimod L-arginine and its observed and potential impurities in accordance with ICH M7(R1), "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk." The assessment identified eight potentially genotoxic compounds: (b) (4)

(b) (4)

(b) (4) was determined to be non-mutagenic in a definitive bacterial reverse mutation assay and is therefore being controlled in the etrasimod drug substance as a non-mutagenic impurity (Class 5) in accordance with ICH Q3A.

(b) (4)
(b) (4) was identified in silico as a Class 2 impurity and was positive for genotoxicity in a definitive bacterial reverse mutation assay. Therefore, (b) (4)
(b) (4) in accordance with ICH M7(R1) option 2.

(b) (4)

(b) (4) are potential impurities in starting material (b) (4) and have been identified as a potential ICH M7(R1) Class 3 impurities. (b) (4) is controlled to the non-

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specified impurity limit of (b) (4) ppm in the starting material (b) (4), while (b) (4) is controlled by specification to a level of $\leq$ (b) (4) % in the starting material. The acceptable daily intakes for (b) (4) individually are limited to not more than (b) (4) $\mu\text{g}/\text{day}$ per ICH M7(R1) guidance on (b) (4) ($\mu\text{g}/\text{day}$), equivalent to a maximum limit of not more than (b) (4) ppm based on the clinical dose of etrasimod of 2 mg/day. The control strategies described are aligned with ICH M7(R1) option 2 for (b) (4) and option 3 for (b) (4)

Three organic impurities have been observed in the etrasimod drug substance and/or drug product: (b) (4) while (b) (4) is a process-related impurity (b) (4) (b) (4) are both degradation products. Based on in silico mutagenicity assessments, (b) (4) were determined to be Class 5 impurities and are treated as non-mutagenic impurities in accordance with ICH M7(R1).

6 Clinical Pharmacology

6.1. Executive Summary

Etrasimod is a sphingosine 1-phosphate (S1P) receptor modulator, which binds to S1P receptors 1, 4, and 5. It is a new molecular entity with a proposed indication for the treatment of moderately to severely active ulcerative colitis (UC) in adults.

In support of this application, the safety and effectiveness of etrasimod were assessed in phase 3 trials APD334-301 and APD334-302 in subjects 16 years of age and older with moderately to severely active UC. (b) (4)

The clinical pharmacology review evaluated the appropriateness of the general dosing instructions, dosage for specific patient populations, drug-drug interaction (DDI), as well as population PK (PopPK), and exposure-response (E-R) analyses.

Overall, the proposed oral dosage at 2 mg daily (QD) for the general adult patient population is supported by a significantly higher proportion of subjects achieving clinical remission at Weeks 12 and 52 compared to placebo as well as an acceptable safety profile. No dosage adjustment is recommended for patients with mild to severe renal impairment or mild to moderate hepatic impairment. However, use of etrasimod in patients with severe hepatic impairment is not recommended.

Co-administration of etrasimod with the following drugs is not recommended:

- Drugs that are moderate or strong inhibitors of both CYP2C9 and CYP3A4.
- Drugs that are combined CYP3A4 (strong), CYP2C8 (moderate) and CYP2C9 (moderate) inducer.
- Drugs that are moderate or strong CYP2C8 or CYP3A4 inhibitor in patients who are known to be CYP2C9 poor metabolizers.

6.1.1. Recommendations

The Office of Clinical Pharmacology has reviewed this NDA submission and found it acceptable for approval for the treatment of adult patients with moderately to severely active UC from a clinical pharmacology standpoint.

6.1.2. Key Review Issues

Key review issues with specific recommendations and comments are summarized in Table 11 below.

Table 11. Review Issues and Recommendations

Review Issues	Recommendations and Comments
Pivotal evidence of effectiveness	The effectiveness of etrasimod for adult patients was supported by the efficacy results from the phase 3 trials APD334-301 and APD334-302. A greater proportion of subjects treated with etrasimod 2 mg QD achieved clinical remission at Week 12 and Week 52 (the primary efficacy endpoint for induction and maintenance periods, respectively), compared to placebo.
General dosing instructions	The proposed oral dosage of 2 mg QD for adult patients with moderately to severely active UC is acceptable.
Dosing in patient subgroups (intrinsic and extrinsic factors)	Etrasimod can be taken with or without food. <u>Hepatic impairment</u> No dosage adjustment is recommended for patients with mild to moderate hepatic impairment. Use of etrasimod in patients with severe hepatic impairment is not recommended based on 57% higher mean etrasimod exposure in subjects with severe hepatic impairment compared with subjects with normal liver function in combination of limited clinical data at doses higher than 2 mg. <u>Renal impairment</u> No dosage adjustment is needed for patients with any degree of renal impairment. The effect of hemodialysis on the PK of etrasimod has not been evaluated. <u>Drug Interactions</u> Co-administration of etrasimod with agents that are moderate or strong inhibitors of both CYP2C9 and CYP3A4 (e.g., fluconazole) is not recommended. Co-administration of etrasimod with a drug that is an inducer of multiple etrasimod metabolizing enzymes, such as rifampin which induces CYP3A4 (strong), CYP2C8 (moderate) and CYP2C9 (moderate), is not recommended. In patients who are known to be CYP2C9 poor metabolizers, co-administration of etrasimod with drugs that are a moderate or strong inhibitor of CYP2C8 or CYP3A4 is not recommended.
Bridge between the “to-be-marketed” and clinical trial formulations	The 2-mg tablet formulation used in two phase 3 clinical trials was different from the to-be-marketed formulation (2 mg tablets) and has been adequately bridged to the to-be-marketed formulation by an in vivo relative bioavailability study (Study APD334-114).

Source: Reviewer’s table

Abbreviations: DDI, drug-drug interaction; PK, pharmacokinetics; UC, Ulcerative colitis

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Pharmacology

Etrasimod is a S1P receptor modulator, which binds with high affinity to S1P receptors 1, 4, and 5. Etrasimod partially and reversibly blocks the capacity of lymphocytes to egress from lymphoid organs, reducing the number of lymphocytes in the peripheral blood, thereby lowering the number of activated lymphocytes in the tissue. However, the mechanism by which etrasimod exerts therapeutic effects in UC is not fully known.

Clinical Pharmacokinetics

Clinical pharmacokinetics of etrasimod have been characterized in healthy subjects and subjects with UC and briefly summarized below. Overall, the pharmacokinetics of etrasimod is similar in healthy subjects and subjects with UC.

Absorption

Following oral administration, the T_{max} of etrasimod is approximately 4 hours (ranging 2 – 8 hours). Following single dose administration from 0.1 mg to 5 mg, the C_{max} and AUC for etrasimod increase proportionally by 59 and 55-fold, respectively, with a 50-fold increase in dose. Following the multiple dose administration from 0.7 mg QD to 2 mg QD, the C_{max} and AUC_{tau} at steady-state increased more than dose proportionally by 3.67 and 3.63-fold, respectively, with a 2.86-fold increase in dose. Steady state was reached by 7 days following 2 mg QD dosing, with a mean C_{max} of 113 ng/mL and AUC_{tau} of 2162 h*ng/mL.

Food effect

No clinically significant differences in the C_{max} and AUC of etrasimod were observed following administration with a high-fat, high-calorie meal, compared to the fasted conditions (< 2%). Food intake can result in delayed absorption (the median T_{max}) by 2 hours.

Distribution

The mean apparent volume of distribution of etrasimod (V_z/F) is 66 L. Human plasma proteins binding of etrasimod is approximately 97.9%.

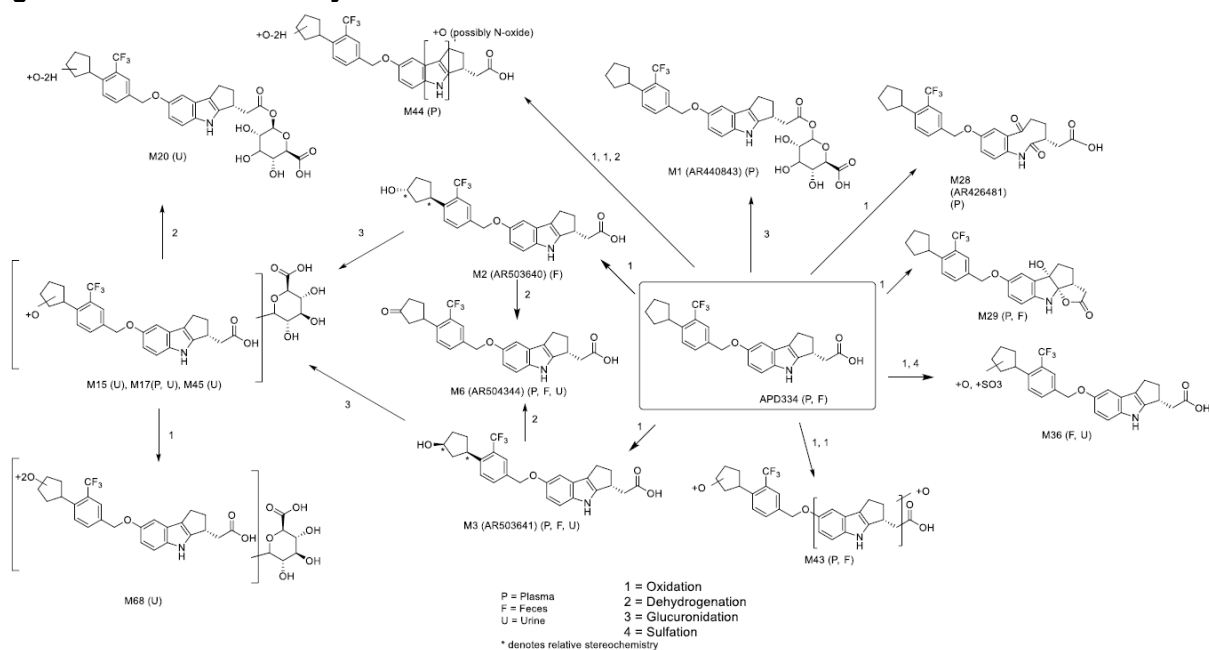
Elimination

The mean plasma elimination $t_{1/2}$ of etrasimod is approximately 30 hours. The $T_{1/2}$ of the two most abundant metabolites, M3 and M6, ranged from 46 to 83 hours and 52 to 70 hours, respectively.

Metabolism

Etrasimod is extensively metabolized by multiple enzymes via oxidation, dehydrogenation, glucuronidation, sulfation and a combination of these reactions, though unchanged etrasimod is the main circulating component in plasma as shown in Figure 2. In vitro assays indicate that etrasimod is metabolized via CYP2C8 (38%), CYP2C9 (37%), and CYP3A4 (22%), and with minor contribution (1%) via CYP2C19 and CYP2J2. The two most abundant circulating metabolites of etrasimod, AR503641 (M3) and AR504344 (M6), accounted for <20% of the parent AUC as well as <10% of total radioactivity in human plasma. In vitro studies indicated that M3 was formed primarily by CYP2C9*1 and to a lesser extent by CYP2C8 and CYP2C19. M3 was further metabolized to M6.

Figure 2. Metabolic Pathways for Etrasimod and Metabolites in Human



Source: Clinical Study Report APD334-107, Figure 5

Etrasimod has one chiral center. Analysis of cross-subject pooled plasma samples obtained from Study APD334-005 indicated that etrasimod does not undergo any in vivo stereoconversion to its opposite enantiomer, AR401967.

Excretion

Following a single oral dose of radiolabeled etrasimod 2 mg, approximately 82% of the administered radioactivity was recovered in feces and 4.9% in urine over 336 hours. 57.2% of the total administered radioactivity was recovered in feces by 144 hours post-dose. In feces, 11.2% was unchanged etrasimod and 41% was metabolites. Unchanged etrasimod was not detected in urine.

PK in Subjects with Ulcerative Colitis

The PK of etrasimod was characterized in subjects with UC by population PK analysis using sparse PK samples collected in phase 2 and phase 3 trials.

In subjects with UC, the model estimated mean apparent total clearance (CL/F) of etrasimod after oral administration was 1.06 L/h, mean apparent volume of distribution at steady state (V_{ss}/F) was 70 L for a 70 kg subject, and the mean effective half-life (t_{1/2,eff}) was 33.9 hours. PopPK estimated that subjects with UC would have slightly higher C_{max,ss} and AUC_{ss} (about 10%) compared to healthy subjects of the same body weight, following 2 mg QD dosing. Hence, disease status is not expected to have a clinically significant effect on etrasimod PK exposure in subjects with UC.

Pharmacodynamics

In subjects with UC, mean lymphocyte counts decreased to approximately 50% of baseline at 2 weeks after treatment initiation and the lower lymphocyte counts were maintained during treatment. After discontinuing etrasimod, the median time for peripheral blood lymphocytes to return to the normal range was 2.6 weeks, with approximately 90% of subjects in the normal range within 4.7 weeks in phase 3 trials.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The recommended dosage regimen for adult patients with moderate to severe UC is 2 mg QD without regards of food.

The effectiveness of etrasimod in adult patients was supported by the efficacy results from the phase 3 trials APD334-301 and APD334-302. A greater proportion of subjects treated with etrasimod 2 mg QD achieved clinical remission at Week 12 compared to placebo (27.4% versus 7.5% in APD334-301; 26.2% versus 15.2% in APD334-302) during the initial treatment period. Similarly, a greater proportion of subjects treated with etrasimod 2 mg QD achieved clinical remission at Week 52 compared to placebo (32.5% versus 6.7%) in APD334-301. APD334-301 was a treat-through design in which all patients continued assigned treatment without rerandomization of responders following assessment of early response at Week 12.

The Applicant has proposed a dosage of 2 mg QD in adults [REDACTED] (b) (4) [REDACTED] PK, clinical efficacy, and safety data are available from only 1 pediatric subject (16 years-old, female, body weight of 42 kg) received etrasimod 2 mg QD. Although adolescents aged 16 to less than 18 years of age were allowed to enroll in phase 3 trials APD334-301 and APD334-302, a total three adolescent patients were enrolled, and two subjects received placebo. No other clinical trials enrolled adolescent subjects.

See Section 8 Statistical and Clinical and Evaluation of this multi-discipline review for the related efficacy and safety data.

Therapeutic Individualization

Renal Impairment

No dose adjustment is needed in patients with renal impairment. Based on a dedicated renal impairment study, renal impairment has no clinically important effects on the PK of etrasimod. The effect of dialysis on etrasimod was not assessed.

Hepatic Impairment

No dosage adjustment is needed for patients with mild to moderate hepatic impairment (Child-Pugh A and B). Use of etrasimod in patients with severe hepatic impairment (Child-Pugh C) is not recommended based on 57% higher etrasimod exposure compared with subjects with normal liver function given multiple doses higher than 2 mg have not been studied.

Drug-Drug Interactions

Effects of Other Drugs on Etrasimod:

- In vitro data suggest that etrasimod is mainly metabolized via CYP2C8, CYP2C9, and CYP3A4, and with only minor contribution from CYP2C19 and CYP2J2.
- CYP2C8 or CYP3A4 inhibitor alone: Only moderate effect of a strong inhibitor of a single CYP enzyme was observed. Overall, the risk for clinically relevant DDIs appears low with concomitant medications that inhibit only CYP2C8 or CYP3A4.
 - **CYP3A4:** Itraconazole (a strong inhibitor of CYP3A4) had little impact on the C_{max} of etrasimod (increased by 6%) but increased AUC_{0-inf} of etrasimod by 32%.
 - **CYP2C8:** Gemfibrozil (a strong inhibitor of CYP2C8) had little impact on the C_{max} of etrasimod (increased by 12%) but increased AUC_{0-inf} of etrasimod by 36%. Thus, no dose adjustment is warranted.
- Drugs that are both CYP2C9 and CYP3A4 inhibitors: The AUC of etrasimod increased by 84% when co-administered with fluconazole (a moderate inhibitor of CYP2C9 and CYP3A4, and strong inhibitor of CYP2C19). While fluconazole inhibits multiple CYP enzymes, it appears that the effect of fluconazole is mainly by inhibition of CYP2C9 given a strong CYP3A4

inhibitor (itraconazole) increased AUC by 32%, although additive effects of moderate inhibition of both CYP2C9 and CYP3A4 cannot be ruled out. Thus, co-administration of etrasimod with a drug that is a dual inhibitor of CYP2C9 and CYP3A4 (e.g., fluconazole) is not recommended.

- Rifampin: The AUC of etrasimod decreased by 49% when co-administered with rifampin (a moderate inducer of CYP2C8 and CYP2C9 and a strong inducer of CYP2C19 and CYP3A4). Thus, co-administration of etrasimod with a drug that is a combined CYP3A4 (strong), CYP2C8 (moderate) and CYP2C9 (moderate) inducer (i.e., rifampin) is not recommended.

Additionally, administration of etrasimod in patients who are CYP2C9 poor metabolizers and receiving CYP2C8 or CYP3A4 moderate or strong inhibitors is not recommended. In patients who are intermediate CYP2C9 metabolizers, the effect on etrasimod exposure when co-administered with CYP3A4 or CYP2C8 inhibitors is not known.

Effects of Etrasimod on Other Drugs:

DDI study with oral contraceptive:

Co-administration of etrasimod with an oral contraceptive (containing 30 µg ethinylestradiol [EE] and 150 µg levonorgestrel [LVG]) increased $C_{max,ss}$ and $AUC_{tau,ss}$ of EE by 12% and 24%, respectively, and increased $C_{max,ss}$ and $AUC_{tau,ss}$ of LVG by 22% and 32%, respectively. These changes are not expected to be clinically relevant.

No notable differences in mean concentration-time profiles for follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, and sex hormone binding globulin (SHBG), as well as the incidents of Hoogland scores were observed between when oral contraceptive was administered alone and co-administration of etrasimod and oral contraceptive. Steady-state plasma PK parameters for etrasimod appear generally consistent with previous findings for etrasimod when given alone, in a cross-study comparison. Thus, the review team agrees with the Applicant's proposal that no dosing adjustment is necessary when etrasimod is co-administered with oral contraceptive.

No dosage adjustment based on other intrinsic or extrinsic factors is needed for adult subjects with UC. See details in Section 6.3.2.

Outstanding Issues

There are no outstanding issues that would preclude the approval of etrasimod for the currently proposed indication in adult patients with moderately to severely active UC from a clinical pharmacology perspective.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

The PK of etrasimod were characterized in healthy adult subjects and in subjects with UC. A summary of the general PK of etrasimod is provided in Table 12 below.

Table 12. Summary of General Pharmacology and Pharmacokinetics

Characteristic	Drug Information												
	Pharmacologic Activity												
Established pharmacologic class	Etrasimod is a S1P receptor modulator, which binds with high affinity to S1P receptors 1, 4, and 5.												
Mechanism of action	Etrasimod partially and reversibly blocks the capacity of lymphocytes to egress from lymphoid organs, reducing the number of lymphocytes in peripheral blood, thereby lowering the number of activated lymphocytes in the tissue. However, the exact mechanism by which etrasimod exerts therapeutic effects in UC is not fully known.												
Active moieties	Etrasimod												
QT prolongation	In the thorough QT study, etrasimod was not associated with significant QTc prolonging effect at a suprathreshold dose of 4 mg QD on Day 14 (i.e., 2 mg QD for 7 days, followed by 3 mg QD for 5 days, then 4 mg QD for 2 days). See the IRT-QT review memo by Eliford Kitabi in DARRTS dated March 3, 2023 (DARRTS ID: 5135842)												
	General Information												
Bioanalysis	Plasma etrasimod was measured using validated LC-MS/MS method.												
Healthy subjects versus patients	The PK of etrasimod appears generally similar in healthy subjects and subjects with UC.												
Drug exposure at steady state following the therapeutic dosing regimen	Mean (SD) PK Parameters on Day 21 After 2 mg QD in Healthy Subjects (APD334-002) <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;">C_{max} (ng/mL)</th> <th style="text-align: center;">AUC_{0-24h} (ng·h/mL)</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">113 (27.5)</td> <td style="text-align: center;">2162 (488)</td> </tr> </tbody> </table> Simulated Median (95% Prediction Interval) PK Parameters at Steady State in Subjects with UC by Population PK: <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: center;">$C_{max,ss}$ (ng/mL)</th> <th style="text-align: center;">$AUC_{0-24h,ss}$ (ng·h/mL)</th> <th style="text-align: center;">$C_{trough,ss}$ (ng/mL)</th> <th style="text-align: center;">$t_{1/2,eff}$ (h)</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">99.9 (60.1, 171)</td> <td style="text-align: center;">1950 (1140, 3390)</td> <td style="text-align: center;">63.2 (34.3, 114)</td> <td style="text-align: center;">33.9 (24.2, 48.6)</td> </tr> </tbody> </table>	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)	113 (27.5)	2162 (488)	$C_{max,ss}$ (ng/mL)	$AUC_{0-24h,ss}$ (ng·h/mL)	$C_{trough,ss}$ (ng/mL)	$t_{1/2,eff}$ (h)	99.9 (60.1, 171)	1950 (1140, 3390)	63.2 (34.3, 114)	33.9 (24.2, 48.6)
C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)												
113 (27.5)	2162 (488)												
$C_{max,ss}$ (ng/mL)	$AUC_{0-24h,ss}$ (ng·h/mL)	$C_{trough,ss}$ (ng/mL)	$t_{1/2,eff}$ (h)										
99.9 (60.1, 171)	1950 (1140, 3390)	63.2 (34.3, 114)	33.9 (24.2, 48.6)										
Range of effective dose(s) or exposure	2 mg QD was studied in phase 3 trials APD334-301 and APD334-302.												
Maximally tolerated dose or exposure	The maximum tolerated multiple-dose regimen without dose titration was 2 mg QD. The maximum tolerated multiple-dose regimen with dose titration was 4 mg (i.e., subjects received 2 mg QD for 7 days, followed by 3 mg QD for 5 days, then 4 mg QD for 2 days).												
Dose proportionality	The C_{max} and AUC for etrasimod increase approximately proportionally over the single dose range from 0.1 mg to 5 mg and increased more than dose proportional over the multiple dose range from 0.7 mg QD to 2 mg QD (i.e., C_{max} and AUC_{tau} at steady state increased 3.67-fold and 3.63-fold respectively, with a 2.86-fold increase in dose).												

NDA/BLA Multi-Disciplinary Review and Evaluation NDA 216956
Velsipity (etrasimod)

Characteristic	Drug Information	
Accumulation	The mean accumulation ratios for C_{max} and AUC_{0-24h} of etrasimod at steady state was 2.7 and 3, respectively, in healthy subjects following 2 mg QD dosing.	
Bridge between to-be marketed and clinical trial formulations	Bridge between the to-be-marketed and clinical trial formulations has been established in a relative bioavailability study (APD334-114). Systemic exposures were equivalent between the formulation used in the phase 3 trials and the to-be-marketed formulation.	
Absorption		
Bioavailability	The absolute oral bioavailability of etrasimod has not been determined.	
T_{max}	Following oral administration, the median T_{max} of etrasimod is approximately 4 hours (ranging 2 – 8 hours).	
Food effect (fed/fasted) geometric least square mean and 90% CI	Coadministration with a high-fat, high-calorie meal (consisted of 800 to 1000 calories) did not alter the extent of absorption of etrasimod following administration of a single 2 mg tablet (to-be-marketed formulation). Median T_{max} of etrasimod under fed condition was delayed by 2 hours compared to fasted condition.	
Effect of Food on PK Parameters Following Administration of Etrasimod 2 mg Tablet (Study APD334-114)		
	Geometric LS Mean	
PK Parameters	Ratio Fed/Fasted (%)	90% CI
C_{max}	99.3	94.4 – 104.5
AUC_{0-t}	101.8	98.6 – 105.2
AUC_{0-inf}	101.8	98.4 – 105.4
Distribution		
Volume of distribution	The mean apparent volume of distribution of etrasimod (V_z/F) is 66 L.	
Plasma protein binding	Human plasma proteins binding of etrasimod is approximately 97.9%.	
Drug as substrate of transporters	Based on the in vitro studies, etrasimod is not a substrate or inhibitor of human ABC transporters (P-gp, BCRP, and BSEP), and SLC transporters (OATP1B1, OATP1B3, OAT1/3, OCT1, OCT2, and MATE1/2 K).	
Elimination		
Mass balance results	Fecal (hepatobiliary) excretion was the predominant route of elimination for etrasimod. Following a single oral dose of radiolabeled etrasimod 2 mg, approximately 82% of the radioactivity was recovered in feces and 4.9% in urine over 336 hours. Total administered radioactivity was recovered in urine and feces through the last collection interval (336 hours postdose) with 3.00% of the total administered radioactivity recovered in urine within the first 72 hours postdose and 57.2% of the total administered radioactivity recovered in feces by 144 hours postdose. Total of 11, 21, and 9 metabolites were observed in plasma, urine, and feces, respectively. In feces, metabolites M3 (AR503641) and M36 (APD334-oxy sulfate) accounted for a mean of 22.1% and 18.9% of the dose (total administered radioactivity), respectively, and unchanged etrasimod accounted for a mean of 11.2% of the dose. Unchanged etrasimod was not detected in urine. No major circulating metabolites were observed. The two most abundant circulating metabolites of etrasimod, AR503641 (M3) and AR504344 (M6) accounted for <20% of the parent AUC as well as <10% of total radioactivity in human plasma.	

NDA/BLA Multi-Disciplinary Review and Evaluation NDA 216956
Velsipity (etrasimod)

Characteristic	Drug Information
Half-life	The mean plasma elimination $t_{1/2}$ of etrasimod is approximately 30 hours.
Metabolic pathway(s)	Etrasimod is extensively metabolized by multiple enzymes via oxidation, dehydrogenation, glucuronidation, sulfation and a combination of these reactions, though unchanged etrasimod is the main circulating component in plasma. In vitro assays indicate that etrasimod is mainly metabolized via CYP2C9, CYP2C8, and CYP3A4.
Primary excretion pathways (% dose)	Fecal excretion was the predominant route of excretion. See Mass balance results above.
Intrinsic Factors and Specific Populations	
Body weight	In population PK analysis, body weight was a significant covariate of etrasimod PK with lower etrasimod exposure associated with higher body weight. The $C_{trough,ss}$ for subjects with body weights of 50 kg and 110 kg were estimated to be 1.20 and 0.779 times, respectively, that of a 70 kg subject.
Age/sex/race/ethnicity	The PK of etrasimod would not be expected to be significantly affected by age, sex, or race/ethnicity.
Renal impairment	Etrasimod C_{max} and AUC were comparable between subjects with severe renal impairment/ESRD and subjects with normal renal function.
Hepatic impairment	Etrasimod AUC parameters were 13%, 29%, and 57% higher in subjects with mild, moderate, and severe hepatic impairment (Child-Pugh A, B, and C, respectively), respectively, compared with subjects with normal liver function.
Drug Interaction Liability Effects of Etrasimod on Other Drugs	
Inhibition/induction of metabolism	In vitro data suggested that the major human enzymes involved in the metabolism of etrasimod are CYP2C8, CYP2C9, and CYP3A4 and to a lesser extent CYP2C19 and CYP2J2. Etrasimod (10 μM) was not a direct or time-dependent inhibitor of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 or CYP3A4/5. Etrasimod is not an inducer of human CYP1A2, CYP2B6, and CYP3A4 based on the fold-change method as mRNA evaluations. Etrasimod was not an inhibitor of UGT1A3, UGT1A4, UGT1A9, UGT2B7, or UGT2B17 in vitro. No clinically meaningful effects of etrasimod on the steady-state C_{max} and AUC of oral contraceptive (30 μg ethinylestradiol and 150 μg levonorgestrel) were observed when co-administered with etrasimod 2 mg QD.
Inhibition/induction of transporter systems	In vitro studies suggest that etrasimod is not an inhibitor of human ABC transporters (P-gp, BCRP, and BSEP) or SLC transporters (OATP1B1, OATP1B3, OAT1/3, OCT1, OCT2, and MATE1/2 K).

Source: Data combined from NDA 216956 submission.

Abbreviations: SD = standard deviation; UC = ulcerative colitis; QD = once daily; PK = pharmacokinetics; $AUC_{r,ss}$ = area under the concentration time curve during one dosing interval at steady state; CL/F = apparent total body clearance of drug calculated after extra-vascular administration; $C_{max,ss}$ = maximum observed drug concentration during a dosing interval at steady state; $t_{1/2}$ = half-life of elimination phase; V/F = apparent total volume of distribution

Based on PopPK analysis, disease status is only expected to have a minimal effect on etrasimod PK exposure in subjects with UC. Healthy subjects have slightly smaller (within 10%) mean etrasimod $C_{max,ss}$ and AUC_{ss} compared to UC patient of the same body weight, following 2 mg QD dosing.

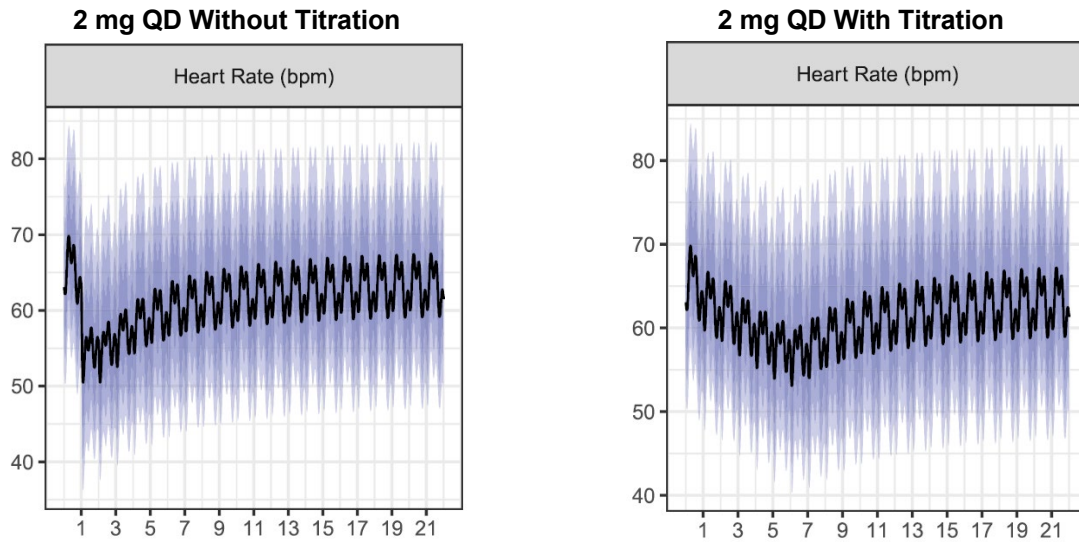
Heart Rate Reduction

As a class, S1P1 agonists are known to affect heart rate especially following the first dose. A dose-dependent reduction in heart rate (HR) by etrasimod was observed with the maximum decline in HR from predose baseline observed from 2 to 4 hours postdose across clinical studies. Of note, median T_{max} is 4 hours. Maximal mean HR decrease from baseline ranged from -3.3 to -19.4 bpm in healthy subjects following the first 2 mg dose of etrasimod. In subjects with UC, maximal mean HR change from baseline ranged from -7.3 to -9.4 bpm following the first 2 mg dose of etrasimod in phase 2 and 3 trials.

Of note, several different etrasimod dose titration regimens were evaluated in phase 1 study APD334-110 and compared with dosing regimen without up-titration. Under dose titration regimens, i.e., initiating with a low (0.25-0.5 mg) starting dose followed by up-titration to 2 mg over a period of one week or less, as expected minimal HR-lowering effect was observed after the first dose on Day 1. The maximal HR-lowering effect was delayed and observed at 2 mg at the end of titration. Additionally, similar findings were also described using population PK/PD HR modeling as shown in Figure 3. The model estimated that while the 2 mg QD with titration regimen reduced the HR effect on the first day of treatment, the model predicted nadir, reached after 5 days of treatment, was similar to that of the 2 mg QD without titration regimen nadir reached on Day 1.

As such, etrasimod dosages of 1 mg QD and 2 mg QD without dose titration were used in phase 2 trials and subsequently 2 mg QD without dose titration was used in phase 3 trials in subjects with UC.

Figure 3. Simulated Heart Rate in Subjects with UC Following 2 mg QD With vs. Without Dose Titration for 21 Days



Source: Applicant's Population PK/PD Report ARE0301H-HR, Figures 25 and 26.

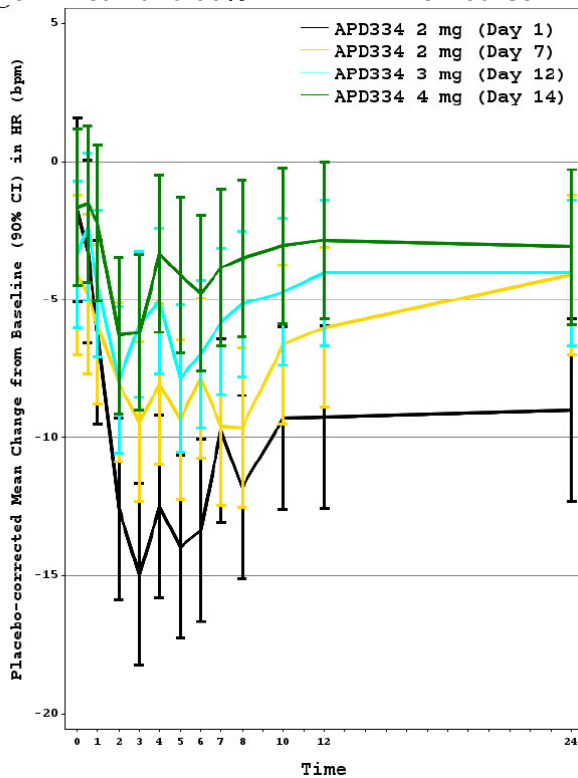
2 mg QD without titration. for 21 days

2 mg QD with titration: 0.25 mg QD on Days 1 and 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5, then 2 mg QD on Days 6-21

The solid lines represent the median. The shaded areas represent 50% (darker blue color), 75%, and 90% (lighter blue color) prediction intervals for data simulated under the model.

On the other hand, when dose was titrated up from 2 mg to 4 mg (APD334-008 in healthy subjects: 2 mg on Day 1-7, 3 mg on Day 12, and 4 mg on Day 14), the HR lowering effect was greatest on Day 1 at 2 mg and subsequent dose increase to 3 mg and 4 mg did not enhance the effects as shown in Figure 4.

Figure 4. Mean and 90% CI $\Delta\Delta$ HR Time Course



Source: FDA Interdisciplinary Review Team for QT Studies Consultation Review, Figure 2

6.3.2. Clinical Pharmacology Questions

Does the Clinical Pharmacology Program Provide Supportive Evidence of Effectiveness?

The primary evidence of effectiveness of 2 mg etrasimod QD was supported by a significantly higher proportion of adult subjects achieving clinical remission at Week 12 and Week 52 compared to placebo in the placebo-controlled phase 3 trials. See Section 8.1 of this multi-disciplinary review for the related efficacy data.

For adolescent subjects aged 16 years and older, there were very limited data from the phase 3 trials for this age group. Overall, there was only one pediatric subject that received etrasimod 2 mg QD in phase 3 trials APD334-301 and APD334-302.

Is the Proposed Dosing Regimen Appropriate for the General Patient Population for Which the Indication is Being Sought?

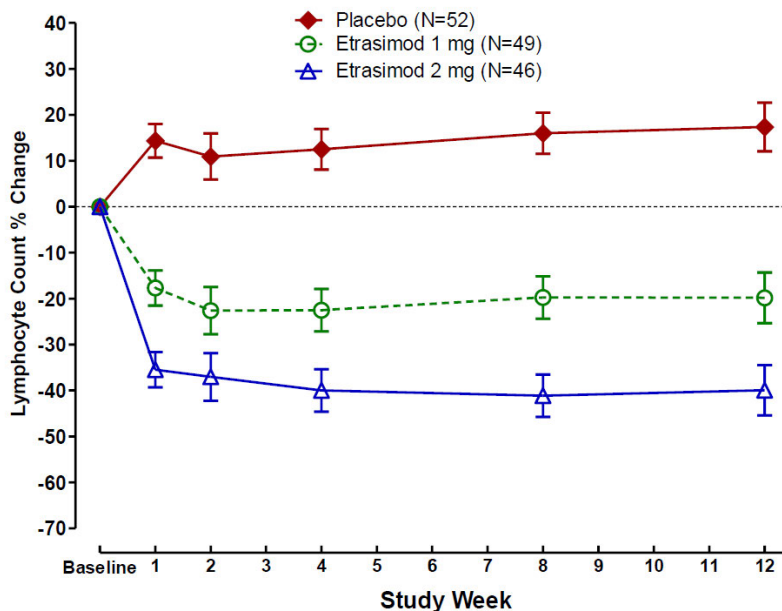
Yes. The proposed oral dosage of 2 mg QD for the general adult patient population is appropriate.

Dose-Response Relationship

In a phase 2 dose-ranging study APD334-003, etrasimod at 1 mg and 2 mg QD, or placebo, were studied in subjects with moderately to severely active UC for 12 weeks. Both doses showed numerically better efficacy based on the primary efficacy endpoint of the study (i.e., change from baseline in the modified Mayo score) compared to placebo at Week 12. Only the 2-mg dose was statistically significantly different from placebo for the primary efficacy endpoint. In addition, there was a dose-dependent increase in the proportion of subjects who achieved endoscopic improvement, and greater decrease from baseline in rectal bleeding and stool frequency subscores and the total Mayo score between 1 mg and 2 mg doses.

The effects of etrasimod on lymphocyte counts were also assessed in this phase 2 study. Reduction of circulating lymphocyte counts was observed in both etrasimod dose groups at Week 12. Numerically greater reductions in lymphocyte counts from baseline were observed for the 2 mg dose compared to the 1 mg dose. The mean percent changes from baseline in absolute lymphocyte count (ALC) versus time profiles are presented in Figure 5. It should be noted that the clinical relevance of reduction in circulating lymphocyte counts with UC is not fully understood.

Figure 5. Mean (SE) Percent Change from Baseline in Lymphocyte Count



Source: Phase 2 trial APD334-003 report, Figure 5.
Abbreviations: SE = standard error

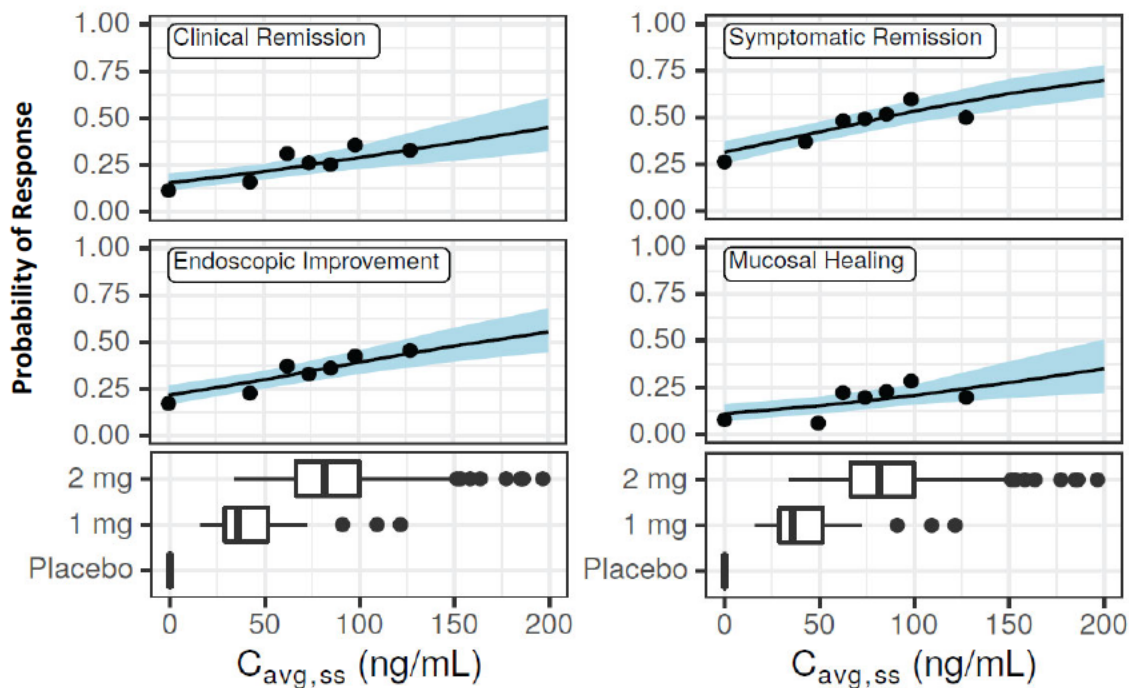
Although the data described above are supportive of the proposed 2 mg dose for induction, no dose exploration was performed for etrasimod for the treatment beyond week 12 in the phase 2 study and doses higher than 2 mg were not studied.

As mentioned above, in the phase 3 trials, etrasimod 2 mg QD was supported by a significantly higher proportion of subjects achieving clinical remission at Week 12 and Week 52. Refer to Section 8.1 for more details.

Exposure-Response Relationship

The exposure-response relationships for the observed rates of clinical remission at Week 12 and several Applicant-proposed secondary efficacy endpoints (i.e., endoscopic improvement, symptomatic remission, and mucosal healing)¹ at Week 12 were explored using data from the phase 2 trial APD334-003 and the phase 3 trials APD334-301 and APD334-302 in adult subjects with UC using population PK/PD analysis, with individual PK parameter estimates from the final PopPK model. The exposure-response relationships are presented in Figure 6.

Figure 6. Exposure-Response Relationship for Predicted and Observed Probability of Efficacy Responses at Week 12 in Adult Subjects With UC



Source: Modified from Exposure-Response Modeling and Simulation Report ARE0301H-UC-Efficacy, Figure S41.
 Abbreviations: $C_{avg,ss}$ = individual *post hoc* estimates of average concentration during the dosing interval at steady state
 Black points are the observed proportions of patients achieving relevant improvements for placebo and each of the six exposure ($C_{avg,ss}$) bins.
 Solid black lines and shaded blue regions represent the medians and 95% prediction intervals of simulated proportions, respectively.
 Distributions of $C_{avg,ss}$ are represented by the boxplots, with median values as a solid line in the center of the box and boxes indicating the inter-quartile range (IQR) with whiskers extending to 1.5*IQR.

Consistent with observed data, population PK/PD analyses suggest that there is an apparent positive exposure-response relationship for clinical remission at Week 12, as well as for several

¹Refer to Section 8.1.2 for discussion on the acceptability of the proposed secondary endpoints for inclusion in labeling.

Applicant-proposed secondary endpoints (endoscopic improvement, symptomatic remission, and mucosal healing)¹ at Week 12. There appeared to be a trend toward greater efficacy response with higher etrasimod exposure. Overall, exposure-response analysis estimated a higher proportion of patients achieving improvements in efficacy response in the etrasimod-treated group as compared to placebo.

Of note, the overall incidence of treatment-emergent adverse events (TEAEs) appeared to be higher in the etrasimod group (71.3%) compared to placebo (56.3%) in the phase 3 trial APD334-301, although most TEAEs were Grade 1 or Grade 2 in severity. The incidence of severe TEAEs, serious TEAEs, TEAEs leading to study drug discontinuation were similar between the two treatment groups in the phase 3 trial APD334-301. Additionally, the overall incidence of TEAEs were similar between the etrasimod group (47.1%) and placebo (46.6%) in phase 3 trial APD334-302.

Overall, the proposed dosage of 2 mg QD is considered appropriate for the treatment of adult patients with moderately to severely active UC based on the efficacy data in the phase 3 clinical trials. See Section 8 of this multidisciplinary review for the related efficacy and safety data.

Is an Alternative Dosing Regimen or Management Strategy Required for Subpopulations Based on Intrinsic Patient Factors?

Etrasimod is not recommended in patients with severe hepatic impairment. No dosage adjustment is needed based on other intrinsic factors.

Hepatic Impairment

Based on a dedicated hepatic impairment study, after single dose administration of 2 mg, mean C_{max} of etrasimod was generally similar between subjects with hepatic impairment and matched subjects with normal liver function; however, mean AUC_{0-inf} were 13%, 29%, and 57% higher in subjects with mild, moderate, and severe hepatic impairment, respectively, compared with subjects with normal liver function. Mean $T_{1/2}$ increased as hepatic function decreased, ranging from 43.9 to 59.5 hours in normal matched controls and for 55.7 hours in subjects with mild hepatic impairment to 76.5 hours for subjects with severe hepatic impairment.

The geometric mean AUC of M3 increased 3-fold and M6 increased 2-fold in the severe hepatic impairment group compared to matched-control.

The summary of PK parameters for etrasimod is presented in Table 13.

Table 13. Summary of Etrasimod Plasma Pharmacokinetic Parameters in Subjects with Hepatic Impairment (Child-Pugh A, B, and C) and Subjects with Normal Hepatic Function

Parameter	Mild Hepatic Impairment	Matched Controls	Moderate Hepatic Impairment	Matched Controls	Severe Hepatic Impairment	Matched Controls
	(N=8)	(N=8)	(N=8)	(N=8)	(N=6)	(N=6)
C _{max} (ng/mL)	31.6 (28.7)	33.4 (15.2)	31.5 (30.5)	35.3 (26.3)	42.0 (22.5)	40.5 (29.5)
t _{max} (h)	5.00 (2.00-12.00)	5.00 (1.98-8.08)	8.00 (4.00-12.00)	4.00 (1.98-8.00)	7.00 (4.00-12.00)	5.00 (1.98-8.00)
AUC _{0-t} (h*ng/mL)	1350 (33.7)	1320 (20.5)	1710 (30.9)	1370 (21.3)	2570 (26.9) ^a	1710 (23.5) ^a
AUC ₀₋₂₄ (h*ng/mL)	561 (30.0)	576 (15.9)	614 (29.9)	611 (20.1)	809 (20.7)	703 (22.2)
AUC _{0-∞} (h*ng/mL)	1390 (34.1)	1370 (20.1)	1760 (31.1)	1410 (20.3)	2730 (26.4)	1740 (20.6)
t _{1/2} (h)	55.7 (33.8)	43.9 (14.9)	69.7 (39.2)	49.0 (16.6)	76.5 (15.0)	59.5 (14.8)
CL/F (L/h)	1.44 (34.1)	1.46 (20.1)	1.13 (31.1)	1.42 (20.3)	0.732 (26.4)	1.15 (20.6)
V _z /F (L)	102 (30.8)	88.4 (19.8)	102 (28.6)	95.4 (24.7)	79.3 (35.8)	95.9 (11.7)

Source: Clinical Study Report APD334-108, Table 9.

^a n=5

Note: Geometric mean (geometric CV%) results are presented unless otherwise indicated.

Median (minimum-maximum) is presented for T_{max}. Arithmetic mean (standard deviation) presented for T_{1/2}.

There was an unexpected decreasing trend in %fu with decreasing liver function (i.e., %fu of 4.35%, 3.84%, and 2.59% for subjects with mild, moderate, and severe hepatic impairment, respectively), while the mean %fu remained similar across the matched healthy control subjects (%fu ranging 4.67% to 5.11%). The unbound AUC_{0-inf} in subjects with severe hepatic impairment (AUC_{0-inf,u} of 70.8 ng·h/mL) appeared lower compared to that in matched subjects with normal liver function (AUC_{0-inf,u} of 81.1 ng·h/mL). The reasons for this slight decreasing trend in %fu is unknown.

Overall, the differences in AUC_{0-inf} for subjects with mild and moderate hepatic impairment compared to healthy controls are not expected to be clinically meaningful to warrant dose adjustment. However, etrasimod is not recommended in patients with severe hepatic impairment, considering the extent of increase in AUC (57% higher) as well as lack of data from phase 3 trials on subjects with severe hepatic impairment.

Renal Impairment

Based on a dedicated renal impairment study, renal impairment does not have clinically significant effects on the PK of etrasimod. The mean C_{max} and AUC of etrasimod were similar between subjects with severe renal impairment/end-stage renal disease (ESRD) and subjects with normal renal function as shown in Table 14. Of note, the effect of hemodialysis on the PK of etrasimod has not been evaluated.

Table 14. Summary of Etrasimod Plasma Pharmacokinetic Parameters in Subjects with Renal Impairment and Subjects with Normal Renal Function

Parameter	Statistics	Severe Renal Impairment (N = 8)	Normal Renal Function (N = 8)
T _{max} (h)	Median (range)	4.0 (2.0 - 6.0)	2.0 (2.0 - 12.0)
C _{max} (ng/mL)	Mean (SD)	31.5 (10.4)	35.2 (6.53)
AUC _{0-t} (ng·h/mL)	Mean (SD)	1501 (533)	1606 (697)
AUC _{0-inf} (ng·h/mL)	Mean (SD)	1550 (537)	1662 (734)

Note: Severe renal impairment: severe impairment and/or end-stage renal disease (estimated glomerular filtration rate $\leq$ 29 mL/min; either requiring hemodialysis or not).

Source: Modified from Clinical Study Report APD334-112, Table 8.

Patients $\geq$ 65 Years of Age

Based on population PK analysis, steady state PK estimates (C_{max,ss}, AUC_{ss}, and C_{trough,ss}) of etrasimod in subjects over 65 years of age were similar (within 5%) to younger adult subjects. Thus, dose reduction for UC patients aged 65 years and older is deemed not necessary based on the available data.

Body Weight

Dose adjustment based on body weight is not needed for adult patients with UC.

In population PK analysis, covariate effects for body weight were included on both clearance and volume terms. Model estimated the C_{max,ss}, AUC_{ss}, and C_{trough,ss} values for etrasimod in a typical 50 kg subject are 125%, 123%, and 120%, respectively, of those from a reference 70 kg subject. Model predicted C_{max,ss}, AUC_{ss}, and C_{trough,ss} values for etrasimod in a typical 110 kg subject are 74.1%, 75.7%, and 77.9%, respectively, of those from a reference 70 kg subject. Hence, the effect of body weight on etrasimod PK is not expected to be clinically meaningful to warrant dose adjustment across the typical body weight range in adults. Of note, body weight ranged 41.4 kg to 138 kg in subjects with UC who received 2 mg QD dosing in phase 3 trials APD334-301 and APD334-302.

Pharmacogenetics

Etrasimod is extensively metabolized by multiple CYP enzymes in the liver. In vitro studies demonstrated that etrasimod was predominantly metabolized by CYP2C8, CYP2C9, CYP3A4, and with minor contribution via CYP2C19 and CYP2J2 (1% each).

The CYP2C9 gene is highly polymorphic. Individuals could be categorized into normal metabolizers (NM), intermediate metabolizers (IM), and poor metabolizers (PM) based on CYP2C9 genotypes (*1, *2, or *3). The Applicant studied metabolism by using an in vitro recombinant CYP2C9 enzyme test. However, the Applicant did not submit a pharmacogenetic report or data for CYP2C9 genotypes from in vivo studies in the original submission package.

The conversion of CYP2C9 genotype to phenotype relies on categorizing alleles into functional status based on assigned "activity value" ranging from 0-1 (e.g., 0 for no function, 0.5 for

decreased function, and 1.0 for normal function), which are summarized into an “activity score (AS)” for each diplotype (see Table 15). The CYP2C9 phenotype frequencies differ across ancestrally diverse populations (see Table 16).

Table 15. Clinical Pharmacogenetics Implementation Consortium Standard for CYP2C9 Genotype to Phenotype Translation Using Activity Scores^a (AS)

Phenotypes	AS Definition	Examples of Diplotypes
Normal Metabolizer (NM)	2	*1/*1
Intermediate Metabolizer (IM)	1.5	*1/*2
	1	*1/*3, *2/*2
Poor Metabolizer (PM)	0.5	*2/*3
	0	*3/*3

Source: Reviewer-generated table based on PMID: 32189324.

Other no function and decreased function alleles are more prevalent in Non-White populations (e.g., *5, *6, *8, *11) than *2 and *3 alleles.

^a0 for no function, 0.5 for decreased function, and 1.0 for normal function

Table 16. Frequency of the CYP2C9 Phenotypes Across Racial/Ethnic Populations

Racial/Ethnic Populations	Phenotype Frequency (%) Based On AS		
	NM	IM	PM
African-American/Afro-Caribbean	76	24	0.1
American [†]	83	16	<0.1
Central/South Asian	60	36	3.8
East Asian	84	15	0.1
European	63	35	2.6
Latino	74	25	0.1
Near Eastern	61	36	3.0
Oceanian	91	9	0.1
Sub-Saharan African	73	26	0.1

Source: Reviewer-generated table based on [https://cpicpgx.org/guidelines/cpic-guideline-for-nsaids-based-on-cyp2c9-genotype/Racial/Ethnic Population categorization](https://cpicpgx.org/guidelines/cpic-guideline-for-nsaids-based-on-cyp2c9-genotype/Racial/Ethnic%20Population%20categorization) based on PMID: 30506572.

[†]The American genetic ancestry group includes populations from both North and South America with ancestors predating European colonization, including American Indian, Alaska Native, First Nations, Inuit, and Métis in Canada, and Indigenous peoples of Central and South America.

Clinical drug-drug interaction (DDI) studies with fluconazole (a moderate inhibitor of both CYP2C9 and CYP3A4; study APD334-009) and gemfibrozil (a strong inhibitor of CYP2C8; study APD334-009) increased the AUC of etrasimod by 84% and 36%, respectively. Itraconazole (a strong CYP3A4 inhibitor; study APD334-116) increased the AUC of etrasimod by 32%. Rifampin (a strong inducer of CYP3A4 and moderate inducer of CYP2C8 and CYP2C9; study APD334-009) decreased the AUC of etrasimod by 49%. Based on the results obtained from the DDI study with fluconazole (refer to additional study information below), a similar magnitude of increase in etrasimod exposure is expected for CYP2C9 poor metabolizers with co-administration of moderate or strong inhibitors of CYP2C8 or CYP3A4.

As the results obtained from the DDI studies suggested a potential increase of exposure in subjects with genetically decreased CYP2C9 enzyme activity, an Information Request (IR) was sent to the Applicant (December 13, 2022) requesting available pharmacogenetic data for CYP2C9. According to the Applicant’s response received on January 4, 2023, a CYP2C9 pharmacogenetic study was not conducted for the following reasons:

1. The clinical DDI data were consistent with the in vitro data, indicating involvement of three CYP enzymes and suggests that a single enzyme doesn't dominate the oxidative metabolism of etrasimod.
2. Inter-subject variability across all the etrasimod PK studies was low to moderate (about 27% for CL/F based on a population PK analysis) with no evidence of outlying clusters of subjects with higher exposure.
3. The treatment emergent adverse events (TEAEs) were generally mild to moderate across phase 1 and UC studies.

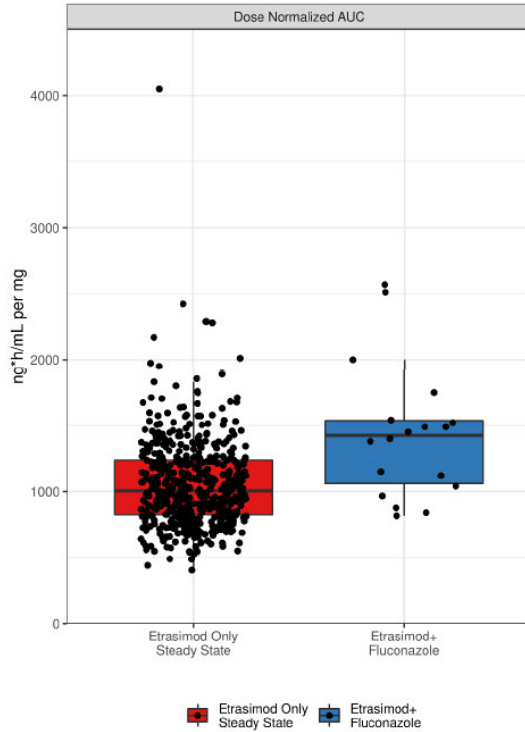
The review team agrees with the above rationales provided by the Applicant. However, considering the expected increase in etrasimod exposure for IM or PM CYP2C9 metabolizers with co-administration of CYP2C8 or CYP3A4 moderate or strong inhibitors, an additional IR was sent on March 6, 2023, requesting recommendations for dosing approaches for patients who are CYP2C9 IMs or PMs and receiving CYP2C8 or CYP3A4 moderate or strong inhibitors. The Applicant's response was received on March 20, 2023. The Applicant added the following language to the label, (b) (4)

The proposed dose recommendation was based on the justification outlined below:

1. The Applicant conducted a review of the published literature on the impact of CYP2C9 allelic variants on in vivo drug clearance. For different CYP2C9 substrates, decreases in clearance in IMs relative to CYP2C9*1/*1 ranged from 3% (torsemide), 49% (celecoxib), and ~68% (S-warfarin). In PMs, clearances are 9% to 33% or as high as ~91% (S-warfarin in CYP2C9*3/*3 subjects), compared to the wild-type. The Applicant indicated that the observed range of impact on clearance for CYP2C9 IM phenotype with concomitant administration of an inhibitor of CYP3A4 or CYP2C8 is expected to result in <50% decrease in etrasimod clearance (<2-fold increase in AUC), relative to wild-type. Whereas in PMs, the decrease in clearance is unlikely to substantially exceed 50%, with an increase in exposure that is 2-fold or slightly higher.
2. In the presence of fluconazole, while CYP2C9 and CYP3A4 pathways are moderately inhibited, a significant extent of CYP2C8 activity is available to metabolize etrasimod. Similarly, in IM or PM of CYP2C9 patients taking CYP3A4 or CYP2C8 inhibitor, the remaining uninhibited metabolic pathways are expected to be fully active. Therefore, the overall inhibition of metabolic activity of etrasimod in PM patients is not expected to substantially exceed 50%, compared to wild type.
3. Based on the Applicant's population PK model, post hoc steady state AUC_{ss} values were generated for each subject receiving etrasimod 2 mg in the UC phase 3 trials. As shown in Figure 7, for the etrasimod single dose, AUC_{inf} in the presence of fluconazole (blue box plot) is within the distribution of AUC_{ss} (red box plot) as determined in subjects with

UC from the phase 3 trials. According to the Applicant, this magnitude of increase in etrasimod AUC is not expected to be a significant safety concern.

Figure 7. Etrasimod AUC for Etrasimod Co-Administered with Fluconazole Compared to Predicted Exposure in UC Subjects Using a Population Pharmacokinetic Model



Source data: Left, Improve repository artifact: CP1:F1-40641155; Right, [Module 5.3.3.4 Study APD334-009 CSR Listing 16.2.6.4](#)

Source: The Applicant PopPK analysis.

The Applicant concluded that the increase in etrasimod exposure in patients with IM or PM CYP2C9 status taking concomitant moderate or strong CYP3A4 or CYP2C8 inhibitors is expected to be in a similar range as that observed in the fluconazole DDI study. Therefore, the recommendation for PM patients should be consistent with recommendation for fluconazole. Due to uncertainty in the range of potential changes in etrasimod exposure, the effect of etrasimod exposure with concomitant use of CYP3A4 or CYP2C8 inhibitors is unknown in CYP2C9 IM patients.

In general, the review team agrees with the Applicant's justification. Pharmacogenomic labeling recommendations will be included in the etrasimod label.

Are There Clinically Relevant Food-Drug or Drug-Drug Interactions, and What is the Appropriate Management Strategy?

The proposed dosing instruction to administer etrasimod with or without food is appropriate.

Drug-Drug Interactions

- Co-administration of etrasimod with drugs that are both moderate CYP2C9 and moderate or strong CYP3A4 inhibitors (e.g., fluconazole) is not recommended.
- Co-administration of etrasimod with a drug that is a combined CYP3A4 (strong), CYP2C8 (moderate) and CYP2C9 (moderate) inducer (e.g., rifampin) is not recommended.
- Co-administration of etrasimod with drugs that are CYP2C8 or CYP3A4 inhibitor in patients who are known to be CYP2C9 poor metabolizers is not recommended. In patients who are intermediate metabolizers of CYP2C9 substrates, the effect on etrasimod exposure when co-administered with CYP3A4 or CYP2C8 inhibitors is not known.

Food Effect

The effect of food on etrasimod PK was evaluated in study APD334-114 using the proposed TBM formulation. No significant food effect on the PK of etrasimod was observed when a single dose of 2 mg etrasimod was administered with a high-fat meal as compared to the fasted state. Geometric mean C_{max} and AUC were similar between the fed state and the fasted state (Table 17). Mean T_{max} was delayed by 2 hours in the fed state (6 h) compared to the fasted state (4 h).

Table 17. Statistical Analysis to Assess the Effect of Food on the PK of Etrasimod

Parameter	2 mg Fasted (N=16)	2 mg Fed (N=15)	Geometric LS Mean Ratio Fed/Fasted (90% CI)
T_{max} (h)	4	6	NA
C_{max} (ng/mL)	41.62	41.34	99.33 (94.40, 104.51)
AUC _{0-t} (ng.h/mL)	1597.01	1625.98	101.81 (98.56, 105.17)
AUC _{0-inf} (ng.h/mL)	1662.69	1692.64	101.80 (98.35, 105.37)

Source: Clinical Study Report APD334-114, Table 7 and Table 8.

Abbreviations: CI = confidence interval; LS = least squares; NA = not applicable; PK = pharmacokinetics

DDI Study with Fluconazole, Gemfibrozil, and Rifampin

A clinical drug interaction study (APD334-009) was conducted to evaluate the effects of fluconazole, gemfibrozil, or rifampin on the PK of etrasimod in healthy adult subjects.

Co-administration of fluconazole 200 mg QD (400 mg on the first day, followed by 200 mg QD for 14 days) with a single dose of etrasimod 1 mg resulted in an increase of 12% in mean C_{max} and an increase of 84% in mean AUC_{0-inf} of etrasimod as shown in Table 18.

Table 18. Statistical Analysis to Assess the Effect of Fluconazole on the PK of Etrasimod

PK Parameter (Unit)	Etrasimod			Etrasimod+ Fluconazole			(Etrasimod + Fluconazole) / Etrasimod	
	N	GM	90% CI	N	GM	90% CI	GMR	90% CI
Analyte: Etrasimod								
C _{max} (ng/mL)	18	18.5	(16.91 - 20.35)	18	20.8	(18.99 - 22.87)	1.12	(1.09 - 1.16)
AUC _{0-168h} (ng*h/mL)	18	708	(635.43 - 788.92)	18	1140	(1026.67 - 1274.67)	1.62	(1.52 - 1.72)
AUC _{0-∞} (ng*h/mL)	18	739	(656.12 - 831.35)	18	1360	(1208.83 - 1531.69)	1.84	(1.71 - 1.99)

Source: Clinical Study Source: Clinical Study Report APD334-009, Table 11.

Abbreviations: PK = pharmacokinetics; GM = geometric least squares mean; GMR = geometric least squares mean ratio; CI = confidence interval

Fluconazole is a moderate inhibitor of CYP2C9 and CYP3A4, and a strong inhibitor of CYP2C19. However, considering in vitro data suggested only a minor contribution (1%) via CYP2C19 metabolism for etrasimod, co-administration of etrasimod with drugs that are both moderate CYP2C9 and moderate or strong CYP3A4 inhibitors is not recommended.

Co-administration of rifampin 600 mg QD for 15 days with a single dose of etrasimod 2 mg resulted in an increase of 24% in mean C_{max} but a decrease of approximately 49% in mean AUC_{0-∞} of etrasimod as shown in Table 19.

Table 19. Statistical Analysis to Assess the Effect of Rifampin on the PK of Etrasimod

PK Parameter (Unit)	Etrasimod			Etrasimod+ Rifampin			(Etrasimod + Rifampin) / Etrasimod	
	N	GM	90% CI	N	GM	90% CI	GMR	90% CI
Analyte: Etrasimod								
C _{max} (ng/mL)	18	27.8	(20.52 - 37.79)	18	34.6	(25.49 - 46.95)	1.24	(0.82 - 1.88)
AUC _{0-168h} (ng*h/mL)	17	1390	(1238.93 - 1568.79)	18	733	(651.79 - 823.74)	0.526	(0.49 - 0.56)
AUC _{0-∞} (ng*h/mL)	17	1460	(1290.22 - 1640.98)	18	737	(654.30 - 830.65)	0.507	(0.47 - 0.54)

Source: Clinical Study Source: Clinical Study Report APD334-009, Table 15.

Abbreviations: PK = pharmacokinetics; GM = geometric least squares mean; GMR = geometric least squares mean ratio; CI = confidence interval

Rifampin is a moderate inducer of CYP1A2, CYP2B6, CYP2C8 and CYP2C9 and a strong inducer of CYP2C19 and CYP3A4. However, considering in vitro data suggested that etrasimod is mainly metabolized by CYP2C8, CYP2C9, and CYP3A4, co-administration of etrasimod with a drug that is a combined CYP3A4 (strong), CYP2C8 (moderate) and CYP2C9 (moderate) inducer (i.e., rifampin) is not recommended

Co-administration of gemfibrozil (a strong inhibitor of CYP2C8 and inhibitor of OATP1B1 and OAT3) 600 mg BID for 15 days with a single dose of etrasimod 1 mg resulted in an increase of 12% in mean C_{max} and an increase of 36% in mean AUC_{0-inf} of etrasimod as shown in Table 20.

Table 20. Statistical Analysis to Assess the Effect of Gemfibrozil on the PK of Etrasimod

PK Parameter (Unit)	Etrasimod			Etrasimod+ Gemfibrozil			(Etrasimod + Gemfibrozil) / Etrasimod	
	N	GM	90% CI	N	GM	90% CI	GMR	90% CI
Analyte: Etrasimod								
C_{max} (ng/mL)	18	18.8	(16.72 - 21.15)	18	21.1	(18.72 - 23.68)	1.12	(1.06 - 1.19)
AUC_{0-168h} (ng*h/mL)	18	736	(653.33 - 828.02)	18	934	(829.99 - 1051.93)	1.27	(1.20 - 1.35)
$AUC_{0-\infty}$ (ng*h/mL)	18	772	(679.22 - 876.85)	18	1050	(920.53 - 1188.38)	1.36	(1.26 - 1.46)

Source: Clinical Study Source: Clinical Study Report APD334-009, Table 14.

Abbreviations: PK = pharmacokinetics; GM = geometric least squares mean; GMR = geometric least squares mean ratio; CI = confidence interval

Considering in vitro data suggested that etrasimod is metabolized by multiple CYP enzymes, the risk for clinically relevant DDIs appears low with concomitant medications that inhibit only a single enzymatic pathway. Overall, the increase in systemic exposure (up to 36%) is not considered clinically significant to warrant dose adjustment when etrasimod is co-administered with a CYP2C8 inhibitor.

DDI Study with Itraconazole

A clinical drug interaction study (APD334-116) was conducted to evaluate the effects of itraconazole on the PK of etrasimod in healthy adult subjects.

Co-administration of itraconazole (a strong inhibitor of CYP3A4) 200 mg QD for 13 days with a single dose of etrasimod 1 mg had no or minimum impact on the mean C_{max} (6% increase) but resulted in an increase of 32% in mean AUC_{0-inf} of etrasimod as shown in Table 21.

Table 21. Statistical Analysis to Assess the Effect of Itraconazole on the PK of Etrasimod

Parameter (unit)	Etrasimod alone				Etrasimod + Itraconazole				(Etrasimod + Itraconazole)/ Etrasimod alone		
	N	n	GM	90% CI	N	n	GM	90% CI	N	GMR	90% CI
C _{max} (ng/mL)	18	18	18.5	(17.23 – 19.88)	18	18	19.6	(18.29 – 21.10)	18	1.06	(1.02 – 1.11)
AUC _{0-168h} (h*ng/mL)	18	18	711	(645.92 – 781.62)	18	18	917	(833.46 – 1008.55)	18	1.29	(1.24 – 1.34)
AUC _{0-last} (h*ng/mL)	18	18	720	(650.98 – 796.11)	18	18	946	(855.14 – 1045.79)	18	1.31	(1.26 – 1.37)
AUC _{0-∞} (h*ng/mL)	18	18	741	(667.70 – 822.02)	18	18	981	(884.29 – 1088.67)	18	1.32	(1.27 – 1.38)

Source: Clinical Study Source: Clinical Study Report APD334-116, Table 11-4.

Abbreviations: PK = pharmacokinetics; GM = geometric least squares mean; GMR = geometric least squares mean ratio; CI = confidence interval

The increase in systemic exposure (up to 32%) is not considered clinically significant to warrant dose adjustment when etrasimod is co-administered with a CYP3A4 inhibitor.

DDI Study with Oral Contraceptive

Study APD334-111 was conducted to evaluate the effects of etrasimod on the PK and PD of an oral contraceptive (30 µg ethinylestradiol [EE] and 150 µg levonorgestrel [LVG]) in healthy premenopausal female subjects.

Co-administration of etrasimod 2 mg QD with the oral contraceptives (OCs) QD at steady state increased the mean C_{max} of EE by 12% and increased the mean AUC_{0-inf} by 24%. For LVG, co-administration of etrasimod 2 mg QD resulted in an increase of the mean C_{max} by 22% and an increase of the mean AUC_{0-inf} by 32% as shown in Table 22.

Table 22. Statistical Analysis to Assess the Effect of Etrasimod on the PK of Ethinylestradiol and Levonorgestrel

Parameter	Geometric LS Mean		Geometric LS Mean Ratio (%) (Test/Reference) [90% CI]
	Test (Etrasimod + OC) (N = 17)	Reference (OC) (N = 21)	
Ethinylestradiol			
AUC _{tau,ss} (h*pg/mL)	1026	827	124.05 (116.36, 132.25)
C _{max,ss} (pg/mL)	95.7	85.5	111.95 (100.94, 124.16)
Levonorgestrel			
AUC _{tau,ss} (h*pg/mL)	127074	96490	131.70 (126.85, 136.73)
C _{max,ss} (pg/mL)	9884	8091	122.16 (115.01, 129.76)

Source: Clinical Study Source: Clinical Study Report APD334-111, Table 10, Table 12.

Abbreviations: PK = pharmacokinetics; LS = least squares; OC = oral contraceptive; CI = confidence interval

Overall, no clinically meaningful effects of etrasimod on the steady-state C_{max} or AUC of EE and LVG were observed when co-administered with etrasimod 2 mg QD.

In addition to the PK assessment, the effect of etrasimod on PD measures of ovarian activity (i.e., follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone, sex hormone binding globulin (SHBG), and transvaginal ultrasound (TVUS)/Hoogland scores) were explored in this study. Graphical assessment suggested that co-administration of etrasimod and OC did not show clinically meaningfully altered hormone concentration levels of FSH, LH, estradiol, and progesterone compared to OC administration alone. A similar increasing trend in SHBG levels was observed in both OC administration alone and co-administration of etrasimod and OC treatment, which is expected for monophasic OC containing both EE and LVG (Abdalla, Shabaan, and Stanczyk 1992). The incidents of Hoogland scores also showed similar trends for both OC treatments.

Smoking Status

Population PK analysis estimated that steady state PK estimates ($C_{max,ss}$, AUC_{ss} , and $C_{trough,ss}$) of etrasimod in a typical tobacco user were similar (within 2%) to those from a reference male non-user of tobacco of the same body weight.

Immune System Effects After Stopping Etrasimod

As lymphocyte count is reported to return to the normal range in 90% of subjects within 4 to 5 weeks of stopping etrasimod in subjects with UC, use of immunosuppressants within this period may lead to an additive effect on the immune system. Monitoring of patients receiving concomitant immunosuppressants for infectious complications during treatment and for 5 weeks after the last dose of etrasimod will be recommended in the label.

Bridge Between the “To-Be-Marketed” and Clinical Trial Formulations

Since the formulation used in the phase 3 trials APD334-301 and APD334-302 is different from the to-be-marketed formulation (2 mg oral tablets), a relative bioavailability study (APD334-114) was conducted to bridge the clinical trial formulation to the to-be-marketed formulation. Bioequivalence criteria were met for systemic exposures between these two formulations. Geometric mean C_{max} and AUC were comparable between the formulation used in the phase 3 trials and the to-be-marketed formulation as shown in Table 23.

Table 23. Statistical Analysis of Relative Bioavailability for the Clinical and To-Be-Marketed Formulations

Parameter	2 mg Clinical Tablet (N=16)	2 mg TBM Tablet (N=15)	Geometric LS Mean Ratio Fed/Fasted (90% CI)
T_{max} (h)	4	4	NA
C_{max} (ng/mL)	42.54	41.62	97.83 (92.94, 102.97)
AUC_{0-t} (ng·h/mL)	1631.21	1597.01	97.90 (94.75, 101.16)
AUC_{0-inf} (ng·h/mL)	1695.59	1662.69	98.06 (94.72, 101.52)

Source: Clinical Study Report APD334-114, Table 7 and Table 8.

Abbreviations: CI = confidence interval; LS = least squares; NA = not applicable; PK = pharmacokinetics; TBM = to be marketed
Median is presented for t_{max} ; geometric least squares mean presented for all other parameters

NDA/BLA Multi-Disciplinary Review and Evaluation NDA 216956
Velsipity (etrasimod)

A request to conduct an inspection of the clinical and bioanalytical sites for Study APD334-114 was submitted to the Office of Study Integrity and Surveillance (OSIS). OSIS has conducted the inspection of the both clinical and bioanalytical sites for Study APD334-114 and recommends accepting the study data for review (see OSIS Memos by Dr. Gajendiran Mahadevan dated June 2, 2023 [DARRTS Reference ID: 5183704], and by Dr. Melkamu Getie Kehtie dated June 27, 2023 [DARRTS Reference ID: 5197761]).

7 Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

Table 24. Clinical Studies Submitted to Support the Efficacy and Safety of Etrasimod

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary Endpoints	No. of Patients Planned; Actual Randomized	No. of Centers and Countries
APD334-301	Adolescent aged 16 years and older and adult subjects with moderately to severely active UC	R, DB, PC, phase 3	- Etrasimod 2 mg PO QD (n=289) - Placebo PO QD (n=144) Duration: 52 weeks	Coprimary: - Clinical remission at Week 12 - Clinical remission at Week 52	Planned: 420 Actual: 433	Centers: 205 Countries: 37
APD334-302	Adolescent aged 16 years and older and adult subjects with moderately to severely active UC	R, DB, PC, phase 3	- Etrasimod 2 mg PO QD (n=238) - Placebo PO QD (n=116) Duration: 12 weeks	Primary: Clinical remission at Week 12	Planned: 330 Actual: 354	Centers: 185 Countries: 39
APD334-303	Adolescent aged 16 years and older and adult subjects with moderately to severely active UC	OLE, phase 3 Single-arm	Etrasimod 2 mg PO QD Duration: Maximum 5 years	Primary: Long-term safety and efficacy	Planned: 912 Actual: 636	Centers: 311 Countries: 40
APD334-003	Adults aged 18 to 80 years old with moderately to severely active UC	R, DB, PC, phase 2	- Etrasimod 1mg PO QD (n=52) - Etrasimod 2 mg PO QD (n=50) - Placebo PO QD (n=54) Duration: 12 weeks	Primary: Clinical remission at Week 12	Planned: 240 Actual: 156	Centers: 87 Countries: 17

NDA/BLA Multi-Disciplinary Review and Evaluation NDA 216956
Velsipity (etrasimod)

Trial Identifier	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary Endpoints	No. of Patients Planned; Actual Randomized	No. of Centers and Countries
APD334-005	Adults aged 18 to 80 years old with moderately to severely active UC	OLE, phase 2	Etrasimod 2 mg PO QD Duration: 34 weeks, extended to 40 weeks with Amendment 2	Primary: Evaluate the long-term safety and tolerability of etrasimod in subjects with UC who have completed APD334-003	Planned: N/A Actual: 118	Centers: 51 Countries: 16
ES102002	Adults aged 18 to 75 years old with moderately to severely active UC	R, DB, PC, phase 3	- Etrasimod 2 mg PO QD - Placebo PO QD Duration: 52 weeks	Primary (induction): - Clinical remission at Week 12 Primary (maintenance): - Clinical remission at Week 40	Planned: Ongoing Actual: 66	Centers: 51 Countries: 3

Source: Reviewer Generated Table, Adapted from Tabular Listing of All Clinical Studies

Abbreviations: R, randomized; DB, double-blind; PC, placebo-controlled; MC, multi center; RW, randomized withdrawal; OLE: Open label extension; ES, endoscopic subscore; IV, intravenous; n, number of patients in specified population or group; MMS, modified Mayo score; PLB, placebo; Q4W, once every 4 weeks; Q12W, once every 12 weeks; R, randomized; RB, rectal bleeding; SC, subcutaneous; SF, stool frequency; UC, ulcerative colitis

7.2. Review Strategy

The clinical development program for etrasimod that supported the determination of safety and effectiveness included one 12-week phase 2 trial (APD334-003), one 12-week phase 3 trial (APD334-302), and one 52-week phase 3 trial (APD334-301).

Efficacy

The determination of effectiveness was based on the two phase 3 trials, APD334-301 and APD334-302. No pooling of data across trials was conducted for the assessment of efficacy. The two trials are summarized as follows:

- APD334-301: A phase 3, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of etrasimod 2 mg for the treatment of moderately to severely active UC. The trial was a “treat-through” design for a duration of 52 weeks with an initial treatment period of 12 weeks, at which time endpoints were assessed (i.e., Week 12). Subjects then continued their blinded, randomized treatment for an additional 40 weeks, at which time endpoints were assessed (i.e., Week 52). After Week 12, subjects who met the prespecified definition of UC worsening² were eligible to leave APD334-301 and enter the open-label extension study (APD334-303).
- APD334-302: A phase 3, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of etrasimod 2 mg for the treatment of moderately to severely active UC. The trial duration was 12 weeks.

During the review, several issues were identified which resulted in the primary efficacy analyses being conducted in a redefined population (“FDA Preferred Analysis Population”) as explained below.

Disease Severity Criteria

APD334-301 and APD334-302 defined moderately to severely active UC as a modified Mayo Score (mMS) of 4 to 9, including an endoscopic subscore (ES) of at least 2 and rectal bleeding subscore (RBS) of at least 1. Prior to completion of the Applicant’s phase 3 program, the Division communicated to the Applicant that the Division’s recommended definition of moderately to severely active UC includes subjects with a mMS of 5 to 9 and an ES of at least 2. The Division raised concerns that a mMS of 4 may result in the enrollment of subjects with mild symptoms (e.g., a subject with a SFS of 1, RBS of 1, and ES of 2), in whom achieving the

² Subjects with disease worsening were defined as having at least 1 of the following criteria: 1) absolute rectal bleeding subscore (RBS) ≥ 2 on 2-consecutive days and confirmation of endoscopic subscore (ES) ≥ 2 , 2) absolute RBS + stool frequency subscore (SFS) ≥ 4 on 2-consecutive days and confirmation of ES ≥ 2 or 3) absolute RBS ≥ 2 or RBS + SFS ≥ 4 (in any order) on 2-consecutive days and confirmation of ES ≥ 2 .

definition of remission (which may include a SFS of 1) may not represent a clinically meaningful improvement. The Applicant performed the requested additional analyses using the Division's recommended definition of moderately to severely active UC, and the results of analyses in this population are the focus of this multidisciplinary review.

Pediatric Subjects

APD334-301 and APD334-302 enrolled subjects 16 to 80 years of age inclusive, (b) (4)

However, only 1 adolescent subject (who received placebo) was enrolled in APD334-301 and only 2 adolescent subjects (1 placebo, 1 etrasimod) were enrolled in APD334-302. Therefore, given data were available for only one adolescent subject who received etrasimod, the Division communicated to the Applicant (b) (4) the results of analyses in adults are the focus of this multidisciplinary review. The efficacy populations used in this review are shown in Table 25.

Table 25. Efficacy Populations APD334-301 and APD334-302

Population	Definition	Number of Subjects APD334-301	Number of Subjects APD334-302
Full Analysis Set (FAS)	All randomized subjects	N=433	N=354
FAS with mMS 5-9	All randomized subjects with baseline mMS of 5-9 and an ES of at least 2	N=409	N=334
FDA Preferred Analysis Population	All randomized adult subjects with baseline mMS of 5-9 and an ES of at least 2	N=408	N=333 ¹

¹Two adolescent subjects were enrolled in APD334-302; however, only 1 subject had a baseline MMS of 5-9 with an ES of at least 2.

Safety

The primary safety analyses were performed on the 52-week trial APD334-301, and on pooled safety data from the 12-week trials APD334-302 and APD334-003. The pooling of the data from APD334-302 and APD334-003 for subjects that received the same dose of etrasimod or placebo was appropriate as the study design, demographics, baseline disease characteristics, and prior and concomitant therapy were similar between the two trials. Pooling of the 12-week trials provided an assessment of adverse events in a larger safety database, as compared to evaluating each study individually.

The safety of etrasimod was assessed in the safety population, which included all randomized subjects who received at least one dose of study treatment. For trials APD334-301 and APD334-302, the safety population is the same as the FAS because all randomized subjects received at least one dose of study treatment.

Additionally, The Applicant submitted pooled safety data from the integrated summary of safety (ISS). The ISS consisted of safety data from the placebo-controlled parent studies

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APD334-301, APD334-302, and APD334-003, as well as their open-label extension studies APD334-303 (for APD334-301 and APD334-302) and APD334-005 (for APD334-003). The ISS also included data from the open-label portion of an ongoing placebo-controlled trial conducted in East Asia, ES101002. The open-label extension studies are described below.

- Safety Information from Open-Label Studies
 - ES101002: A phase 3, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of etrasimod 2 mg for the treatment of moderately to severely active UC in East Asia. This trial was ongoing at the time of the NDA submission; however, data from the open-label portion of the trial were included. Subjects that were nonresponders at Week 12 and those that experienced disease worsening during ES101002 were eligible to enter the open-label treatment period with a treatment duration of up to 40 weeks.
 - APD334-005: An open-label extension study for subjects that completed APD334-003. All subjects who completed APD334-003 and met the eligibility criteria for the APD334-005 extension study had the option to enroll and receive open-label etrasimod 2 mg once daily for 34 weeks, which was later extended to 40 weeks under Protocol Amendment 2.
 - APD334-303: An open-label extension study for subjects that completed APD334-301, APD334-302, and APD334-308. Subjects from the ongoing studies APD334-210 (a 52-week study in moderately active UC) and APD334-203 (a 12-week study in Japanese subjects with moderately to severely active UC) were also eligible to enroll in APD334-303. All subjects received open-label etrasimod 2 mg once daily for up to 5 years.

Given the open-label nature of the data from ES101002, APD334-005, and APD334-303, results were not considered to provide substantial evidence of effectiveness. Additionally, APD334-308 was not submitted as part of the original NDA submission and consisted of summary safety information; as such, data from APD334-308 was not considered to provide substantial evidence of effectiveness. However, safety data were reviewed to assess for rare events given the extended duration of the open-label studies.

8 Statistical and Clinical and Evaluation

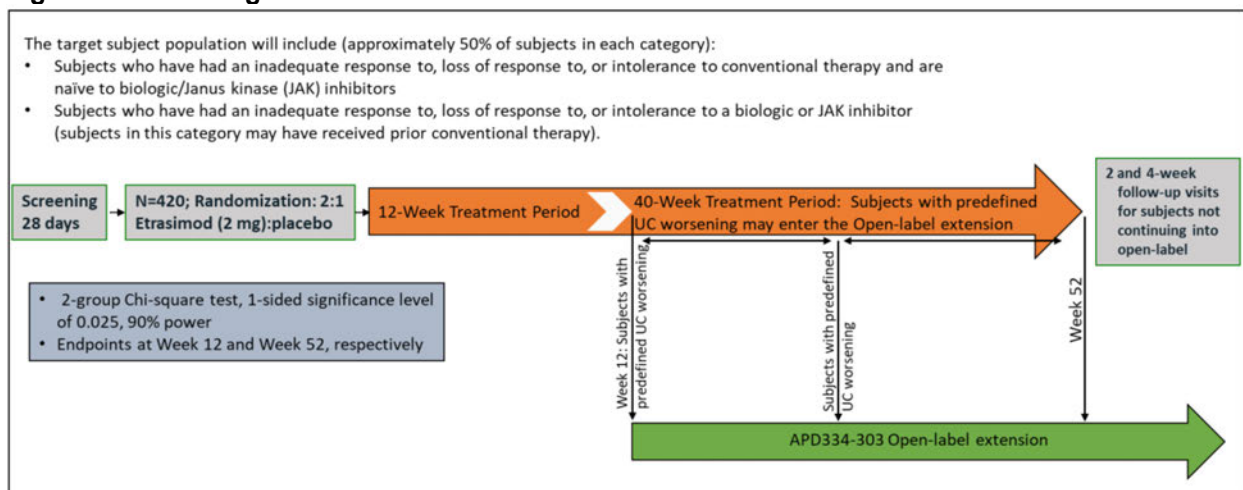
8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. APD334-301

Trial Design

APD334-301 was a multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of etrasimod 2 mg over 52 weeks. The trial population included subjects with a modified Mayo Score (mMS) of 4 to 9 who had an inadequate response to, loss of response to, or intolerance to at least 1 of the following: oral 5-aminosalicylic acid (5-ASA) compounds, corticosteroids, thiopurines, biologic therapy (TNF blocker, vedolizumab, anti-interleukin [anti-IL] 12/23), or Janus kinase (JAK) inhibitors. A total of 433 subjects, including 1 adolescent, from 37 countries were randomized in a 2:1 ratio to receive either etrasimod 2 mg once daily or matching placebo once daily for up to 52 weeks. The trial included an initial 12-week treatment period, after which, subjects who met the predefined definition for UC worsening² were eligible to enter into the open-label extension trial (APD334-303). All other subjects were to continue on their randomized treatment for an additional 40 weeks. Subjects that met the predefined definition for UC worsening during this 40-week treatment period could switch to the open-label extension trial. Randomization was stratified by prior treatment status (i.e., naïve to biologic or JAK inhibitor therapy at trial entry [yes/no]), baseline corticosteroid use (yes/no), and baseline disease activity (mMS: [4-6] or [7-9]). The overall trial design for APD334-301 is shown in Figure 8.

Figure 8. Trial Design for APD334-301



Source: Applicant's clinical study report for APD334-301, Figure 1 (page 22).
Abbreviation: JAK, Janus kinase; UC, ulcerative colitis

Trial Endpoints

The co-primary efficacy endpoints comparing etrasimod to placebo consisted of:

- The proportion of subjects achieving clinical remission at Week 12
- The proportion of subjects achieving clinical remission at Week 52

Clinical remission was evaluated using the mMS and was defined as a stool frequency subscore (SFS) of 0 or a SFS of 1 with a ≥ 1 point decrease from baseline, rectal bleeding subscore (RBS) of 0, and endoscopic subscore (ES) of 0 or 1 (excluding friability). The Applicant's definition of clinical remission differed from the Agency's preferred definition of clinical remission of SFS of 0 or 1 (i.e., no requirement for a ≥ 1 point decrease from baseline), RBS of 0, and ES of 0 or 1 (excluding friability). The multiplicity-controlled secondary endpoints and definitions are shown in Table 26 and were subject to overall type I error control (tested based on a gatekeeping approach). Refer to Section 16.4.1 for additional details on the multiple testing procedure.

Table 26. Multiplicity-Controlled Secondary Efficacy Endpoints for Trial APD334-301

Secondary Endpoint	Definition
Endoscopic improvement at Week 12	ES ≤ 1 (excluding friability)
Endoscopic improvement at Week 52	
Symptomatic remission at Week 12	SFS = 0 (or = 1 with a ≥ 1 point decrease from baseline) and RBS of 0
Symptomatic remission at Week 52	
Corticosteroid-free clinical remission at Week 52	Clinical remission at Week 52 and who had not been receiving corticosteroids for ≥ 12 weeks prior to Week 52
Mucosal healing at Week 12	ES of ≤ 1 (excluding friability) with histologic remission measured by a Geboes Index score < 2.0
Mucosal healing at Week 52	
Sustained clinical remission at Week 52	Clinical remission at both Week 12 and Week 52

Source: Review team's table

Abbreviation: ES, endoscopic subscore; SFS, stool frequency subscore; RBS, rectal bleeding subscore

Statistical Analysis Plan

Populations

The Applicant's primary efficacy analyses were based on subjects in the full analysis set (FAS) with baseline mMS of 5 to 9 including an ES of at least 2. The FAS consisted of all randomized subjects who received at least 1 dose of trial treatment. (b) (4)

The single adolescent subject was excluded from the efficacy analysis population, resulting in the "FDA Preferred Analysis Population." The Division's efficacy analyses (in addition to confirming the Applicant's analyses) focus on efficacy in the FDA preferred analysis population, defined as all adult subjects in the FAS with baseline mMS of 5 to 9 including an ES of at least 2. The safety population included all randomized subjects (including adolescents and

subjects with mMS of 4) who received at least 1 dose of trial treatment, which is used for safety-related analysis.

Sample Size

The trial was powered based on assumed clinical remission rates at Week 52 of 23.5% for etrasimod versus 10% for placebo. A sample size of 420 (280 etrasimod, 140 placebo) was expected to provide 93.4% power to demonstrate that etrasimod is superior to placebo in achieving the clinical remission at Week 52, as assessed using a 2-group Fisher's exact test with a 1-sided significance level of 0.025. With this sample size, there was 96% power to detect a difference of 12.5% in the other primary endpoint of clinical remission at Week 12, assuming a placebo rate of 6%. The lower bound of overall power for both primary endpoints were at least 90%.

Calculation of SFS and RBS

The coprimary efficacy endpoints were based on the mMS which contains three subscore components: SFS, RBS, and ES. On visits when the mMS was calculated, SFS and RBS were derived by averaging the scores from the 3 most recent consecutive days within the 7 days prior to the day of bowel preparation (excluding the day of bowel preparation) and rounding to the nearest integer. On visits without endoscopy, SFS and RBS were derived by averaging the scores from the 3 most recent consecutive days within the 7 days prior to the date of visit and rounding to the nearest integer. If 3 consecutive days were not available, then the average of the 2 most recent consecutive days within the 7 days would be used. Sensitivity analyses were conducted that considered subjects with only 2 consecutive days available as non-responders. The outcomes of these analyses were consistent with the primary efficacy results.

Analyses of Efficacy Endpoints

For assessments of the coprimary endpoints and multiplicity-controlled secondary endpoints, the estimated difference in proportions between arms and the corresponding 95% confidence interval (CI) were computed based on the stratified risk difference using the Cochran-Mantel-Haenszel (CMH) weights. The analyses adjusted for the following randomization stratification factors: naïve to biologic or JAK inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (mMS: [4-6] or [7-9]).

Estimands and Intercurrent Events

The estimand corresponding to the co-primary efficacy endpoint of clinical remission at Week 12 evaluated the difference in the proportion of subjects in clinical remission at Week 12 for etrasimod 2 mg versus placebo in subjects with baseline mMS of 5 to 9 in the FAS population. For the intercurrent events of 1) premature treatment discontinuation for worsening of disease, lack of efficacy, or adverse event related to UC, 2) initiation of a rescue medication for UC, 3) an increase in dose over baseline levels in subjects' existing UC medication, or 4) rescue medical procedure during the trial, subjects were considered non-responders. The other co-

primary endpoint at Week 52 and multiplicity-controlled secondary endpoints used the same estimand as the co-primary endpoint at Week 12.

Handling of Missing Data

For analyses of the coprimary efficacy endpoints and multiplicity-controlled secondary endpoints, missing data were imputed using non-responder imputation (NRI). Sensitivity analyses for all multiplicity-controlled endpoints included multiple imputation under missing at random (MAR), tipping point analysis, multiple imputation with Copy Reference (CR) under missing not at random (MNAR), and a hybrid imputation approach with multiple imputation used to handle missing endoscopy data due to the impact of the COVID-19 pandemic, and NRI for missing data not due to the COVID-19 pandemic.

Multiplicity Adjustment

A gatekeeping multiple testing procedure was specified to control the overall Type I error rate at 2-sided alpha of 0.05 for all coprimary endpoints and major secondary endpoints. Refer to Figure 29 in the Appendix Section 16.4.1 for the details on the gatekeeping procedure.

Protocol Amendments

The original study protocol for APD334-301 had four amendments. A summary of changes for each amendment are described in Table 27.

Table 27. Summary of Changes for APD334-301 Protocol Amendments

Amendment	Date	Summary of Changes
1.0	March 05, 2019	Additional procedures were added to the cardiac monitoring required on Day 1 after the first dose of study treatment. Post-dose safety vital signs, 12-lead ECGs, monitoring of subjects who do not meet the discharge criteria, and discontinuation rules were revised to align with FDA feedback.
2.0	December 20, 2019	- Criteria for early (i.e., prior to Week 52) enrollment in the open-label study and eligibility criteria (e.g., list of prior therapy failures or non-response, contraception use, cardiovascular disease history, and prior therapy washout period) were updated. - Additional instructions for safety monitoring related to 12-lead ECG, pulmonary function tests (PFTs), and ophthalmoscopy and optical coherence tomography (OCT) testing were added. - Further instructions for QuantiFERON TB Gold and tuberculin skin test were added. - A 4-Week Follow-Up visit was added.
3.0	February 7, 2020	- Clarification and further instructions for tuberculosis testing and an update for the tuberculosis screening questionnaire was made. - A correction was made to the entry criteria for enrollment in the OLE study. - Lack of efficacy was added as a reason a subject may discontinue from double-blind treatment. - Clarifications were made to ophthalmoscopy and OCT testing.
4.0	December 22, 2020	- Guidance for conducting virtual and offsite visits was updated in the protocol. - Information on anti-arrhythmic drugs was added to prohibited concomitant therapies - The corticosteroid-free remission secondary endpoint was updated to evaluate corticosteroid-free remission in all subjects rather than those on corticosteroids at baseline. - Language was updated regarding timing of screening OCTs and PFTs. - An exclusion criterion was added regarding treatment with topical rectal Chinese medicine, enemas or suppositories prior to randomization.

None of the changes had a major impact on the overall study design, efficacy assessments, or negatively impacted collection of safety data.

8.1.2. Trial Results (APD334-301)

Patient Disposition

The “FDA Preferred Analysis Population” consisted of a total of 408 subjects, 274 in the etrasimod 2 mg group and 134 in the placebo group; 55% (n=150) in the etrasimod group and

28% (n=38) in the placebo group completed the trial. The disposition of subjects in the “FDA Preferred Analysis Population” was similar to the FAS.

In both the placebo and etrasimod groups, the most frequently reported reasons for trial discontinuation were disease worsening, withdrawal by subject, and adverse events. Table 28 summarizes subject disposition by treatment group.

Table 28. Subject Disposition, FDA Preferred Analysis Population, APD334-301

Subject Disposition	Etrasimod 2 mg N=274 n (%)	Placebo N=134 n (%)
Completed the trial	150 (54.7)	38 (28.4)
Discontinued the trial	124 (45.3)	96 (71.6)
Disease worsening ¹	78 (28.5)	73 (54.5)
Withdrawal by subject	22 (8.0)	8 (6.0)
Adverse event	10 (3.6)	4 (3.0)
Lack of efficacy	7 (2.6)	4 (3.0)
Physician decision	2 (0.7)	2 (1.5)
Other ²	2 (0.7)	2 (1.5)
Pregnancy	2 (0.7)	0
Lost to follow-up	1 (0.4)	2 (1.5)
Protocol deviation	1 (0.4)	0

Source: Reviewer generated table using JMP based on the Applicant submitted data adsl.xpt

¹ Subjects with disease worsening were defined as having at least 1 of the following criteria: 1) absolute RBS ≥ 2 on 2-consecutive days and confirmation of ES ≥ 2 , 2) absolute RBS + SFS ≥ 4 on 2-consecutive days and confirmation of ES ≥ 2 , or 3) absolute RBS ≥ 2 or RBS + SFS ≥ 4 (in any order) on 2-consecutive days and confirmation of ES ≥ 2 .

²Other included migration to USA (1), randomized in error (1), departure to another country (1), unable to return to the country due to COVID-19 pandemic (1)

³ Wald-type confidence interval using normal distribution approximation

Abbreviations: CI, confidence interval; N, number of subjects in treatment arm; n, number of subjects in specified population or group

Protocol Violations/Deviations

A total of 212 (52%) subjects had at least 1 important protocol deviation, including 145 (53%) subjects in the etrasimod group and 67 (50%) subjects in the placebo group. The most frequently reported (> 5% subjects) important protocol deviations by category were “Study Procedures” (e.g., tests were not performed during the trial due to COVID-19, subjects did not return IP bottle due to non-compliance), “Laboratory Assessment”, “Eligibility and Entry Criteria” (e.g., tests were not performed during screening visit due to COVID-19, subjects were randomized into the trial despite meeting exclusion criteria due to an oversight), “Concomitant Medication” (e.g., baseline corticosteroid dose was changed, corticosteroids were not tapered after Week 12), and “Visit Schedule.” The most common protocol deviations in both cohorts were “Study Procedures and Laboratory Assessment”, followed by “Eligibility and Entry Criteria” in the etrasimod group, and “Concomitant medication” in the placebo group. The number and distribution of protocol deviations were similar across both groups.

Table of Demographic Characteristics

The demographic characteristics are summarized by treatment group in Table 29. Overall, the

majority of patients were male (53% and 59% in etrasimod and placebo groups, respectively) and most were White (89% in both treatment groups). The mean age at consent was approximately 42 years and 39 years for etrasimod and placebo groups, respectively, with a range in ages from 18-78 years for both groups. The mean BMI was 25kg/m² in both groups, ranging from 13kg/m² to 51kg/m² in the etrasimod group, and 16kg/m² to 50 kg/m² in the placebo group. Demographic characteristics were generally similar between treatment groups.

It is notable that most subjects were enrolled outside the U.S. However, as there are no well-established differences in UC disease severity or response to treatment by different ethnic groups, and the disease activity scores and documentation of prior treatment failure were standardized by the protocol, it is reasonable to generalize the result to the U.S. population.

Table 29. Baseline Demographic Characteristics, FDA Preferred Analysis Population, APD334-301

Demographic Parameters	Etrasimod 2 mg (N=274)	Placebo (N=134)	Total (N=408)
Sex			
Male, n (%)	144 (52.6)	79 (59.0)	223 (54.7)
Female, n (%)	130 (47.4)	55 (41.0)	185 (45.3)
Age			
Mean years (SD)	41.6 (14.0)	38.8 (13.9)	40.7 (14.1)
Median (years)	40	35	38
Min, max (years)	18, 78	18, 78	18, 78
Age group			
18 – 64 years, n (%)	257 (93.8)	124 (92.5)	381 (93.4)
≥ 65 years, n (%)	17 (6.2)	10 (7.5)	27 (6.6)
BMI (kg/m²)			
Mean (SD)	25.4 (5.6)	25.3 (5.3)	25.3 (5.5)
Median	24.5	25.2	24.6
Min, max	13.0, 51.1	16.2, 49.7	13.0, 51.1
Race			
White, n (%)	244 (89.1)	119 (88.8)	363 (89.0)
Black or African American	6 (2.2)	3 (2.2)	9 (2.2)
Asian, n (%)	19 (6.9)	9 (6.7)	28 (6.9)
American Indian or Alaska Native, n (%)	1 (0.3)	3 (2.2)	4 (1.0)
Native Hawaiian or Other Pacific Islander, n (%)	0	0	0
Not reported, n (%)	4 (1.5)	0	4 (1.0)
Ethnicity			
Hispanic or Latino, n (%)	12 (4.4)	7 (5.2)	19 (4.4)
Not Hispanic or Latino, n (%)	260 (94.9)	126 (94.0)	386 (94.9)
Not reported, n (%)	1 (0.4)	1 (0.7)	2 (0.5)
Unknown, n (%)	1 (0.4)	0	1 (0.2)
Region			
Eastern Europe, n (%)	171 (62.4)	81 (60.4)	252 (61.8)
North America, n (%)	47 (17.2)	29 (21.6)	76 (18.6)
Western Europe, n (%)	23 (8.4)	11 (8.2)	34 (8.3)
Other, n (%)	33 (12.0)	13 (9.7)	46 (11.3)

Source: Reviewer generated table based on the Applicant submitted data adsl.xpt

Abbreviations: N, number of subjects in treatment group; n, number of subjects with given characteristic; SD, standard deviation
Eastern Europe: Bulgaria, Belarus, Croatia, Czech Republic, Estonia, Georgia, Hungary, Latvia, Lithuania, Moldova, Poland, Romania, Russian Federation, Serbia, Slovakia, Ukraine

Other: Australia, Chile, India, Israel, Mexico, South Africa, South Korea, Thailand, Turkey;

North America: Canada, United States of America;

Western Europe: Austria, Belgium, Denmark, France, Germany, Italy, Netherlands, Portugal, Spain, United Kingdom.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Other baseline characteristics including baseline mMS, total Mayo score, duration of disease, and prior treatment are shown in Table 30. Overall, the baseline demographic characteristics were similar between treatment groups.

Table 30. Baseline Disease Characteristics, FDA Preferred Analysis Population, APD334-301

Parameters	Etrasimod 2		
	mg (N=274)	Placebo (N=134)	Total (N=408)
Naïve to biologic or JAK inhibitor therapy			
Yes, n (%)	194 (70.8)	93 (69.4)	287 (70.3)
No, n (%)	80 (29.2)	41 (30.6)	121 (29.7)
Exposure to >1 biologic or JAK inhibitor therapy			
Yes, n (%)	39 (14.2)	18 (13.4)	57 (14.0)
No, n (%)	235 (85.8)	116 (86.6)	351 (86.0)
Baseline corticosteroid use			
Yes, n (%)	87 (31.2)	40 (29.9)	127 (31.1)
No, n (%)	187 (68.2)	94 (70.1)	281 (68.9)
Baseline oral 5-aminosalicylic acid compounds use			
Yes, n (%)	188 (68.6)	88 (65.7)	276 (67.6)
No, n (%)	86 (31.4)	46 (34.3)	132 (32.4)
Baseline mMS			
Mean (SD)	6.9 (1.0)	6.8 (1.0)	6.9 (1.0)
Median	7	7	7
Min, max	5, 9	5, 9	5, 9
Baseline mMS group			
5 to 6, n (%)	98 (35.8)	48 (35.8)	146 (35.8)
7 to 9, n (%)	176 (64.2)	86 (64.2)	262 (64.2)
Disease extent ¹			
Left-sided colitis/proctosigmoiditis	161 (59.2)	81 (60.9)	242 (59.8)
Pancolitis	92 (33.8)	46 (34.6)	138 (34.1)
Isolated proctitis	19 (7.0)	6 (4.5)	25 (6.2)

Source: Reviewer generated table based on the Applicant submitted data adsl.xpt, and adcm.xpt

¹ Information obtained from patients' medical history included in case report forms

Abbreviations: N, number of subjects in treatment group; n, number of subjects with given characteristic; SD, standard deviation; mMS, modified Mayo score

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Overall compliance was calculated as the total number of tablets taken divided by the total number of tablets expected during the treatment period. Subjects who took less than 80% or more than 120% of investigational drug during the entire treatment period were considered non-compliant. Treatment compliance was 98% for the etrasimod and placebo groups, respectively.

Subjects must have had inadequate response to, loss of response to, or intolerance to at least 1 prior approved therapy for UC, including oral 5-ASAs, corticosteroids, thiopurines, biologics, and JAK inhibitors. Oral 5-ASA, AZA, 6-MP, oral corticosteroids, or medicinal probiotics were permitted at screening and as per the inclusion criteria (Trial APD334-301 Protocol Section 4.1). Concomitant treatments for UC were required to be stable prior to and during the trial and

could not be initiated during screening or the treatment periods. Immunosuppressive agents such as oral AZA and 6-MP were to be discontinued ≥ 2 weeks prior to randomization. The majority of subjects received at least 1 concomitant medication for UC during the trial (89% in the etrasimod group and 88% in the placebo group), the majority of which were oral 5-ASAs.

Efficacy Results – Co-Primary Endpoints

A total of 433 subjects were in the FAS population, among which 409 subjects had baseline mMS of 5 to 9, including an ES of at least 2. The Applicant's pre-specified primary analysis was performed on subjects in the FAS population with baseline mMS of 5 to 9, including an ES of at least 2, which included one adolescent subject. (b) (4)

The adolescent subject was excluded from the efficacy analysis population, resulting in a total of 408 subjects (i.e., the "FDA Preferred Analysis Population"). Unless otherwise specified, all efficacy results in this review are based on the FDA Preferred Analysis Population defined as all adult subjects in the FAS with baseline mMS of 5 to 9, including an ES of at least 2.

The Applicant's definition of clinical remission was different from the FDA preferred definition of clinical remission. Refer to Section 8.1.1 for details. The review team recommended the FDA preferred definition for assessment of the coprimary endpoint and for labeling, because the definition corresponds to the Agency's currently recommended definition of clinical remission as described in the draft guidance for industry *Ulcerative Colitis: Developing Drugs for Treatment* (April 2022).³ Unless otherwise specified, the FDA preferred definition of clinical remission is used in all applicable efficacy analyses.

On December 18, 2022, FDA sent an information request to the Applicant, asking the Applicant to provide new analyses on all multiplicity-controlled endpoints based on adult subjects in the FAS with baseline mMS of 5 to 9, including an ES of at least 2, and based on the FDA preferred definition of clinical remission. On Jan 11, 2023, the updated results were submitted and verified by the review team.

The primary efficacy results demonstrate superiority of etrasimod 2 mg compared to placebo in achieving clinical remission at Week 12 and Week 52, as summarized in Table 31. The estimated difference between etrasimod compared to placebo in the percentage of subjects achieving clinical remission was 20.1% at Week 12 and 25.7% at Week 52, with p-values less than 0.0001 for both comparisons.

The percentage of subjects with missing data was 9.9% for clinical remission at Week 12 and 44.5% for clinical remission at Week 52 in the etrasimod 2 mg group. The percent missing was 11.2% for clinical remission at Week 12 and 73.1% for clinical remission at Week 52 in the

³ When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the FDA guidance web page at <https://www.fda.gov/regulatory-information/search-fda-guidance-documents>.

placebo group. For the endpoint of clinical remission at Week 12, most of the missing data were due to trial discontinuation, with the main reasons for discontinuation being adverse events or withdrawal by subject. For clinical remission at Week 52, most of the missing data were due to trial discontinuation with the main reason for discontinuation being disease worsening in both arms. A higher dropout rate was observed in the placebo arm compared to the etrasimod 2 mg arm. A non-responder imputation approach was used to handle missing data regardless of the reason for dropout. As UC is a chronic condition and etrasimod is intended as a chronic treatment, subjects who prematurely discontinued treatment would not experience a long-term benefit from the trial treatment. Therefore, non-responder imputation was considered appropriate for handling missing data due to dropout.

Table 31. Results for Coprimary Endpoints, FDA Preferred Analysis Population, APD334-301

Parameter	Etrasimod 2 mg (N=274)	Placebo (N=134)
Clinical Remission at Week 12, n (%)	75 (27.4)	10 (7.5 ¹)
Missing data, n (%)	27 (9.9)	15 (11.2)
Treatment Difference ² , (95% CI)		20.1 (13.2, 27.0)
P-value ³		< 0.0001
Clinical Remission at Week 52, n (%)	89 (32.5 ⁴)	9 (6.7)
Missing data, n (%)	122 (44.5)	98 (73.1)
Treatment Difference ² , (95% CI)		25.7 (18.7, 32.7)
P-value ³		< 0.0001

Source: Review team's analysis using Applicant submitted data adeff.xpt, adqs.xpt, and adsl.xpt; based on the Applicant's information request response submitted on January 17, 2023

¹ The percentage is 7.46% and is rounded in the label as 7%.

² Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

³ The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

⁴ The percentage is 32.48% and is rounded in the label as 32%.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

Efficacy Results – Secondary and Other Relevant Endpoints

Efficacy results for the multiplicity-controlled endpoints proposed for labeling by the Applicant are presented in Table 32 assessed within the FDA preferred analysis population. The adjusted treatment differences between the etrasimod arm and the placebo arm were statistically significant for all the multiplicity-controlled secondary endpoints, with all p-values < 0.001.

Table 32. Results for Multiplicity-Controlled Secondary Endpoints, FDA Preferred Analysis Population, APD334-301

Endpoints	Etrasimod 2 mg n (%) (N=274)	Placebo n (%) (N=134)	Treatment Difference¹ versus Placebo (95% CI)	P-value²
Endoscopic improvement at Week 12	96 (35.0)	19 (14.2)	21.10 (12.92, 29.27)	<0.001
Endoscopic improvement at Week 52	102 (37.2)	14 (10.4)	26.65 (18.93, 34.37)	<0.001
Symptomatic remission at Week 12	126 (46.0)	29 (21.6)	24.42 (15.30, 33.54)	<0.001
Symptomatic remission at Week 52	119 (43.4)	25 (18.7)	24.77 (16.02, 33.51)	<0.001
Corticosteroid-free clinical remission ³ at Week 52	89 (32.5 ⁴)	9 (6.7)	25.74 (18.73, 32.74)	<0.001
Mucosal healing ⁵ at Week 12	58 (21.2)	6 (4.5 ⁶)	16.85 (10.73, 22.96)	<0.001
Mucosal healing ⁵ at Week 52	73 (26.6)	11 (8.2)	18.35 (11.33, 25.37)	<0.001
Sustained clinical remission at Week 52 ⁷	50 (18.2)	3 (2.2)	16.21 (10.98, 21.43)	<0.001

Source: Review team's analysis using Applicant submitted data adefx.xpt, adsl.xpt, and adqs.xpt; based on the Applicant's information request response submitted on January 17, 2023

¹ Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

² The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

³ Corticosteroid-free clinical remission is defined as clinical remission at Week 52 without receiving corticosteroids for at least 12 weeks prior to Week 52.

⁴ The percentage is 32.48% and is rounded in the label as 32%.

⁵ The endpoint will be called "histologic-endoscopic mucosal improvement" in the label.

⁶ The percentage is 4.47% and is rounded in the label as 4%.

⁷ Sustained clinical remission at Week 52 is defined as achievement of clinical remission at both Week 12 and Week 52. This endpoint will be called "maintenance of clinical remission" in the label.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

The efficacy results for endpoints that were not multiplicity-controlled but were proposed for labeling by the Applicant are displayed in Table 33. The etrasimod arm showed numerically higher response/remission rates compared to the placebo arm for all endpoints listed in Table 33, nominal p-values were less than 0.001.

Table 33. Results for Additional Non-Multiplicity-Controlled Endpoints, FDA Preferred Analysis Population, APD334-301

Endpoints	Etrasimod 2 mg n (%) (N=274)	Placebo n (%) (N=134)	Treatment Difference ¹ vs. Placebo (95% CI)	Nominal P-value ²
Clinical response ³ at Week 12	171 (62.4)	46 (34.3)	28.05 (18.26, 37.84)	<0.001
Clinical response ³ at Week 52	132 (48.2)	31 (23.1)	24.81 (15.64, 33.99)	<0.001
Endoscopic remission ⁴ at Week 12	40 (14.6)	6 (4.5 ⁵)	10.21 (4.69, 15.73)	<0.001
Endoscopic remission ⁴ at Week 52	72 (26.3)	8 (6.0)	20.37 (13.75, 26.98)	<0.001
Endoscopic remission ⁴ at both Week 12 and Week 52	29 (10.6)	2 (1.5 ⁶)	9.16 (4.91, 13.40)	<0.001
Endoscopic remission and Geboes histologic score < 2.0 at Week 12	29 (10.6)	2 (1.5)	9.15 (4.95, 13.36)	<0.001
Endoscopic remission and Geboes histologic score < 2.0 at Week 52	50 (18.2)	7 (5.2)	13.10 (7.18, 19.02)	<0.001
Corticosteroid-free clinical remission ⁷ at Week 52 among subjects treated with corticosteroids at baseline	(N = 87) 27 (31.0)	(N = 40) 3 (7.5 ⁸)	23.0 (10.2, 35.9)	<0.001

Source: Review team's analysis using Applicant submitted data adefx.xpt, adsl.xpt, and adqs.xpt

¹ Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

² The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

³ Clinical response was defined as a ≥ 2 point and $\geq 30\%$ decrease from baseline in mMS, and a ≥ 1 point decrease from baseline in RBS or an absolute RBS ≤ 1 .

⁴ Endoscopic remission was defined as ES of 0.

⁵ The percentage is 4.48% and is rounded in the label as 4%.

⁶ The percentage is 1.49% and is rounded in the label as 1%.

⁷ Corticosteroid-free clinical remission is defined as clinical remission at Week 52 without receiving corticosteroids for at least 12 weeks prior to Week 52.

⁸ The percentage is 7.5% and is rounded in the label as 8%.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

Discussion of Primary and Secondary Endpoints Proposed for Labeling

Clinical Remission

The Applicant's proposed definition of clinical remission differs from the FDA preferred definition of clinical remission due to the requirement for subjects to demonstrate at least 1-point decrease from baseline in the SFS. The efficacy results for clinical remission using the FDA preferred definition and the Applicant's definition were similar and both were statistically significant. To align with the Division's current thinking, the team concluded that the efficacy endpoint of clinical remission based on the FDA preferred definition will be included in the label to replace the Applicant's prespecified coprimary endpoints of clinical remission (which

includes a requirement of at least 1-point decrease from baseline in the SFS).

Endoscopic Improvement

The Applicant's proposed definition of endoscopic improvement aligns with the Division's recommended definition. Achievement of resolution of gross inflammation that can be visualized on endoscopy is a clinically relevant outcome of interest, as patients who achieve endoscopic improvement have been shown to have better outcomes than those with continued active inflammation. Therefore, this endpoint will be included in labeling.

Symptomatic Remission

The Applicant defined symptomatic remission as a SFS of 0 (or 1 with at least 1-point decrease from baseline), and RBS of 0 on the mMS. Therefore, symptomatic remission is the same as clinical remission (based on the Applicant's definition) without the endoscopy assessment. There is no consensus on the definition of the term "symptomatic remission" and the concept is redundant to the primary endpoint given that improvement in stool frequency and rectal bleeding are incorporated in clinical remission. Therefore, the endpoint of symptomatic remission will not be included in labeling. However, there was a trend toward more subjects experiencing improvement in SFS at Week 2 and RBS at Week 4. This information is useful to clinicians to help characterize the anticipated onset of improvement in symptoms. Therefore, this information will be included descriptively in labeling. Refer to the Discussion of Additional Endpoints below.

Histologic-Endoscopic Mucosal Improvement

The endpoint definition for "mucosal healing" represents an important clinical concept, incorporating both improvement of the endoscopic appearance of the mucosa (ES $\leq$ 1 excluding friability) and improvement in histologic abnormalities, including absence of any neutrophilic inflammation (Geboes score $<$ 2.0). This combination represents an important clinical endpoint and will be reflected in labeling. However, there is no standard definition for "mucosal healing" and the definition varies depending on the source. There are also uncertainties regarding the assessment of the histologic component of this endpoint, including the potential limitation of obtaining biopsies from a single segment of the colon, as the degree of histologic inflammation may vary by location of the biopsies. Although improvement in histologic findings may be observed in the location from which the biopsy is taken, there is uncertainty as to whether other locations in the colon have also improved. Therefore, the endpoint will be described in labeling as histologic-endoscopic mucosal improvement, rather than "mucosal healing."

The Applicant utilized a definition of ES of 0 or 1 (excluding friability) and a Geboes score of $<$ 2.0, which represents no neutrophils in the epithelial crypts or lamina propria, no increase in eosinophils, and no crypt destruction, erosions, ulcerations, or granulation tissue.

The Applicant proposed that the clinical relevance of this definition was supported by literature. For example, the Applicant described that the improvement, and resolution, of

inflammation in UC, as measured by a histologic assessment and endoscopic measures [respectively], compared to endoscopic measures alone, is associated with lower rates of clinical relapse, corticosteroid use, hospitalization, and colectomy. Furthermore, the Applicant stated that a “using very conservative standards (i.e., Geboes < 2.0, indicating neutrophil absence in the epithelium and no increase detected in the lamina propria) is supported by International Consensus and Position Papers.”

Although the review team agrees with this justification, there were some important limitations to the histologic evaluation in this program. Thirty-three subjects (approximately 8%) entered the trial with a Geboes score of <2.0 on the baseline biopsy, thus meeting the histologic component of the endpoint definition. Therefore, it was unclear whether achieving histologic-endoscopic mucosal improvement represents an improvement for subjects with a Geboes score of <2.0 at baseline. The review team conducted a sensitivity analysis in which this endpoint was reanalyzed only in the subgroup of subjects with a baseline Geboes score of ≥ 2.0 . Results were consistent with those obtained in the FDA preferred analysis population, and therefore the team concluded that it is reasonable to include this endpoint in the label.

Additionally, although investigators were instructed to perform biopsies on the mucosa “most representative of the inflammation seen in the region,” there may be variation in histologic findings due to sampling. UC is generally considered to involve continuous segments of the GI tract; however, discontinuous patterns of inflammation may also be observed, particularly in subjects with a prior history of treatment (Bernstein et al. 1995). Furthermore, the clinical significance of achieving “histologic improvement” (in addition to endoscopic improvement) has not been well characterized and is limited by the various definitions used in the literature to define this concept (Battat et al. 2019). Due to these limitation, the label will indicate that the relationship between any histologic-endoscopic mucosal improvement endpoint and disease progression and longer-term outcomes beyond 52 weeks has not been assessed.

Discussion of Additional Endpoints

Clinical Response

Clinical response at Week 12 is a clinically meaningful endpoint. Although clinical response at Week 12 was not multiplicity-controlled, the etrasimod group had a numerically higher proportion of clinical responders in comparison with those treated with placebo, with an observed p-value less than 0.001. Clinical response at Week 52 is less clinically important than clinical response at Week 12 as subjects were expected to achieve clinical remission at Week 52, which has a more stringent definition. Therefore, language describing only the results of clinical response at Week 12 will be included in labeling.

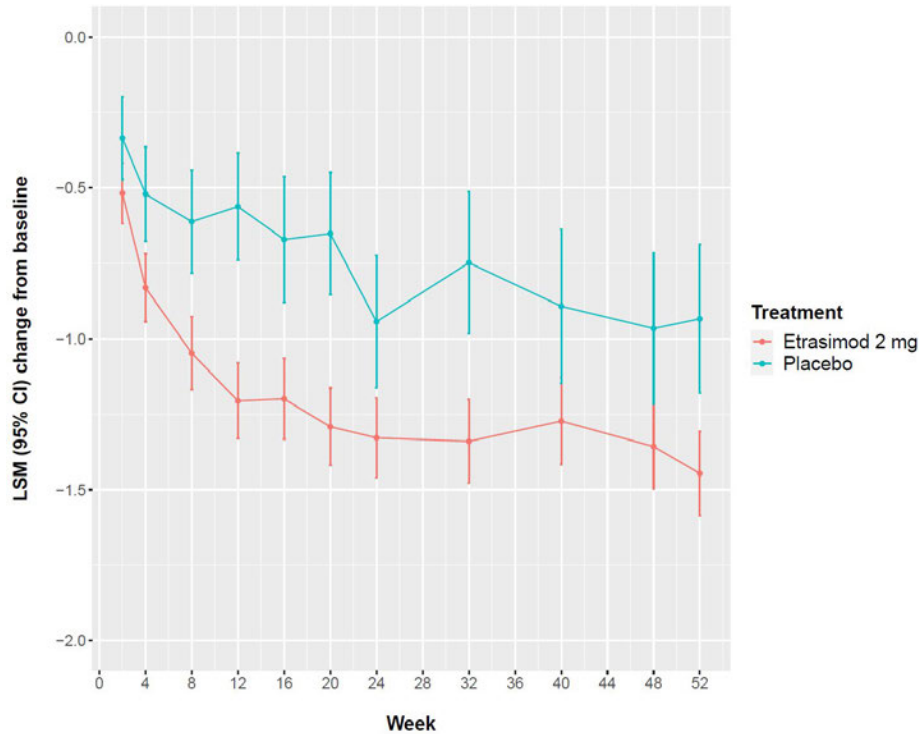
Stool Frequency and Rectal Bleeding Subscores

Individual analyses of the SFS and RBS of the mMS in APD334-301 showed evidence that subjects treated with etrasimod reported symptomatic relief after only a few weeks of dosing (Figure 9 and Figure 10). Subjects in the etrasimod treatment group showed a nominally

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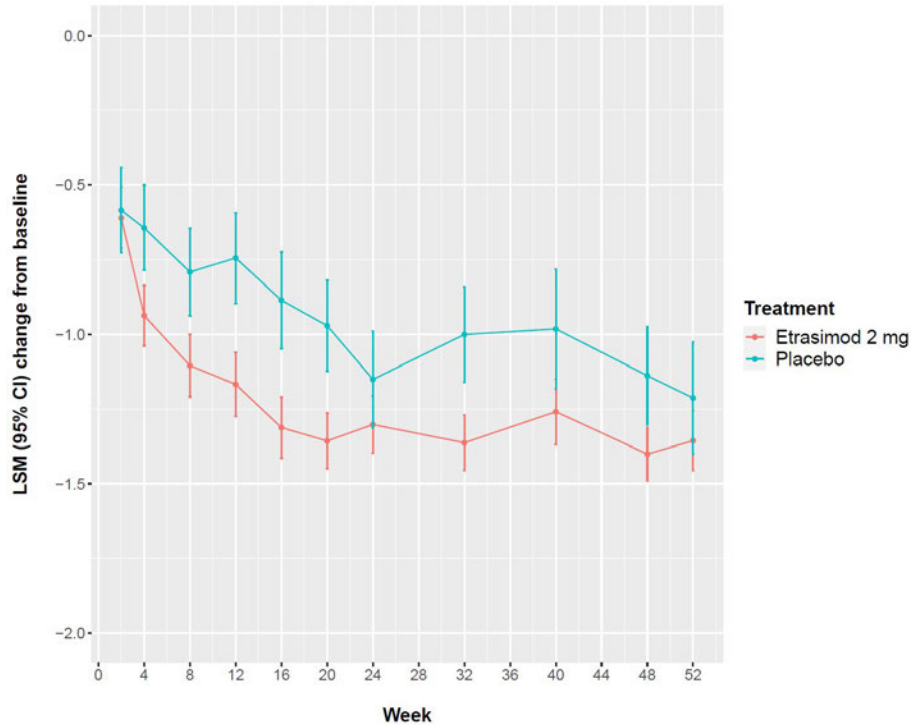
significant and greater mean reduction in SFS as early as Week 2 (nominal p-value = 0.0258) and in RBS as early as Week 4 (nominal p-value = 0.0007) compared to the placebo group based on the estimates from a mixed-effect model with repeated measures (MMRM). The improvement from baseline in SFS and RBS was sustained in the etrasimod group starting from Week 20.

Figure 9. Stool Frequency Subscore Change from Baseline, FDA Preferred Analysis Population, APD334-301



Source: Reviewer generated figure based on the Applicant submitted data adqs.xpt.
Estimates are from a mixed-effect model with repeated measures for change from baseline with a covariate for baseline score, and factors for naive to biologic/JAK inhibitor therapy at trial entry (Yes/No), baseline corticosteroid use (Yes/No), baseline disease activity (mMS: 4 to 6 or 7 to 9), treatment, visit, treatment by visit interaction.
Abbreviations: LSM, least-square mean; CI, confidence interval.

Figure 10. Rectal Bleeding Subscore Change from Baseline, FDA Preferred Analysis Population, APD334-301



Source: Reviewer generated figure based on the Applicant submitted data adqs.xpt. Estimates are from a mixed-effect model with repeated measures for change from baseline with a covariate for baseline score, and factors for naive to biologic/JAK inhibitor therapy at trial entry (Yes/No), baseline corticosteroid use (Yes/No), baseline disease activity (mMS: 4 to 6 or 7 to 9), treatment, visit, treatment by visit interaction. Abbreviations: LSM, least-square mean; CI, confidence interval.

Endoscopic Remission

The Applicant's definition of endoscopic remission is consistent with the Division's preferred definition of endoscopic remission. Although the endoscopic remission endpoint was not multiplicity-controlled, this endpoint demonstrated a nominally significant effect and represents a clinically relevant endpoint that is informative to prescribers as part of their clinical practice. Therefore, the results of endoscopic remission (at Week 12 and both Weeks 12 and 52) will be included descriptively in labeling.

Endoscopic Remission and Geboes Histologic Score < 2.0

This endpoint is similar to histologic-endoscopic mucosal improvement but included a more stringent definition of resolution of endoscopic findings, characterized by normal appearing mucosa (ES = 0). However, this endpoint was not pre-specified in the protocol or in the SAP. Therefore, this endpoint will not be included in labeling.

Efficacy Results – Secondary or Exploratory COA (PRO) Endpoints

Bowel Urgency and Abdominal Pain

Bowel urgency is a common symptom of UC and is associated with reduced quality of life (Dubinsky et al. 2022). The Applicant assessed bowel urgency severity with the Urgency Numeric Rating Scale (NRS) patient-reported outcome (PRO) measure, a single-item PRO measure assessing severity of a subject's urgency (the sudden or immediate need) to have a bowel movement within the past 24 hours using an 11-point NRS ranging from 0 ("No urgency") to 10 ("Worst possible urgency").

Abdominal pain is another common symptom of UC that is an important treatment objective for patients. The Applicant assessed abdominal pain with the Abdominal Pain NRS PRO measure, a single item PRO measure assessing severity of a subject's abdominal pain within the past 24 hours using an 11-point NRS ranging from 0 ("No Pain") to 10 ("Pain as Bad as Can Imagine").

The Urgency NRS and Abdominal Pain NRS were collected at baseline, Week 12, and Week 52. An ad hoc analysis of the proportion of subjects with "absence of urgency" defined as an Urgency NRS score of 0 and "absence of abdominal pain" defined as an Abdominal Pain NRS score of 0 was performed at Week 12 and Week 52. Of note, no data were collected between these timepoints.

A greater proportion of subjects treated with etrasimod 2 mg reported an Urgency NRS of 0 than placebo at Week 12 (19.3% vs 7.4%) and Week 52 (18.6% vs 8.1%). A greater proportion of subjects treated with etrasimod 2 mg reported an Abdominal Pain NRS of 0 than placebo at Week 12 (26.6% vs 13.3%) and Week 52 (21.5% vs 7.4%).

Although bowel urgency and abdominal pain are common and important symptoms of UC, the results of the Urgency NRS and Abdominal Pain NRS are not recommended for inclusion in labeling due to the ad hoc nature of the analyses (i.e., not multiplicity controlled and performed after unblinding). Additionally, given the recall period of "the past 24 hours" for the bowel urgency and abdominal pain assessments, data collected at baseline, Week 12, and Week 52 would only be representative of patients' experiences in the past 24 hours at each respective assessment time point. Therefore, the results are difficult to interpret as UC is a chronic disease that can relapse and remit.

Inflammatory Bowel Disease Questionnaire

The inflammatory bowel disease questionnaire (IBDQ) is an instrument used to assess health-related quality of life. The Division had previously determined that IBDQ is not appropriate to support labeling claims. The instrument combines many concepts and impacts, and some of the questions are double-barreled or otherwise unclear. Therefore, the results of the IBDQ are not recommended for inclusion in labeling.

Additional Analyses Conducted on the Individual Trial

Efficacy results were investigated by prior biologic or JAK inhibitor exposure status for multiplicity-controlled endpoints in APD334-301, as summarized in Table 34. Although the trial was not powered to detect a significant difference for each subgroup, the treatment differences were consistent with the primary analysis. The etrasimod group consistently showed higher remission and response rates compared to the placebo group across all subgroups. In general, the observed remission and response rates were higher in the subgroup with no prior biologic/JAK inhibitor exposure versus the subgroup with prior biologic/JAK inhibitor exposure for both etrasimod and placebo, which was expected because patients with prior biologic/JAK inhibitor exposure are typically more refractory to treatment. The subgroup with no prior exposure to biologics/JAK inhibitors generally had a larger treatment difference between etrasimod and placebo, though the estimated treatment differences for endoscopic improvement and mucosal healing endpoints were similar across subgroups.

Table 34. Subgroup Analyses, No Prior Biologic/JAK Inhibitor Exposure Versus Prior Biologic or JAK Inhibitor Exposure, FDA Preferred Analysis Population, APD334-301

Endpoints	Etrasimod 2 mg (N=274)		Placebo (N=134)		Treatment Difference (95% CI) ¹
	n/N	%	n/N	%	
Clinical remission at Week 12					
No prior biologic/JAK inhibitor exposure	61/194	31.4	9/93	9.7	21.8 (12.9, 30.6)
Prior biologic or JAK inhibitor exposure	14/80	17.5 ²	1/41	2.4	15.1 (5.5, 24.6)
Clinical remission at Week 52					
No prior biologic/JAK inhibitor exposure	72/194	37.1	7/93	7.5 ³	29.6 (20.9, 38.2)
Prior biologic or JAK inhibitor exposure	17/80	21.2	2/41	4.9	16.4 (5.2, 27.5)
Endoscopic improvement at Week 12					
No prior biologic/JAK inhibitor exposure	76/194	39.2	17/93	18.3	20.9 (10.5, 31.3)
Prior biologic or JAK inhibitor exposure	20/80	25.0	2/41	4.9	20.1 (8.6, 31.7)
Endoscopic improvement at Week 52					
No prior biologic/JAK inhibitor exposure	78/194	40.2	12/93	12.9	27.3 (17.6, 37.0)
Prior biologic or JAK inhibitor exposure	24/80	30.0	2/41	4.9	25.1 (13.1, 37.1)
Symptomatic remission at Week 12					
No prior biologic/JAK inhibitor exposure	101/194	52.1	22/93	23.7	28.4 (17.3, 39.5)
Prior biologic or JAK inhibitor exposure	25/80	31.2	7/41	17.1	14.2 (-1.2, 29.5)
Symptomatic remission at Week 52					
No prior biologic/JAK inhibitor exposure	97/194	50.0	19/93	20.4	29.6 (18.8, 40.4)
Prior biologic or JAK inhibitor exposure	22/80	27.5	6/41	14.6	12.9 (-1.7, 27.4)
Corticosteroid-free clinical remission at Week 52					
No prior biologic/JAK inhibitor exposure	72/194	37.1	7/93	7.5 ³	29.6 (20.9, 38.2)
Prior biologic or JAK inhibitor exposure	17/80	21.2	2/41	4.9	16.4 (5.2, 27.5)
Mucosal healing ⁴ at Week 12					
No prior biologic/JAK inhibitor exposure	47/194	24.2	6/93	6.5 ⁵	17.8 (9.9, 25.6)
Prior biologic or JAK inhibitor exposure	11/80	13.8	0/41	0	13.8 (6.2, 21.3)

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Endpoints	Etrasimod 2 mg (N=274)		Placebo (N=134)		Treatment Difference (95% CI) ¹
	n/N	%	n/N	%	
Mucosal healing ⁴ at Week 52					
No prior biologic/JAK inhibitor exposure	55/194	28.4	10/93	10.8	17.6 (8.7, 26.5)
Prior biologic or JAK inhibitor exposure	18/80	22.5 ⁶	1/41	2.4	20.1 (9.8, 30.4)
Sustained clinical remission at Week 52 ⁶					
No prior biologic/JAK inhibitor exposure	42/194	21.6	2/93	2.2	19.5 (13.0, 26.0)
Prior biologic or JAK inhibitor exposure	8/80	10.0	1/41	2.4	7.6 (-0.5, 15.7)

Source: Review team's analysis based on Applicant submitted data adeff.xpt, adsl.xpt, and adqs.xpt.

¹ The 95% confidence intervals of the treatment effects were constructed by the Wald approach.

² The percentage is 17.5% and is rounded in the label as 18%.

³ The percentage is 7.53% and is rounded in the label as 8%.

⁴ The endpoint will be called "histologic-endoscopic mucosal improvement" in the label.

⁵ The percentage is 6.45% and is rounded in the label as 6%.

⁶ The percentage is 22.5% and is rounded in the label as 23%.

⁶ Sustained clinical remission at Week 52 is defined as achievement of clinical remission at both Week 12 and Week 52. This endpoint will be called "maintenance of clinical remission" in the label.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

The subgroup analyses for efficacy conducted by use of corticosteroids at baseline for the co-primary endpoints were presented in Table 35. The etrasimod group showed higher remission rates compared to the placebo group across all subgroups. The treatment difference at Week 12 is small in the group with use of corticosteroids at baseline compared to the group who did not use corticosteroids at baseline. The smaller treatment difference is mostly due to a numerically higher remission rate for placebo subjects who used corticosteroids at baseline compared to those who did not use corticosteroids at baseline. However, a larger benefit was demonstrated in the subgroup with baseline corticosteroids use at Week 52, indicating long-term benefit of etrasimod in subjects who used corticosteroids at baseline.

Table 35. Subgroup Analyses, Coprimary Endpoints, by Corticosteroids Use at Baseline, FDA Preferred Analysis Population, APD334-301

Endpoints	Etrasimod 2 mg (N=274)		Placebo (N=134)		Treatment Difference (95% CI) ¹
	n/N	%	n/N	%	
Clinical remission at Week 12					
Corticosteroids use at baseline	27/87	31.0	7/40	17.5	13.5 (-1.7, 28.8)
No corticosteroids use at baseline	48/187	25.7	3/94	3.2	22.5 (15.3, 29.7)
Clinical remission at Week 52					
Corticosteroids use at baseline	27/87	31.0	3/40	7.5	23.5 (10.8, 36.2)
No corticosteroids use at baseline	62/187	33.2	6/94	6.4	26.8 (18.4, 35.1)

Source: Review team's analysis based on Applicant submitted data adefx.xpt, adsl.xpt, and adqs.xpt.

¹ The 95% confidence intervals of the treatment effects were constructed by the Wald approach.

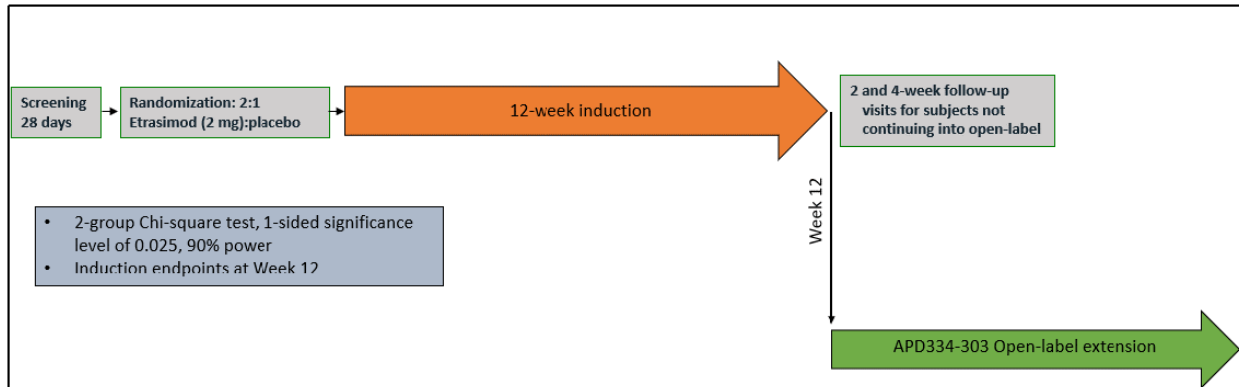
Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

8.1.3. APD334-302

Trial Design

Trial APD334-302 was a multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of etrasimod 2 mg, compared with placebo, over a 12-week induction period. The trial population included subjects with a modified Mayo Score (mMS) of 4 to 9 who had an inadequate response to, loss of response to, or intolerance to at least 1 of the following: oral 5-aminosalicylic acid (5-ASA) compounds, corticosteroids, thiopurines, biologic therapy (TNF blocker, vedolizumab, anti-IL 12/23), or Janus kinase (JAK) inhibitors. A total of 354 subjects, including 2 adolescent subjects, from 39 countries were randomized in a 2:1 ratio to receive either etrasimod 2 mg once daily or matching placebo once daily for up to 12 weeks. Randomization was stratified by prior treatment status (i.e., naïve to biologic or JAK inhibitor therapy at trial entry [yes/no]), baseline corticosteroid use (yes/no), and baseline disease activity (mMS: [4-6] or [7-9]). The overall trial design for APD334-302 is shown in Figure 11.

Figure 11. Trial Design for APD334-302



Source: clinical study report for APD334-302, Figure 1 (page 20).
Abbreviation: JAK, Janus kinase; UC, ulcerative colitis

Trial Endpoints

The primary efficacy endpoint was the proportion of subjects achieving clinical remission at Week 12. The Applicant’s definition was the same as APD334-301; refer to Section 8.1.1 for additional details. The multiplicity-controlled secondary efficacy endpoints are shown in Table 36 and were subject to overall type I error control (tested based on a prespecified gatekeeping approach). Refer to Section 16.4.1 for additional details on the multiple testing procedure.

Table 36. Multiplicity-Controlled Secondary Efficacy Endpoints for Study APD334-302

Secondary Endpoint	Definition
Endoscopic improvement at Week 12	ES ≤ 1 (excluding friability)
Symptomatic remission at Week 12	SFS = 0 (or = 1 with a ≥ 1 point decrease from baseline) and RBS of 0
Mucosal healing at Week 12	ES of ≤ 1 (excluding friability) with histologic remission measured by a Geboes Index score < 2.0

Source: Review team’s table

Abbreviation: ES, endoscopic subscore; SFS, stool frequency subscore; RBS, rectal bleeding subscore

Statistical Analysis Plan

Populations

The Applicant’s primary efficacy analyses were based on subjects in the FAS with baseline mMS of 5 to 9. The FAS consisted of all randomized subjects who received at least 1 dose of trial treatment. (b) (4)

The two adolescent subjects were excluded from the efficacy analysis population, resulting in the “FDA Preferred Analysis Population.” The safety population included all randomized subjects (including adolescents and subjects with mMS of 4) who received at least 1 dose of trial treatment, which is used for safety-related analysis.

Sample Size

Based on a 2-sided Fisher's exact test with a 1-sided significance level of 0.025, and a 2:1 randomization ratio, a sample size of 330 subjects (220 etrasimod, 110 placebo) was expected to provide at least 90% power to detect a difference of 12.5% in the primary endpoint of clinical remission between the etrasimod treatment group and the placebo treatment group, assuming a 6.0% remission rate for the placebo group.

Calculation of SFS and RBS

Similar to APD334-301, the primary efficacy endpoint for APD334-302 was evaluated using the mMS, which contains three components: SFS, RBS, and ES. For the SFS and RBS, the average of the most recent 3 consecutive days within the 7 days prior to the day of bowel preparation (excluding the day of bowel preparation) was rounded to the nearest integer to calculate the weekly score for each subject. If 3 consecutive days were not available, then the average of the 2 most recent consecutive days within the 7-day period was used. Sensitivity analyses were conducted that considered subjects with only two consecutive days as non-responders. The outcomes of these analyses were consistent with the primary efficacy results.

Analyses of Efficacy Endpoints

For assessments of the primary endpoint and multiplicity-controlled secondary endpoints, the estimated difference in proportions between arms and the corresponding 95% confidence interval were computed based on the stratified risk difference using the Cochran-Mantel-Haenszel (CMH) weights. The analyses adjusted for the following randomization stratification factors: naïve to biologic or JAK inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (mMS: [4-6] or [7-9]).

Estimands and Intercurrent Events

Similar to APD334-301, the estimand corresponding to the primary efficacy endpoint of clinical remission at Week 12 evaluated the difference in the proportion of subjects in clinical remission at Week 12 for etrasimod 2 mg versus placebo in subjects with baseline mMS of 5 to 9 in the FAS population. For the intercurrent events of 1) premature treatment discontinuation for worsening of disease, lack of efficacy, or adverse event related to UC, 2) initiation of a rescue medication for UC, 3) an increase in dose over baseline levels in subjects' existing UC medication, or 4) rescue medical procedure during the trial, subjects were considered non-responders.

Handling of Missing Data

For analyses of the efficacy endpoint and multiplicity-controlled secondary endpoints, missing data were imputed using non-responder imputation (NRI). Sensitivity analyses for all multiplicity-controlled endpoints included multiple imputation under missing at random (MAR), tipping point analysis, multiple imputation with Copy Reference (CR) under missing not at

random (MNAR), and a hybrid imputation with multiple imputation used to handle missing endoscopy data due to the impact of the COVID-19 pandemic and NRI used for missing data not due to the COVID-19 pandemic.

Multiplicity Adjustment

A gatekeeping multiple testing procedure was specified to control the overall Type I error rate at 2-sided alpha of 0.05, for all co-primary endpoints and major secondary endpoints. Refer to Figure 30 in Section 16.4.1 for the details of the gatekeeping procedure.

Protocol Amendments

The original study protocol for APD334-302 had three amendments. A summary of changes for each amendment are described in Table 37.

Table 37. Summary of Changes for APD334-302 Protocol Amendments

Amendment	Date	Summary of Changes
1.0	March 5, 2019	• Additional monitoring procedures were added to the cardiac monitoring required on Day 1 after the first dose of study treatment. Post-dose safety vital signs, 12-lead ECGs, monitoring of subjects who do not meet the discharge criteria, and discontinuation rules were revised to align with FDA feedback.
2.0	February 7, 2020	• Eligibility criteria related to contraceptive use was updated. • Additional instructions for safety monitoring related to ophthalmoscopy and optical coherence tomography (OCT) testing were included. • The prohibition of concomitant use of QT prolonging drugs was removed based on the results of the QT study (Study APD334-008). • An additional safety follow-up visit was added at 4 weeks. • Clarification and further instructions for tuberculosis testing were added and the tuberculosis screening questionnaire was updated.
3.0	February 22, 2021	• Guidance for conducting virtual and offsite visits was updated in the protocol. • Anti-arrhythmic drugs were added to prohibited concomitant therapies. • Language was updated regarding timing of screening OCTs and pulmonary function tests (PFTs). • An exclusion criterion was added regarding treatment with topical rectal Chinese medicine, enemas, or suppositories prior to randomization.

None of the changes had a major impact on the overall study design, efficacy assessments, or negatively impacted collection of safety data.

8.1.4. Trial Results (APD334-302)

Patient Disposition

The “FDA Preferred Analysis Population” consisted of a total of 333 subjects, 221 in the etrasimod 2 mg group and 112 in the placebo group, 89% (N=197) of whom completed the trial in the etrasimod group and 88% (N=99) in the placebo group. The disposition of subjects in the “FDA Preferred Analysis Population” was similar to the FAS.

The most frequently reported reason for trial discontinuation in the etrasimod group was due to an adverse event. In the placebo group, the most frequently reported reason for trial discontinuation was withdrawal by subject. Table 38 summarizes subject disposition by treatment group.

Table 38. Subject Disposition, FDA Preferred Analysis Population, APD334-302

Subject Disposition	Etrasimod 2 mg	Placebo
	N=221 n (%)	N=112 n (%)
Completed the trial	197 (89.1)	99 (88.4)
Discontinued the trial	24 (10.9)	13 (11.6)
Adverse event	9 (4.1)	0
Withdrawal by subject	5 (2.3)	8 (7.1)
Physician decision	4 (1.8)	2 (1.8)
Lack of efficacy	4 (1.8)	0
Other	1 (0.5)	1 (0.9)
Protocol deviation	1 (0.5)	1 (0.9)
Lost to follow-up	0	1 (0.9)

Source: adsl.xpt; JMP

¹ Other included **not meeting concomitant treatment criteria (1), did not follow-up due to COVID-19 pandemic (1)**

² **Wald-type confidence interval using normal distribution approximation**

Abbreviations: CI, confidence interval; N, number of subjects in treatment arm; n, number of subjects in specified population or group

Protocol Violations/Deviations

A total of 71 (21%) subjects had at least 1 important protocol deviation, including 50 (23%) subjects in the etrasimod group and 21 (19%) subjects in the placebo group. The most frequently reported (> 5% subjects) important protocol deviations by category were “Study Procedures” (e.g., tests or assessments were not performed during the trial), “Laboratory Assessment”, “Eligibility and Entry Criteria” (e.g., subjects were randomized into the trial despite meeting exclusion criteria due to an oversight.), and “Visit Schedule.” The most common protocol deviations in both cohorts were “Study Procedures.” The number and distribution of protocol deviations were similar across both groups.

Table of Demographic Characteristics

Baseline demographic characteristics from the FDA preferred analysis population are presented in Table 39. Overall, the baseline demographics were similar to APD334-301. The majority of patients were male (57% and 63% in etrasimod and placebo, respectively) and most were

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White (75% and 78% in etrasimod and placebo groups, respectively). The mean age at consent was approximately 41 years for etrasimod and placebo groups, with a range in ages from 18-73 years and 18-72 years, respectively. The mean BMI was 24kg/m² and 25 kg/m² in etrasimod and placebo groups, respectively, ranging from 16kg/m² to 40kg/m² in the etrasimod group, and 15kg/m² to 39kg/m² in the placebo group. Demographic characteristics were generally similar among treatment groups.

Similar to APD334-301, the majority of subjects were enrolled outside the U.S. However, as there are no well-established differences in UC disease severity or response to treatment by different ethnic groups, and the disease activity scores and documentation of prior treatment failure were standardized by the protocol, it is reasonable to generalize the result to the U.S. population.

Table 39. Baseline Demographic Characteristics, FDA Preferred Analysis Population, APD334-302

Demographic Parameters	Etrasimod 2 mg (N=221)	Placebo (N=112)	Total (N=333)
Sex			
Male, n (%)	125 (56.6)	70 (62.5)	195 (58.6)
Female, n (%)	96 (43.4)	42 (37.5)	138 (41.4)
Age			
Mean years (SD)	40.5 (13.6)	40.7 (13.2)	40.6 (13.4)
Median (years)	38	38.5	38
Min, max (years)	18, 73	18, 72	18, 73
Age Group			
18 to 64 years, n (%)	210 (95.0)	106 (94.6)	316 (94.9)
≥ 65 years, n (%)	11 (5.0)	6 (5.4)	17 (5.1)
BMI (kg/m²)			
Mean (SD)	24.4 (4.9)	25.4 (4.4)	24.7 (4.7)
Median	23.5	24.4	24.0
Min, max	15.8, 40.4	15.4, 39.1	15.4, 40.4
Race			
White, n (%)	166 (75.1)	87 (77.7)	253 (76.0)
Black or African American	2 (0.9)	2 (1.8)	4 (1.2)
Asian, n (%)	41 (18.6)	22 (19.6)	63 (18.9)
American Indian or Alaska Native, n (%)	5 (2.3)	1 (0.9)	6 (1.8)
Native Hawaiian or Other Pacific Islander, n (%)	0	0	0
Multiple	1 (0.5)	0	1 (0.3)
Not reported, n (%)	6 (2.7)	0	6 (1.8)
Ethnicity			
Hispanic or Latino, n (%)	9 (4.1)	9 (8.0)	18 (5.4)
Not Hispanic or Latino, n (%)	210 (95.0)	103 (92.0)	313 (94.0)
Not reported, n (%)	1 (0.5)	0	1 (0.3)
Unknown, n (%)	1 (0.5)	0	1 (0.3)

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Demographic Parameters	Etrasimod 2 mg (N=221)	Placebo (N=112)	Total (N=333)
Region			
Eastern Europe, n (%)	124 (56.1)	61 (54.5)	185 (55.6)
North America, n (%)	20 (9.0)	29 (8.0)	29 (8.7)
Western Europe, n (%)	17 (7.7)	5 (4.5)	22 (6.6)
Other, n (%)	60 (27.1)	37 (33.0)	97 (29.1)

Source: adsl.xpt; Software: R

Abbreviations: N, number of subjects in treatment group; n, number of subjects with given characteristic; SD, standard deviation
Eastern Europe: Bulgaria, Belarus, Croatia, Czech Republic, Estonia, Georgia, Hungary, Lithuania, Moldova, Poland, Romania, Russian Federation, Serbia, Slovakia, Ukraine

Other: Argentina, Chile, India, Israel, Japan, Lebanon, Mexico, South Africa, South Korea, Thailand, Turkey; North America: United States of America; Western Europe: Austria, Belgium, Denmark, France, Germany, Italy, Portugal, United Kingdom.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Baseline disease characteristics from the FDA preferred analysis population were generally balanced between treatment groups, as summarized in Table 40.

Table 40. Baseline Disease Characteristics, FDA Preferred Analysis Population, APD334-302

Parameters	Etrasimod 2 mg (N=221)	Placebo (N=112)	Total (N=333)
Naïve to biologic or JAK inhibitor therapy, actual			
Yes, n (%)	147 (66.5)	74 (66.1)	221 (66.4)
No, n (%)	74 (33.5)	38 (33.9)	112 (33.6)
Exposure to >1 biologic or JAK inhibitor therapy			
Yes, n (%)	40 (18.1)	18 (16.1)	58 (17.4)
No, n (%)	181 (81.9)	94 (83.9)	275 (82.6)
Baseline corticosteroid use, actual			
Yes, n (%)	60 (27.1)	32 (28.6)	92 (27.6)
No, n (%)	161 (72.9)	80 (71.4)	241 (72.4)
Baseline oral 5-aminosalicylic acid compounds use			
Yes, n (%)	137 (62.0)	82 (73.2)	219 (65.8)
No, n (%)	84 (38.0)	30 (26.8)	114 (34.2)
Baseline MMS			
Mean (SD)	6.7 (1.1)	6.7 (1.1)	6.7 (1.1)
Median	7	7	7
Min, max	5, 9	5, 9	5, 9
Baseline MMS group			
5 to 6, n (%)	92 (41.6)	49 (43.8)	141 (42.3)
7 to 9, n (%)	129 (58.4)	64 (56.3)	192 (57.7)
Disease extent ¹			
Left-sided colitis/proctosigmoiditis	135 (61.1)	61 (54.5)	196 (58.9)
Pancolitis	74 (33.5)	40 (35.7)	114 (34.2)
Isolated proctitis	12 (5.4)	11 (9.8)	23 (6.9)

Source: Reviewer generated table based on the Applicant submitted data adsl.xpt, and adcm.xpt

¹ Information obtained from patients' medical history included in case report forms

Abbreviations: N, number of subjects in treatment group; n, number of subjects with given characteristic; SD, standard deviation; mMS,

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Overall compliance was calculated as the total number of tablets taken divided by the total number of tablets expected during the treatment period. Subjects who took less than 80% or more than 120% of investigational drug during the entire treatment period were considered non-compliant. Treatment compliance was 99.6% for the etrasimod and 98.3% for the placebo group, respectively.

Subjects must have had inadequate response to, loss of response to, or intolerance to at least 1 prior approved therapy for UC, including oral ASAs, corticosteroids, thiopurines, biologics, and JAK inhibitors. Oral 5-ASA, AZA, 6-MP, oral corticosteroids, or medicinal probiotics were permitted at screening and as per the inclusion criteria (Trial APD334-302 Protocol Section 4.1). Concomitant treatments for UC were required to be stable prior to and during the trial and could not be initiated during screening or the treatment periods. Immunosuppressive agents such as oral AZA and 6-MP were to be discontinued ≥ 2 weeks prior to randomization. The majority of subjects received at least 1 concomitant medication for UC during the trial (93% in the etrasimod group and 89% in the placebo group), the majority of which were oral 5-ASAs.

Efficacy Results – Primary Endpoint

As discussed in Section 8.1.2, unless otherwise specified, all efficacy results in this review are based on the FDA Preferred Analysis Population defined as all adult subjects in the FAS with baseline mMS of 5 to 9. The FDA preferred definition of clinical remission was used in the analyses of clinical remission related endpoints.

The Applicant's efficacy results were confirmed by the review team. As shown in Table 41, the etrasimod treatment group had a statistically higher proportion of subjects achieving clinical remission at Week 12 (26.2%) in comparison with those treated with placebo (15.2%), p-value = 0.010.

Table 41. Results for the Primary Endpoint, FDA Preferred Analysis Population, APD334-302

Parameter	Etrasimod 2 mg (N=221)	Placebo (N=112)
Clinical Remission at Week 12, n (%)	58 (26.2)	17 (15.2)
Missing data, n (%)	22 (10.0)	11 (9.8)
Treatment Difference ¹ , (95% CI)		11.2 (2.7, 19.8)
P-value ²		0.010

Source: Review team's analysis using Applicant submitted data adefx.xpt, adqs.xpt, and adsl.xpt; based on the Applicant's information request response submitted on January 17, 2023

¹ Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

² The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

Of note, the treatment difference observed for APD334-302 for clinical remission at Week 12 was smaller than the treatment difference observed for APD334-301 for clinical remission at Week 12. Specifically, the proportion of patients in clinical remission at Week 12 was higher in

the placebo group (15.2%) in APD334-302 versus the placebo group (7.5%) in APD334-301. However, baseline demographic and disease characteristics were similar between APD334-301 and APD334-302. In response to an information request by the review team, the Applicant analyzed potentially contributing factors, but did not identify any covariates that fully explained the difference observed between the clinical remission rates of the two trials. The difference in placebo response was thus attributed to the intrinsic variability of the placebo response rate, which has been described to range from 5% to 25% in the literature (Jairath et al. 2017).

Twenty-two subjects (10%) in the etrasimod group and 11 subjects (9.8%) in the placebo group had missing values. Most missing data was due to trial dropout with main reason due to adverse events for the etrasimod group and withdrawal by subject for the placebo group. Similar to APD334-301, non-responder imputation was applied to handle missing data. The results from the pre-specified missing data sensitivity analyses (described in Section 8.1.3) supported the conclusions of the primary analysis.

Efficacy Results – Secondary and Other Relevant Endpoints

All of the multiplicity-controlled endpoints proposed for labeling by the Applicant showed statistically significant results for the etrasimod group compared to placebo, as summarized in Table 42.

Table 42. Results for Multiplicity-Controlled Secondary Endpoints, FDA Preferred Analysis Population, APD334-302

Endpoints	Etrasimod 2 mg n (%) (N=221)	Placebo n (%) (N=112)	Treatment Difference¹ vs. Placebo (95% CI)	P-value²
Endoscopic improvement at Week 12	67 (30.3)	21 (18.8)	11.84 (2.73,20.96)	0.011
Symptomatic remission at Week 12	104 (47.1)	33 (29.5)	17.75 (7.08, 28.42)	0.001
Mucosal healing ³ at Week 12	36 (16.3)	10 (8.9)	7.55 (0.59, 14.51)	0.034

Source: Review team's analysis using Applicant submitted data adeff.xpt, adsl.xpt, and adqs.xpt; based on the Applicant's information request response submitted on January 17, 2023

¹ Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

² The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

³ The endpoint will be called "endoscopic-histologic mucosal improvement" in the label.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

The efficacy results for endpoints that were non-multiplicity-controlled but were proposed for labeling by the Applicant are displayed in Table 43. Although these endpoints were not multiplicity-controlled, the etrasimod arm showed numerically higher response/remission rates compared to the placebo arm for all endpoints listed in Table 43; nominal p-values were less than 0.03.

Table 43. Results for Additional Non-Multiplicity-Controlled Endpoints, FDA Preferred Analysis Population, APD334-302

Endpoints	Etrasimod 2 mg n (%) (N=221)	Placebo n (%) (N=112)	Treatment	Nominal P-value ²
			Difference ¹ vs. Placebo (95% CI)	
Clinical response ³ at Week 12	137 (62.0)	46 (41.1)	21.11 (10.04, 32.19)	<0.001
Endoscopic remission ⁴ at Week 12	37 (16.7)	9 (8.0)	8.88 (1.94, 15.83)	0.012
Endoscopic remission and Geboes histologic score < 2.0 at Week 12	23 (10.4)	5 (4.5)	6.09 (0.64, 11.53)	0.028

Source: Review team's analysis using Applicant submitted data adefx.xpt, adsl.xpt, and adqs.xpt

¹ Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

² The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

³ Clinical response was defined as a ≥ 2 point and $\geq 30\%$ decrease from baseline in mMS, and a ≥ 1 point decrease from baseline in RBS or an absolute RBS ≤ 1 .

⁴ Endoscopic remission was defined as ES of 0.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

Discussion of Primary and Secondary Endpoints Proposed for Labeling

Symptomatic Remission

As previously discussed in this document, symptomatic remission is not considered appropriate for inclusion in labeling because symptomatic remission is redundant to clinical remission. However, as discussed in Section 8.1.2 for APD334-301, information related to improvements in SFS, and RBS is useful to clinicians to help characterize the anticipated onset of improvement in symptoms. Refer to the Discussion of Additional Endpoints below.

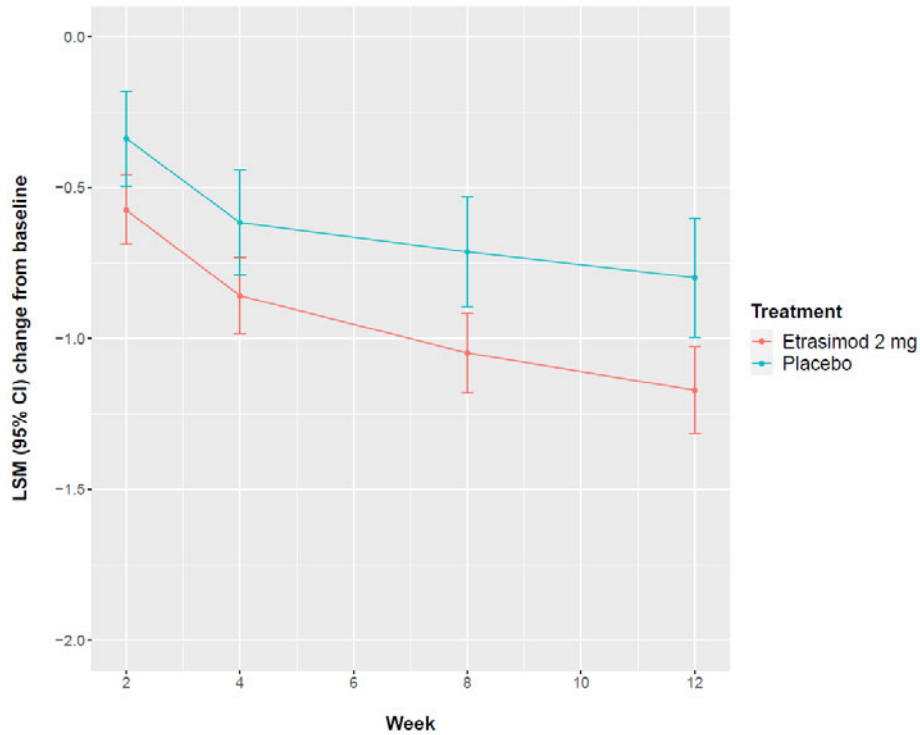
The consideration for other multiplicity-controlled endpoints (i.e., endoscopic improvement and histologic-endoscopic mucosal improvement) is similar to those described for APD334-301. Refer to Section 8.1.2.

Discussion of Additional Endpoints

Stool Frequency and Rectal Bleeding Subscores

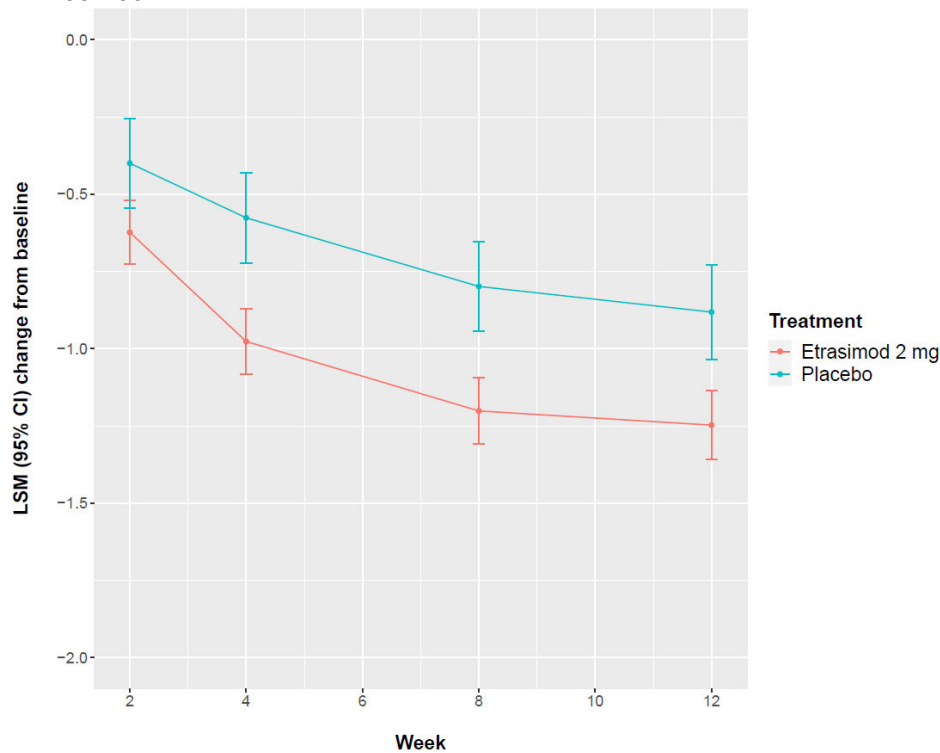
Individual analyses of the SFS and RBS of the mMS in APD334-302 provided evidence that subjects treated with etrasimod reported symptomatic relief within a few weeks of treatment (Figure 12 and Figure 13). In comparison to the placebo group, subjects in the etrasimod treatment group exhibited a nominally significantly greater mean reduction in SFS (nominal p-value = 0.0128) and RBS (nominal p-value = 0.0107) as early as Week 2 based on estimates derived from an MMRM.

Figure 12. Stool Frequency Subscore Change from Baseline, FDA Preferred Analysis Population, APD334-302



Source: Reviewer generated figure based on the Applicant submitted data adqs.xpt.
Estimates are from a mixed-effect model with repeated measures for change from baseline with a covariate for baseline score, and factors for naive to biologic/JAK inhibitor therapy at trial entry (Yes/No), baseline corticosteroid use (Yes/No), baseline disease activity (mMS: 4 to 6 or 7 to 9), treatment, visit, treatment by visit interaction.
Abbreviations: LSM, least-square mean; CI, confidence interval.

Figure 13. Rectal Bleeding Subscore Change from Baseline, FDA Preferred Analysis Population, APD334-302



Source: Reviewer generated figure based on the Applicant submitted data adqs.xpt.

Estimates are from a mixed-effect model with repeated measures for change from baseline with a covariate for baseline score, and factors for naive to biologic/JAK inhibitor therapy at trial entry (Yes/No), baseline corticosteroid use (Yes/No), baseline disease activity (mMS: 4 to 6 or 7 to 9), treatment, visit, treatment by visit interaction.

Abbreviations: LSM, least-square mean; CI, confidence interval.

For APD334-302, there was a trend toward more subjects experiencing improvement in SFS and RBS at Week 2. Given that it was not until Week 4 that a significant difference between etrasimod and placebo for the RBS was observed in APD334-301, the review team recommended aligning the timepoint between APD334-301 and APD334-302 for the RBS due to the importance of replication to ensure observed results are not due to chance. Therefore, decreases in RBS being observed as early as Week 4 will be descriptively included in labeling.

The considerations for other endpoints that are not controlled for multiplicity (i.e., clinical response, endoscopic remission, and endoscopic remission and Geboes histologic score <2.0) are similar to those outlined for APD334-301. Refer to Section 8.1.2 for discussion regarding the endpoints proposed for labeling.

Efficacy Results – Secondary or Exploratory COA (PRO) Endpoints

Refer to Section 8.1.2. for discussion regarding the Urgency NRS and Abdominal Pain NRS. Similar to APD334-301, the Urgency NRS and Abdominal Pain NRS were collected at baseline and Week 12. An ad hoc analysis of the proportion of subjects with “absence of urgency” defined as an Urgency NRS score of 0 and “absence of abdominal pain” defined as an

Abdominal Pain NRS score of 0 was performed at Week 12. Of note, no data were collected between these timepoints.

A greater proportion of subjects treated with etrasimod 2 mg reported an Urgency NRS of 0 than placebo at Week 12 (21.2% vs 11.6%). A greater proportion of subjects treated with etrasimod 2 mg reported an Abdominal Pain NRS of 0 than placebo at Week 12 (31.5% vs 17.9%).

Similar to APD334-301, although bowel urgency and abdominal pain are common and important symptoms of UC, the results of the Urgency NRS and Abdominal Pain NRS are not recommended for inclusion in labeling due to the ad hoc nature of the analyses (i.e., not multiplicity controlled and performed after unblinding). Refer to Section 8.1.2 for further discussion of these endpoints.

Additional Analyses Conducted on the Individual Trial

The efficacy results for multiplicity-controlled endpoints in APD334-302 were analyzed based on prior biologic or JAK inhibitor exposure status, as presented in Table 44. While the trial was not designed to detect a significant difference for each subgroup, the treatment differences observed were in line with the primary analysis. Across all subgroups, the etrasimod groups consistently demonstrated numerically higher rates of remission and response compared to the placebo. The subgroup of subjects without prior exposure to biologic or JAK inhibitors exhibited numerically higher remission and response rates than those with prior exposure.

Table 44. Subgroup Analyses, No Prior Biologic/JAK Inhibitor Exposure Versus Prior Biologic or JAK Inhibitor Exposure, FDA Preferred Analysis Population, APD334-302

Endpoints	Etrasimod 2 mg (N=221)		Placebo (N=112)		Treatment Difference (95% CI) ¹
	n/N	%	n/N	%	
Clinical remission at Week 12					
No prior biologic/JAK inhibitor exposure	44/147	29.9	12/74	16.2	13.7 (2.5, 24.9)
Prior biologic or JAK inhibitor exposure	14/74	18.9	5/38	13.2	5.8 (-8.2, 19.7)
Endoscopic improvement at Week 12					
No prior biologic/JAK inhibitor exposure	50/147	34.0	14/74	18.9	15.1 (3.3, 26.9)
Prior biologic or JAK inhibitor exposure	17/74	23.0	7/38	18.4	4.6 (-11.1, 20.2)
Symptomatic remission at Week 12					
No prior biologic/JAK inhibitor exposure	73/147	49.7	23/74	31.1	18.6 (5.3, 31.9)
Prior biologic or JAK inhibitor exposure	31/74	41.9	10/38	26.3	15.6 (-2.4, 33.5)
Mucosal healing ² at Week 12					
No prior biologic/JAK inhibitor exposure	28/147	19.0	8/74	10.8	8.2 (-1.3, 17.7)
Prior biologic or JAK inhibitor exposure	8/74	10.8	2/38	5.3	5.6 (-4.5, 15.6)

Source: Review team's analysis based on Applicant submitted data adefx.xpt, adsl.xpt, and adqs.xpt.

¹ The 95% confidence intervals of the treatment effects were constructed by the Wald approach.

² The endpoint will be called "histologic-endoscopic mucosal improvement" in the label.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

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Table 45 presents the results of subgroup analyses for efficacy based on the use of corticosteroids at baseline for the primary endpoint. For each subgroup, the etrasimod groups demonstrated numerically higher remission rates compared to the placebo. The observed treatment differences were similar across subgroups.

Table 45. Subgroup Analyses, Primary Endpoint, by Corticosteroids Use at Baseline, FDA Preferred Analysis Population, APD334-302

Endpoints	Etrasimod 2 mg (N=221)		Placebo (N=112)		Treatment Difference (95% CI) ¹
	n/N	%	n/N	%	
Clinical remission at Week 12					
Corticosteroids use at baseline	19/60	31.7	7/32	21.9	9.8 (-8.8, 28.3)
No corticosteroids use at baseline	39/161	24.2	10/80	12.5	11.7 (1.9, 21.5)

Source: Review team's analysis based on Applicant submitted data adefx.xpt, adsl.xpt, and adqs.xpt.

¹ The 95% confidence intervals of the treatment effects were constructed by the Wald approach.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

8.1.5. Assessment of Efficacy Across Trials

Subpopulations

Subgroup analyses were conducted to assess the potential for differences in the treatment effect at Week 12 and at Week 52 for various demographic characteristics. Overall, the etrasimod group consistently showed higher clinical remission rates compared to placebo across all subgroups, as detailed in Table 46, Table 47, and Table 48. In APD334-301, female subjects within the etrasimod group had a clinical remission rate approximately 17% higher at Week 12 compared to the male subjects; however, the difference was not evident in APD334-302. In the placebo group of APD334-302, female subjects had around 10% clinical remission rate higher at Week 12 compared to male subjects; however, the discrepancy was not evident in APD334-301. Such variations might be attributed to the constrained sample sizes of the subgroups.

Table 46. Subgroup Analyses by Demographic Characteristics, Clinical Remission at Week 12, APD334-301

Group	Etrasimod 2 mg		Placebo SC		Treatment Difference (95% CI) ¹
	n/N	%	n/N	%	
Sex					
Male	28/144	19.4	7/79	8.9	10.6 (1.6, 19.6)
Female	47/130	36.2	3/55	5.5	30.7 (20.5, 40.9)
Age					
<65	73/257	28.4	10/124	8.1	20.3 (13.0, 27.6)
≥65	2/17	11.8	0/10	0	11.8 (-3.5, 27.1)
Race					
White	67/244	27.5	10/119	8.4	19.1 (11.6, 26.6)
American Indian or Alaska Native	1/1	100	0/3	0	NA
Asian	4/19	21.1	0/9	0	21.1 (2.7, 39.4)
Black or African American	2/6	33.3	0/3	0	33.3 (-4.4, 71.0)
Ethnicity					
Hispanic or Latino	3/12	25.0	0/7	0	25.0 (0.5, 49.5)
Not Hispanic or Latino	72/260	27.7	10/126	7.9	19.8 (12.6, 27.0)

Source: Reviewer generated table based on Applicant submitted data adeff.xpt, adqs.xpt, and adsl.xpt.

¹ The 95% confidence intervals of the treatment effects were constructed by Wald approach.

Abbreviations: N, number of subjects in the subgroup; QD, once daily; n, number of events; CI, confidence interval

Table 47. Subgroup Analyses by Demographic Characteristics, Clinical Remission at Week 52, APD334-301

Group	Etrasimod 2 mg		Placebo SC		Treatment Difference (95% CI) ¹
	n/N	%	n/N	%	
Sex					
Male	42/144	29.2	5/79	6.3	22.8 (13.7, 32.0)
Female	47/130	36.2	4/55	7.3	28.9 (18.1, 39.6)
Age					
<65	79/257	30.7	9/124	7.3	23.5 (16.2, 30.7)
≥65	10/17	58.5	0/10	0	58.8 (35.4, 82.2)
Race					
White	80/244	32.8	7/119	5.9	26.9 (19.6, 34.1)
American Indian or Alaska Native	0/1	0	0/3	0	NA
Asian	6/19	31.6	1/9	11.1	20.5 (-8.8, 49.8)
Black or African American	2/6	33.3	1/3	33.3	0 (-65.3, 65.3)
Ethnicity					
Hispanic or Latino	2/12	16.7	0/7	0	16.7 (-4.4, 37.8)
Not Hispanic or Latino	87/260	33.5	9/126	7.1	26.3 (19.0, 33.6)

Source: Reviewer generated table based on Applicant submitted data adeff.xpt, adqs.xpt, and adsl.xpt.

¹ The 95% confidence intervals of the treatment effects were constructed by Wald approach.

Abbreviations: N, number of subjects in the subgroup; QD, once daily; n, number of events; CI, confidence interval

Table 48. Subgroup Analyses by Demographic Characteristics, Clinical Remission at Week 12, APD334-302

Group	Etrasimod 2 mg		Placebo SC		Treatment Difference (95% CI) ¹
	n/N	%	n/N	%	
Sex					
Male	32/125	25.6	8/70	11.4	14.2 (3.5, 24.9)
Female	26/96	27.1	9/42	21.4	5.7 (-9.6, 20.9)
Age					
<65	56/210	26.7	17/106	16.0	10.6 (1.4, 19.8)
≥65	2/11	18.2	0/6	0	18.2 (-4.6, 41.0)
Race					
White	46/166	27.7	16/87	18.4	9.3 (-1.3, 19.9)
American Indian or Alaska Native	3/5	60.0	0/1	0	60.0 (17.1, 102.9)
Asian	7/41	17.1	1/22	4.5	12.5 (-1.9, 27.0)
Black or African American	1/2	50.0	0/2	0	50.0 (-19.3, 119.3)
Multiple	0/1	0	0	NA	NA
Ethnicity					
Hispanic or Latino	7/9	77.8	2/9	22.2	55.6 (17.1, 94.0)
Not Hispanic or Latino	50/210	23.8	15/103	14.6	9.2 (0.3, 18.2)

Source: Reviewer generated table based on Applicant submitted data adeff.xpt, adqs.xpt, and adsl.xpt.

¹ The 95% confidence intervals of the treatment effects were constructed by Wald approach.

Abbreviations: N, number of subjects in the subgroup; QD, once daily; n, number of events; CI, confidence interval

8.1.6. Conclusions on Effectiveness

Overall, the submission contains substantial evidence of effectiveness for etrasimod 2 mg daily based on data from two adequate and well-controlled investigations. The results of the 52-week trial (APD334-301) were statistically and clinically significant compared to placebo on the primary and multiple major secondary endpoints. Similarly, the results for the 12-week trial (APD334-302) were statistically and clinically significant compared to placebo on the primary

and multiple major secondary endpoints. The results of both trials were robust to subgroup analyses, including for subjects with prior biologic or JAKi failure, baseline corticosteroid use, and mMS greater than 5, supporting that clinical benefit was observed among the trial population. The submitted evidence supports the conclusion that etrasimod is effective in the treatment of moderately to severely active UC in adult patients.

8.2. Review of Safety

8.2.1. Safety Review Approach

The safety evaluation of etrasimod for the treatment of moderately to severely active UC focused on data from the randomized, double-blind, placebo-controlled trials APD334-301, APD334-302, and APD334-003, and is supplemented by additional data from an integrated summary of safety (ISS). The ISS was a pool of controlled data from studies in subjects with UC including APD334-301, APD334-302, and APD334-003 as well as uncontrolled data from the open-label extension studies APD334-303, APD334-005, and ES101002.

APD334-301 was a phase 3, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of etrasimod 2 mg for the treatment of moderately to severely active UC. Eligible subjects were randomized in a 2:1 ratio to receive either etrasimod 2 mg once daily or matching placebo once daily for up to 52 weeks. After Week 12, subjects who met the prespecified definition of UC worsening were eligible to enter the open-label extension study (APD334-303).

Safety data from APD334-302 and APD334-003 were pooled (“12-Week Safety Pool”) because the two study designs and populations were sufficiently similar, to better assess the adverse event profile for the 12-week trials. APD334-302 was a phase 3, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of etrasimod 2 mg for the treatment of moderately to severely active UC. Eligible subjects were randomized in a 2:1 ratio to receive either etrasimod 2 mg once daily or matching placebo once daily for up to 12 weeks. APD334-003 was a phase 2, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of etrasimod 2 mg and 1 mg for the treatment of moderately to severely active UC. Eligible subjects were randomized in a 1:1:1 ratio to receive either etrasimod 2 mg once daily, etrasimod 1 mg once daily, or matching placebo for up to 12 weeks. The 1 mg etrasimod arm of APD334-003 was not pooled because this dose was not evaluated in APD334-301 or APD334-302. Due to the differences in randomization ratios, pooled analysis results were adjusted for the study size.

The ISS consisted of controlled and uncontrolled data from studies in subjects with UC, as described previously. Since much of the data in the ISS was uncontrolled, the ISS was not considered to provide substantial evidence of effectiveness or safety. However, safety data were reviewed to assess for rare events given the extended duration of treatment for the open-label studies.

Clinical trial data were analyzed using JMP and JMP Clinical software. All safety assessments and conclusions are those of the clinical review team unless otherwise specified. The clinical review team recoded several adverse event terms using logical groupings in order to better ascertain the safety profile of etrasimod; refer to Table 107 in Section 16.5 of the Appendix. The clinical review team did not identify any major data quality or integrity issues that precluded performing a thorough safety review.

8.2.2. Review of the Safety Database

Overall Exposure

The overall safety database that was the focus of the safety review consisted of 891 subjects as described in Table 49. A total of 577 subjects received etrasimod 2 mg. A total of 3 adolescent subjects were enrolled in the overall safety database. Data from these adolescent subjects were included in the safety analyses; however, due to the limited data, the efficacy analyses were focused on adult subjects, and it was not recommended to include adolescents in the proposed indication.

Table 49. Overview of the Overall Safety Database

Trial	Etrasimod 2 mg	Placebo
Overall Safety Database	577	314
APD334-301	289	144
12-Week Safety Pool	288	170
APD334-302	238	116
APD334-003	50	54

Source: adsl.xpt; Software: JMP

The maximum duration of exposure was 52 weeks during APD334-301 and 12 weeks during APD334-302 and APD334-003. Subjects that completed at least 26 weeks of treatment were from APD334-301 and included 185 (64%) of 289 subjects in the etrasimod group and 56 (39%) of 144 subjects in the placebo group. Subjects that completed at least 52 weeks of treatment were also from APD334-301 and included 132 (46%) of 289 subjects in the etrasimod group and 40 (28%) of 144 subjects in the placebo group.

Table 50 summarizes the duration of exposure for the overall safety database that includes all three studies, APD334-301, APD 334-302, and APD334-003.

Table 50. Duration of Exposure, Overall Safety Database

Parameter	Etrasimod 2 mg N=577	Placebo N=314
Duration of treatment, weeks		
Mean (SD)	25 (19)	19 (15)
Median (Min, max)	14 (0, 59)	13 (2, 58)
Total exposure (subject years)	277	115

Parameter	Etrasimod 2 mg N=577	Placebo N=314
Subjects treated, by duration, n (%)		
≥12 weeks ¹	507 (88)	267 (85)
≥26 weeks ²	185 (32)	56 (18)
≥52 weeks ²	132 (23)	40 (13)

Adapted from Table 6, Summary of Clinical Safety.

¹ Subjects contributed exposure from APD334-301, APD334-302, and APD334-003

² Subjects contributed exposure from APD334-301

Abbreviations: N, number of subjects in treatment arm

Adequacy of the Safety Database

A significant dropout rate was observed during the 52-week treat-through trial APD334-301. The most common reason for study discontinuation was disease worsening, which was predefined in the protocol to ensure subjects who were not receiving benefit could leave the trial to receive additional treatment. Refer to Section 8.1.2 for details regarding subject disposition. Additional safety data from open label studies (ES101002, APD334-005, and APD334-303) and the Japanese study APD334-308 provided supportive safety information. This supportive safety information included longer-term data from subjects that completed 12 weeks of treatment in APD334-302 and APD334-003 and data from additional subjects enrolled in ES101002 and APD334-308. Refer to Section 7.2 for additional details.

Given the duration of exposures in the trials, the safety database is deemed sufficient to characterize the safety of etrasimod in adults with moderately to severely active UC when used through 52 weeks. It is acknowledged that a pre-market database in UC contains limited data to fully assess the potential for rare events, particularly those of long latency (e.g., malignancy). Nevertheless, the size and scope of the submitted safety database is acceptable to inform a risk-benefit assessment for the proposed indication of the treatment of moderately to severely active UC.

8.2.3. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

No major issues regarding data integrity or submission quality were identified.

Categorization of Adverse Events

A treatment emergent adverse event (TEAE) was defined as any adverse event that started or worsened in severity on or after the first dose of study treatment. Terms are coded using MedDRA version 24.1. Serious adverse events (SAEs) were appropriately defined consistent with 21 CFR 312.32(a) and reported as required. The baseline period was the screening period.

All AEs, including SAEs, were monitored until the subject discontinued and/or completed the study. The adverse event reporting period for safety surveillance began when the subject was initially included in the study (date of first signature of informed consent) and continued up to

30 days after the last administration of study treatment. If an adverse event was not resolved or stabilized by that time, the Applicant in consultation with the Investigator decided whether to continue to monitor the adverse event or closeout the event in the database if no further follow-up was necessary. Any SAE suspected to be related to the study treatment was reported whenever it occurred, irrespective of the time elapsed since the last administration of study treatment. Any SAE that was ongoing when the subject completed the study or discontinued the study was followed by the Investigator until the event resolved, stabilized, or returned to baseline status.

All AEs were graded as mild, moderate, or severe as per the Investigator's judgement. The causal relationship between the investigational drug and the AE was characterized by the Investigator as related or not related, according to definitions outlined in the study protocol.

Adverse events of special interest (AESIs) were specified as being of special interest based on prior experience with other agents acting via a similar mechanism; these included bradycardia, heart conduction abnormalities (2nd degree and higher AV block), elevated blood pressure, macular edema, serious or opportunistic infection, pulmonary effects, hepatic effects, posterior reversible encephalopathy syndrome (PRES), and malignancy. Within the category of infections, severe infections, opportunistic infections (including progressive multifocal leukoencephalopathy), and herpes simplex and herpes zoster were considered AESIs. Refer to Section 8.2.4.4 for AESI definitions used in the trials.

Routine Clinical Tests

For each subject, the Applicant collected laboratory tests, radiologic tests, electrocardiograms (ECGs), and ophthalmoscopy according to the Schedule of Activities defined in the study protocol.

Screening samples for complete blood count (CBC) with differential, platelet count, lymphocyte counts, T lymphocytes, B lymphocytes, natural killer lymphocytes (TBNK), serum chemistry, virology, thyroid panel, coagulation, urinalysis, high-sensitivity C-reactive protein (hs-CRP), TB screen, and stool sample were obtained and reviewed prior to randomization. Safety endpoints included the incidence and severity of laboratory abnormalities and change from baseline in laboratory values. Refer to Table 108 in Section 16.5 of the Appendix for details on the laboratory tests performed during the trials.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days after the last dose of study treatment were repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor. AE included clinically relevant adverse changes in laboratory parameters over the course of the study.

Total white blood cell (WBC), neutrophil, lymphocyte, and TBNK counts were available for review prior to randomization. Since lymphopenia is a known class effect of S1P receptor

modulators, Investigators were blinded to the complete blood count lab results after randomization. An unblinded Medical Monitor assessed WBC differential lab results and alerted the Investigator if (1) ANC < 1000 cells/ μ L and/or (2) ALC <200 cells/ μ L.

All laboratory assessments required by the protocol were performed by a central laboratory unless otherwise stated.

8.2.4. Safety Results for Trial APD334-301

Overview of Adverse Events

A summary of AEs reported during trial APD334-301 is provided in Table 51. Overall, there was a higher rate of AEs in the etrasimod group vs. placebo, with most AEs in both groups being mild or moderate. The proportion of subjects who reported serious adverse events (SAEs) and AEs leading to discontinuation was similar between etrasimod and placebo.

Table 51. Overview of Adverse Events, Safety Population, APD334-301

Event Category	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)
SAE	20 (6.9)	9 (6.3)
SAEs with fatal outcome	0	0
AE leading to permanent discontinuation of study drug	12 (4.2)	7 (4.9)
AE leading to interruption of study drug	19 (6.6)	4 (2.8)
Any TEAE	206 (71.3)	81 (56.3)
Maximum intensity for any TEAE		
Severe	21 (7.3)	11 (7.6)
Moderate	84 (29.1)	30 (20.8)
Mild	101 (34.9)	40 (27.8)

Source: Reviewer analysis using Applicant submitted data, adae.xpt and adsl.xpt

Abbreviations: AE, adverse event; N, number of subjects in treatment arm; n, number of subjects with at least one event; SAE, serious adverse event; TEAEs, Treatment emergent adverse events

8.2.4.1. Deaths

There were no deaths during trial APD334-301.

8.2.4.2. Serious Adverse Events

Subjects with at least 1 serious adverse event (SAE) are shown in Table 52. The overall incidence of SAEs was similar between etrasimod and placebo, with 20/289 (7%) of subjects who received etrasimod experiencing at least one SAE, compared to 9/144 (6%) of subjects who received placebo.

Table 52. Subjects with Serious Adverse Events (SAEs) by System Organ Class and Preferred Term, Safety Population, APD334-301

System Organ Class (SOC) Preferred Term (PT)	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)
Subjects with at least one serious adverse event (SAE)	20 (6.9)	9 (6.3)
Gastrointestinal disorders	9 (3.1)	4 (2.8)
Colitis ulcerative	6 (2.1)	3 (2.1)
Abdominal pain	1 (0.3)	0
Mucosal prolapse syndrome	1 (0.3)	0
Proctitis	1 (0.3)	0
Large intestine perforation	0	1 (0.7)
Infections and infestations	3 (1.0)	5 (3.5)
COVID-19	1 (0.3)	1 (0.7)
COVID-19 pneumonia	1 (0.3)	1 (0.7)
Pneumonia bacterial	1 (0.3)	0
Campylobacter infection	0	1 (0.7)
Cellulitis	0	1 (0.7)
Peritonitis	0	1 (0.7)
Blood and lymphatic disorders	1 (0.3)	2 (1.7)
Anemia	2 (0.7)	1 (0.7)
Musculoskeletal and connective tissue disorders	2 (0.7)	0
Arthralgia	1 (0.3)	0
Musculoskeletal chest pain	1 (0.3)	0
Nervous system disorders	2 (0.7)	0
Intracranial pressure increased	1 (0.3)	0
Migraine	1 (0.3)	0
Immune system disorders	1 (0.3)	0
Allergy to arthropod bite	1 (0.3)	0
Investigations	1 (0.3)	0
Hepatic enzyme increased	1 (0.3)	0
Pregnancy, puerperium and perinatal conditions	1 (0.3)	0
Anembryonic gestation	1 (0.3)	0
Surgical and medical procedures	1 (0.3)	0
Breast conserving surgery	1 (0.3)	0

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP

Analysis for both SOC and PT are subject level (i.e., each subject is counted once regardless of the number of reported events).

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; SAE, serious adverse event

The most frequently reported SAE was “colitis ulcerative” (i.e., ulcerative colitis). Ulcerative colitis) and colon perforation were the only SAEs that led to permanent treatment discontinuation, occurring in 1 subject in the etrasimod group and 1 subject in the placebo group. Infections and hepatic enzyme increased were the only SAEs that were also AESIs. Ulcerative colitis and anemia were the only SAEs that occurred in more than 1 etrasimod-treated subject. There were no SAEs that occurred in more than 1 subject in the etrasimod group and that were not reported by at least 1 subject in the placebo group.

SAEs of ulcerative colitis and anemia were considered unlikely to be related to etrasimod as these likely represent worsening of the underlying disease.

Pertinent Narratives for SAEs

The following SAEs occurred only in the etrasimod group and were considered to be possibly related to etrasimod by the review team: bacterial pneumonia, arthralgia, increased intracranial pressure, and hepatic enzyme increased.

- **Bacterial pneumonia:** A 55-year-old White male had an SAE of "pneumonia bacterial" on Study Day 163. On Study Day 158, the subject reported fatigue and body aches, followed by a nonserious AE of pyrexia. The subject continued to have fever and developed headache, nausea, bloating, and abdominal pain through Study Day 163. On Study Day 163, the subject was hospitalized for bacterial pneumonia where a chest X-ray showed mild infiltration on the left, lower fields. The subject was treated with antibiotics and on Study Day 168, the SAE was considered recovered/resolved. Relevant medical history included UC (since 2011) and daily cigarette smoking (since 1985). The only concomitant medication at the onset of the SAE was sulfasalazine. Study treatment was unchanged. The Investigator assessed the event to be not related to study treatment. Given that S1P receptor modulators are known to affect lymphocyte trafficking, it is possible that etrasimod may increase the risk of infection, and the SAE of bacterial pneumonia may possibly be related to etrasimod. The risk of infections will be included in the Warnings and Precautions section of the label.
- **Arthralgia:** A 48-year-old White female had an SAE of "arthralgia" on Study Day 162. On Study Day 137, the subject was placed on bedrest and started on methylprednisolone for a nonserious AE of "UC-associated arthralgia" of the wrist, elbow, and knee. On Study Day 141, the subject complained of pain in the knee, hip, and wrist joints and was hospitalized for an SAE of "arthralgia." During the hospitalization, the subject did not receive additional treatment. Laboratory analyses showed mild anemia and elevated C-reactive protein (CRP). An ultrasound did not demonstrate venous thrombosis in the lower limbs. No action was taken with the study treatment. The subject's last dose of study treatment prior to the SAE of arthralgia was on Study Day 141. The SAE of arthralgia was resolved on Study Day 145. The review team agrees with investigator's assessment that the SAE of arthralgia was likely not related to study treatment and is likely an extraintestinal manifestation of UC. However, arthralgia was reported in a greater proportion of subjects who received etrasimod compared to placebo in APD334-301 (4% etrasimod vs. 2% placebo). While arthralgia is not a known S1P receptor class effect, it has been reported in subjects with UC who received another S1P receptor, ozanimod. Additionally, arthralgia has been reported as an adverse reaction of other IBD therapeutics. Therefore, arthralgia will be included in the Adverse Reactions section of the label.
- **Increased intracranial pressure:** A 63-year-old male had an SAE of "increased intracranial pressure" first reported on Study Day 90. Relevant medical history included ulcerative colitis (since 2019), anal fissure (since 2019), and anemia (since 2019). Concomitant therapy included mesalazine. The subject reported gradual loss of eyesight on Study Day 90 that started on Study Day 58. On Study Day 96, an optical coherence tomography (OCT) was performed, which did not show macular edema. An MRI was performed on Study Day 100,

which was reported as showing no significant abnormalities. The subject was seen by a neurologist on Study Day 105. On Study Day 110, a spinal tap and fluid analysis was performed, and the neurologist diagnosed the subject with increased ICP of unknown origins. The study treatment was interrupted from Study Day 107 to Study Day 114. On Study Day 114, the subject reported that his eyesight had improved but was not fully recovered. On the same day, study treatment resumed. On Study Day 160, an OCT was performed and revealed atrophy of the optic nerve in the left eye. The subject's visual acuity in the left eye had not improved. On Study Day 236, an OCT was performed and showed similar results as on Study Day 160. The SAE of increased intracranial pressure was considered recovered/resolved on Study Day 243, with sequelae of ocular atrophy and partial vision loss.

According to the Applicant, the subject's neurologist attributed the increased ICP as more likely an episode of what could be a chronic process of rise in ICP; however, S1P receptor modulators have been associated with ocular disorders (e.g., macular edema) and disorders of the central nervous system (e.g., PRES, PML). It is also possible the increased ICP was secondary to mesalazine use, in which increased ICP has been reported as an AE. Therefore, a relationship between etrasimod and the SAE of increased intracranial pressure cannot be ruled out.

- **Migraine:** A 52-year-old White female had an SAE of migraine on Study Day 80 requiring hospitalization. The subject's relevant medical history included ulcerative colitis (since 1997), migraine (since 1981), and arthralgia (2019-2020). Concomitant medication ongoing at the onset of the SAE of migraine included rizatriptan, zolmitriptan, paracetamol, and estradiol. It was reported that the subject did not have a history of recurrent migraine episodes with the same symptoms or cluster headaches, and she had not experienced any usual migraine triggers. However, 2 weeks prior to the event, the subject reported recurrent headaches that per the Investigator, were not from the usual triggers that were consistent with her usual migraine headaches; they were much more severe.

The subject's last dose of study treatment (etrasimod 2 mg) prior to the SAE was on Study Day 79, and study treatment was interrupted. A magnetic resonance imaging scan was normal. The SAE was considered resolved on Study Day 81.

On Study Day 89, the subject experienced nonserious AEs of mild vomiting and mild migraine. On Study Day 93, after the SAE resolved, study treatment resumed. Upon interruption, the event abated, but then reappeared after study treatment was reintroduced. On Study Day 116, the subject stopped estradiol treatment that had been initiated on Study Day 71 as hormone replacement. Per the investigator, a gynecologist had advised to stop estradiol treatment due to its side effect profile, which includes migraines. The subject continued to experience mild and moderate AEs of migraine through Study Day 174. The Investigator assessed the event to be unlikely related to study treatment; however, the review team determined that a relationship between etrasimod and the SAE of migraine cannot be ruled out. Although the subject had a history of migraines and

migraine is a potential side effect of the concomitant estradiol, migraine was reported in a greater proportion of subjects who received etrasimod (3/289, 1%) compared to placebo (none) during APD334-301. Therefore, headache, including related terms headache, migraine, tension headache, and sinus headache, will be included as an adverse reaction in the Adverse Reactions section of the label.

- **Hepatic enzyme increased:** A 34-year-old female reported SAEs of “abdominal pain” and “elevated hepatic enzymes” first reported on Study Day 196, requiring hospitalization for abdominal pain. The subject’s relevant medical history included ulcerative colitis (since 2020). Concomitant medications ongoing at the onset of the SAEs included fluticasone/formoterol and levonorgestrel. Baseline alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, alkaline phosphatase, and gamma-glutamyl transferase (GGT) were normal (Study Day 1). On Study Day 195, the subject reported “intense epigastric abdominal pain that was radiating to her back” and exacerbated by eating and drinking. The subject was hospitalized on Study Day 196 for “severe abdominal pain.” The same day, the subject was found to have elevated liver tests during the hospitalization. The subject’s ALT was 3.5 x ULN, AST was 7.9 x ULN, GGT was 1.9 x ULN, and bilirubin was 1.8 x ULN. The subject’s alkaline phosphatase was normal. An ultrasound did not show gallstones or gallbladder inflammation; however, a magnetic resonance cholangiopancreatography was performed which showed stenosis of the posterior right hepatic duct with distal dilation. According to the Investigator, the findings could represent early primary sclerosing cholangitis. The subject received symptomatic treatment for abdominal pain during the hospitalization. On Study Day 202, the subject’s abdominal pain had resolved, and she was discharged from the hospital. On the same day, the subject’s AST and bilirubin had returned to normal though her ALT and GGT remained elevated 2.8 x ULN and 2.2 x ULN, respectively. No action was taken with study treatment due to the events. On Study Day 224, the subject’s hepatic enzymes were normal, and the SAE of hepatic enzyme increased was considered recovered/resolved. The investigator assessed the events to be unlikely related to study treatment; however, liver injury has been reported in other S1P receptor modulators and is an AESI for etrasimod. The risk of liver injury will be communicated in the Warnings and Precautions section of the label.

In summary, the causes of SAEs were varied and the overall frequency of SAEs and SAEs leading to discontinuation were similar between the etrasimod and placebo groups. However, several SAEs were considered likely related to etrasimod due to the mechanism of action of the drug and will be described in labeling.

8.2.4.3. Discontinuations Due to Adverse Events

The overall incidence of AEs leading to discontinuation was similar in the etrasimod and placebo groups with 12/289 (4%) of subjects treated with etrasimod experiencing at least one AE that led to discontinuation, compared to 7/144 (5%) of subjects who received placebo. Ulcerative colitis and colon perforation were the only SAEs that led to permanent treatment discontinuation, occurring in 1 subject in the etrasimod group and 1 subject in the placebo

group (refer to Section 8.2.4.2). Overall, there was no consistent safety signal or specific AE or organ class of AEs leading to discontinuation. Subjects with adverse events leading to treatment discontinuation are shown in Table 53.

Table 53. Subjects With Adverse Events Leading to Treatment Discontinuation by System Organ Class and Preferred Term, Safety Population, APD334-301

System Organ Class (SOC) Preferred Term (PT)	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)
Subjects with at least one AE Leading to Discontinuation	12 (4.2)	7 (4.9)
Gastrointestinal Disorders	6 (2.1)	4 (2.8)
Colitis ulcerative	4 (1.4)	2 (1.4)
ALT increased	1 (0.3)	1 (0.7)
Blood alkaline phosphatase increased	1 (0.3)	0
Large intestine perforation	0	1 (0.7)
Cardiovascular	2 (0.7)	0
Bradycardia	1 (0.3)	0
AV block second degree	1 (0.3)	0
Infections and infestations	2 (0.7)	1 (0.7)
Clostridium difficile infection	1 (0.3)	0
Tuberculosis	0	1 (0.7)
COVID-19	1 (0.3)	0
Blood and lymphatic disorders	0	1 (0.7)
Anemia	0	1 (0.7)
Eye disorders	1 (0.3)	0
Macular edema	1 (0.3)	0
General disorders and administration	1 (0.3)	1 (0.7)
Pyrexia	1 (0.3)	0
Malaise	0	1 (0.7)

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP
Analysis for both SOC and PT are subject level (i.e., each subject is counted once regardless of the number of reported events).
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; AE, adverse event

There were no AEs that led to discontinuation that were reported by more than one subject who received etrasimod, except for ulcerative colitis. However, ulcerative colitis was reported in a similar proportion of subjects in the etrasimod group as in the placebo group and is most likely related to the underlying disease and lack of response to treatment, rather than an adverse event. Narratives for AEs leading to discontinuation of etrasimod that were also AESIs are described below.

AEs Leading to Discontinuation That Were Also AESIs

- **ALT elevated:** An 18-year-old White male with a 10-year history of primary sclerosing cholangitis (PSC) was discontinued from study treatment due to elevated ALT and AST. Concomitant medications included mesalazine and ursodeoxycholic acid. At the baseline visit, mild elevations of ALT and AST (1.7 x ULN and 1.4 x ULN, respectively) were noted. On Study Day 15, both ALT and AST increased to 3.1 x ULN but on Study Day 21, both had returned to baseline levels after the study drug was held from Study Days 18-23.

On Study Day 45, ALT was 8.7 x ULN, and AST was 10.6 x ULN. No treatment was given for the events and study drug was discontinued on Study Day 46. Both ALT and AST decreased significantly on Study Day 50 (2.1 and 1.0 x ULN, respectively). Alkaline phosphatase was mildly elevated throughout the study from 1.1-2.1 x ULN and there was a mild to moderate elevation of GGT (1.8-4.0 x ULN). Bilirubin was consistently normal. On Study Day 65, the events of ALT increased and AST increased were considered recovered/resolved at which time ALT was 1.8 x ULN and AST was 1.2 x ULN (i.e., similar to baseline). The review team determined that the events may have been related to study treatment given the liver enzymes returned to baseline after study drug discontinuation; however, the liver enzyme elevations could have been due to the subject's past medical history of PSC. Recommendations for evaluation of transaminases and bilirubin prior to initiation of treatment and during treatment are listed in the Warnings and Precautions section of the label.

- **Alkaline phosphatase elevated:** A 44-year-old White female had an alkaline phosphatase 1.0 - 1.2 x ULN beginning on Study Day 171. Mild increases were noted in ALT beginning on Study Day 115 (1.5 - 2.7 x ULN) and in AST beginning on Study Day 227 (1.3 - 1.4 x ULN). No transaminase increase reached 3 x ULN. GGT was elevated at screening (5.5 x ULN) and throughout the study (maximum of 21.8 x ULN on Study Day 280). The subject reported no symptoms. Relevant medical history included ulcerative colitis and obesity. Study drug was discontinued due to the event of alkaline phosphatase increased, with the last dose taken on Study Day 293. At the time of the subject's last available report, the events were considered not recovered/not resolved. The investigator assessed the events of GGT increased, ALT increased, and blood alkaline phosphatase increased to be probably related to study treatment. Given that the subject's baseline GGT was elevated, the liver test elevations may not be related to etrasimod; however, S1PR modulators have been associated with a risk of hepatic injury. Therefore, a relationship between the AE of "alkaline phosphatase elevated" and etrasimod cannot be ruled out. The risk of liver injury will be communicated in the Warnings and Precautions section of the label.
- **Bradycardia:** A 51-year-old White male had an AE of bradycardia and dizziness reported on Study Day 2. On Study Day 1, prior to dosing of study treatment, the subject's heart rate (HR) was 72 beats per minute (bpm). On Study Day 2, the subject reported the AEs of bradycardia and dizziness, and his heart rate was 44 bpm. Five hours later, the subject's heart rate was 42 bpm. The subject's blood pressure remained stable throughout and electrocardiograms (ECGs) were normal. No treatment was administered for these events. The subject discontinued study treatment due to the AE of bradycardia and received the last dose of study treatment on Study Day 2. On Study Day 3, the subject's heart rate was 46 and 47 bpm, and the severity of the dizziness had improved. On Study Day 4, the subject's heart rate was 58 bpm, and the bradycardia and dizziness were considered recovered/resolved. Bradycardia after the first dose of S1P receptor modulators is a known class effect; therefore, the AE of bradycardia and dizziness are likely related to etrasimod. The risk of bradyarrhythmia will be described in the Warning and Precaution section of the label.

- **AV block second degree:** A 48-year-old Black or African American female had AEs of bradycardia and first-degree AV block on Study Days 1 and 2. The subject's baseline ECG was normal. At 4 hours post-dose on Study Day 1, the ECG results showed a HR of 49 bpm, and a moderate AE of bradycardia was recorded. The ECG also showed a PR interval > 200 msec (258 msec). The investigator and central ECG reader reported a second-degree AV block, Mobitz type I. No symptomatic AEs were reported, and no treatment was administered for either event. The subject discontinued study treatment due to the AE of "AV block second degree." The subject's last dose of study treatment was taken on Study Day 1. On Study Day 2, repeat ECG showed a normal PR interval (172 msec) and HR (72 bpm), the events of AV block second degree and bradycardia were considered recovered/resolved. The subject had no history of cardiac events and AV conduction delays are a known risk with S1P receptor modulators; therefore, the AE of "AV block second degree" is likely related to etrasimod. The risk of AV conduction delays will be described in the Warning and Precaution section of the label.
- **Macular edema:** A 43-year-old White female had an AE of macular edema on Study Day 75. The subject reported blurred vision in the left eye on Study Day 74. On Study Day 75, ophthalmic examination showed a decrease in visual acuity and macular edema. Treatment was discontinued on Study Day 75 due to the nonserious AE of macular edema. The subject's last dose of study treatment was taken on Study Day 74. Medical history included cerebral infarction and hypertension. Concomitant medications included mesalazine, methylprednisolone, lercanidipine, and perindopril. Macular edema and decreased visual acuity were ongoing at the time of early termination visit (Study Day 81). The AE of macular edema was considered recovered/resolved on Study Day 139, and a follow-up ophthalmologic exam on Study Day 173 was normal. Macular edema has been identified as a risk in other S1P receptor modulators and was reported more frequently in subjects who received etrasimod compared to placebo (18% vs. 8%). The risk of macular edema will be communicated in the Warnings and Precautions section of the label and a baseline evaluation of the fundus near the start of treatment and monitoring during treatment will be recommended. Refer to Section 8.2.6.5 for additional analyses regarding the risk of eye disorders.

8.2.4.4. Adverse Events of Special Interest

At the time of protocol development, progressive multifocal leukoencephalopathy (PML) was identified as an AESI. Additional AESIs were identified by the Applicant as the understanding of the safety profile of etrasimod evolved. The Applicant conducted a retrospective, blinded medical review to identify AESIs based on the mechanism of action of etrasimod, prior experience with other S1PR modulators, and disease-specific potential risks. The following categories and preferred terms were used to query the clinical database, and a blinded medical review of these AEs was conducted to identify which events were AESIs. Refer to Section 8.2.6 for the review team's analysis of adverse events that are of special interest based on known class effects but did not meet the Applicant's criteria for AESI.

- Cardiovascular
 - Bradycardia – if one or more of the following:
 - HR was <40 bpm (with or without symptoms)
 - Event was associated with clinical symptoms (e.g., lightheadedness)
 - Event led to subject withdrawal
 - Atrioventricular (AV) conduction delay – if one or more of the following:
 - AV block was second degree (Mobitz Type I or Type II) or higher
 - PR interval was greater than 230 ms
 - Event was associated with clinical symptoms (e.g., dizziness) considered potentially related based upon medical review
 - Hypertension – if one or more of the following:
 - Sustained systolic BP elevation was greater than 160 mmHg
 - Sustained diastolic BP elevation was greater than 100 mmHg
 - Medication was added for BP control
- Macular edema – defined as an increase in central foveal thickness (CFT) ≥ 40 μm , and/or with associated symptoms or clinically significant abnormal findings
- Hepatic injury
 - Liver transaminases increased – defined as any liver transaminase elevation >5 x ULN or a sustained elevation >3 x ULN
 - Bilirubin elevation – defined as bilirubin >2 x ULN without Gilbert’s syndrome
- Infections
 - Severe infections
 - Opportunistic infections
 - Herpes simplex and herpes zoster
- Pulmonary
 - Airflow obstruction (FEV₁, FVC) – defined as a decrease from baseline of $>25\%$ or accompanied by relevant symptoms
 - Decreased gas exchange (DLCO) – defined as a decrease from baseline of $>25\%$ or accompanied by relevant symptoms
- Posterior reversible encephalopathy syndrome (PRES)
- Malignancy

The AESIs as identified by the Applicant are shown in Table 54. Overall, there were more subjects with at least one AESI in the etrasimod group compared to placebo with 26/289 (9%) of subjects treated with etrasimod experiencing at least one AESI, compared to 10/144 (7%) of subjects who received placebo.

Table 54. Subjects With Adverse Events of Special Interest (AESI), Safety Population, APD334-301

Adverse Event of Special Interest	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)
Subjects with at least one AESI	26 (9.0)	10 (6.9)
Cardiovascular events	12 (4.2)	0
Hypertension ¹	9 (3.1)	0
AV conduction delay ²	2 (0.7)	0
Bradycardia	1 (0.3)	0
Infections (severe, opportunistic, or herpes infections)	8 (2.8)	7 (4.9)
Severe infections ³	3 (1.0)	5 (3.5)
Opportunistic infections ⁴	0	1 (0.7)
Herpes simplex and herpes zoster ⁵	5 (1.7)	1 (0.7)
Liver injury	4 (1.4)	2 (1.4)
Liver transaminases elevation ⁶	4 (1.4)	2 (1.4)
Macular edema	1 (0.3)	0
Pulmonary Disorders	1 (0.3)	1 (0.7)
Airflow obstruction (FEV1, FVC)	1 (0.3)	1 (0.7)

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP

Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).

¹Hypertension includes PTs: hypertension, hypertensive crisis

²AV conduction delay includes PTs: atrioventricular block first degree, atrioventricular block second degree

³Severe infections includes PTs: COVID-19, COVID-19 pneumonia, pneumonia bacterial, pneumonia viral, campylobacter infection, cellulitis, and peritonitis

⁴Opportunistic infections includes PT tuberculosis

⁵Herpes simplex and herpes zoster includes PTs: herpes simplex, oral herpes, and herpes zoster

⁶Liver transaminases elevation includes PTs: ALT increased, AST increased, transaminases increased

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; AESI, adverse event of special interest; FEV1, forced expiratory volume in one second; FVC, forced vital capacity

Cardiovascular adverse events, including hypertension, bradycardia, and AV conduction delay, macular edema, and herpes infections were reported more frequently in the etrasimod group compared to placebo. The overall incidence of liver injury AESIs were balanced between the two groups. No AESIs of PML, bilirubin elevation, PRES, or malignancy were reported. AESIs that were reported more frequently in subjects who received etrasimod compared to placebo are described in detail below.

- **Cardiovascular events:** Cardiovascular events were reported more frequently in subjects who received etrasimod compared to placebo. One AE of bradycardia and 1 AE of AV block second degree led to study discontinuation; refer to Section 8.2.4.3.
 - **AV Block First Degree:** One subject experienced AV block first degree, 4 hours post-dose on Study Day 1. The subject had no history of cardiac disease. Predose on Study Day 1, ECG results showed normal T-wave morphology, sinus rhythm, and atrial premature complexes, which were considered abnormal and clinically significant by the investigator and the central reader. At 4 hours post-dose on Study Day 1, ECG results showed PR interval >200 msec (238 msec, increased from baseline 152 msec), normal T-wave morphology, sinus rhythm, and atrial premature complexes considered abnormal and clinically significant by the investigator and the central reader, and an AESI of AV block first degree was

reported. No treatment was administered for the event and no action was taken with study treatment in response to the event. On Study Day 2, ECG results were normal, and the event was considered recovered/resolved. The Investigator assessed the event to be probably related to study treatment.

- ***Hypertension:*** Hypertension was reported as an adverse reaction in 3% of subjects treated with etrasimod. No subjects treated with placebo met the Applicant's criteria for hypertension. The majority of the events were reported by the Applicant as mild or moderate in severity. There was a small but persistent increase in systolic blood pressure (SBP) compared to baseline in subjects treated with etrasimod. However, the mean change from baseline to Week 52 was 2.2 mmHg in the etrasimod group compared to -1.3 mmHg in the placebo group, the clinical significance of which is unclear.

One subject reported an AE of "hypertensive crisis" on Day 218. The subject's vital signs before and after this episode were all normal. This event was confirmed by the Investigator as not being a "hypertensive crisis" event based on the objective blood pressure measurements and accepted diagnostic definitions. No action was taken with study treatment and the event resolved on the same day without intervention from the Investigator.

Due to the risk of cardiovascular events observed in the etrasimod development program and the known risks described in other S1P receptor modulators, a Warnings and Precautions section will be included in the label regarding the risk of bradyarrhythmia and AV conduction delays, as well as increased blood pressure.

- **Macular edema:** Macular edema was considered an AESI because it has been identified as a risk in other S1P receptor modulators. The Applicant reported one case of a nonserious AE of macular edema in a 43-year-old female treated with etrasimod during APD334-301, which led to study discontinuation. The AE of macular edema is described in detail in Section 8.2.4.3.
- **Infections (severe, opportunistic, or herpes infections)**
 - ***Herpes simplex and herpes zoster:*** Herpes viral infections were reported by more subjects in the etrasimod group compared to placebo (2% vs. 1%). Herpes simplex, including preferred terms herpes simplex and oral herpes, was reported by 4/289 (1%) subjects in the etrasimod group versus 1/144 (1%) in the placebo group. Herpes zoster was reported by 2/289 (1%) subjects in the etrasimod group versus none in the placebo group.

Of the subjects who experienced herpes zoster, one was a 50-year-old White female who on Study Day 395 had a nonserious AE of herpes zoster, followed by a nonserious AE of post-herpetic neuralgia on Study Day 407. The subject

received etrasimod 2 mg up to Study Day 407, the study termination visit. The Investigator assessed the event of herpes zoster to be unlikely related to study treatment. The other subject was a 50-year-old White female who received etrasimod 2 mg up to Study Day 371. On Study Day 229, the subject had an AESI of herpes zoster. No action was taken with study treatment in response to the event. On Study Day 240, the event was considered recovered/resolved. The Investigator assessed the event to be not related to study treatment.

S1P receptor modulators are known to affect lymphocyte trafficking; therefore, etrasimod may increase the risk of infections, including herpes simplex or herpes zoster. Consistent with other S1P receptor modulators, the risk of infections will be described in the Warnings and Precautions section of the label.

8.2.4.5. Treatment Emergent Adverse Events and Adverse Reactions

An overview of treatment emergent adverse events (TEAEs) for APD334-301 is shown in Table 51. Overall, the proportion of subjects with at least one TEAE was greater in subjects who received etrasimod 206/289 (71%) compared to placebo 81/144 (56%). The majority of TEAEs were mild or moderate in severity. The proportion of subjects with a severe TEAE was similar between etrasimod 21/289 (7%) and placebo 11/144 (8%).

Table 55 summarizes the common TEAEs reported at a frequency of at least 2% of subjects in the etrasimod group and greater than placebo during APD334-301. The most common TEAEs were headache, elevated liver tests, dizziness, and arthralgia.

Table 55. Common Treatment-Emergent Adverse Events (TEAEs) Occurring in at Least 2% of Subjects and at a Higher Frequency Than Placebo, Safety Population, APD334-301

Preferred Term	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)
Subjects with at least 1 TEAE	206 (71.3)	81 (56.3)
Headache ¹	27 (9.3)	7 (4.9)
Elevated liver tests ²	17 (5.9)	7 (4.9)
Dizziness ³	15 (5.2)	3 (2.1)
Arthralgia	13 (4.5)	3 (2.1)
Hypertension ⁴	10 (3.5)	1 (0.7)
Urinary tract infection ⁵	10 (3.5)	3 (2.1)
Nausea	9 (3.1)	2 (1.4)

Preferred Term	Etrasimod 2 mg	Placebo
	N=289 n (%)	N=144 n (%)
Hypercholesterolemia ⁶	8 (2.8)	0
Muscle spasms	5 (1.7)	0
Vomiting	5 (1.7)	0
Herpes viral infection ⁷	5 (1.7)	1 (0.7)
Blood creatine phosphokinase increased	5 (1.7)	1 (0.7)

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP

Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).

Gray shading reflects what will be included in the label as the most common adverse reactions

¹Headache includes PTs: headache, migraine, sinus headache and tension headache

²Elevated liver tests includes PTs: ALT increased, AST increased, blood alkaline phosphatase increased, cholestasis, GGT increased, hepatic enzyme increased, hyperbilirubinemia, liver function test increased, and transaminases increased

³Dizziness includes PTs: dizziness, dizziness exertional, and dizziness postural

⁴Hypertension includes PTs: hypertension, blood pressure increased, and hypertensive crisis

⁵Urinary tract infection include PTs: urinary tract infection and cystitis

⁶Hypercholesterolemia includes PTs: hypercholesterolemia and blood cholesterol increased.

⁷Herpes viral infection includes PTs: herpes simplex, herpes zoster, and oral herpes

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; TEAE, treatment emergent adverse event

TEAEs that occurred in at least 2% of subjects treated with etrasimod and more frequently than placebo are described below.

- Headache:** Headache was the most common TEAE reported by subjects during APD334-301. There was an imbalance in the proportion of subjects who received etrasimod that reported headache (9%) compared to placebo (5%). The majority of headache AEs were mild or moderate. One headache was an SAE (preferred term “migraine”) and is described in greater detail in Section 8.2.4.2. No AEs of headache led to study discontinuation. An imbalance was also observed in the pooled data from APD334-302 and APD334-003 (refer to Section 8.2.5.2). The mechanism by which etrasimod may increase the risk of headache is unknown; however, headache is an adverse reaction observed in several other S1P receptor modulators, including Zeposia (ozanimod)⁴, which is approved for the treatment of moderately to severely active UC. Therefore, headache will be included in the Adverse Reactions section of the label.
- Elevated liver tests:** A greater proportion of subjects who received etrasimod had an AE of elevated liver tests (6%) compared to placebo (5%). The majority of elevated liver test AEs were mild or moderate in severity. One AE was considered an SAE and is described in detail in Section 8.2.4.2. Two subjects were discontinued from study treatment due to an AE of elevated liver tests (one subject with ALT increased to 8.7 x ULN and one subject with blood alkaline phosphatase increased to 1.2 x ULN); refer to Section 8.2.4.3 for full details of the AEs. An imbalance was also observed in the pooled data from APD334-302 and APD334-003 (refer to Section 8.2.5.2).

⁴ See Zeposia (ozanimod) at <https://www.accessdata.fda.gov/scripts/cder/daf/>

Liver injury is described in labeling as a Warnings and Precautions for other S1P receptor modulators, including Zeposia (ozanimod), which is approved for the treatment of moderately to severely UC. Although the mechanism by which etrasimod might cause liver injury is not known, a similar Warnings and Precautions will be included in the etrasimod label. Refer to Section 8.2.6.4 for additional analyses that support the potential of hepatotoxicity with etrasimod.

- **Dizziness:** A greater proportion of subjects who received etrasimod had an AE of dizziness (5%) compared to placebo (2%). All the AEs of dizziness were mild or moderate in severity. No AEs were considered SAEs, and none led to study discontinuation. Approximately half (7/15) of the AEs of dizziness were reported within the first 2 days of treatment with etrasimod and may be related to the transient bradycardia observed with etrasimod initiation. Dizziness has also been observed in trials of other S1P receptor modulators. Taken together, the review team recommends including dizziness in the Adverse Reactions section of the label.
- **Arthralgia:** A greater proportion of subjects who received etrasimod had an AE of arthralgia (4%) compared to placebo (2%). All the arthralgia AEs were mild or moderate in severity. One AE of arthralgia was considered an SAE and is described in detail in Section 8.2.4.2. None of the arthralgia AEs led to study discontinuation. Arthralgia can be an extra-intestinal manifestation of inflammatory bowel disease, including UC; however, arthralgia has been reported as an adverse reaction in other S1P receptor modulators, including Zeposia (ozanimod), which is approved for the treatment of moderately to severely active UC. Arthralgia will be included in the Adverse Reactions section of the label.
- **Hypertension:** A greater proportion of subjects who received etrasimod had an AE of hypertension (3%) compared to placebo (1%). Most hypertension AEs were reviewed in Section 8.2.4.4 as hypertension with a sustained systolic BP elevation greater than 160 mmHg was considered an AESI. Two additional AEs of hypertension (1 preferred term “blood pressure increased” in the etrasimod group and 1 preferred term “hypertension” in the placebo group) were identified as TEAEs but not considered AESIs by the Applicant. Both AEs were either mild or moderate and did not result in study discontinuation. Therefore, the two additional AEs of hypertension do not change the analysis of the risk of hypertension due to etrasimod as described in the previous section. A Warnings and Precautions section will be included in the label regarding the risk of increased blood pressure, and hypertension will be included in the Adverse Reactions section of the label.
- **Urinary Tract Infection:** A greater proportion of subjects who received etrasimod had an AE of urinary tract infection (UTI) compared to placebo (3% and 2%, respectively). No events of UTI were SAEs or led to study discontinuation or interruption. UTI was reported as a frequent adverse reaction in subjects with multiple sclerosis who received

S1P receptor modulators Zeposia (ozanimod)⁴ and Ponvory (ponesimod)⁵. Given that etrasimod may increase the risk of infection, UTI will be included in the Adverse Reactions section of the label.

- **Nausea:** A greater proportion of subjects who received etrasimod had an AE of nausea (3%) compared to placebo (1%). Most nausea AEs were mild in severity, with only one considered moderate. None of the AEs were SAEs, led to study discontinuation, or a pause in study treatment. An imbalance was also observed in APD334-302 and APD334-003 as described in Section 8.2.5.5. Nausea has also been reported as an adverse reaction in other S1PR modulators, including Zeposia (ozanimod), which is approved for the treatment of moderately to severely active UC. Taken together, nausea will be included in the Adverse Reactions section of the label.
- **Hypercholesterolemia:** A greater proportion of subjects who received etrasimod had an AE of hypercholesterolemia (3%) compared to placebo (0%). Most AEs were mild, only one AE was considered moderate. All subjects with hypercholesterolemia AEs had cholesterol levels that remained < 2 x ULN. Hypercholesterolemia has been reported as an adverse reaction in another S1P receptor modulator, Ponvory (ponesimod). Although the clinical significance of the degree of hypercholesterolemia is uncertain, the review team considered it important to inform patients and prescribers of this potential risk. Therefore, hypercholesterolemia will be included in the Adverse Reactions section of the label.
- **Herpes viral infection:** The AEs of herpes viral infection were considered AESI and are described in detail in Section 8.2.4.4.
- **Blood creatine phosphokinase increased:** A greater proportion of subjects who received etrasimod had an AE of increased blood creatine phosphokinase (CPK) (2%) compared to placebo (1%). All the AEs were mild or moderate, none of the AEs were SAEs, and none of the AEs led to study discontinuation. Elevation of CPK resolved in all subjects despite continued treatment with etrasimod. There were no other AEs related to rhabdomyolysis (e.g., rhabdomyolysis, myoglobinuria, chromaturia) reported in these subjects. CPK elevation has not been reported as an adverse reaction in other S1P receptor modulators. Therefore, the increased CPK is not likely to be related to etrasimod. The review team does not recommend its inclusion in labeling.
- **Muscle spasms and vomiting:** A greater proportion of subjects who received etrasimod had an AE of muscle spasms (2%) and vomiting (2%) compared to placebo (none for both). All of the AEs were mild or moderate, none of the AEs were SAEs, and none of the AEs led to study discontinuation. One AE of vomiting led to study drug interruption for 1 day. Only 1 subject who reported an AE of vomiting also reported an AE of nausea on

⁵ See Ponvory (ponesimod) at <https://www.accessdata.fda.gov/scripts/cder/daf/>

the same day. Therefore, the AEs of muscle spasm and vomiting are likely not clinically significant, and the review team did not recommend inclusion in labeling.

8.2.4.6. Laboratory Findings, Vital Signs, and Electrocardiograms

Laboratory Findings

Routine hematology and chemistry clinical laboratory testing were performed during Screening, at Baseline, and throughout the study from Weeks 2 to 52, and at 2- and 4-week follow-up visits (Weeks 54 and 56), early termination visit, and/or end of treatment visit according to the Schedule of Activities.

No clinically meaningful differences between etrasimod and placebo were observed in hematology and chemistry parameters except for lymphopenia. However, lymphopenia was anticipated based on the mechanism of action of the drug. Additional safety analyses for lymphopenia are described in detail in Section 8.2.6.2.

Vital Signs

Due to the anticipated effects of etrasimod on bradyarrhythmia and atrioventricular conduction delays, first-dose cardiac monitoring was implemented during APD334-301. Subjects were monitored in-clinic for at least 4 hours with the following monitoring:

- Baseline vital signs and 12-lead ECG at pre-randomization
- Subjects remained under observation at the site for at least 4 hours after the first dose of study treatment.
- At Hours 1, 2, and 3 post-dose, the HR and systolic/diastolic BP were assessed. If the subject had a HR <50 bpm or if cardiovascular symptoms developed, then the subject would remain closely monitored and a 12-lead ECG would be performed.
- At Hour 4, a discharge assessment was performed including heart rate, systolic/diastolic BP, and a 12-lead ECG. Subjects could be discharged from the clinic if they met the discharge criteria (Table 56).
- If subjects did not meet the discharge criteria at Hour 4, extended cardiac monitoring was performed including:
 - Hourly vital signs and 12-lead ECG until the subject meets the discharge criteria.
 - Any subject who required extended monitoring on Day 1 must return on Day 2 for the second dose and were re-monitored as on Day 1.

Table 56. Discharge Criteria After Cardiac Monitoring

Subjects will be released from the clinical site after dosing on Day 1 (but no sooner than 4 hours post-dose) when they fulfill the following discharge criteria:
• Heart rate $\geq$ 50 bpm or no more than 10 bpm lower than the pre-dose (baseline) value
• No evidence of second-degree AV block or higher
• No cardiac symptoms (eg, chest pain, dizziness, palpitations, lightheadedness, shortness of breath, or syncope)

Source: Clinical Study Report, Phase 3, 52-Week Study in UC (ELEVATE UC 52), 16.1.1 Protocol and Protocol Amendments, Table 4

APD334-301 included discontinuation criteria related to post-dose cardiac monitoring, as described in Table 57.

Table 57. Discontinuation of Study Treatment-Related to Post-Dose Cardiac Monitoring

Reasons for Study Treatment Discontinuation Related to Post-Dose Cardiac Monitoring^a
• Subjects who have a cardiovascular treatment-related symptomatic event (eg, chest pain, dizziness, palpitations, lightheadedness, shortness of breath, or syncope) associated with reduction of the heart rate or associated with clinically relevant 12-lead ECG changes at any time during the 4-hour monitoring period on Day 1 or Day 2 (as applicable).
• Subjects who have not met the discharge criteria on Day 1 after $\geq$ 4 hours of extended monitoring, or Day 2 by 4 hours post-dose.

Source: Clinical Study Report, Phase 3, 52-Week Study in UC (ELEVATE UC 52), 16.1.1 Protocol and Protocol Amendments, Table 4

Additionally, subjects underwent the same first-dose cardiac monitoring procedures as was performed for the original treatment initiation if study treatment dosing was interrupted for $\geq$ 2 consecutive days within the first week of treatment or $\geq$ 7 days after the first week of treatment.

Bradycardia was reported by a greater proportion of subjects who were treated with etrasimod compared to placebo. The impact of etrasimod on bradyarrhythmia is discussed in detail in Section 8.2.6.2. Similarly, hypertension was an AESI and observed in a greater proportion of subjects treated with etrasimod compared to placebo. Refer to Section 8.2.4.4 for additional details.

No other clinically meaningful differences between etrasimod and placebo were observed in other routine vital signs during APD334-301.

Electrocardiograms (ECGs)

During APD334-301, 12-lead ECGs were obtained during the first-dose cardiac monitoring as described previously. Additionally, 12-lead ECGs were obtained at Week 12, Week 52, and during unscheduled visits. Bradyarrhythmia and AV conduction delays are described in Section

8.2.4.4 and Section 8.2.6.2. Overall, the number of abnormal and clinically significant ECGs (e.g., bradycardia, first-degree AV block, second-degree AV block, completed right bundle branch block, left bundle branch block, sinus tachycardia, atrial fibrillation) was balanced between etrasimod (13%) and placebo (14%).

QT

During APD334-301, no clinically significant trends for QT prolongation were observed. The QT prolongation potential of etrasimod was assessed in a thorough QT (TQT) study. The results from the TQT study indicated that etrasimod was not associated with significant QTc prolonging effect. Refer to the QT-IRT Review by Eliford Kitabi dated March 3, 2023 (DARRTS ID: 5135842).

8.2.5. Safety Results for Pooled Data from APD334-302 and APD334-003

Overview of Adverse Events

A summary of AEs reported during trials APD334-303 and APD334-003 is provided in Table 58. Overall, a greater proportion of subjects who received placebo reported SAEs, AEs leading to study drug discontinuation, severe TEAEs, and TEAEs compared to subjects who received etrasimod.

Table 58. Overview of Adverse Events, Safety Population, APD334-302 and APD334-003

Event Category	Etrasimod 2 mg	Placebo
	N=288 n (%)	N=170 n (%)
SAE	6 (1.9)	8 (4.1)
SAEs with fatal outcome	0	0
AE leading to permanent discontinuation of study drug	17 (6.1)	1 (0.6)
AE leading to interruption of study drug	6 (2.3)	4 (2.4)
Any TEAE	140 (49.3)	81 (47.4)
Maximum intensity for any TEAE		
Severe	10 (3.5)	8 (4.1)
Moderate	54 (19.2)	31 (18.2)
Mild	76 (26.5)	42 (25.1)

Source: adae.xpt and adsl.xpt

Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).

Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

Abbreviations: AE, adverse event; N, number of subjects in treatment arm; n, number of subjects with at least one event; SAE, serious adverse event; TEAEs, Treatment emergent adverse events

8.2.5.1. Deaths

There were no deaths during trials APD334-302 or APD334-003.

8.2.5.2. Serious Adverse Events

Subjects with at least 1 serious adverse event (SAE) are shown in Table 59. Overall, SAEs were reported by a greater proportion of subjects who received placebo than were treated with etrasimod, with 8/170 (4%) of subjects who received placebo reporting at least one SAE

compared to 6/288 (2%) of subjects treated with etrasimod. Ulcerative colitis was the only SAE experienced by subjects in both groups and the incidence was similar between the groups.

Table 59. Subjects With Serious Adverse Event (SAE) by System Organ Class and Preferred Term, Safety Population, APD334-302 and APD334-003

System Organ Class (SOC) Preferred Term (PT)	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Subjects with at least one serious adverse event (SAE)	6 (1.9)	8 (4.1)
Gastrointestinal disorders	3 (0.9)	6 (3.0)
Colitis ulcerative	3 (0.9)	3 (1.4)
Abdominal pain ¹	0	2 (1.1)
Duodenal ulcer perforation	0	1 (0.5)
Cardiac disorders		
Coronary artery disease	1 (0.3)	0
Injury, poisoning and procedural complications		
Hepatobiliary procedural complication	1 (0.3)	0
Nervous system disorders		
Migraine	1 (0.3)	0
Hepatobiliary disorders		
Jaundice	0	1 (0.5)
Blood and lymphatic disorders		
Anemia	0	1 (0.6)
Renal and urinary disorders		
Hydronephrosis	0	1 (0.5)

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP

Analysis for both SOC and PT are subject level (i.e., each subject is counted once regardless of the number of reported events).

Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

¹Abdominal pain includes PTs abdominal pain and abdominal pain upper.

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; SAE, serious adverse event

Two subjects that received etrasimod experienced SAEs that led to study discontinuation, both due to ulcerative colitis. SAEs of ulcerative colitis were considered unlikely to be related to etrasimod as these represent worsening of the underlying disease. Pertinent narratives for SAEs are described below.

Pertinent Narratives for SAEs

- Coronary artery disease:** During APD334-302, a 62-year-old female was hospitalized for an SAE of coronary artery disease on Study Day 15. Relevant medical history included dyslipidemia (since 1975), obesity (since 1980), sleeve gastrectomy (2020), ulcerative colitis (since 2017), and hypertension (since 2021). Concomitant medications ongoing at the onset of the event of coronary artery disease included paracetamol and tramadol. The subject's last dose of study treatment prior to the onset of the SAE was on Study Day 14. On Study Day 15, the subject reported symptoms of dyspnea on exertion for last few months. The subject underwent coronary angioplasty on Study Day 15. Study treatment dosing was not changed due to the event. The event was considered recovered/resolved on Study Day 16 and the subject was discharged from the hospital. The review team considered the SAE as unlikely to be related to etrasimod given the

subject's past medical history and lack of other coronary artery disease events reported in the etrasimod development program.

- **Hepatobiliary procedural complication:** During APD334-302, a 29-year-old White male underwent an endoscopic retrograde cholangiopancreatography (ERCP) with stent placement on Study Day 9 for the nonserious adverse event of primary sclerosing cholangitis, diagnosed on Study Day -3 by magnetic resonance cholangiopancreatography. Approximately 1.5 hours post ERCP procedure, the subject reported abdominal pain (5/10 on the pain scale) and was admitted to the hospital for pain control. The subject was diagnosed with post-ERCP pancreatitis, although no laboratory values of pancreatic enzymes were provided by the Applicant. No action was taken with study treatment due to this event. On Study Day 10, the SAE was considered recovered/resolved and the subject was discharged. The subject's last dose of study treatment prior to the SAE was on Study Day 8. Relevant medical history included iron deficiency (date not available), ulcerative colitis (since 2008), and osteopenia (since 2017). Concomitant medications ongoing at the onset of the event of hepatobiliary procedural complication included mesalazine. The review team considered the SAE as unlikely to be related to etrasimod, as post-ERCP pancreatitis is a well-described potential complication of ERCP. Further, the ERCP was performed due to PSC, which was diagnosed prior to initiating study treatment.
- **Migraine:** During APD334-302, a 28-year-old White female with a history of migraine and intermittent headache reported an SAE of migraine leading to hospitalization and study treatment interruption. The event was first reported on Study Day 9 when the subject presented to the emergency department with frontal headache and associated monocular visual blurring, vomiting, a tingling sensation in her arm, and dysphasia. Computed tomography (CT) of the head and neck and magnetic resonance imaging (MRI) of the brain were normal. The last dose of study drug prior to the SAE onset was on Study Day 8. Concomitant medications at the onset of the event included drospirenone/ethinylestradiol betadex clathrate, mesalazine, and paracetamol. On Study Day 10, the SAE of severe migraine was considered recovered/resolved and study treatment was resumed on Study Day 11. The Investigator assessed the event as unlikely to be related to study treatment; however, the review team determined that a relationship between etrasimod and the SAE of migraine cannot be ruled out. Although the subject had a past medical history of migraine, an imbalance in headache AEs (including migraine) was observed for APD334-301 (refer to Section 8.2.4.5) and APD334-302 and APD334-003 (refer to Section 8.2.5.5). Therefore, headache, including related terms headache, migraine, tension headache, and sinus headache, will be included as an adverse reaction in the Adverse Reactions section of the label.

8.2.5.3. Discontinuations Due to Adverse Events

The overall incidence of AEs leading to discontinuation was greater in the etrasimod group compared to the placebo groups with 17/289 (6%) of subjects treated with etrasimod experiencing at least one AE that led to discontinuation, compared to 1/170 (1%) of subjects who received placebo. Subjects with AEs leading to treatment discontinuation are shown in Table 60.

Table 60. Subjects With Adverse Events Leading to Treatment Discontinuation by System Organ Class and Preferred Term, Safety Population, APD334-302 and APD334-003

System Organ Class (SOC) Preferred Term (PT)	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Subjects with at least one AE leading to discontinuation	17 (6.1)	1 (0.6)
Gastrointestinal disorders	10 (3.5)	1 (0.6)
Colitis ulcerative	8 (2.9)	0
Liver function test abnormal	1 (0.3)	0
Diarrhea	1 (0.3)	0
Abdominal pain	0	1 (0.6)
Cardiovascular	5 (1.8)	0
Bradycardia ¹	3 (0.9)	0
Atrioventricular block first degree	1 (0.3)	0
Electrocardiogram T-wave abnormal	1 (0.5)	0
General disorders and administration site		
Weight decreased	1 (0.3)	0
Nervous system disorders		
Neurological symptom	1 (0.5)	0

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP
Analysis for both SOC and PT are subject level (i.e., each subject is counted once regardless of the number of reported events).
Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

¹ Bradycardia includes the preferred terms bradycardia and sinus bradycardia

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; AE, adverse event

The most common AE that led to study discontinuation was ulcerative colitis. Two of the AEs of ulcerative colitis were SAEs. However, AEs of ulcerative colitis are most likely related to the underlying disease and lack of response to treatment, rather than an adverse event. Four subjects discontinued from the study due to AEs that were considered AESIs, one AE of liver function test abnormal, one AV block first degree, and three AEs of bradycardia. These AEs are described in detail below.

- Liver function test abnormal:** During APD334-302, a 36-year-old Asian female experienced both AST and ALT elevations to 3.1 x ULN and 5.8 x ULN, respectively, on Study Day 15. On Study Day 19, the subject discontinued study treatment due to the AE of liver function test abnormal. The AST remained elevated to ≥ 4 x ULN and ALT to > 6 x ULN through Study Day 28, after which both returned to normal on Study Day 36 and remained there through the final visit on Study Day 50. No elevation in bilirubin was observed. Azathioprine had been discontinued 14 days before randomization, and concomitant medications included propranolol for migraine and oral contraceptive. No

treatment was administered for the event. Given S1P receptor modulators have been associated with liver enzyme elevations and TEAEs of elevated liver tests were observed in the etrasimod development program, the review team considered this AE as likely related to study treatment. Elevated liver tests were a common adverse reaction during APD334-302 and APD334-003 and will be included in the Adverse Reactions section of the label. Additionally, a Warnings and Precautions regarding liver injury will also be included in the label.

- **Bradycardia and AV Block First-degree:** The following AEs related to bradyarrhythmia and AV conduction delays led to study discontinuation. All AEs occurred during APD334-302.
 - **Bradycardia:** 3 subjects treated with etrasimod discontinued the study due to AESIs of bradycardia.
 - The first subject was a 73-year-old White female with a longstanding history of hypertension. Heart rates on Study Day -28 and predose on Study Day 1 were 61 bpm and 66 bpm, respectively. Baseline ECG on Study Day -28 was interpreted as normal. On Study Day 1, the subject had a moderate AE of “sinus bradycardia” (HR of 49 bpm) and discontinued study treatment on the same day. No treatment was given for the event. There were no associated symptomatic adverse events reported and no changes to the subject’s blood pressure. The subject’s only dose of study treatment was taken on Study Day 1. The subject was monitored on Study Day 2 (after discontinuing treatment) where the HR was 48-53 bpm. On Study Day 3, the event was considered recovered/resolved with HR of 56 bpm. The event was assessed to be related to study treatment.
 - The second subject was a 43-year-old White female who experienced a mild AE of “sinus bradycardia” 4 hours after receiving etrasimod (HR of 45 bpm) on Study Day 1. A repeat ECG showed a HR of 51 bpm on Study Day 1, and the event was considered recovered/resolved. The subject had repeat vital signs and ECG performed at the Early Termination visit on Study Day 6, which showed a HR of 60 bpm.
 - The third subject was a 23-year-old Black male who experienced a mild AE of bradycardia after receiving etrasimod with a pre-dose HR of 60 bpm and a 4-hour post-dose HR of 45 bpm on Study Day 1. Two additional hours of monitoring on Day 1 had similar heart rates of 49 bpm and 50 bpm, again without symptoms. On Study Day 1, the event was considered recovered/resolved. No action was taken with study drug at that time. On Study Day 2, the subject had a mild AE of bradycardia with a pre-dose HR of 51 bpm and a 1-hour post-dose HR of 45 bpm. The subject also had nonserious mild AEs of dizziness and palpitations on Study Day 2. The AEs

of bradycardia, dizziness, and palpitations resolved the same day (Study Day 2). The last dose of study drug was taken on Study Day 2. No treatment was given for the event. At the Early Termination visit on Study Day 17, the ECG recorded a HR of 63 bpm.

- AV block first-degree: A 35-year-old White male with a history of hypertension was diagnosed with a moderate AE of AV block first degree with ECG results that showed AV first degree, ventricular hypertrophy, normal T-wave morphology, sinus rhythm, and a heart rate of 58 bpm at pre-dose on Study Day 1. At 4 hours post-dose on Study Day 1, the atrioventricular block first degree was again noted. On Study Day 14, the ECG was reported as abnormal with first-degree AV block. The subject discontinued study treatment due to the first-degree AV block (which had not changed from pre-dose). No treatment was administered for either event. Given the AV block was identified prior to initiating treatment with etrasimod, the AE is unlikely to be related to etrasimod.
- ECG T wave abnormal: During APD334-003, a 48-year-old white female experienced a non-specific T wave abnormality on ECG during the screening period. On Study Day 1, a non-specific T wave abnormality was documented 2 hours post-dose, with the consideration of ischemia. The subsequent ECG results remained unchanged throughout the rest of the day. However, the last ECG at 8 hours post-dose was considered clinically significant by the investigator and was reported as an adverse event of an ECG T wave abnormal (reported term: worsening of T wave changes). There were no symptoms recorded by the Investigator and the patient's blood pressure (113/68 mmHg) was stable. No treatment was reported for the event. The study drug was interrupted because of the abnormal ECG T wave. The subject was subsequently evaluated with a stress test, echocardiogram, cardiologist evaluation, and cardiac CT calcium score obtained between Day 15 and Day 27, which were all normal. Prior medical history included cholelithiasis, dyspepsia, gastritis, arthralgia, headache, and chronic tobacco and caffeine intake. The subject did not take any medications for UC at the time of screening. Prior medication included oral 5-aminosalicylates. The study drug was re-introduced on Day 36 with a pre-dose ECG finding of nonspecific T wave abnormality that remained constant throughout the day. At the time of the event, the patient was not taking any concomitant medications. The study drug was permanently discontinued because of the adverse event of ECG T wave abnormal. On Study Day 382, the event of ECG T wave abnormal was considered recovered/resolved. Given that the ECG T wave abnormality was identified prior to initiating treatment with etrasimod, it is unlikely that that the AE was related to the study drug.

Due to the risk of cardiovascular events as observed in the etrasimod development program and the known risks described in other S1PR modulators, a Warnings and Precautions will be

included in the label regarding the risk of bradyarrhythmia and atrioventricular conduction delays, as well as increased blood pressure.

- **Neurological symptom:** During APD334-003, A 59-year-old White female with a history of pancolitis experienced an AE of “neurological symptom” described as multiple small-focal neurological symptoms by the Investigator on Study Day 85. At the time of the event, the patient was also experiencing nonserious AEs of hypertension and urinary tract infection. The patient’s last dose of study drug was taken on Study Day 84. On Study Day 125, the event of neurological symptom was considered recovered/resolved. Medical history also included affect lability, hypertension, hypercholesterolemia, chronic gastritis, duodenal papillitis, arthritis, goiter, and chorioretinal atrophy. Concomitant medications included mesalazine, alimemazine tartrate, valsartan, diltiazem, indapamide, algeldrate/magnesium hydroxide, pancreatin, levothyroxine, and ciprofloxacin. No treatment was reported for the event. The Investigator assessed the event of neurological symptom to be related to study drug. A follow up report was solicited by the Applicant and received approximately 3 years and 7 months after study completion. At that time, the subject reported ongoing “transient symptoms” (slight staggering when walking, loss of sensitivity in fingers and toes). After study completion, she was never evaluated by a neurologist and had no imaging studies. Hypertension has been controlled since medications were adjusted around the time of study completion. The review team considered that the neurological symptoms may be related to the subject’s past medical history and other concomitant medications; however, details are lacking regarding the subject’s symptoms due to unavailability of additional information. Therefore, a relationship between etrasimod and the AE of neurological symptom cannot be determined.

8.2.5.4. Adverse Events of Special Interest

Similar to APD334-301, PML was identified as an AESI for APD334-302 and APD334-003 at the time of protocol development. APD334-302 and APD334-003 had a similar retrospective, blinded medical review to identify additional AESIs as described for APD334-301; refer to Section 8.2.4.4 for additional details. The review team performed additional analyses of adverse events that are of special interest based on known class effects but that did not meet the Applicant’s criteria for AESI; refer to Section 8.2.6.

The AESIs as identified by the Applicant for APD334-302 and APD334-302 are shown in Table 61. Overall, there were more subjects with at least one AESI in the etrasimod group compared to placebo with 15/288 (5%) of subjects treated with etrasimod experiencing at least one AESI, compared to 5/170 (3%) of subjects treated with placebo.

Table 61. Subjects With Adverse Events of Special Interest (AESI), Safety Population, APD334-302 and APD334-003

Adverse Event of Special Interest	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Subjects with at least one AESI	15 (5.1)	5 (3.0)
Cardiovascular events	10 (3.5)	3 (1.8)
Bradycardia ¹	4 (1.4)	0
Hypertension ²	5 (1.8)	3 (1.8)
AV conduction delay ³	2 (0.8)	0
Liver injury	3 (0.9)	0
Liver transaminases elevation ⁴	2 (0.6)	0
Bilirubin elevation ⁵	1 (0.3)	0
Infections (severe, opportunistic, or herpes infections)	2 (0.6)	2 (1.3)
Opportunistic infections ⁷	1 (0.3)	0
Herpes simplex and herpes zoster ⁸	1 (0.3)	2 (1.3)

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP

Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).

Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

¹ Bradycardia includes PTs: bradycardia and sinus bradycardia.

² Hypertension includes PTs: hypertension, blood pressure increased, and essential hypertension.

³ AV conduction delay includes PTs: atrioventricular block first degree, atrioventricular block second degree

⁴ Liver transaminases elevation includes PTs: alanine aminotransferase increased and aspartate aminotransferase increased.

⁵ Bilirubin elevation includes the PT liver function test abnormal

⁶ Severe infections were defined by the Applicant as events in the “Infections and Infestations” MedDRA SOC with severity reported as Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (death).

⁷ Opportunistic infections include the PT cytomegalovirus infection

⁸ Herpes simplex and herpes zoster includes PTs: herpes simplex, oral herpes, and herpes zoster.

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; AESI, adverse event of special interest

Cardiovascular AESIs, including hypertension, bradycardia, and AV conduction delay, and liver injury AESIs were reported more frequently in the etrasimod group compared to placebo. No AESIs of PML, severe infection, macular edema, pulmonary disorders, PRES, or malignancy were reported. Refer to Section 8.2.6 for additional analyses of AEs of special interest that did not meet the Applicant’s criteria for AESI. AESIs that were reported more frequently in subjects who received etrasimod compared to placebo are described in detail below.

- **Cardiovascular events:** All AESIs of bradycardia and AV conduction delay occurred on Day 1 or Day 2. No cardiovascular events were SAEs. Cardiovascular events led to discontinuation of treatment in 4 subjects all of whom received etrasimod in APD334-302; 3 subjects with bradycardia and 1 subject with AV conduction delay. These AEs that led to discontinuation are described in detail in Section 8.2.5.3.

Due to the risk of cardiovascular events, specifically bradyarrhythmia, AV conduction delay, and hypertension, observed in the etrasimod development program and the known risks described in other S1P receptor modulators, a Warnings and Precautions will be described in the label regarding these risks.

- **Liver injury:** The overall incidence of liver injury AESIs was low at 1% in the etrasimod group; however, there were no subjects with AESIs of liver injury in the placebo group. No liver injury AESIs were SAEs and none of the AESIs were considered severe in severity. Additionally, there were no cases that raised concern for drug-induced liver injury, including no Hy's Law cases. One AESI of liver transaminase elevation (PT "liver function test abnormal") in the etrasimod group of APD334-302 led to treatment discontinuation and is described in detail in Section 8.2.5.3. A Warnings and Precautions regarding liver injury will also be included in labeling, aligned with other S1P receptor modulators.
- **Opportunistic infection:** One subject who received etrasimod in APD334-302 reported an AESI of an opportunistic infection, which was cytomegalovirus (CMV) infection. The subject was a 68-year-old Asian male who had withdrawn from the study on Study Day 20 due to "withdrawal by subject or parent/guardian." On Study Day 36, the subject reported a worsening of bowel movement frequency, and a CMV stool antigen test was performed and was positive. An AESI of CMV infection was reported on the same day. At the time of the CMV AESI, the subject had been receiving rectal budesonide since Study Day 20 (day of study discontinuation) and had just started vedolizumab on Study Day 36 (the day of the AESI). Given CMV is a common infection observed in patients with UC and AESI was reported after the subject had discontinued etrasimod, the relationship between etrasimod and the AESI is uncertain.

8.2.5.5. Treatment Emergent Adverse Events and Adverse Reactions

An overview of treatment emergent adverse events (TEAEs) for the pooled data for APD334-302 and APD334-003 is shown in Table 58. Overall, the proportion of subjects with at least one TEAE was similar in subjects who received etrasimod (140/288, 49%) compared to placebo (81/170, 47%). The majority of TEAEs were mild or moderate in severity. The number of subjects who reported a severe TEAE was similar between etrasimod (10/288, 4%) and placebo (8/170, 4%).

TEAEs that occurred in at least 2% of subjects during APD334-302 and APD334-003 and at a higher frequency than placebo are shown in Table 62. Headache and elevated liver tests were the most common TEAEs during APD334-302 and APD334-003, which was similar to APD334-301.

Table 62. Treatment-Emergent Adverse Events (TEAEs) Occurring in at Least 2% of Subjects and at a Higher Frequency Than Placebo, Safety Population, APD334-302 and APD334-003

Preferred Term	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Subjects with at least 1 TEAE	140 (48.6)	81 (47.3)
Headache ¹	17 (5.9)	7 (4.3)
Elevated liver tests ²	15 (5.1)	1 (0.5)
Colitis ulcerative	11 (3.8)	5 (2.5)
Nausea	11 (3.7)	4 (2.2)
Pyrexia	10 (3.5)	4 (2.4)
Bradycardia ⁶	8 (2.9)	0
Urinary tract infection ³	8 (2.9)	0
Vomiting	6 (2.1)	2 (1.3)
Blood creatine phosphokinase increased ⁴	5 (1.9)	1 (0.6)
Diarrhea ⁵	5 (1.8)	0
Abdominal distention	5 (1.6)	0

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP

Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).

Gray shading reflects what will be included in the label as the most common adverse reactions

Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

¹ Headache includes PTs: headache, migraine, and sinus headache.

² Elevated liver tests includes PTs: ALT increased, AST increased, blood alkaline phosphatase increased, blood bilirubin increased, cholestasis, GGT increased, hepatic enzyme increased, hepatic function abnormal, liver function test abnormal, and transaminases increased.

³ Urinary tract infection includes PTs: urinary tract infection, cystitis, and genitourinary tract infection.

⁴ Blood creatine phosphokinase increased includes PTs: Blood creatine phosphokinase increased, and blood creatine phosphokinase MB increased.

⁵ Diarrhea includes PTs: diarrhea and frequent bowel movements.

⁶ Bradycardia includes PTs: bradycardia, sinus bradycardia, and heart rate decreased.

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; TEAE, treatment emergent adverse event

TEAEs that occurred in at least 2% of subjects treated with etrasimod and more frequently than placebo are described below.

- Headache:** Headache was the most common TEAE reported by subjects during APD334-302 and APD334-003. A greater proportion of subjects who received etrasimod (6%) reported a TEAE of headache compared to placebo (4%). The majority of headache TEAEs were mild or moderate in severity. One subject reported a severe TEAE of headache, which was considered an SAE (preferred term “migraine”), which is described in detail in Section 8.2.5.2. No headache TEAEs led to study discontinuation. An imbalance was also observed in APD334-301 (refer to Section 8.2.5.5). The mechanism by which etrasimod may increase the risk of headache is unknown; however, headache is an adverse reaction observed in several other S1P receptor modulators, including Zeposia (ozanimod), which is approved for the treatment of moderately to severely active UC. Therefore, headache will be included in the Adverse Reactions section of the label.
- Elevated liver tests:** A greater proportion of subjects who received etrasimod had a TEAE of elevated liver tests (5%) compared to placebo (<1%). All of the elevated liver test TEAEs were mild or moderate in severity. None of the TEAEs were considered an

SAE. One subject was discontinued from study treatment due to a TEAE of elevated liver tests (preferred term “liver function test abnormal”); refer to Section 8.2.5.3. An imbalance was also observed in APD334-301 (refer to Section 8.2.4.5). Liver injury is a Warnings and Precautions for other S1P receptor modulators, including Zeposia, which is approved for the treatment of moderately to severely active UC. Although the mechanism by which etrasimod might cause liver injury is not known, a similar Warnings and Precautions will be included in the etrasimod label. Refer to Section 8.2.6.4 for additional hepatotoxicity analyses.

- **Colitis ulcerative:** Although a greater proportion of subjects who received etrasimod had a TEAE of “colitis ulcerative” (i.e., ulcerative colitis) (4%) compared to placebo (3%), the difference was small. Three TEAEs of ulcerative colitis were SAEs. Two SAEs and 6 non-serious TEAEs of ulcerative colitis led to study discontinuation. The review team determined that TEAEs of ulcerative colitis are likely to be related to the underlying disease/lack of response to therapy and unlikely to represent a drug effect and does not recommend inclusion in the Adverse Reactions section of the label.
- **Nausea:** A greater proportion of subjects who received etrasimod had a TEAE of nausea (4%) compared to placebo (2%). The majority of the TEAEs of nausea were mild or moderate, one TEAE was severe. None of the TEAEs of nausea were considered SAEs, led to study discontinuation, or a pause in treatment. An imbalance was also observed in APD334-301 as described in Section 8.2.4.5. Nausea has also been reported as an adverse reaction in other S1P receptor modulators, including Zeposia, which is approved for the treatment of moderately to severely active UC. Taken together, nausea will be included in the Adverse Reaction sections of the label.
- **Pyrexia:** Although a greater proportion of subjects who received etrasimod had a TEAE of pyrexia (3%) compared to placebo (2%), the difference was small. The majority of TEAEs of pyrexia were associated with other TEAEs that may explain the pyrexia (e.g., recent COVID-19 vaccination, diarrhea, upper respiratory tract infection). The review team determined that pyrexia was unlikely to be related to etrasimod and does not recommend inclusion in the Adverse Reactions section of the label.
- **Urinary tract infection:** A greater proportion of subjects who received etrasimod had a TEAE of urinary tract infection (3%) compared to placebo (0%). All of the urinary tract infection TEAEs were mild or moderate. None of the TEAEs were considered SAEs or led to study discontinuation. The mechanism of action of etrasimod (i.e., inhibition of lymphocyte trafficking) has been associated with an increased risk of infection. Aligned with other S1P receptor modulators, the label for etrasimod will include a Warnings and Precautions regarding infection. Additionally, urinary tract infections will be included in the Adverse Reactions section of the label.
- **Bradycardia:** A greater proportion of subjects who received etrasimod had a TEAE of bradycardia (3%) compared to placebo (0%). Four TEAEs of bradycardia were considered

AESIs and are described in Section 8.2.5.4. An additional four TEAEs of bradycardia were not reported as AESIs because they did not meet the Applicant's criteria. Three subjects discontinued the study due to TEAEs of bradycardia, as described in Section 8.2.5.3. The mechanism of action of etrasimod has been associated with bradyarrhythmia and a Warnings and Precautions regarding bradyarrhythmia will be included in labeling. Additionally, bradycardia will be described in the Adverse Reactions section of the label.

- **Blood creatine phosphokinase increased:** A greater proportion of subjects who received etrasimod had an AE of blood creatine phosphokinase (CPK) increased (2%) compared to placebo (1%). All of the AEs were mild or moderate, none of the AEs were SAEs, and none of the AEs led to study discontinuation. All the subjects had normal CPK following the AE of increased CPK. There were no other AEs related to rhabdomyolysis (e.g., rhabdomyolysis, myoglobinuria, chromaturia) reported in these subjects. Therefore, the increased CPK is not likely to be related to etrasimod. The review team did not recommend inclusion in labeling.
- **Vomiting, abdominal distension, and diarrhea:** Vomiting, abdominal distension, and diarrhea were each reported by more subjects who received etrasimod compared to placebo. However, the majority of TEAEs of vomiting, abdominal distension, and diarrhea were reported in association with additional TEAEs (e.g., pyrexia, abdominal pain, ulcerative colitis, gastroenteritis) that may explain the reported TEAEs. The review team determined that vomiting, abdominal distension, and diarrhea were unlikely to be related to etrasimod and does not recommend inclusion in the Adverse Reactions section of the label.

8.2.5.6. Laboratory Findings, Vital Signs, and Electrocardiograms

Laboratory Findings

Routine hematology and chemistry clinical laboratory testing were performed during Screening, at Baseline, and throughout the study from Weeks 2 to 12, and at 2-week follow-up visits (Week 14), early termination visit, and/or end of treatment visit according to the Schedule of Activities.

No clinically meaningful differences between etrasimod and placebo were observed in hematology and chemistry parameters except for lymphopenia. However, lymphopenia was anticipated based on the mechanism of action of the drug. Additional safety analyses for lymphopenia are described in detail in Section 8.2.6.2.

Vital Signs

First-dose cardiac monitoring was performed during APD334-302, similar to APD334-301. Refer to Section 8.2.4.6, Vital Signs for further details. During APD334-003, subjects had 24-hour Holter monitoring. Bradycardia was reported by a greater proportion of subjects who were treated with etrasimod compared to placebo, as described previously in Section 8.2.5.4 and

Section 8.2.5.5. The impact of etrasimod on bradyarrhythmia is also discussed in detail in Section 8.2.6.3.

No other clinically meaningful differences between etrasimod and placebo were observed in other routine vital signs during APD334-302 and APD334-003.

Electrocardiograms (ECGs)

During APD334-302, 12-lead ECGs were obtained during the first-dose cardiac monitoring as described previously. Additionally, 12-lead ECGs were obtained at Week 12 and during unscheduled visits. During APD334-003, Holter monitoring was performed during the first 24-hours of treatment and 12-lead ECGs were obtained at screening, Weeks 0, 1, 2, 4, 8, and 12 and during unscheduled visits. Bradyarrhythmia and AV conduction delays are described in Section 8.2.5.4 and Section 8.2.6.2. Overall, the number of abnormal and clinically significant ECGs (e.g., bradycardia, first-degree AV block, second-degree AV block, complete right bundle branch block, left bundle branch block, sinus tachycardia, atrial fibrillation) was balanced between etrasimod (8%) and placebo (11%) for APD334-302 and no clinically meaningful changes in ECG parameters were reported for APD334-003.

QT

During APD334-302 and APD334-003, no clinically significant trends for QT prolongation were observed. The QT prolongation potential of etrasimod was assessed in a thorough QT (TQT) study. The results from the TQT study indicated that etrasimod was not associated with significant QTc prolonging effect. Refer to the QT-IRT Review (DARRTS ID: 5135842) by Eliford Kitabi dated March 3, 2023.

8.2.6. Analysis of Submission-Specific Safety Issues

8.2.6.1. Infections

S1P receptor modulators have been associated with an increased risk of infection due to the mechanism of action of the drug, which causes a reduction in peripheral blood lymphocytes. The Applicant evaluated the risk of severe infections, opportunistic infections, and herpes simplex and herpes zoster infections; refer to Section 8.2.4.4 and Section 8.2.5.4 for details of these analyses. To further characterize the potential risk of infection, the review team conducted additional safety analyses.

Table 63. Summary of Infection TEAEs, Safety Population, APD334-301 and Pooled APD334-302 and APD334-003

Characteristic	APD334-301		APD334-302 and APD334-003	
	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Subjects with at least 1 infection	72 (24.9)	32 (22.2)	38 (14.0)	20 (11.8)
Maximum severity for any infection				
Mild	45 (15.6)	19 (13.2)	21 (7.4)	14 (8.3)
Moderate	24 (8.3)	8 (5.6)	17 (6.7)	6 (3.5)
Severe	3 (1.0)	5 (3.5)	0	0

Source: Reviewer analysis using Applicant submitted data, adae.xpt and adsl.xpt
Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).
Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event

Infections that were reported in at least 2% of subjects and at a higher frequency than placebo were urinary tract infection and herpes viral infection in APD334-301, and urinary tract infection in APD334-302 and APD334-003. Refer to Section 8.2.4.5 and 8.2.5.5 for details regarding urinary tract infections and herpes viral infections

In summary, the overall incidence of infections was slightly higher in the etrasimod treatment group than placebo; however, most infections were mild or moderate in severity, were not serious, and did not lead to study discontinuation. However, based on the mechanism of action of the drug and the observed imbalance in infections, a Warnings and Precautions regarding infection will be included in labeling. Additionally, urinary tract infection and herpes viral infection will be described in the Adverse Reactions section of the label to inform patients and prescribers of the specific risk of these infections.

8.2.6.2. Lymphopenia

Lymphopenia is a known S1P receptor modulator class effect due to the mechanism of action, which reduces lymphocyte egress from lymph nodes. Since etrasimod was expected to cause lymphopenia and may cause neutropenia, investigators were blinded to the complete blood count lab results in order to preserve the blind. An unblinded Medical Monitor assessed WBC differential lab results and alerted the Investigator if (1) ANC <1000 cells/ μ L and/or (2) ALC <200 cells/ μ L. As a result, the incidence of TEAEs of lymphopenia and neutropenia may not be an accurate reflection of lymphopenia and neutropenia during studies APD334-301, APD334-302, and APD334-003 because the TEAE threshold for the investigators was high.

An analysis of the incidence of lymphopenia and neutropenia based on laboratory data for APD334-301 and pooled data for APD334-302 and APD334-003 is shown in Table 64.

Table 64. Subjects With Lymphopenia and Neutropenia, Safety Population, APD334-301 and Pooled APD334-302 and APD334-003

Characteristic	APD334-301		APD334-302 and APD334-003	
	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Absolute Lymphocyte Count (cells/ μ L)				
<800-500	207 (71.6)	16 (11.1)	183 (64.0)	15 (8.4)
<500-200	127 (43.9)	4 (2.8)	85 (28.5)	2 (1.3)
<200	16 (5.5)	0	2 (0.6)	0
Absolute Neutrophil Count (cells/ μ L) ¹				
<1500-1000	29 (10.0)	11 (7.6)	13 (4.7)	6 (3.1)
<1000-500	6 (2.1)	2 (1.4)	2 (0.6)	1 (0.7)
<500	0	0	1 (0.3)	0

Source: Reviewer analysis using Applicant submitted data, ad b.xpt; Software: JMP
Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).
Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.
¹ Neutrophils included search values “neutrophils” and “left shift neutrophils.”
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event

As expected, lymphopenia was more common in the subjects who received etrasimod compared to placebo. An ALC of <200 cells/ μ L was observed in 16/289 (5.5%) and 2/288 (0.6%) subjects who received etrasimod during APD334-301 and APD334-302 and APD334-003, respectively versus none in placebo. Among subjects with an ALC of <200 cells/ μ L, rhinitis, COVID-19, and tinea each occurred once in 3 subjects, and upper respiratory tract infection occurred twice in a fourth subject; all were reported as mild in severity and no infection in a subject with ALC <200 cells/ μ L was serious or led to study discontinuation. As described in Section 16.3.2, the median time for peripheral blood lymphocytes to return to the normal range was 2.6 weeks, with approximately 90% of subjects in the normal range within 4.7 weeks.

Neutropenia was generally similar between subjects who received etrasimod compared to placebo, only 1 subject (0.3%) who received etrasimod had an ANC of <500 cells/ μ L versus none in placebo and this subject experienced no TEAEs of infection.

Although the overall incidence of infections was greater in the etrasimod group compared to placebo during APD334-301 and pooled data from APD334-302 and APD334-003, the difference between the two groups was small (25% etrasimod vs. 22% placebo in APD334-301, and 14% vs. 12% in pooled APD334-302 and APD334-003). Refer to Section 8.2.6.1 for further analysis of infection risk with etrasimod.

Based on the available data and consistent with the mechanism of action of the drug, lymphopenia, including severe lymphopenia (<200 cells/ μ L), was observed in subjects who received etrasimod. However, the overall rate of infection between etrasimod and placebo was only slightly higher in etrasimod compared to placebo. Additionally, the majority of infections, even for subjects with severe lymphopenia, were mild, not serious, and did not lead to study discontinuation. Therefore, to address the risk of lymphopenia due to etrasimod, the Warnings and Precautions section of the label will describe the reduction in peripheral blood lymphocyte count and the risk of infection in subjects in APD334-301 and pooled studies APD334-302 and

APD334-003. The label will include recommendations to obtain a baseline complete blood count, including lymphocyte count, and to consider interruption in treatment with etrasimod if a patient develops a serious infection. This information in the label is similar to other S1P receptor modulators.

8.2.6.3. *Bradycardia and Atrioventricular Conduction Delays*

Cardiovascular events including bradycardia, AV conduction delays, and hypertension are known class effects in S1P receptor modulators thought to be related to their effect on S1P receptors found on atrial myocytes and smooth muscle cells (i.e., S1P₁, S1P₂, and S1P₃). Transient lowering of the heart rate and atrioventricular conduction delay were observed with the first dose of etrasimod and when therapy was reinitiated and occurred more frequently in subjects treated with etrasimod versus placebo. The largest heart rate reduction was observed 3 hours after the first etrasimod dose -7.2 (8.98) bpm compared to 0.4 (7.93) bpm in the placebo group. These effects were self-limited and resolved within 8 hours without the need for intervention. Refer to Section 6.3.1 for additional details regarding etrasimod's heart rate reduction effects.

The Applicant considered cardiovascular events as adverse events of special interest (AESIs) if the preferred term met specific criteria, which was reviewed by a blinded medical reviewer to determine whether the AE was considered an AESI. Refer to the description in Section 8.2.4.4 for details on the criteria used by the Applicant to define AESIs.⁶

The review team analyzed the available safety data to characterize the potential risk of bradycardia and AV conduction delay. These events were identified as those with preferred terms bradycardia, sinus bradycardia, heart rate decreased, atrioventricular block first degree, and atrioventricular block second degree. There was no requirement for a specific HR threshold or associated clinical symptoms, which differs from the Applicant's AESI analysis. The incidence of cardiovascular events identified by the review team during APD334-301 and APD334-302 and APD334-003 are shown in Table 65.

⁶ The review team considered that the risk of hypertension was sufficiently characterized by the AESI and TEAE analyses performed for APD334-301 and pooled data from APD334-302 and APD334-003. Additional analyses in this section were not performed. Refer to the respective sections for additional details regarding hypertension.

Table 65. Bradyarrhythmia and Atrioventricular Conduction Delay TEAEs, Safety Population, APD334-301 and Pooled APD334-302 and APD334-003

Preferred Term	APD334-301		APD334-302 and APD334-003	
	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Bradycardia ¹	4 (1.4)	0	8 (2.9)	0
AV conduction delay ²	2 (0.7)	0	2 (0.8)	0

Source: Reviewer analysis using Applicant submitted data, adae.xpt; Software: JMP

Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).

Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

¹ Bradycardia includes PTs: bradycardia, sinus bradycardia, and heart rate decreased.

² AV conduction delay includes PTs: atrioventricular block first degree, atrioventricular block second degree. Subjects that experienced AV conduction delay after being dosed with etrasimod were included.

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; TEAE, treatment emergent adverse event

The review team's broader criteria identified an increased number of subjects with bradycardia in the etrasimod groups of APD334-301 and pooled APD334-302 and APD334-003 compared to the number of subjects reported by the Applicant as AESIs. However, the overall trend between the review team's analysis and the Applicant's AESI analysis was similar.

- **Bradycardia:** During APD334-301, 4 subjects (1%) treated with etrasimod reported AEs of bradycardia, 3 of which occurred on Study Day 1. One subject (<1%) treated with etrasimod reported bradycardia on Study Day 2. In the placebo group, no AEs of bradycardia were reported. During APD334-302 and APD334-003, 8 subjects (2.8%) treated with etrasimod reported AEs of bradycardia, which all occurred on Study Day 1. One subject (0.3%) treated with etrasimod reported bradycardia on Study Day 2 after having reported bradycardia on Study Day 1. In the placebo group, no AEs of bradycardia were reported.

As described previously, no bradycardia TEAEs were SAEs. A total of four bradycardia TEAEs led to study discontinuation (1 during APD334-301 and 3 during APD334-302/APD334-003), which are described in Sections 8.2.4.3 and 8.2.5.3.

- **AV Conduction Delay:** During APD334-301, 2 subjects (1%) in the etrasimod group had AV conduction delay (1 subject with first-degree AV block and 1 subject with second-degree AV block, Mobitz type I) compared to none in placebo. Both events occurred on Day 1. During APD334-302 and APD334-003, 2 subjects (1%) in the etrasimod group had AV conduction delay (1 subject with first-degree AV block and 1 subject with second-degree AV block, Mobitz type I) compared to none in placebo. Both events occurred on Day 1.

As described previously, no AV conduction delays were SAEs. Two subjects (1 from APD334-301 and 1 from APD334-302/APD334-003) were discontinued due to TEAEs of AV conduction delay (both first-degree AV blocks), which are described in Sections 8.2.4.3 and 8.2.5.3.

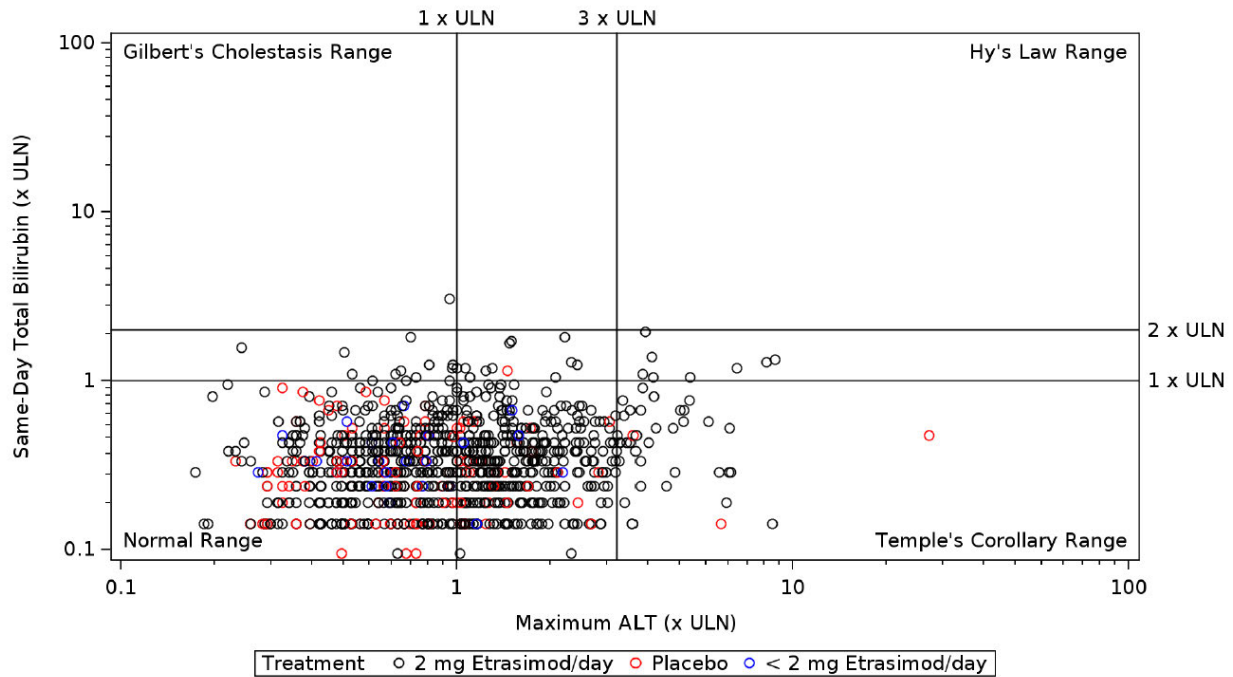
In summary, treatment with etrasimod resulted in a transient decrease in heart rate and an increased risk of AV conduction abnormalities (first-degree and second-degree [Mobitz Type I] AV block). Bradycardia after the first dose is a known class effect of S1P receptor modulators. Bradycardia and AV conduction delays were transient, mostly asymptomatic, and most episodes resolved within 8 hours. Therefore, the Warnings and Precautions section of the label will describe the risk of bradyarrhythmia and AV conduction delays, aligned with other currently approved S1P receptor modulators.

8.2.6.4. Hepatotoxicity

Liver injury has been reported in S1P receptor modulators and a Warnings and Precautions is described in the labels of all approved S1P receptor modulators. The mechanism of liver injury has not been well described; however, liver injury may be caused by a toxic or immunogenic intermediate because of extensive metabolism in the liver. During the etrasimod development program, the Applicant defined liver injury as an AESI for TEAEs that met certain criteria. Overall, elevations in liver transaminases (AST and/or ALT) occurred more frequently in subjects who received etrasimod compared to placebo. These criteria and AESI analyses are described in detail in Section 8.2.4.4 and Section 8.2.5.4. The review team conducted additional analyses to evaluate for Hy's law cases and the risk of elevated liver tests not limited to the Applicant's definition of "hepatic injury."

As shown in Figure 14, there were no Hy's Law cases reported in the integrated summary of safety, which included data from the "All UC Pool," which included APD334-301, APD334-302, and APD334-003; the open-label extension studies APD334-303 and APD334-005; and open-label data from ES101002, APD334-301, APD334-302, APD334-003.

Figure 14. eDISH Plot of Maximum ALT and Same-Day Total Bilirubin, Safety Population, All UC Pool



Source: Figure 14.3.18.2.3, Integrated Summary of Safety, Pfizer Inc.
Abbreviations: ULN, upper limit of normal; ALT, alanine aminotransferase

The review team analyzed laboratory data to evaluate the risk of elevated liver tests. Table 66 shows the number of subjects with an increase in the liver enzyme tests according to various cut-offs with respect to the upper limit of normal (ULN) for APD334-301 and pooled data from APD334-302 and APD334-003.

Table 66. Subjects with Elevated Liver Tests, Safety Population, APD334-301 and Pooled APD334-302 and APD334-003

Abnormal Liver Laboratory Parameter	APD334-301		APD334-302 and APD334-003	
	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Alanine aminotransferase				
>3 x ULN	13 (4.5)	3 (2.1)	8 (2.8)	1 (0.6)
>5 x ULN	3 (1.0)	2 (1.4)	2 (0.7)	0
>10 x ULN	0	0	0	0
>20 x ULN	0	1 (0.7)	0	0
Aspartate aminotransferase				
>3 x ULN	5 (1.7)	3 (2.1)	3 (1)	1 (0.6)
>5 x ULN	0	0	0	0
>10 x ULN	1 (0.3)	1 (0.7)	0	0
>20 x ULN	0	0	0	0
Alkaline phosphatase				
>2 x ULN	9 (3.1)	0	7 (2.4)	1 (0.6)
>5 x ULN	1 (0.3)	0	0	0

Abnormal Liver Laboratory Parameter	APD334-301		APD334-302 and APD334-003	
	Etrasimod 2 mg N=289 n (%)	Placebo N=144 n (%)	Etrasimod 2 mg N=288 n (%)	Placebo N=170 n (%)
Total bilirubin				
>2 x ULN	2 (0.7)	1 (0.7)	4 (1.4)	0
>5 x ULN	0	0	0	0
Gamma-glutamyltransferase				
>2 x ULN	38 (13.1)	6 (4.2)	28 (9.7)	3 (1.8)
>5 x ULN	12 (4.2)	1 (0.7)	7 (2.4)	0

Source: Reviewer analysis using ad b.xpt; Software: JMP

Analysis is subject level (i.e., each subject is counted once regardless of the number of reported events).

Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with at least one event; ULN, upper limit of normal

In APD334-301, elevations in ALT >3.0 x ULN, alkaline phosphatase >2.0 x ULN, and GGT occurred at a higher frequency in the etrasimod group compared to placebo. Similarly, in pooled data from APD334-302 and APD334-003, elevations in ALT >3.0 x ULN and GGT occurred at a higher frequency in the etrasimod group compared to placebo. One (<1%) subject who received etrasimod reported an SAE of hepatic enzymes increased during APD334-301 (refer to Section 8.2.4.2). In APD334-301, the proportion of subjects that discontinued treatment due to liver enzyme elevations (i.e., ALT and alkaline phosphatase) was balanced between the etrasimod and placebo groups (refer to Section 8.2.4.3). In APD334-302 and APD334-003, there were no SAEs related to increased hepatic enzymes and 1 (<1%) subject in the etrasimod group discontinued treatment due to elevated AST and ALT compared to none in the placebo group (refer to Section 8.2.5.3).

In summary, elevated liver tests was observed more frequently in the etrasimod group compared to placebo but there were no Hy's Law cases, and the majority were not serious or led to study discontinuation. Additionally, most AEs that were either serious or led to study discontinuation were considered unlikely to be related due to other underlying conditions (e.g., primary sclerosing cholangitis). However, elevations in liver enzymes and liver injury have been reported in S1P receptor modulators and will be included in etrasimod's Warnings and Precautions section, which will describe the rate of ALT >3.0 x ULN, recommend testing liver enzymes and bilirubin levels before initiation of treatment and if concern for liver injury is present during treatment, and to discontinue etrasimod if liver injury is confirmed. Additionally, elevated liver tests, which includes elevated ALT, AST, GGT, alkaline phosphatase, bilirubin, transaminases, and liver function test, will be included in the Adverse Reactions section of the label.

8.2.6.5. Ophthalmologic Findings

To characterize the potential risk of macular edema and other ophthalmologic findings, the review team analyzed the results of ophthalmologic examinations, including ophthalmoscopy, optical coherence tomography (OCT), visual acuity measurements, and slit lamp examinations. The analysis of the results of ophthalmologic evaluations were limited to APD334-301 because it was the only completed, 52-week, randomized comparison of etrasimod versus placebo.

Additionally, the assessment of ophthalmologic findings was limited due to missing ophthalmologic evaluations. Refer to the Ophthalmology Consult Review by Dr. Wiley Chambers for full details of the review (DARRTS ID: 5106241).

Macular Edema

Macular edema has been reported in other S1P receptor modulators and observed during the etrasimod development program. The mechanism by which S1P receptor modulators may cause macular edema is not well described but may be due to effects on endothelial cells. The Applicant defined macular edema as “an increase in CFT $\geq 40 \mu\text{m}$ and/or with associated symptoms or clinically significant abnormal findings.” Based on this definition, 1 case of macular edema in a subject who received etrasimod during APD334-301 was reported, which led to study discontinuation. This AE of macular edema is described in detail in Section 8.2.4.4.

In subjects that had OCT macular thickness reported at baseline, 16/95 (17%) of subjects in the etrasimod group compared to 2/26 (8%) in the placebo group had increases of $\geq 40 \mu\text{m}$, which was the predefined OCT cut-off by the Applicant for macular edema. The review team did not consider a requirement for “associated symptoms” to be relevant because most cases of macular edema are asymptomatic. Furthermore, a decrease in visual acuity due to macular edema may not be noticed if it occurs in one eye.

On October 10, 2023, the Applicant submitted additional analyses of OCT findings, in which they claimed they verified the CFT data by reviewing the case report forms (CRFs) and OCT reports that were available for APD334-301. However, the Applicant did not submit the CRFs or OCT reports and several reports were missing; therefore, the review team could not verify the additional analyses. The analyses raised concerns about the integrity of the OCT data because nearly half (i.e., 41.7%) were identified by the Applicant to be inaccurate readings of CFT. Due to the data quality issues, the review team was unable to agree with the Applicant to report the rate of macular edema in the proposed label.

Given an imbalance was observed in the originally submitted OCT analyses and S1P receptor modulators are associated with macular edema, a Warnings and Precautions regarding macular edema will describe the association of etrasimod with an increased risk of macular edema and recommend that patients be evaluated near the start of therapy and periodically during treatment for macular edema. Additionally, consideration to discontinue etrasimod if macular edema develops will be included.

Decrease in Visual Acuity

The review team defined a decrease in visual acuity as a clinically significant decrease in visual acuity (i.e., doubling of the visual angle). For subjects with a baseline and follow-up examination, a decrease in visual acuity was reported in 4/156 (3%) of subjects who received etrasimod compared to no subjects who received placebo. Based on these findings, the decrease in visual acuity will be described in the Adverse Reactions section of the label.

8.2.6.6. Respiratory Effects

S1P receptor modulators have been associated with changes to pulmonary function including forced expiratory volume over 1 second (FEV1) and forced vital capacity (FVC). The Applicant evaluated the risk of pulmonary function changes as AEs as described in Sections 8.2.4.4 and 8.2.5.4. However, the review team conducted a separate analysis of the pulmonary function data to better characterize the potential risk of pulmonary function changes. The analysis focused on data from APD334-301 and APD334-302 because pulmonary function testing was not performed during APD334-003. Refer to the Pulmonology Consult Review by Dr. Rekha Jhamnani for full details of the review (DARRTS ID: 5186355)

During APD334-301 and APD334-302, there were no respiratory SAEs, and no respiratory TEAEs that led to treatment interruption or discontinuation in either study. In Study APD334-301 the mean difference in change from baseline in FEV1 is shown in Table 67. A greater decrease in FEV1 was observed in the etrasimod group compared to placebo at Week 12, which was nominally significant. A difference was not present at Week 52; however, there was significant dropout in both the etrasimod and placebo groups between Week 12 and Week 52 that limited the interpretation of the Week 52 results. No difference was observed between etrasimod and placebo for FVC (not shown). Asthma and chronic obstructive pulmonary disease (COPD) subjects were included in the study, but the small sample size limited conclusions on pulmonary function effects in this population.

Table 67. Change From Baseline in FEV1, APD334-301, Safety Population

Parameter Timepoint Statistic	Placebo (N=144)		Etrasimod 2 mg (N=289)	
	Actual Value	Change from Baseline	Actual Value	Change from Baseline
Forced Expiratory Volume in 1 Second (L)				
Baseline				
n	141		284	
Mean (SD)	3.717 (0.9328)		3.522 (0.9537)	
Median	3.560		3.475	
Min, Max	1.75, 6.33		1.55, 7.30	
Week 12				
n	120	118	248	244
Mean (SD)	3.771 (0.9392)	0.010 (0.3274)	3.407 (0.9689)	-0.068 (0.3441)
Median	3.730	0.010	3.320	-0.050
Min, Max	1.69, 5.68	-1.41, 0.88	1.52, 8.00	-2.28, 1.41
Mean Diff (95% CI) ^a				-0.079 (-0.152, -0.005)
Week 32				
n	9	8	13	12
Mean (SD)	3.561 (0.8740)	-0.425 (0.7830)	2.728 (0.6299)	-0.098 (0.2025)
Median	3.760	-0.100	2.560	-0.155
Min, Max	2.13, 4.77	-1.95, 0.39	1.74, 4.02	-0.32, 0.30
Mean Diff (95% CI) ^a				0.328 (-0.331, 0.986)
Week 52				
n	43	42	153	150
Mean (SD)	3.555 (0.9265)	-0.092 (0.4479)	3.293 (0.9673)	-0.068 (0.4203)
Median	3.470	-0.065	3.160	-0.080
Min, Max	2.07, 5.49	-1.16, 0.96	1.50, 6.35	-2.64, 1.45
Mean Diff (95% CI) ^a				0.024 (-0.130, 0.178)

Adapted from Table 0029b.1, Applicant response to information request, received March 15, 2023.

^a Confidence interval is based on SAS procedure proc t-test with Satterthwaite method for variance estimate. Abbreviations: CI, confidence interval; FEV1, forced expiratory volume in 1 second; SD, standard deviation

In Study APD334-302, there were no pulmonary function tests that were significantly different between the etrasimod group and placebo. As in Study APD334-301, asthma and COPD subjects were included in the study, but the small sample size limited conclusions on pulmonary function effects.

Based on these findings the Warnings and Precautions section and the Clinical Pharmacology section of the label will reflect the observed changes in FEV1.

8.2.7. Safety Results for Integrated Summary of Safety

The Applicant submitted an integrated summary of safety (ISS), which was an analysis that pooled data from controlled and uncontrolled studies of subjects with UC. The dataset included APD334-301, APD334-302, and APD334-003 as well as the open-label extension (OLE) studies APD334-303, APD334-005, and ES101002. Refer to Section 7.1 for details regarding the studies included in the ISS. Given that much of the data was uncontrolled, safety information was considered supportive, and the review team focused on the assessment for rare events, given the extended duration of the open-label studies. Although etrasimod 1 mg was evaluated in APD334-003, the review team focused on safety assessments for etrasimod 2 mg because there were a limited number of subjects who received etrasimod 1 mg (i.e., 42 subjects for 12 weeks) and there were no additional safety findings for the etrasimod 1 mg group.

Table 68 shows the duration of exposure to etrasimod 2 mg in the overall safety database used for the primary assessment of safety and ISS.

Table 68. Duration of Exposure to Etrasimod 2 mg

Parameter	Overall Safety Database		ISS
	N=577	N=942	
Subjects treated with etrasimod 2 mg, by duration, n			
≥12 weeks	507	828	
≥26 weeks	185	666	
≥52 weeks	132	281	
≥104 weeks	0	27	

Adapted from Table 3, Summary of Clinical Safety.
Abbreviations: N, number of subjects in treatment arm

8.2.7.1. Deaths

One death was reported in the ISS from APD334-303 due to an SAE of “neuroendocrine tumor of unknown primary site.” The subject was a 33-year-old White male who was enrolled in APD334-301 where he received placebo. He was subsequently enrolled in APD334-303 where he received open-label etrasimod 2 mg for a total of 24 weeks. On Study Day 170, etrasimod 2 mg was stopped due to lack of efficacy. The subject was subsequently started on IV methylprednisolone (Study Day 171) followed by vedolizumab and rectal mesalazine (Study Day 185) for the treatment of his ulcerative colitis. The subject’s last study visit was conducted on Study Day 189. However, on Study Day 194 the subject had an unscheduled visit for a laboratory abnormality (GGT, not reported) which prompted an abdominal ultrasound (US). At

the time, the subject reported abdominal pain with cramping as well as bloating, nausea, vomiting, and loose stool. The abdominal US showed a large number of abdominal lymph nodes and liver lesions. Further imaging identified multiple lesions in the liver and bones (vertebrae, pelvis, sternum, and femur). Upon further testing, the subject was diagnosed with poorly differentiated neuroendocrine carcinoma of unknown origin. The subject initiated treatment on Study Day 207 for the neuroendocrine carcinoma; however, the subject died on Study Day 212. The Investigator and Sponsor assessed the event of neuroendocrine tumor as unlikely to be related to study treatment. Given the short duration of exposure to etrasimod, the review team assessed the event as unlikely to be related. S1P receptor modulators have been associated with cases of malignancies. Therefore, the label will include a Warnings and Precautions regarding the risk of malignancies.

8.2.7.2. *Serious Adverse Events*

In the ISS, there were 50 subjects who received etrasimod 2 mg and reported at least 1 SAE that was not previously reported as an SAE during their parent study. There were 41 unique reports of SAEs that were not previously reported during APD334-301, APD334-302, and APD334-003. Most SAEs were reported by 1 subject. The only SAEs that were reported by more than 1 subject included: ulcerative colitis, COVID-19, anemia, gastroenteritis, iron deficiency anemia, migraine, and transient ischemic attack. One SAE led to death, which is described in the previous section. SAEs that led to study discontinuation were reported by 12 subjects and were not previously reported in the parent studies. These included atrial fibrillation (1), ulcerative colitis (9), herpes simplex meningitis (1), and transient ischemic attack (1). SAEs (other than ulcerative colitis) that led to study discontinuation are described below.

- **Herpes simplex meningitis:** An SAE of herpes simplex (HSV) meningitis was reported by a 66-year-old White male who had received placebo treatment in parent study APD334-301 and then received etrasimod 2 mg up to Study Day 12 of APD334-303. On Study Day 7, the subject reported symptoms of headache, nausea, blurry vision, and hypoaesthesia. On Study Day 9, the subject presented to the emergency room (ER) with similar complaints. In the ER, chest X-ray and head/brain CT were normal; however, a lumbar puncture and examination of cerebrospinal fluid (CSF) showed lymphocytes and CSF HSV 1/2 IgG mildly elevated (which may indicate past or recent HSV infection). Of note, HSV 1/2 PCR was not detected. Brain MRI and cervical spine CT did not show evidence to suggest HSV encephalitis or meningitis. In consultation with an infectious disease specialist, the subject received acyclovir. The Investigator discontinued etrasimod due to the SAE of HSV meningitis on Study Day 18 and the last dose of study treatment was taken on Study Day 12. The Investigator and Applicant assessed the SAE as unlikely to be related to study treatment and due to the open-label design of the study the results are difficult to interpret. However, etrasimod may increase the susceptibility to infections, and herpes viral infections have been reported with S1P receptor modulators. Therefore, the label will include a Warnings and Precautions regarding the risk of infections, including herpes viral infections.

- **Atrial fibrillation:** During APD334-005, a 69-year-old White male who received placebo in APD334-003 presented on Study Day 197 with complaints of chest pain and shortness of breath. The subject had a past medical history of obesity and hyperglycemia. ECG results were considered abnormal with atrial fibrillation with controlled ventricular response, and the subject was hospitalized. Cardioversion was performed successfully, and the subject was discharged from the hospital on Study Day 198. Etrasimod was permanently withdrawn due to the AE of atrial fibrillation. The Investigator and Applicant reported the AE as not related to etrasimod. Atrial fibrillation is not a known risk of S1P receptor modulators, and the effects of S1P receptor modulators on the cardiovascular system have been related to bradyarrhythmia, AV conduction delays, and hypertension. The label will include a Warnings and Precautions regarding the risk of bradycardia and AV conduction delay.
- **Transient ischemic attack (TIA):** A 54-year-old male with a history of hypertension was hospitalized for Grade 2 TIA on Study Day 18 of APD334-303. The subject received etrasimod 2 mg in parent study APD334-301 prior to enrollment in APD334-303. On Study Day 15, the subject went to the hospital with symptoms suspicious for a TIA. Concomitant medications included sulfasalazine, nebivolol, and barnidipine. On Study Day 22, brain magnetic resonance imaging and magnetic resonance angiography did not show any acute cerebrovascular lesions of recent significance and the event was considered recovered/resolved. The subject withdrew from the study and study treatment (etrasimod) was discontinued due to an SAE of moderate TIA. TIA is not a known risk of S1P receptor modulators, and the results are difficult to interpret due to the open-label study design. However, hypertension was observed in the etrasimod development program and, therefore, a Warnings and Precautions regarding hypertension will be included in the label.

Overall, the unique SAEs reported in the ISS did not identify a new safety signal. Most subjects had a past medical history that may have contributed to the SAE. The majority of SAEs were reported by one subject. Additionally, due to the open-label nature of the extension studies (from which, unique subjects were identified), the safety information is challenging to interpret without a comparator arm.

8.2.7.3. Adverse Events that Led to Study Discontinuation

In the ISS, there were 21 subjects treated with etrasimod 2 mg who reported TEAEs that led to study discontinuation. TEAEs that led to study discontinuation and occurred in more than 2 subjects were ulcerative colitis, lymphopenia, and headache. TEAEs that led to study discontinuation that were also SAEs (other than ulcerative colitis) are described in the previous section. There were no TEAEs that led to study discontinuation during the extension studies APD334-303, APD334-005, and ES101002 that were considered AESIs. Overall, the TEAEs that led to study discontinuation in the ISS were similar to APD334-301, APD334-302, and APD334-003.

8.2.7.4. Treatment Emergent Adverse Events of Special Interest

The ISS was reviewed for AESIs that have been previously described in Section 8.2.4.4. No TEAEs of PML, respiratory disorders, or PRES were reported in the ISS. Pertinent TEAEs corresponding to the AESI categories previously described, are noted below.

- **Bradycardia, AV Conduction Delay, and Hypertension:** TEAEs of AV conduction delay (AV block first degree and AV block second degree) were reported in 3 subjects in the ISS that were not reported during the parent studies. All 3 subjects were switched from placebo to etrasimod 2 mg during either APD334-303, APD334-005, or ES101002. Additionally, all 3 subjects reported the AESI on the first day of treatment with etrasimod 2 mg, which is consistent with TEAEs observed in APD334-301, APD334-302, and APD334-003. No new subjects reported AESIs of bradycardia in the ISS. Two additional subjects reported a TEAE of hypertension in the ISS; however, neither TEAEs were SAEs nor led to study discontinuation.
- **Ophthalmologic Findings:** One additional subject reported a TEAE of “cystoid macular oedema” in the ISS that was not previously reported in APD334-301, APD334-302, or APD334-003. The subject was a 58-year-old White woman who received placebo in parent study APD334-301, and etrasimod 2 mg up to study day 248 in APD334-303. On Study Day 85, the subject had a moderate AESI of “cystoid macular oedema” according to findings from retinal photographs, which were reported as abnormal and clinically significant for the left eye. On Study Day 120, the event was considered recovered/resolved. A dilated fundoscopic examination on this day was reported as abnormal clinically significant for epiretinal fibrosis and mild hyperopia; remaining structures in that examination were deemed normal at that time. On Study Day 169, OCT and retinal photographic findings were reported as abnormal clinically significant for paramacular retinal edema of the left eye. On Study Day 248, the subject had a moderate AESI of cystoid macular edema. There were no ophthalmic examination findings reported from this day. Study drug was discontinued due to the event with the last dose on Study Day 248. At the time of the subject’s last available report, the outcome of the event of cystoid macular edema was unknown.
- **Hepatotoxicity:** There were no TEAEs that met Hy’s law criteria during the ISS. There were 27 additional subjects who reported TEAEs related to elevated liver tests (preferred terms: ALT increased, AST increased, GGT increased, alkaline phosphatase increased, elevated liver enzymes, transaminases increased, and bilirubin increased). The majority of TEAEs were mild or moderate in severity and no subjects were discontinued from the study due to the TEAEs of elevated liver enzymes.
- **Infections:** In the ISS, there were 8 severe infections in subjects that received etrasimod 2 mg, which included COVID-19 (3), pneumonia (2), appendicitis (1), gastroenteritis (1), and pyelonephritis (1). Two subjects who received etrasimod 2 mg reported TEAEs that led to study discontinuation that had not been reported previously, herpes simplex

meningitis (described in Section 8.2.7.2) and latent tuberculosis (TB). The subject with latent tuberculosis had been screened with a “tuberculosis test” and chest X-ray during screening for APD334-301; however, an Interferon Gamma Release Assay was performed on Study Day 385, prior to initiating etrasimod 2 mg for APD334-303, which was positive. The subject was asymptomatic and was treated for latent TB but was discontinued from the study due to the AE of latent TB. Overall, the types of infection-related TEAEs reported in the ISS were similar to those described in APD334-301, APD334-302, and APD334-003.

- **Malignancy:** Two subjects reported one TEAE of malignancy in the ISS, which were not previously reported. One subject that received etrasimod 2 mg in parent study APD334-301 and continued on etrasimod 2 mg in APD334-303, reported a TEAE of “malignant melanoma” from the verbatim reported term of “Melanocytic mole manifestation.” However, this TEAE was incorrectly coded and was corrected to the term melanocytic nevus (i.e., not a malignancy). The second TEAE of malignancy was a neuroendocrine tumor resulting in death, which is described in Section 8.2.7.1.

8.2.7.5. Treatment Emergent Adverse Events

The TEAEs reported in the ISS were generally similar to those reported in APD334-301, APD334-302, and APD334-003. The majority of TEAEs were mild or moderate in severity. The most common TEAEs in the ISS (reported by at least 10 subjects who received etrasimod 2 mg) were ulcerative colitis, COVID-19, lymphopenia, headache, upper respiratory tract infection, and GGT increased.

8.2.7.6. Summary of Integrated Summary of Safety

Overall, safety data from the ISS did not identify any new safety signals. The reported AEs were generally similar to those in the placebo-controlled studies APD334-301, APD334-302, and APD334-003; however, interpretation of the results of the ISS is limited by the lack of a comparator arm and the open-label nature of APD334-303, APD334-005, and ES101002.

8.2.8. Safety Analyses by Demographic Subgroups

Table 69 shows an overview of SAEs by demographic subgroup and Table 70 shows an overview of TEAEs by demographic subgroup for 52-week study APD334-301.

Table 69. Overview of Serious Adverse Events by Demographic Subgroup, Safety Population, APD334-301

Characteristic	Etrasimod 2 mg N=289 n/Ns (%)	Placebo N=144 n/Ns (%)	Treatment Difference (%)
Sex, n (%)			
Female	9/137 (6.6)	2/56 (3.6)	3.0
Male	11/152 (7.2)	7/88 (8.0)	-0.7

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Characteristic	Etrasimod 2 mg N=289 n/Ns (%)	Placebo N=144 n/Ns (%)	Treatment Difference (%)
Age group, years, n (%)			
<65	19/272 (7.0)	9/134 (6.7)	0.3
≥65	1/17 (5.9)	0/10 (0)	5.9
Age group ≥75, years, n (%)			
≥75	0/2 (0)	0/1 (0)	-
Race, n (%)			
American Indian or Alaska Native	0/1 (0)	1/3 (33.3)	-33.3
Asian	3/22 (13.6)	0/9 (0)	13.6
Black or African American	0/6 (0)	0/3 (0)	-
Multiple	0/0 (0)	0/0 (0)	-
Native Hawaiian or Other Pacific Islander	0/0 (0)	0/0 (0)	-
White	17/256 (6.6)	8/129 (6.2)	0.4
Not Reported	0/4 (0)	0/0 (0)	-
Ethnicity, n (%)			
Hispanic or Latino	1/12 (8.3)	2/7 (28.6)	-20.2
Not Hispanic or Latino	19/275 (6.9)	7/136 (5.1)	1.8
Not Reported or Unknown	0/2 (0)	0/1 (0)	-

Source: Reviewer analysis using Applicant submitted data, adae.xpt

Treatment difference is etrasimod – placebo

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; Ns, total number of subjects for each specific subgroup and were assigned to that specific arm

Table 70. Overview of Treatment Emergent Adverse Events by Demographic Subgroup, Safety Population, APD334-301

Characteristic	Etrasimod 2 mg N=289 n/Ns (%)	Placebo N=144 n/Ns (%)	Treatment Difference (%)
Sex, n (%)			
Female	101/137 (73.7)	34/56 (60.7)	13.0
Male	105/152 (69.1)	47/88 (53.4)	15.7
Age group, years, n (%)			
<65	194/272 (71.3)	78/134 (58.2)	13.1
≥65	12/17 (70.6)	3/10 (30)	40.6
Age group ≥75, years, n (%)			
≥75	0/2 (0)	0/1 (0)	-
Race, n (%)			
American Indian or Alaska Native	0/1 (0)	2/3 (66.7)	-66.7
Asian	15/22 (68.2)	7/9 (77.8)	-9.6
Black or African American	5/6 (83.3)	2/3 (66.7)	16.6
Multiple	0/0 (0)	0/0 (0)	-
Native Hawaiian or Other Pacific Islander	0/0 (0)	0/0 (0)	-
White	183/256 (71.5)	70/129 (54.3)	17.2
Not Reported	3/4 (75)	0/0 (0)	75
Ethnicity, n (%)			
Hispanic or Latino	7/12 (58.3)	7/7 (100)	41.7
Not Hispanic or Latino	205/275 (74.5)	76/136 (55.9)	18.6
Not Reported or Unknown	0/2 (0)	1/1 (100)	-100

Source: Reviewer analysis using Applicant submitted data, adae.xpt

Treatment difference is etrasimod – placebo

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; Ns, total number of subjects for each specific subgroup and were assigned to that specific arm

Table 71 shows an overview of SAEs by demographic subgroup and Table 72 shows an overview of TEAEs by demographic subgroup for pooled data from APD334-301 and APD334-003.

Table 71. Overview of Serious Adverse Events by Demographic Subgroup, Safety Population, APD334-302 and APD334-003

Characteristic	Etrasimod 2 mg N=288 n/Ns (%)	Placebo N=170 n/Ns (%)	Treatment Difference (%)
Sex, n (%)			
Female	3/126 (2.2)	3/65 (3.4)	-1.2
Male	3/162 (1.7)	5/105 (4.4)	-2.7
Age group, years, n (%)			
<65	6/275 (2.0)	7/159 (3.9)	-1.9
≥65	0/13 (0)	1/11 (5.0)	-5.0
Age group ≥75, years, n (%)			
≥75	0/0 (0)	0/0 (0)	-
Race, n (%)			
American Indian or Alaska Native	0/6 (0)	0/1 (0)	-
Asian	0/48 (0)	0/27 (0)	-
Black or African American	0/2 (0)	0/3 (0)	-
Multiple	0/1 (0)	0/0 (0)	-
Native Hawaiian or Other Pacific Islander	0/0 (0)	0/0 (0)	-
White	5/225 (2.1)	8/139 (4.7)	-2.6
Not Reported	1/6 (12.5)	0/0 (0)	12.5
Ethnicity, n (%)			
Hispanic or Latino	0/11 (0)	1/12 (8.3)	-8.3
Not Hispanic or Latino	6/275 (2.0)	7/158 (3.9)	-1.9
Not Reported or Unknown	0/2 (0)	0/0 (0)	-

Source: Reviewer analysis using Applicant submitted data, adae.xpt

Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

Treatment difference is etrasimod – placebo

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; Ns, total number of subjects for each specific subgroup and were assigned to that specific arm

Table 72. Overview of Treatment Emergent Adverse Events by Demographic Subgroup, Safety Population, APD334-302 and APD334-003

Characteristic	Etrasimod 2 mg N=288 n/Ns (%)	Placebo N=170 n/Ns (%)	Treatment Difference (%)
Sex, n (%)			
Female	67/126 (53.5)	30/65 (46.3)	7.2
Male	73/162 (46.1)	51/105 (48.2)	-2.1
Age group, years, n (%)			
<65	134/275 (49.5)	75/159 (46.7)	2.8
≥65	6/13 (37.5)	6/11 (67.5)	-30.0
Age group ≥75, years, n (%)			
≥75	0/0 (0)	0/0 (0)	-

Characteristic	Etrasimod 2 mg N=288 n/Ns (%)	Placebo N=170 n/Ns (%)	Treatment Difference (%)
Race, n (%)			
American Indian or Alaska Native	3/6 (37.5)	1/1 (75.0)	-37.5
Asian	26/48 (64.9)	13/27 (39.0)	25.9
Black or African American	2/2 (75.0)	3/3 (100.0)	-25.0
Multiple	1/1 (75.0)	0/0 (0)	75.0
Native Hawaiian or Other Pacific Islander	0/0 (0)	0/0 (0)	-
White	105/225 (47.0)	64/139 (45.1)	1.9
Not Reported	3/6 (37.5)	0/0 (0)	37.5
Ethnicity, n (%)			
Hispanic or Latino	8/11 (77.5)	5/12 (41.7)	35.8
Not Hispanic or Latino	138/275 (50.6)	81/158 (51.7)	-1.1
Not Reported or Unknown	1/2 (75.0)	0/0 (0)	75.0

Source: Reviewer analysis using Applicant submitted data, adae.xpt

Proportions are adjusted for different randomization ratios of 302 and 003 by CMH weights.

Treatment difference is etrasimod – placebo

Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; Ns, total number of subjects for each specific subgroup and were assigned to that specific arm

Overall, there were no notable differences between the treatment groups within the demographic subgroups. Although no discernable differences were observed, the interpretability of subgroup analyses were limited due to the small number of non-White subjects, subjects aged 65 years of age or older, and Hispanic or Latino subjects.

8.2.9. Specific Safety Studies/Clinical Trials

No specific safety studies or clinical trials were performed.

8.2.10. 120-Day Safety Update Report

A 120-day safety update report (SUR) was provided by the Applicant that incorporated safety information and additional integrated cumulative clinical study safety data from February 1, 2022, to August 30, 2022. The safety update includes cumulative safety data from ongoing studies in subjects with UC (APD334-303 and ES101002), as well as from other indications APD334-202 (a phase 2, 14-Week induction study in subjects with Crohn's disease [CD]), and APD334-205 (a phase 2, 28-week OLE study in subjects with alopecia areata). The review team focused on the data available in subjects with UC to evaluate for rare events or events that may have a longer latency period.

As shown in Table 73, during the SUR, an additional 221 subjects had at least 52 weeks of exposure and 90 subjects had at least 104 weeks of exposure to etrasimod 2 mg as compared to the ISS.

Table 73. Duration of Exposure to Etrasimod 2 mg

Parameter	ISS N=942	SUR N = 1037
Subjects treated with etrasimod 2 mg, by duration, n ≥12 weeks	828	914

Parameter	ISS	SUR
	N=942	N = 1037
≥26 weeks	666	785
≥52 weeks	281	502
≥104 weeks	27	117

Adapted from Table 2, 120-Day Safety Update.
Abbreviations: N, number of subjects in treatment arm

Deaths

There were no additional deaths reported during the SUR.

Serious Adverse Events

SAEs were reported in an additional 27 subjects with UC in the SUR. The most frequent SAEs were ulcerative colitis (9) and COVID-19/COVID-19 pneumonia (2). No other SAEs were reported in more than a single subject during the SUR.

Discontinuation Due to Adverse Events

Twenty-five additional subjects discontinued study treatment due to an AE during the SUR. The most frequently reported TEAEs occurring in ≥ 2 subjects in the etrasimod 2 mg group that led to permanent study treatment discontinuation were colitis ulcerative (13), lymphocyte count decreased (3), and weight decreased (1).

Treatment Emergent Adverse Events of Special Interest

The SUR included AESIs that have been previously described in Section 8.2.4.4. No TEAEs of PML, respiratory disorders, or PRES were reported in the SUR. Pertinent TEAEs corresponding to the AESI categories previously described, are noted below.

Bradycardia, AV Conduction Delay, and Hypertension

- **Bradycardia:** Three additional subjects not previously reported in the ISS, experienced TEAEs of sinus bradycardia on Day 1. All sinus bradycardia TEAEs were asymptomatic and none of these subjects experienced an HR < 50 bpm.
- **AV Conduction Delay:** No additional subjects with UC reported TEAEs related to AV conduction delay.
- **Acute myocardial infarction:** One subject that received etrasimod 2 mg experienced an SAE of acute myocardial infarction Grade 3 on Day 27 of ES101002. Prior to receiving open-label etrasimod 2 mg in ES101002, the subject received blinded treatment in the double-blind period of the study. Medical history included alcohol and tobacco use. Study treatment was discontinued due to the AE of acute myocardial infarction.
- **Hypertension:** TEAEs of hypertension were reported in 8 additional subjects during the SUR. None of the TEAEs were SAEs or led to study discontinuation.

Infections and Opportunistic Infections

The most common Infection TEAEs reported by subjects treated with etrasimod 2 mg during the SUR were COVID-19, upper respiratory tract infection, nasopharyngitis, urinary tract infection, bronchitis, and gastroenteritis. There were no additional TEAEs of PML, cryptococcus, nasopharyngitis, urinary tract infection, bronchitis, or gastroenteritis that occurred in UC subjects during the SUR.

- **Opportunistic Infections:** One subject with UC that received etrasimod 2 mg reported an TEAE of opportunistic infection of esophageal candidiasis on Study Day 42. The event was preceded by an SAE of rectal hemorrhage and study treatment withdrawal on Study Day 35 prior to SDEI onset.
- **Severe Infections:** Six additional subjects in the etrasimod 2 mg group reported an TEAE of severe infection. The types of infections were varied. Previously unreported serious infection PTs included bacterial superinfection, anal abscess, colonic abscess, diverticulitis, and vestibular neuronitis.
- **Herpes Viral Infections:** One additional subject each in the etrasimod 2 mg group experienced TEAEs oral herpes and herpes zoster. Both TEAEs were not SAEs and did not lead to study discontinuation.
- **Lymphopenia:** During the SUR, 108 UC subjects treated with etrasimod 2 mg reported TEAEs of lymphopenia (PTs: lymphopenia, lymphocyte count, lymphocyte percentage decreased, and CD4 lymphocytes decreased). Three subjects were discontinued from treatment due to the TEAEs of decreased lymphocyte count.

Hepatic Injury

Twelve additional subjects experienced TEAEs related to hepatic injury. TEAEs reported by > 1 subject were: hepatic function abnormal (6) and cholangitis sclerosing (2). One subject in the etrasimod 2 mg group reported 1 SAE of cholelithiasis that led to temporary interruption of study treatment. There were no AEs that led to study treatment discontinuation and no TEAEs that met Hy's law criteria.

Treatment Emergent Adverse Events

The most frequently reported TEAEs during the SUR were colitis ulcerative, COVID-19, lymphopenia, anemia, lymphocyte count decreased, and headache. These TEAEs are similar to those reported in the placebo-controlled studies APD334-301, APD334-302, and APD334-003 as well as the ISS.

Summary of the 120-Day Safety Report

The SUR did not identify any new safety signals; however, the interpretation of data from the SUR is limited due to the lack of a comparator arm. Overall, similar TEAEs were reported,

consistent with data from the primary safety analysis, performed with placebo-controlled data from APD334-301, APD334-302, and APD334-003.

8.2.11. Human Reproduction and Pregnancy

Pregnancy

Pregnant women were excluded from clinical trials with etrasimod during the clinical development program. At the time of the SUR a total of 11 pregnancy exposure cases were reported with outcomes including: normal livebirth (2), preterm birth (1), ongoing (6), elective abortion (1), and spontaneous abortion (1). Overall, these limited available human data are insufficient to evaluate for a drug-associated risk of major birth defects, miscarriage, or other adverse maternal or fetal outcomes.

Etrasimod is indicated for a condition that would be expected to be seen in females of reproductive potential (including during pregnancy), and current data are insufficient to inform women regarding etrasimod use during pregnancy. Therefore, labeling will include that based on findings from animal studies, etrasimod may cause fetal harm when administered to a pregnant woman. In addition, postmarketing requirements will be issued for the Applicant to conduct a pregnancy exposure registry and a complimentary study of a different design (such as a claims database study). Language regarding the pregnancy exposure registry should be added to Subsection 8.1 and Section 17 of labeling once the study protocol has been finalized and registry contact information established.

Lactation

Lactating women were excluded from clinical trials with etrasimod. At the time of the 120-day SUR, no lactation exposure cases were reported. Etrasimod is indicated for a condition that would be expected to be seen in females of reproductive potential (including during lactation), and there are no current data available to inform women regarding etrasimod use during lactation. Therefore, a PMR will be issued for the Applicant to conduct a clinical lactation study with etrasimod to inform labeling.

Females and Males of Reproductive Potential

There are no available human data on the effects of etrasimod on male or female fertility. Based on animal data, etrasimod may cause fetal harm when administered to pregnant women. Therefore, labeling will include that females of reproductive potential should be counseled on the potential for a serious risk to the fetus and the need for effective contraception during treatment with etrasimod and for one week following the last dose.

8.2.12. Pediatrics and Assessment of Effects on Growth

Insufficient pediatric data were submitted as part of this BLA to support an indication in pediatric subjects or to inform an assessment of the impact of etrasimod on growth. At the

time of this review, pediatric trials were ongoing. Refer to Section 13 Postmarketing Requirements and Commitments.

8.2.13. Overdose, Drug Abuse Potential, Withdrawal, and Rebound

There are no specific concerns about drug abuse potential or withdrawal with etrasimod use in UC.

8.2.14. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

No postmarketing experience is available because etrasimod is not currently approved in any country.

Expectations on Safety in the Postmarket Setting

Refer to Section 13 for details regarding postmarketing requirements and commitments agreed upon with the Applicant.

8.3. Conclusions and Recommendations

The Applicant submitted the results of two adequate and well-controlled phase 3 trials (APD334-301 and APD334-302) to establish the substantial evidence of effectiveness. The review team agreed with the Applicant's conclusion that a clinically meaningful and statistically significant benefit was demonstrated on the primary endpoints and multiple secondary endpoints for APD334-301 and APD334-302.

The Applicant submitted data from the 52-week phase 3 trial APD334-301, the 12-week phase 3 trial APD334-302, and 12-week phase 2 trial APD334-003 to support the safety of etrasimod 2 mg in subjects with moderately to severely active UC. The safety profile of etrasimod 2 mg once daily for up to 52 weeks was acceptable and supports approval of the proposed dosing regimen. There are several known potential risks related to the S1P receptor modulator class which were observed in the etrasimod development program (i.e., infections, bradyarrhythmia and atrioventricular conduction delays, liver injury, macular edema, increased blood pressure, and respiratory effects), which were consistent with other S1P receptor modulators. The labeling is considered adequate to communicate these risks and provide appropriate risk mitigation.

9 Advisory Committee Meeting and Other External Consultations

An Advisory Committee meeting was not held to discuss this application as the application did not raise significant safety or efficacy issues that were unexpected for a drug of this class to warrant input from an Advisory Committee.

10 Pediatrics

Pediatric subjects were included in APD334-301 and APD334-302, which enrolled subjects 16 to 80 years of age inclusive, (b) (4)

However, only 1 adolescent subject (who received placebo) was enrolled in APD334-301, and only 2 adolescent subjects (1 placebo, 1 etrasimod) were enrolled in ADP334-302. Therefore, given data were available for only one adolescent subject who received etrasimod, the Division communicated to the Applicant that (b) (4)

Postmarketing requirements under the Pediatric Research Equity Act (PREA) will be issued; refer to Section 13.

The Applicant submitted an amended agreed initial pediatric study plan (iPSP). In the amended agreed iPSP, the Applicant requested a waiver for pediatric assessment for the birth to <2 years of age pediatric age group due to studies being impossible or highly impracticable. The Applicant also requested a deferral of pediatric studies because the adult studies were completed prior to completion of the pediatric studies. The review team agreed with the waiver and deferral requests.

In the amended agreed iPSP, the Applicant proposed two pediatric clinical studies. At the time of the review of the NDA submission, a one-year, phase 2, multicenter, open-label, single-arm, sentinel dose cohort study to evaluate the efficacy, PK, and safety of etrasimod in adolescent subjects 12 to <18 years of age with moderately to severely active UC was ongoing (i.e., APD334-207). (b) (4)

(refer to Section 7.2).

Given the lack of data available for etrasimod in pediatric subjects with UC, the review team anticipates that data from APD334-207 may be necessary to inform the design of the future pediatric program, particularly to inform dose selection for the second pediatric study (i.e., APD334-208). Therefore, the PREA PMRs will include one PMR to finish the ongoing pediatric study APD334-207 and a second PMR to conduct a one-year study to evaluate the safety, efficacy, pharmacokinetics, and pharmacodynamics of etrasimod in pediatric patients 2 to 17 years of age with moderately to severely active ulcerative colitis. The PREA PMRs are described in Section 13.

11 Labeling Recommendations

11.1 Prescription Drug Labeling

Table 74 is a summary of the high-level changes to the U.S. prescribing information that were negotiated during the review cycle.

Table 74. Prescribing Information

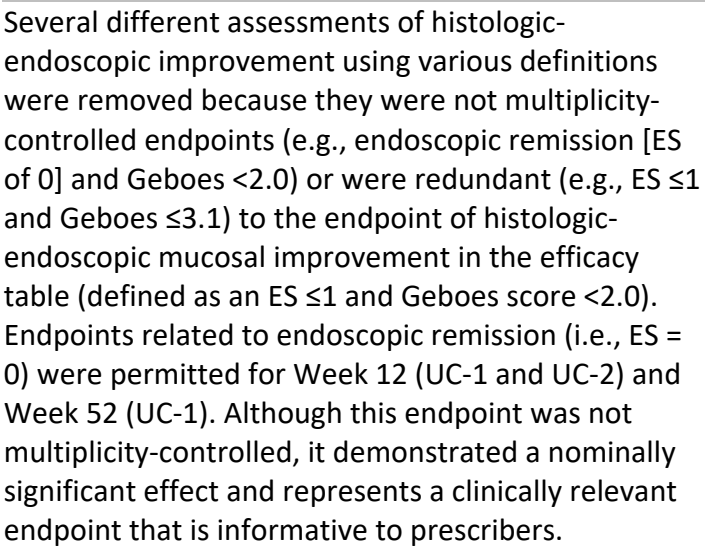
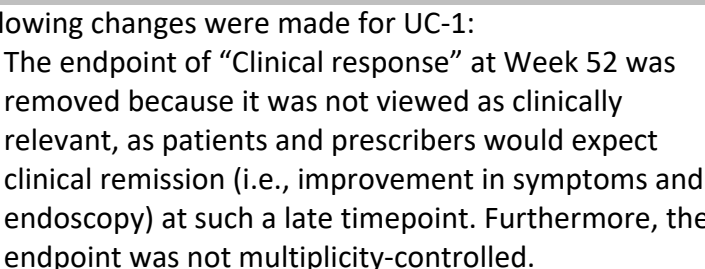
Full Prescribing Information Sections ¹	High-Level Summary of the Major Issues with the Applicant's Proposed Draft PI and How Those Major Issues Were Addressed in the Finalized PI ²
INDICATIONS AND USAGE	(b) (4)
DOSAGE AND ADMINISTRATION	The following assessments and vaccinations prior to initiation of treatment with etrasimod were added: • Ophthalmologic assessment: A baseline evaluation of the fundus, including the macula, near the start of treatment in all subjects was added to align with other S1P receptor modulators and to monitor for macular edema, which was observed during the etrasimod development program. Refer to Sections 8.2.4, 8.2.5, and 8.2.6.5. • A recommendation to test for varicella zoster virus (VZV) antibodies in patients without a full course of vaccination against VZV and vaccinate antibody negative patients before starting treatment was added to align with other S1P receptor modulators and describe the risk of herpes viral infections. • Skin examination: A baseline skin examination was added to mitigate the potential risk of skin malignancy.
WARNINGS AND PRECAUTIONS	No new subsections were added to the labeling proposed by the Applicant. However, the safety results in the individual subsections were updated and included the addition of pooled data from APD334-302 (i.e., "UC-2") and APD334-003 (i.e., "UC-3"). Finally, the order of the subsections within this section were reordered in decreasing order of clinical importance for patients

	and prescribers. The following subsections had revisions that were noteworthy: <i>Infections</i> • The most common infections were updated to include urinary tract infections and herpes viral infections. Refer to Section 8.2.4.5 and Section 8.2.5.5. • A description of the risk of progressive multifocal leukoencephalopathy (PML) was updated to align with other S1P receptor modulators. In addition, information related to immune reconstitution inflammatory syndrome (IRIS) in subjects with multiple sclerosis treated with S1P receptor modulators who developed PML and subsequently discontinued treated was added to align with a Safety Labeling Change for the S1P receptor modulator class for PML-IRIS that was approved on August 10, 2023. • Language regarding vaccination against VZV was added to align with the DOSAGE AND ADMINISTRATION section. • Information related to cryptococcal infection was added to align with other S1P receptor modulators. <i>Bradycardia and Atrioventricular Conduction Delays</i> •  (b) (4) <i>Liver Injury</i> • The number of subjects with ALT > 3 x ULN was updated to reflect the review teams analyses. <i>Macular Edema</i> • A recommendation to obtain a baseline evaluation of the fundus, including the macula, near the start of treatment, if there are any changes in vision, periodically while receiving treatment, and any time there is a change in vision was added. Refer to Section 8.2.6.5. • A recommendation to consider discontinuing etrasimod if macular edema develops was also added. <i>Fetal Risk</i>

	- Language was edited to align with other S1P receptor modulators. <i>Malignancies</i> - A recommendation to perform skin examinations at the start of treatment and periodically during treatment was added to align with other S1P receptor modulators. <i>Respiratory Effects</i> - The language in this section was updated based on the review team’s analyses of respiratory safety data. The decline in absolute FEV1 was reported for UC-1. Additionally, spirometric evaluation of respiratory function is recommended during treatment with etrasimod if clinically indicated. Refer to Section 8.2.6.6.
5 ADVERSE REACTIONS <i>Clinical Trials Experience</i>	- This section was updated to describe the results of APD334-301 (i.e., UC-1, a 52-week trial) and pooled data from APD334-302 and APD334-003 (i.e., UC-2 and UC-3, two 12-week trials). The review team did not agree with pooling data from APD334-301, APD334-302, and APD334-003, due to the different durations of exposure of the trials. - The most common adverse reactions (≥2%) that were added are: - Study UC-1: headache, elevated liver tests, dizziness, arthralgia, hypertension, urinary tract infection, nausea, hypercholesterolemia, and herpes viral infection. Refer to Section 8.2.4.5. - Studies UC-2 and UC-3: headache, elevated liver tests, nausea, bradycardia, and urinary tract infection. Refer to Section 8.2.5.5. - A description of ophthalmologic findings, specifically, a decrease in visual acuity in the subset of subjects with a baseline and follow-up exam, was added for UC-1. Refer to Section 8.2.6.5.
USE IN SPECIFIC POPULATIONS	<i>Pregnancy</i> Contact information for a pregnancy registry, a postmarketing requirement (PMR 4499-4), was added. Refer to Section 13.

	• A “clinical considerations” subsection was added to inform patients and prescribers of the risks associated with etrasimod and the risks associated with IBD as it pertains to pregnant women. <i>Pediatric Use</i> • The Pediatric Use section was updated to state that the safety and effectiveness of etrasimod in pediatric patients have not been established (b) (4) <i>Hepatic Impairment</i> • This subsection was added to describe the impact of hepatic impairment on etrasimod exposure and that use in patients with severe hepatic impairment is not recommended. <i>CYP2C9 Poor Metabolizers</i> • A new subsection was added with the recommendation to avoid concomitant use of Velsipity in subjects who are poor CYP2C9 metabolizers and receiving concomitant moderate to strong CYP2C8 or CYP3A4 inhibitors because of increased etrasimod exposure.
CLINICAL PHARMACOLOGY	<i>Mechanism of Action</i> • The mechanism of action section was modified to describe that etrasimod has minimal activity on S1P₃ and no activity on S1P₂, rather than “no activity” on S1P₂ and S1P₃. • (b) (4) <i>Pharmacodynamics</i> • The median time for peripheral blood lymphocytes to return to baseline and the normal range was updated to reflect data in subjects with UC, rather than a population PK model. Refer to Section 16.3.2. <i>Pharmacokinetics</i>

	•  (b) (4) • Data from drug-drug interaction studies with itraconazole and gemfibrozil were added. <i>Pharmacogenomics</i> • This subsection was added to describe the potential impact of genetic variants of CYP2C9 alleles on CYP2C9 activity, including the potential for decreased clearance of etrasimod in CYP2C9 intermediate and poor metabolizers when used concomitantly with moderate to strong inhibitors of CYP2C8 or CYP3A4.
CLINICAL STUDIES	• Demographic information for UC-1 and UC-2 was added to describe the trial populations. • The following changes were made to the results from UC-1 and UC-2: ○ The number of subjects in each treatment arm were updated to match the review team’s analysis, which included the removal of 1 adolescent subject. ○ The proportion of subjects meeting efficacy endpoints were updated according to the review teams analyses. Refer to Section 8.1.2 and 8.1.4. ○ The endpoint of “symptomatic remission” was removed because it is not considered to provide additional, clinically meaningful information versus “clinical remission.” Information regarding improvements in stool frequency and rectal bleeding are described individually below the table in the <i>Stool Frequency and Rectal Bleeding Subscores</i>. ○ The endpoint of “clinical response” at Week 12 was moved out of the efficacy endpoints table and added below the table in text. This endpoint was not multiplicity-controlled; however, given this endpoint is important to patients and prescribers, the review team agreed with including it in the label. ○ A description of a supplementary analysis of the primary endpoint of clinical remission in a subgroup of subjects with an mMS of 4 to 9 was removed, as this population was not consistent with the preferred

	definition of moderately to severely active UC. ○  (b) (4) ○  ○ Several different assessments of histologic-endoscopic improvement using various definitions were removed because they were not multiplicity-controlled endpoints (e.g., endoscopic remission [ES of 0] and Geboes <2.0) or were redundant (e.g., ES ≤1 and Geboes ≤3.1) to the endpoint of histologic-endoscopic mucosal improvement in the efficacy table (defined as an ES ≤1 and Geboes score <2.0). ○ Endpoints related to endoscopic remission (i.e., ES = 0) were permitted for Week 12 (UC-1 and UC-2) and Week 52 (UC-1). Although this endpoint was not multiplicity-controlled, it demonstrated a nominally significant effect and represents a clinically relevant endpoint that is informative to prescribers. ○  (b) (4) ○  ● The following changes were made for UC-1: ○ The endpoint of “Clinical response” at Week 52 was removed because it was not viewed as clinically relevant, as patients and prescribers would expect clinical remission (i.e., improvement in symptoms and endoscopy) at such a late timepoint. Furthermore, the endpoint was not multiplicity-controlled.

	○  (b) (4) corticosteroid-free clinical remission for all subjects was reported in the efficacy endpoints table.

¹ Some sections may not be included because those sections may not have major issues (or changes).

² The finalized PI is the PI that will be approved or is close to being approved

11.2. Medication Guide

A Medication Guide (MG) was proposed by the Applicant and deemed necessary by the review team for etrasimod to convey information necessary to inform patients of the risk of serious adverse reactions. MGs are required for all other drugs in the S1P receptor modulator class.

Updates were made to the proposed MG to be consistent with the prescribing information.

12 Risk Evaluation and Mitigation Strategies (REMS)

The risks of etrasimod are adequately communicated in the prescribing information. A REMS is not considered necessary at this time.

13 Postmarketing Requirements and Commitment

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients (which includes new salts and new fixed-combinations), new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication in pediatric patients unless this requirement is waived, deferred, or inapplicable. NDA 216956 triggers PREA because etrasimod is a new active ingredient. The following PREA PMRs will be issued.

4499-1 Complete the ongoing phase 2 study to evaluate the safety, efficacy, pharmacokinetics, and pharmacodynamics of Velsipity (etrasimod) in pediatric patients 12 to less than 18 years of age with moderately to severely active ulcerative colitis.

Study Completion: November 2025
Final Report Submission: May 2026

4499-2 Conduct a one-year study to evaluate the safety, efficacy, pharmacokinetics, and pharmacodynamics of Velsipity (etrasimod) in pediatric patients 2 to less than 18 years of age with moderately to severely active ulcerative colitis.

Final Protocol Submission: February 2024
Study Completion: February 2028
Final Report Submission: August 2028

The following postmarketing requirements (PMRs) under Section 505(o)(3) of FDAAA will be issued at the time of approval of the application.

4499-3 Perform a lactation study (milk only) in lactating women who have received Velsipity (etrasimod), regardless of indication, to assess concentrations of etrasimod in breast milk using a validated assay and to assess the effects on the breastfed infant.

Draft Protocol Submission: June 2024
Final Protocol Submission: November 2024
Study Completion: November 2026
Final Report Completion: November 2027

4499-4 Collect data from a prospective pregnancy exposure registry, preferably a disease-based multiproduct pregnancy registry, using a cohort analysis that compares the maternal, fetal, and infant outcomes of women exposed to etrasimod regardless of indication during pregnancy with unexposed comparator

population(s) in a timely manner. Align the study protocol with protocol(s) outside the US to reach the target sample size. The registry will identify and record pregnancy complications, major and minor congenital malformations, spontaneous abortion, stillbirths, elective terminations, preterm births, small-for-gestational-age births, and any other adverse outcomes, including postnatal growth and development. Outcomes described in the protocol will be assessed throughout pregnancy. Infant outcomes, including effects on postnatal growth and development, will be assessed through at least the first year of life.

Draft Protocol Submission: May 2024
Final Protocol Submission: January 2025
Study Completion: September 2032
Final Report Submission: September 2033

4944-5 Conduct an additional pregnancy study that uses a different design from the prospective pregnancy registry established to fulfill postmarketing requirement 4499-4 (for example a retrospective cohort study using claims or electronic medical record data or a case-control study) to assess major congenital malformations, spontaneous abortions, stillbirths, small for gestational age births, and preterm births in women exposed to etrasimod regardless of indication during pregnancy compared to an unexposed control population.

Draft Protocol Submission: May 2024
Final Protocol Submission: January 2025
Study Completion: March 2031
Final Report Submission: March 2032

14 Division Director (Clinical) Comments

I concur with the recommendation of the review team to approve NDA for Velsipity (etrasimod) for the treatment of moderately to severely active ulcerative colitis (UC) in adults. Etrasimod, a new molecular entity (NME), is a sphingosine 1-phosphate (S1P) receptor modulator that binds with high affinity to S1P receptors 1, 4, and 5. Other S1P modulators have been approved for UC, multiple sclerosis, psoriasis, and graft-versus-host disease. The recommended dosage is 2 mg once daily.

As discussed in detail in this multi-disciplinary review, the benefits of etrasimod outweigh the risks for the indication sought. The NDA submission included the results of two adequate and well-controlled phase 3 trials (APD334-301 and APD334-302), which formed the basis of the substantial evidence of effectiveness. The co-primary endpoints of clinical remission at Week 12 and Week 52 in APD334-301 and clinical remission at Week 12 in APD334-301, all assessed by

modified Mayo score, achieved statistical significance. Secondary endpoints also supported the primary efficacy analyses in both trials.

The safety profile was generally consistent with the known adverse event profile for S1P receptor modulators. Some of the adverse events of special interest, such as infections including herpes zoster, liver injury, and malignancies, are shared across multiple drugs used to treat UC; however, other observed or potential risks, such as bradyarrhythmia and atrioventricular conduction delays, macular edema, fetal risk, and respiratory effects, are unique to this drug class. The most common adverse reactions reported in clinical trials of patients with UC include headache, elevated liver tests, and dizziness. Product Labeling (including Contraindications and Warnings and Precautions) and Medication Guide are adequate to communicate these known and potential risks to healthcare providers and patients, respectively. A REMS will not be required.

Postmarketing required studies, under Section 505(o)(3) of the Federal Food, Drug, and Cosmetic Act, will assess the pregnancy outcomes following etrasimod exposure during pregnancy and the effect of etrasimod on lactation. In addition, the Applicant agreed to conduct postmarketing required studies, under the Pediatric Research Equity Act, to assess the safety, efficacy, pharmacokinetics, and pharmacodynamics of etrasimod in pediatric patients 12 to 17 years of age with moderately to severely active UC; and the safety, efficacy, pharmacokinetics, and pharmacodynamics of etrasimod in pediatric patients 2 to 17 years of age with moderately to severely active UC.

15 Office Director (or Designated Signatory Authority) Comments

I concur with the review team's assessment of the application, labeling recommendations, and the recommendation to approve etrasimod, a sphingosine 1-phosphate (S1P) receptor modulator, for the treatment of moderately to severely active ulcerative colitis (UC) in adults, as detailed in this document.

The regulatory action is Approval of NDA 216956 for Velsipity (etrasimod) for the treatment of moderately to severely active ulcerative colitis (UC) in adults.

A REMS is not warranted at this time. Postmarketing required (PMR) studies will include those required under PREA, as well as pregnancy and lactation studies, as detailed in Section 13.

16 Appendices

16.1. References

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16.2. Financial Disclosure

The Applicant provided financial disclosures for the following covered clinical studies.

Study APD334-301: A phase 3, randomized, double-blind, placebo-controlled, 52-week study to assess the efficacy and safety of etrasimod in subjects with moderately to severely active ulcerative colitis.

Study APD334-302: A phase 3, randomized, double-blind, placebo-controlled, 12-week study to assess the efficacy and safety of etrasimod in subjects with moderately to severely active ulcerative colitis.

Covered Clinical Study (Name and/or Number): APD334-301 and APD334-302

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: <u>301: 793 / 302: 933</u>		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): <u>0</u>		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>9</u>		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: <u>9</u> Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in Study: _____ Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) <u>0</u>		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

16.3. OCP Appendices (Technical Documents Supporting OCP Recommendations)

16.3.1. Population PK Analysis

The Applicant's population PK (PopPK) analysis included pooled PK data from 15 phase 1 studies, 2 phase 2 studies (APD334-003 and APD334-005), and 2 phase 3 studies (APD334-301 and APD334-302) in patients with moderately to severely active UC as shown in Table 75. The demographic information of the subjects included in the population PK analysis are shown in Table 76 and Table 77.

Table 75. Summary of Phase 2 and 3 Clinical Trials in Patients with Moderate to Severe Ulcerative Colitis Included in the Population PK

Study	Design	Number of Subjects	Dose Regimen	Planned Observations
APD334-003	Randomized, multiple-dose study in adult UC patients	156 (50-54 per DG, 54 on PBO, 15 discontinued)	QD dosing of either PBO, 1, or 2 mg (capsule) for 12 weeks	Predose and at 2- and 6-h postdose on Week 0 (Day 1), and predose at Weeks 1, 2, 4, 8, 12 (n = 8)
APD334-005	Randomized, multiple-dose study in adult UC patients	118 (6 on PBO, 21 discontinued)	QD dosing of PBO or 2 mg etrasimod (tablet) for a total of 46 weeks (52 weeks for subjects enrolled under protocol Amendment 2), including the 12 weeks of dosing in APD334-003	Predose and at 2 and 6 h postdose on the first day of study drug treatment (Day 1); and predose on Weeks 13, 14, 16, 24, 32, 40 and 46/EOT, and at the 2-week follow-up For subjects enrolled under protocol Amendment 2, samples were also collected 8 h after dosing on Week 12, Day 1, prior to dosing at Weeks 20, 28, 36, 44, 48, and 52/EOT and no sample was collected at Week 46 (n = 11)
APD334-301	Randomized, multiple-dose study in adult UC patients	433 (144 on PBO)	PBO or 2 mg (tablet) QD for up to 52 weeks Subjects may enter Study APD334-303 after 12 weeks of treatment if UC condition has not improved or worsened	Predose and 4 h postdose on Week 0/Day 1, and predose on Weeks 2, 4, 8, 12, 16, 20, 24, 32, 40, 48, and 52 For subjects not entering APD334-303: at 2-week and 4-week follow-up visits (n = up to 15)
APD334-302	Randomized, multiple-dose study in adult UC patients	354 (116 on PBO)	PBO or 2 mg (tablet) QD for 12 weeks Subjects may enter Study APD334-303 after 12 weeks of treatment if eligibility criteria are met	Predose and 4 h postdose on Week 0/Day 1, and predose on Weeks 2, 4, 8, and 12 For subjects not entering APD334-303: at 2-week and 4-week follow-up visits (n = up to 8)

Source: Population PK Report ARE0301H-UC-PK-Report, Table 2, Table 3
Abbreviations: PK = pharmacokinetics; PBO = placebo; QD = once daily

Table 76. Summary of Key Categorical Demographics for Subjects Included in Population Pharmacokinetic Analysis

	Summary n = 1079
Sex	
Female	474 (43.9)
Male	605 (56.1)
Patient Status	
Healthy Volunteer	411 (38.1)
Ulcerative Colitis Patient	668 (61.9)
Race	
Black Or African American	129 (12.0)
White	786 (72.8)
American Indian Or Alaska Native	17 (1.6)
Asian	129 (12.0)
Multiple Or Other Or Unknown	18 (1.7)
Ethnicity	
Other Or Unknown	826 (76.6)
Hispanic Or Latino	175 (16.2)
Japanese	52 (4.8)
Chinese	26 (2.4)
Tobacco Use	
False	928 (86.0)
True	135 (12.5)
Missing	16 (1.5)
Bariatric Surgery	
False	1079 (100.0)
Hepatic Impairment	
Normal	389 (36.1)
Missing	668 (61.9)
Mild	8 (0.7)
Moderate	8 (0.7)
Severe	6 (0.6)

Source: Population PK Report ARE0301H-UC-PK-Report, Table 4
Note: Summary is count (percent)

Table 77. Summary of Key Continuous Demographics for Subjects Included in Population Pharmacokinetic Analysis

Variable	n	Mean	Median	SD	Min / Max	Missing (%)
AGE	1079	39.3	37.0	13.0	16.0 / 78.0	0.00
HT	1079	170	170	9.64	140 / 198	0.00
WT	1079	74.1	72.6	15.8	41.4 / 138	0.00
BMI	1079	25.6	24.9	4.96	13.0 / 51.1	0.00
MMS	1079	4.09	5.00	3.35	0.00 / 9.00	0.00
DURDIS	1079	4.58	1.64	6.65	0.00 / 38.0	0.00
CPSCORE	-	-	-	-	-	100
PT	910	11.7	11.6	1.18	8.40 / 16.5	15.7
INR	944	1.03	1.00	0.106	0.760 / 2.00	12.5
BILI	1079	0.452	0.351	0.394	0.0877 / 5.40	0.00
ALT	1079	17.9	16.0	10.1	4.00 / 96.0	0.00
AST	1079	17.9	16.0	8.92	4.00 / 158	0.00
ALB	1079	4.26	4.30	0.438	2.50 / 5.90	0.00
SCR	1057	0.884	0.826	0.563	0.452 / 10.4	2.04
EGFR	1057	102	100	23.2	8.09 / 204	2.04

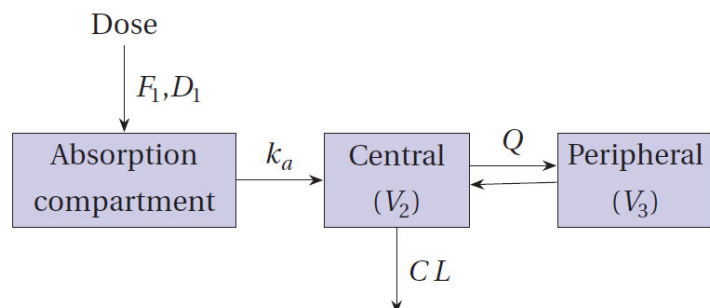
Source: Population PK Report ARE0301H-UC-PK-Report, Table 6

Abbreviations: SD: standard deviation, Min: minimum, Max: maximum, AGE: age (year), HT: height (cm), WT: weight (kg), BMI: body mass index (kg/m²), MMS: Modified Mayo Clinic Score, DURDIS: duration of disease (year), CPSCORE: Child-Pugh score, PT: prothrombin time (seconds), INR: International Normalized Ratio, BILI: total bilirubin (mg/dL), ALT: alanine aminotransferase (U/L), AST: aspartate aminotransferase (U/L), ALB: albumin (g/dL), SCR: serum creatinine (mg/dL), EGFR: estimated glomerular filtration rate (mL/min)

The final PopPK dataset included a total of 16,205 quantifiable etrasimod plasma concentrations from 1079 subjects.

The basic PopPK structure model consisted of a two-compartment model with sequential zero- and first-order absorption and first-order elimination as shown in Figure 15.

Figure 15. Schematic Representation of the Structural Population Pharmacokinetic Model of Etrasimod



Source: Population PK Report ARE0301H-UC-PK-Report, Figure 1

Abbreviations: F1: bioavailability to the absorption compartment; D1: zero-order absorption duration; ka: first-order absorption rate constant; CL: systemic clearance; V2: central volume of distribution; Q: intercompartmental clearance; V3: peripheral volume of distribution

The base model also included fixed weight effects on clearance and volume parameters:

$$CL_{\text{Weight}} = 0.75 \cdot \log\left(\frac{WT_i}{70}\right)$$

$$V_{\text{Weight}} = \log\left(\frac{WT_i}{70}\right)$$

Additionally, for covariate analyses, age, sex, patient status, ethnicity (Japanese, Chinese, or other ethnicities), race (Black or African American versus other races), baseline bilirubin, eGFR, tobacco use, and formulations were tested for their potential influence on the apparent clearance, volume of distribution, bioavailability, first-order absorption rate constant (k_a), and zero-order absorption duration (D_1) of etrasimod.

Briefly, a covariate modeling approach emphasizing parameter estimation rather than stepwise hypothesis testing was implemented as the primary covariate analysis methodology. A full model was constructed to avoid correlation or collinearity in predictors (covariates with correlation coefficients $> |0.6|$ were not simultaneously included as potential predictors). Population parameters, including fixed effects parameters (covariate coefficients and structural model parameters), and random effects parameters were estimated. An exploratory assessment of any remaining trends was conducted by graphical inspection of all covariate effects.

The final PopPK model that best described etrasimod PK was a two-compartment model with sequential zero- and first-order absorption. The PK parameter estimates from the final PopPK model are summarized in Table 78, Table 79, Table 80.

Table 78. Summary of Structural Parameter Estimates for the Final PopPK Model

		Estimand	Estimate	95% CI	Bulk ESS	Tail ESS	$\hat{R}$
CL/F	Apparent clearance (L/h)	$\exp(\theta_1)$	1.06	(1.03, 1.09)	4011	3956	1.00
V_2/F	Apparent central volume (L)	$\exp(\theta_2)$	44.8	(44.2, 45.6)	3010	3893	1.00
Q/F	Intercompartmental clearance (L/h)	$\exp(\theta_3)$	0.160	(0.153, 0.168)	970	2008	1.01
V_3/F	Peripheral volume (L)	$\exp(\theta_4)$	25.2	(24.0, 26.4)	1153	2072	1.00
k_a	1st-order absorption rate constant (1/h)	$\exp(\theta_5)$	1.57	(1.43, 1.74)	581	1540	1.00
D_1	0th-order absorption duration (h)	$\exp(\theta_6)$	1.05	(0.962, 1.14)	228	568	1.02

Source: Population PK Report ARE0301H-UC-PK-Report, Table 7

Abbreviations: CI = confidence interval; ESS = effective sample size; $\hat{R}$ = Gelman-Rubin diagnostic

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Velsipity (etrasimod)

Table 79. Summary of Structural Covariate Estimates for the Final PopPK Model

		Estimand	Estimate	95% CI	Bulk ESS	Tail ESS	$\hat{R}$
CL_{Weight}	Effect of body weight on apparent clearance and apparent intercompartmental clearance	θ_7	0.616	(0.538, 0.697)	3686	3930	1.00
V_{Weight}	Effect of body weight on apparent central and peripheral volume	θ_8	0.858	(0.793, 0.922)	3461	3925	1.00
CL_{Age}	Effect of age on apparent clearance	θ_9	-0.0742	(-0.118, -0.0306)	3655	3970	1.00
CL_{Female}	Effect of female sex on apparent clearance	$\exp(\theta_{10})$	0.939	(0.910, 0.968)	4009	3930	1.00
CL_{Healthy}	Effect of healthy subject on apparent clearance	$\exp(\theta_{11})$	1.10	(1.07, 1.14)	3479	3887	1.00
CL_{Japanese}	Effect of Japanese ethnicity on apparent clearance	$\exp(\theta_{12})$	0.995	(0.935, 1.06)	4293	3799	1.00
CL_{Chinese}	Effect of Chinese ethnicity on apparent clearance	$\exp(\theta_{13})$	0.980	(0.902, 1.06)	4161	3949	1.00
$CL_{\text{Black/AA}}$	Effect of Black or African American race on apparent clearance	$\exp(\theta_{14})$	0.967	(0.924, 1.01)	3869	3889	1.00
$CL_{\text{Bilirubin}}$	Effect of bilirubin on apparent clearance	θ_{15}	-0.0414	(-0.0674, -0.0150)	3741	3625	1.00
CL_{eGFR}	Effect of estimated glomerular filtration rate on apparent clearance	θ_{16}	0.0947	(0.0284, 0.161)	3719	3812	1.00
CL_{Tobacco}	Effect of tobacco use on apparent clearance	$\exp(\theta_{17})$	1.01	(0.968, 1.06)	4004	4015	1.00
F_{Capsule}	Effect of capsule formulation on bioavailability	$\exp(\theta_{18})$	1.03	(1.01, 1.05)	1349	2286	1.00
$k_{a\text{Capsule}}$	Effect of capsule formulation on 1st-order absorption rate constant	$\exp(\theta_{19})$	0.675	(0.582, 0.784)	429	1302	1.00
$D_{1\text{Capsule}}$	Effect of capsule formulation on 0th-order absorption duration	$\exp(\theta_{20})$	1.44	(1.24, 1.68)	279	622	1.01
F_{Solution}	Effect of solution formulation on bioavailability	$\exp(\theta_{21})$	0.853	(0.797, 0.909)	571	1364	1.01
$k_{a\text{Solution}}$	Effect of solution formulation on 1st-order absorption rate constant	$\exp(\theta_{22})$	0.724	(0.525, 1.08)	1160	1827	1.00
$D_{1\text{Solution}}$	Effect of solution formulation on 0th-order absorption duration	$\exp(\theta_{23})$	0.615	(0.376, 0.956)	643	1362	1.00

Source: Population PK Report ARE0301H-UC-PK-Report, Table 7
Abbreviations: CI = confidence interval; ESS = effective sample size; $\hat{R}$ = Gelman-Rubin diagnostic

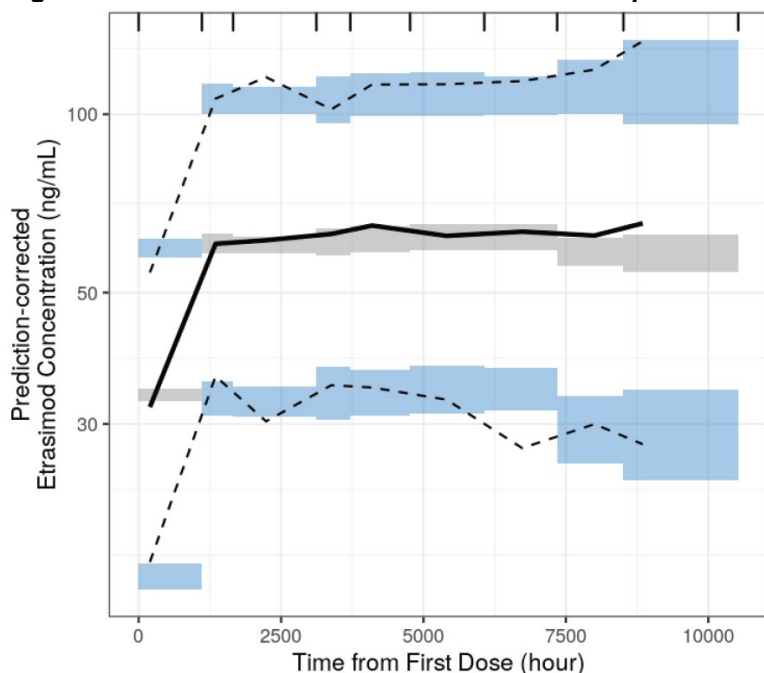
Table 80. Summary of Random Effect Parameter Estimates for the Final PopPK Model

		Estimand	Estimate	95% CI	Shrinkage (%)	Bulk ESS	Tail ESS	$\hat{R}$
IIV-CL/F	IIV on Apparent clearance	$\omega_{1,1}$	0.0686 [CV=26.6%]	(0.0624, 0.0754)	4.12	3929	3776	1.00
IIV-V ₂ /F	IIV on Apparent central volume	$\omega_{2,2}$	0.0371 [CV=19.4%]	(0.0329, 0.0417)	17.5	4176	3783	1.00
IIV-k _a	IIV on 1st-order absorption rate constant	$\omega_{5,5}$	0.319 [CV=61.3%]	(0.244, 0.408)	50.1	1463	2581	1.00
IIV-CL-V ₂	Covariance between IIV-CL and IIV-V ₂	$\omega_{2,1}$	0.0334 [Corr=0.662]	(0.0290, 0.0383)		4045	3872	1.00
IOV-D ₁	IOV on 0th-order absorption duration (h)	$\omega_{7,7}$	0.588 [CV=89.4%]	(0.507, 0.677)	43.9	1120	2386	1.01
RUV-prop	Proportional RUV	$\sigma_{1,1}$	0.0277 [CV=16.7%]	(0.0270, 0.0285)		3101	3878	1.00
RUV-add	Additive RUV	$\sigma_{2,2}$	0.0501 [SD=0.224 ng/mL]	(0.0383, 0.0649)		2283	3498	1.00

Source: Population PK Report ARE0301H-UC-PK-Report, Table 9
Abbreviations: CI = confidence interval; Corr = Correlation coefficient; CV = coefficient of variation; IIV = interindividual variability; RUV = random unexplained variability; ESS = effective sample size; $\hat{R}$ = Gelman-Rubin diagnostic

Figure 16 displays a visual predictive check of the final population PK model for etrasimod.

Figure 16. Visual Predictive Check of the Final PopPK Model for Etrasimod



Source: Population PK Report ARE0301H-UC-PK-Report, Figure S163
Note: Black lines represent the median (solid), 5th and 95th percentiles (dashed) of the observed data. Blue and grey shaded regions represent the 95% confidence intervals of the corresponding (i.e., 5th, 50th, and 95th) percentiles.

Overall, the PopPK model estimated the typical values of CL/F and V_{ss}/F of etrasimod were 1.06 L/h and 70 L, respectively, for a 70 kg subject. The mean effective half-life ($t_{1/2,eff}$) was 33.9 hours in patients with UC. Mean etrasimod PK parameters in patients with UC estimated using PopPK analysis are summarized in Table 12.

PopPK estimates that healthy subjects have slightly smaller (within 10%) mean etrasimod $C_{max,ss}$ and AUC_{ss} compared to reference UC patient of the same body weight, following 2 mg QD dosing. Hence, disease status is not expected to have a clinically significant effect on etrasimod PK exposure in subjects with UC.

Effect of Body Weight on PK

Body weight was identified as a statistically significant covariate on both clearance and volume for etrasimod. Etrasimod exposures are higher in subjects with lower body weight and lower in subjects with higher body weight. Based on popPK analysis, the predicted $C_{max,ss}$, AUC_{ss} , and $C_{trough,ss}$ values for etrasimod in a typical 50 kg subject are 125, 123, and 120%, respectively, of those from a reference 70 kg subject. The predicted $C_{max,ss}$, AUC_{ss} , and $C_{trough,ss}$ values for etrasimod in a typical 110 kg subject are 74.1, 75.7, and 77.9%, respectively, of those from a reference 70 kg subject. Hence, the effect of body weight on PK is not considered to be clinically significant to warrant dose adjustment across the typical body weight range in adults.

Smoking Status

Population PK analysis estimated that steady state PK estimates ($C_{max,ss}$, AUC_{ss} , and $C_{trough,ss}$) of etrasimod in a typical tobacco user are 99.1, 98.8, and 98.5%, respectively, of those from a reference male non-user of tobacco of the same body weight.

In addition, PopPK estimates that there are no meaningful differences in the PK of etrasimod based on sex, age, race, or ethnicity in adults.

16.3.2. Exposure-Response Analysis

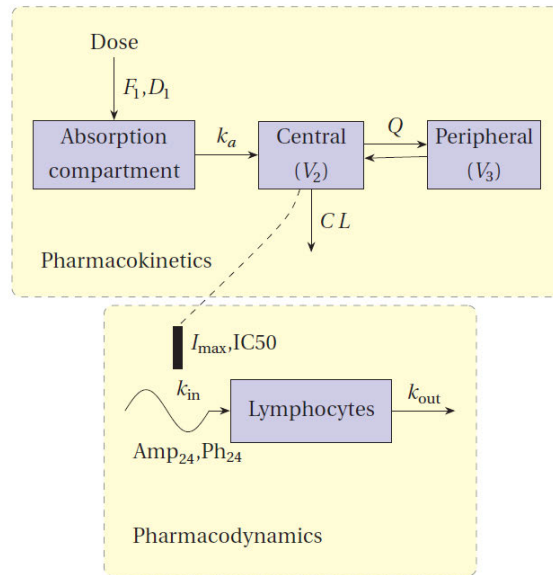
Population PK-PD analyses were conducted to characterize the exposure-response (E-R) relationships between etrasimod and lymphocyte count, clinical efficacy endpoints (clinical remission, endoscopic improvement, symptomatic remission, and mucosal healing at Week 12), and heart rate.

The Applicant conducted population E-R analyses using data from 15 phase 1 studies in adult healthy volunteers and adults with hepatic or renal impairment, two phase 2 studies in adult patients with UC, and two phase 3 studies in adult patients with UC. Post hoc estimates of PK parameters for each subject from the final PopPK model were included in the PK-PD analysis.

Lymphocyte Count

Exploratory graphical evaluations of the observed lymphocyte count versus time and relationships between exposure and response were initially performed prior to analysis (Figure 17). An indirect response model with a circadian rhythm for lymphocyte egress over a 24-hour period was used to describe the etrasimod and lymphocyte relationship.

Figure 17. Base Lymphocyte Model Diagram



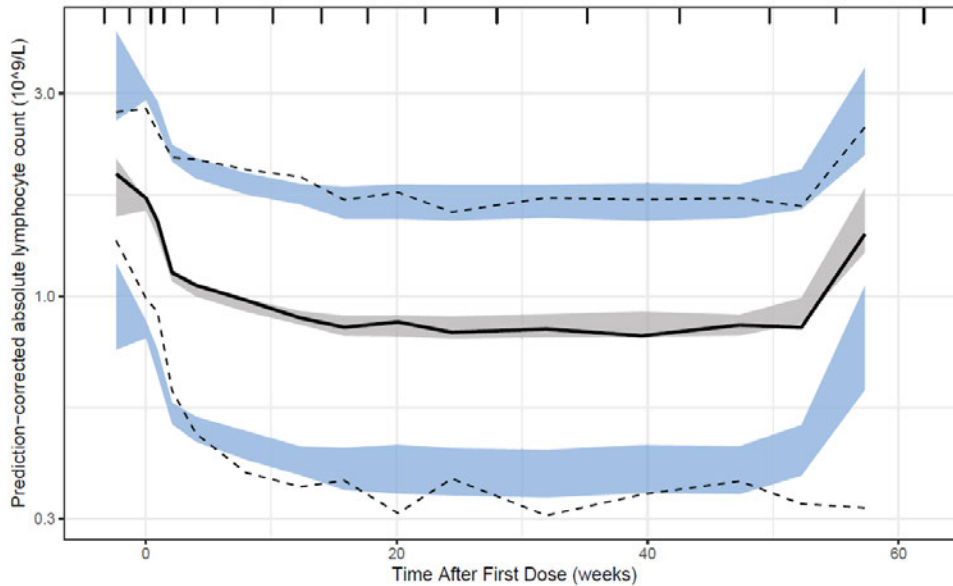
CL : systemic clearance	k_{in} : egress rate constant
V_2 : central volume of distribution	k_{out} : removal rate constant
Q : intercompartmental clearance	I_{max} : maximum inhibition
V_3 : peripheral volume of distribution	IC_{50} : concentration required to achieve half the maximum inhibition
k_a : first-order absorption rate constant	Amp_{24} : amplitude for 24-hour period
D_1 : zero-order absorption duration	Ph_{24} : phase for 24-hour period
F_1 : bioavailability to the absorption compartment	

Source: Population PK-PD ARE0301H-UC-Lymphocytes Report, Figure 1

The covariates tested in the full model included age, height, body weight, body mass index (BMI), sex, race, ethnicity (Western, Japanese, and Chinese), patient status (UC versus healthy, subscores and composite scores at baseline, baseline disease activity (modified Mayo score), and duration of disease), tobacco use at baseline, and concomitant and prior medications.

Overall, visual inspection indicates that the PK-PD model for lymphocyte count is generally predictive for the central tendency of lymphocyte count since the median of the observed lymphocyte are generally within the 95% CI of the simulated data (Figure 18).

Figure 18. Visual Predictive Check for Absolute Lymphocyte Count

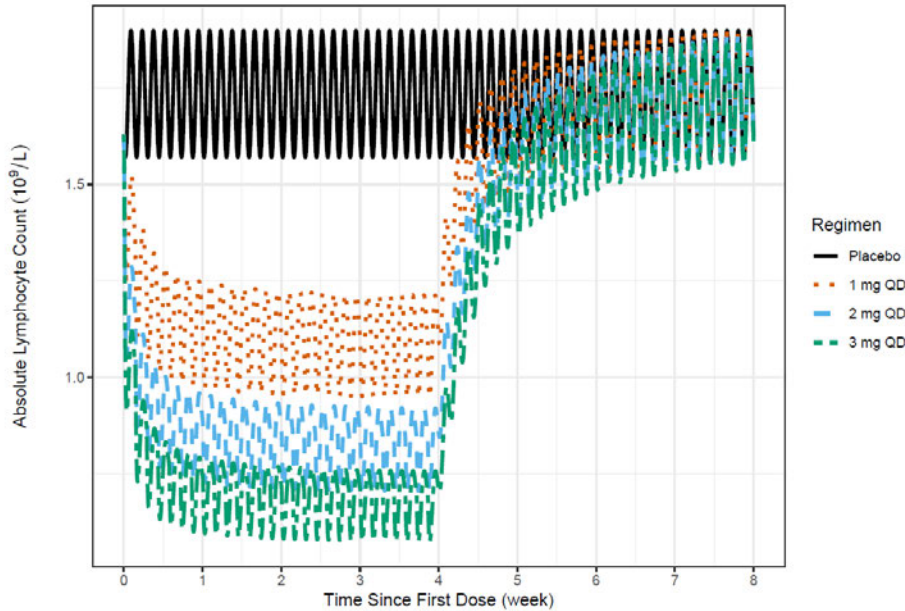


Source: Population PK-PD ARE0301H-UC-Lymphocytes Report, Figure S104

Black lines represent the median (solid), 5th and 95th percentiles (dashed) of the observed data. Blue and grey shaded regions represent the 95% confidence intervals of the corresponding (i.e., 5th, 50th, and 95th) percentiles.

Simulations were performed to predict the typical time to return to a normal level of lymphocytes after discontinuation of etrasimod dosing at steady state (Figure 19). As expected, higher doses increased suppression of lymphocyte egress. Lymphocyte returned to near (within 10% of) baseline within one to two weeks following discontinuation of steady-state dosing.

Figure 19. Predicted Typical Lymphocyte Count Versus Time Profile for Placebo and 1, 2, and 3 mg Doses of Etrasimod Administered QD for 4 Weeks



Source: Population PK-PD ARE0301H-UC-Lymphocytes Report Addendum, Figure 2
Simulations were performed for a reference subject, i.e., a 35-year-old, 70 kg, male patient with UC, not taking concomitant medication, who was neither Japanese nor Chinese and was not Black or African American and represent a typical median response.
Abbreviations: QD = once daily

Additionally, the predicted number of days required to return to normal lymphocyte levels following discontinuation of steady-state dosing at 2 mg QD is summarized in Table 81 for "normal" thresholds of $1.0 \times 10^9/L$, as well as for a threshold of 90% of baseline in both healthy subjects and UC patients.

Table 81. Predicted Time (Days) to Return to Normal Lymphocyte Range and to 90% of Baseline by Subject Type After Discontinuation of 2 mg QD Dosing at Steady State

Threshold	Median	90% of Subjects
Subjects with UC		
$1.0 \times 10^9/L$	1	8
90% of Baseline	11	20
Healthy subjects		
$1.0 \times 10^9/L$	1	6
90% of Baseline	10	21

Source: Adapted from Population PK-PD ARE0301H-UC-Lymphocytes Report Addendum 1, Table 2
Numbers represent days
Abbreviations: UC = ulcerative colitis

Clinical Efficacy Endpoints

Etrasimod exposure-response analyses for key primary (clinical remission) and secondary efficacy endpoints (endoscopic improvement, symptomatic remission, and mucosal healing) after 12 weeks of treatment with etrasimod were performed using data from one phase 2 study (APD334-003) and two phase 3 studies (APD334-301 and APD334-302) in adult patients with

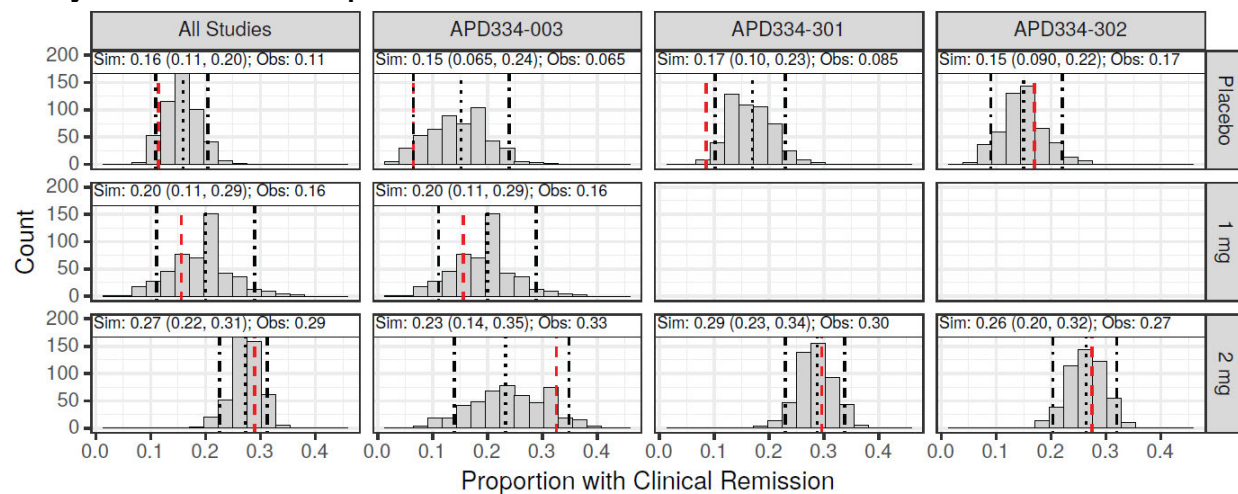
UC. Post hoc estimates of PK parameters for each subject from the final PopPK model were included in the PK-PD analysis.

Exploratory graphical evaluations were performed initially to assess possible trends in the data prior to analysis. For each endpoint, logistic regression analyses were performed to develop a model describing the relationship between the steady state exposure of etrasimod ($C_{avg,ss}$), and the probability of achieving a clinical endpoint.

The following covariates were tested in the full model: age, sex, race, ethnicity (Western, Japanese, and Chinese), body weight, tobacco use at baseline, naïve to biologic/JAK inhibitor therapy at study entry (Yes/No), concomitant corticosteroid use, disease status [composite scores at baseline, baseline disease activity (MMS)].

Visual inspection of the E-R models for clinical remission at Week 12 (as presented in Figure 20) and key secondary efficacy endpoints at Week 12 (not presented in this review) appear to be generally predictive.

Figure 20. Visual Predictive Check of Clinical Remission Proportions at Week 12, Stratified by Study and Treatment Group



Source: Population PK-PD ARE0301H-UC-Efficacy Report, Figure S37:

The red dashed lines indicate the observed endpoint proportions. Distributions of simulated proportions are represented by the histograms.

The black dashed and dash-dot lines represent the medians and 90% prediction intervals, respectively. The text annotations show the simulated medians and 90% prediction intervals, respectively, and the observed proportions.

Abbreviations: Sim = simulated; Obs = observed

The E-R relationships for the observed rates of clinical remission at Week 12 and main secondary efficacy endpoints (endoscopic improvement, symptomatic remission, and mucosal healing) at Week 12 are presented in Figure 6.

Overall, the final models suggest that there is an apparent positive E-R relationship for clinical remission at Week 12, as well as for the secondary endpoints (endoscopic improvement,

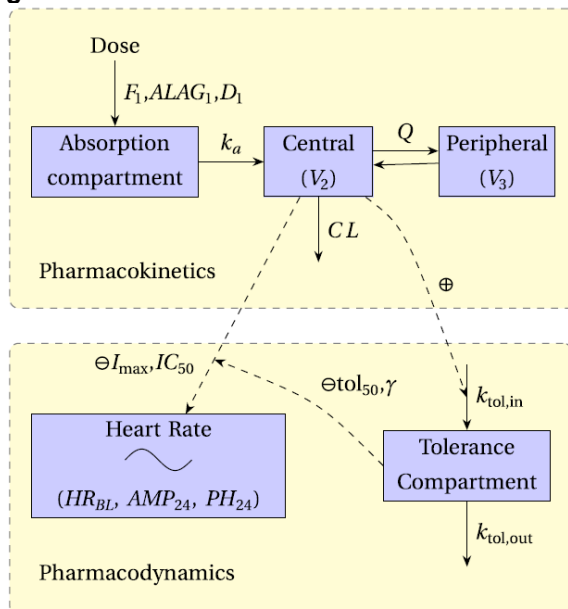
symptomatic remission, and mucosal healing) at Week 12. Across the range of exposures, there appeared to be a trend toward greater efficacy response with higher etrasimod exposure.

Heart Rate

An E-R analysis for heart rate was conducted using heart rate data obtained from 12-lead safety ECG and Holter monitor measurements in two phase 1 studies APD334-008 (QT study) and APD334-110 (cardiodynamic effect study). Post hoc estimates of PK parameters from the final PopPK model were included in the analysis.

Exploratory graphical evaluations of the observed heart rate versus time and relationships between exposure and response (e.g., heart rate nadirs) were performed prior to analysis. Initial modeling was conducted based on a direct effect of etrasimod concentration on heart rate with circadian rhythm and etrasimod-dependent tolerance. Subsequently, other simplified structures for the tolerance mechanism were evaluated in addition to the combination of other circadian rhythms and placebo effects. In the final model (Figure 21), etrasimod concentrations were assumed to decrease heart rate via a direct inhibitory Emax model with a maximum effect that decreased with increasing tolerance.

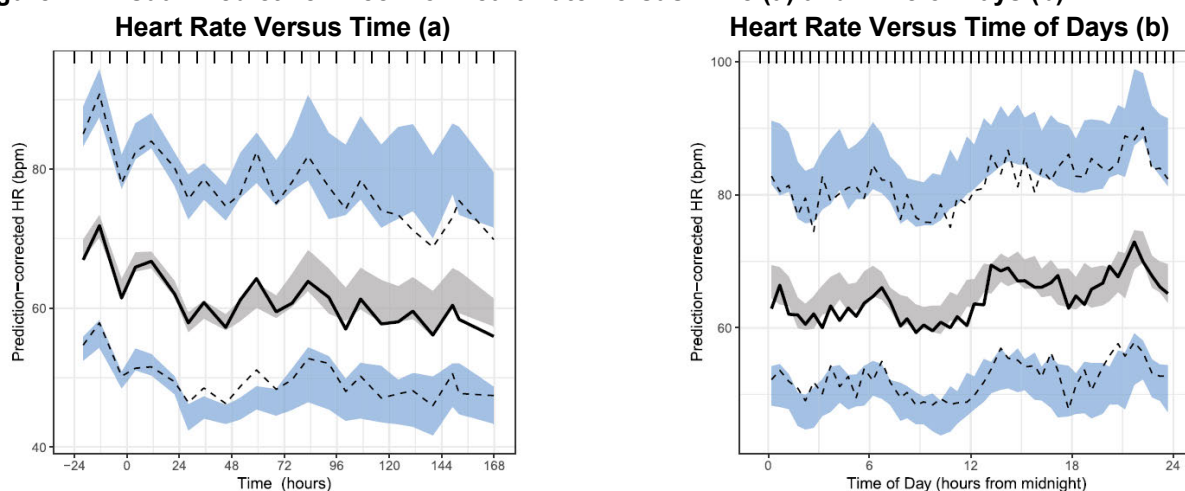
Figure 21. PK-PD Model for Etrasimod Effect on Heart Rate



Source: Population PK-PD ARE0301H-HR Report, Figure 2

Overall, visual inspection indicates that the PK-PD model for heart rate is generally predictive for the central tendency of heart rate since the median of the observed lymphocyte are generally within the 90% CI of the simulated data (Figure 22).

Figure 22. Visual Predictive Check for Heart Rate Versus Time (a) and Time of Days (b)



Source: Population PK-PD ARE0301H-HR Report, Figure 12

Note: The lines represent the median (solid) or 5th/ 95th (dashed) percentiles of the observed data. The shaded areas represent 90% prediction intervals for median (grey) or 5th/ 95th percentiles for data simulated under the model.

Abbreviations: HR = heart rate

Simulations were performed to compare the predicted heart rate in subjects with UC following 2 mg QD for 21 days versus 2 mg QD with dose titration regimen (i.e., 0.25 mg QD on Days 1 and 2, 0.5 mg on Day 3, 0.75 mg on Day 4, 1.25 mg on Day 5, and 2 mg QD on Days 6-21). The model estimated that the extent of maximal HR reduction was generally similar between regimens with and without dose titration although the time to maximal HR reduction was delayed with the regimen with dose titration (reached after 5 days of treatment) compared to that following 2 mg QD without titration (reached on Day 1) (Figure 3).

16.3.3. Drug Interaction Studies

Is Etrasimod a Substrate of CYP Enzymes?

Yes. In vitro studies (Study XT164054 and Study APD334.DMPK.010) indicated that CYP2C8, CYP2C9, and CYP3A4 are the major CYPs involved in the metabolism of etrasimod with fraction metabolized of 0.38, 0.37 and 0.22, respectively, with CYP2C19 and CYP2J2 as minor contributors of 0.01.

Identification of In Vitro Metabolites

In vitro study (MPR-11-005) using human liver microsomes identified five metabolites, M1, M2, M3, M4, and M5. Metabolite M1 was an acyl-glucuronide of etrasimod whereas M2 and M3 were 2 mono-hydroxyl metabolites. M2 was the major metabolite produced by rCYP2C8 and rCYP2C19 whereas M3 was primarily produced by CYP2C9*1 (MPR-16-002). M2 and M3 were further metabolized to glucuronide conjugates, M4 and M5, respectively.

In in vitro study (MPR-16-003), 18 metabolites of etrasimod were identified in human hepatocytes following incubation with [¹⁴C]etrasimod (20 μM). Among them, the hydroxyl (M3) and ketone (M6) metabolites were the most abundant at 4.3% and 9.0% of total radioactivity;

while all other metabolites formed were less than 4% of total radioactivity, including the glucuronide metabolite M1 and other metabolites that included combination of oxidation and glucuronidation.

Identification of Human CYP Enzymes Involved in the Metabolism of Etrasimod

Based on in vitro study (MPR-16-002) using recombinant human CYP enzymes, CYP2C9*1, CYP2C19, and CYP2C8 were the primary enzymes involved in the oxidative metabolism of etrasimod. M2 was the major metabolite produced by rCYP2C8 and rCYP2C19 whereas M3 was primarily produced by CYP2C9*1; and M6 was a minor metabolite of all three rCYPs.

Effect of Other Drugs on Etrasimod PK

Three clinical DDI studies (APD334-009, APD334-111 and APD334-116) were conducted and see Section 6.3.2 above for details.

Concomitant Inducers

In a clinical DDI study (APD334-009), rifampin (a strong inducer of CYP3A4 and CYP2C19, and a moderate inducer of CYP2C8 and CYP2C9) reduced AUC of etrasimod by 49%.

Concomitant Inhibitors

In a clinical DDI study (APD334-111), fluconazole (a moderate inhibitor of CYP2C9 and CYP3A4, and a strong inhibitor of CYP2C19) increased AUC of etrasimod by up to 84%.

In a clinical drug interaction study (APD334-116, co-administration of itraconazole (a strong inhibitor of CYP3A4) 200 mg QD for 13 days with a single dose of etrasimod 1 mg had no or minimum impact on the mean C_{max} (6% increase) but resulted in an increase of 32% in mean AUC_{0-inf} of etrasimod.

Is Etrasimod an Inhibitor and/or an Inducer of CYP Enzymes?

The ability of etrasimod to inhibit or induce the activity of metabolic enzymes was investigated in vitro in HLM (inhibition), rUGTs (inhibition), and human hepatocytes (induction only).

CYP Enzyme/UGT Inhibition

- Etrasimod did not inhibit human CYPs or UGTs (see comments below for details).
- Two in vitro studies (Study MPR-11-004; Study XT165088) were conducted to evaluate the potential of etrasimod to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5. Activity was assessed at concentrations of etrasimod up to 50 μ M using selective probe substrates. There was little or no direct or time dependent inhibition by etrasimod (10 μ M) with any of the CYP enzymes evaluated, except for CYP2C8. Etrasimod reversibly inhibited CYP2C8 with an IC_{50} of 4.4 μ M (amodiaquine as the in vitro substrate). However,

the calculated AUCR (ratio of area under the concentration-time curve) was < 1.25 (using repaglinide as the sensitive clinical CYP2C8 substrate).

- Etrasimod also reversibly inhibited CYP2C8 with an IC₅₀ value of 34.7 μM in the study with paclitaxel as a probe substrate (Study MPR-11-004). However, the calculated AUCR value of 1.14 for CYP2C8 inhibition was less than the threshold value of 1.25 that would necessitate conduct of a clinical DDI study. Thus, etrasimod is not likely to be a clinically relevant CYP2C8 inhibitor.
- In Study XT165088, etrasimod did not inhibit UGT1A3, UGT1A4, UGT1A9, UGT2B7, or UGT2B17. However, etrasimod directly inhibited UGT1A1 and UGT1A6-mediated activities up to 28% and 48%, respectively, at 10 μM which greatly exceeds the typical observed mean steady-state C_{max} for etrasimod (2 mg once daily) of 0.247 μM [113 ng/mL]. Hence, etrasimod is unlikely to potentially produce clinically meaningful inhibition of these UGTs. No clinical study has been conducted to assess the impact of etrasimod on UGTs.

CYP Enzyme Induction

- Etrasimod is not an inducer of human CYP1A2, CYP2B6, and CYP3A4 based on the fold-change method as mRNA evaluations (see comments below for details).
- In vitro study using human hepatocytes (No. XT163079) showed that etrasimod (up to 10 μM) caused no significant induction of CYP1A2 mRNA levels; however, in one of three preparations up to 3.73-fold and 8.94-fold increase was observed for CYP2B6 and CYP3A4 mRNA, respectively, but the increases were less than 11% and 10% as effective as the positive controls (phenobarbital for CYP2B6 and rifampin for CYP3A4).
- In addition, the Applicant also assessed the potential for induction by estimating drug concentration in the liver by using two methods per the Guidance:
 - Method 1: $I_h = f_{u,p} \times [C_{max} + (F_a \times F_g \times k_a \times Dose)/Q_h/R_B]$, where I_h is the hepatic drug concentration, $f_{u,p}$ is the unbound fraction in plasma, C_{max} is the steady state total maximal concentration in plasma, F_a is the fraction absorbed, F_g is the intestinal availability, k_a is the absorption rate constant, Q_h is the hepatic blood flow rate, and R_B is the blood-to-plasma concentration ratio
 - Method 2: $I_{max,u} \times 30$, where $I_{max,u}$ is the maximal unbound plasma concentration of the interacting drug at steady state
- Based on the two methods above, following etrasimod 2 mg once daily, steady-state etrasimod liver concentration was estimated to be 6.8 nM (I_h) or 156 nM ($I_{max,u} \times 30$) while in vitro variable induction potential was shown at 1 μM in human hepatocytes: mRNA increase of 3.44 -fold in one donor's samples while < 2-fold in samples from two other donors.
- Taken together, the potential for etrasimod to induce CYP enzymes at clinically relevant concentrations is likely low.

Is the Drug a Substrate and/or an Inhibitor of Transport Processes?

- Study XT168106 was designed to evaluate etrasimod (as etrasimod L-arginine) as an inhibitor and a substrate of human transporters (i.e., P-gp, BCRP, BSEP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K).
- Etrasimod (up to 10 µM) inhibited less than 50% of activity towards BSEP, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K but inhibited P-gp, BCRP, and OATP1B1 with IC₅₀ values of ~100, 35.7, and 10 µM, respectively (concentrations of 45,750, 16,333, and 4575 ng/mL respectively). The results are summarized in Table 82 and Table 83.
- The Applicant also conducted a definitive bidirectional Caco-2 study to confirm the membrane passive permeability of etrasimod and potential as a substrate for P-gp and BCRP (APD334.DMPK.007). The efflux ratio for etrasimod (0.5 and 5 µM) was ≤ 2, indicating it is not a substrate for either P-gp or BCRP.
- Therefore, etrasimod is not a substrate or an inhibitor of human ABC transporters (P-gp, BCRP, and BSEP), SLC transporters (OATP1B1, OATP1B3, OAT1/3, OCT1, OCT2, and MATE1/2-K). Overall, etrasimod is not expected to have transporter mediated clinical DDIs.

Table 82. Potential for Etrasimod to Inhibit Transporter Activity In Vitro

Transporter	Test System	Substrate	Etrasimod Concentrations	IC ₅₀ (µM)
P-gp	Caco-2	Digoxin (10 µM)	10 and 100 µM	~ 100 ^a
BCRP	MDCKII-BCRP	Prazosin (1 µM)	0.03 to 30 µM	35.7
BSEP	Vesicles	[³ H]Taurocholic acid (0.4 µM)	1 and 10 µM	>10 ^a
OATP1B1	HEK293	[³ H]Estradiol-17β-glucuronide (50 nM)		~10 ^a
OATP1B3				>10 ^a
OAT1		[³ H]p-Aminohippurate (1 µM)		>10 ^a
OAT3		[³ H]Estrone-3-sulfate (50 nM)		>10 ^a
OCT1		[¹⁴ C]Tetratethylammonium bromide (5 µM)		>10 ^a
OCT2		[¹⁴ C]Metformin (10 µM)		>10 ^a
MATE1				>10 ^a
MATE2-K				>10 ^a

^a Two test article concentrations were evaluated and < 50% inhibition was observed.

Source: Study XT168106, Tables 1 to 10.

Table 83. Potential for Etrasimod to Be a Transporter Substrate

Transporter	Test System	Etrasimod Concentrations	Potential Substrate (efflux ratio ≥ 2 or uptake and reduced in presence of inhibitor)
P-gp	MDCKII-P-gp	1 and 10 μM	No
BCRP	MDCKII-BCRP		No
OATP1B1	HEK293		No
OATP1B3			No
OAT1			No
OAT3			No
OCT1			No
OCT2			No

Efflux ratio pertains to P-gp and BCRP and uptake pertains to OATP1B1/3, OAT1/3, and OCT1/2.

Source: Study XT168106, Tables 11 to 18.

Metabolite Profiling in Mass Balance Study

Following single-dose oral administration of [^{14}C]etrasimod in humans (human mass balance study, No. APD334-107), etrasimod was primarily eliminated through metabolism; unchanged etrasimod was not detected in urine. Metabolism of etrasimod occurred via oxidation, dehydrogenation, sulfation, and glucuronidation with 11 minor circulating metabolites identified in plasma. Oxidative metabolites, M3 and M6, were the 2 most abundant minor circulating metabolites, with each accounting for < 10% of total radioactivity. M2 was not detected in human plasma. Etrasimod was the predominant and only major circulating compound detected in plasma, representing 49% of plasma total radioactivity.

16.3.4. Pharmacogenomics Analysis

No pharmacogenomics analysis was conducted. In vitro effect of recombinant CYP2C9 was studied with CYP2C9*1 enzyme variant.

16.3.5. Individual Study Review

Hepatic Impairment Study APD334-108

Title: An Open-label, Single-dose, Parallel-group Study to Assess the Pharmacokinetics of Etrasimod in Subjects with Mild, Moderate, and Severe Hepatic Impairment Compared to Healthy Matched-control Subjects

Clinical Site: American Research Corporation at the Texas Liver Institute, San Antonio, Texas
Study Date: 23 January 2019 to 29 August 2019

Study Design

This was an open-label, single-dose, parallel-group study to evaluate the PK as well as the safety and tolerability of etrasimod 2 mg administration in males and females with mild, moderate, or severe hepatic impairment (Child-Pugh A/B/C) as compared to matched-control males and females with normal hepatic function. Subjects were enrolled within the following groups based on their Child-Pugh score:

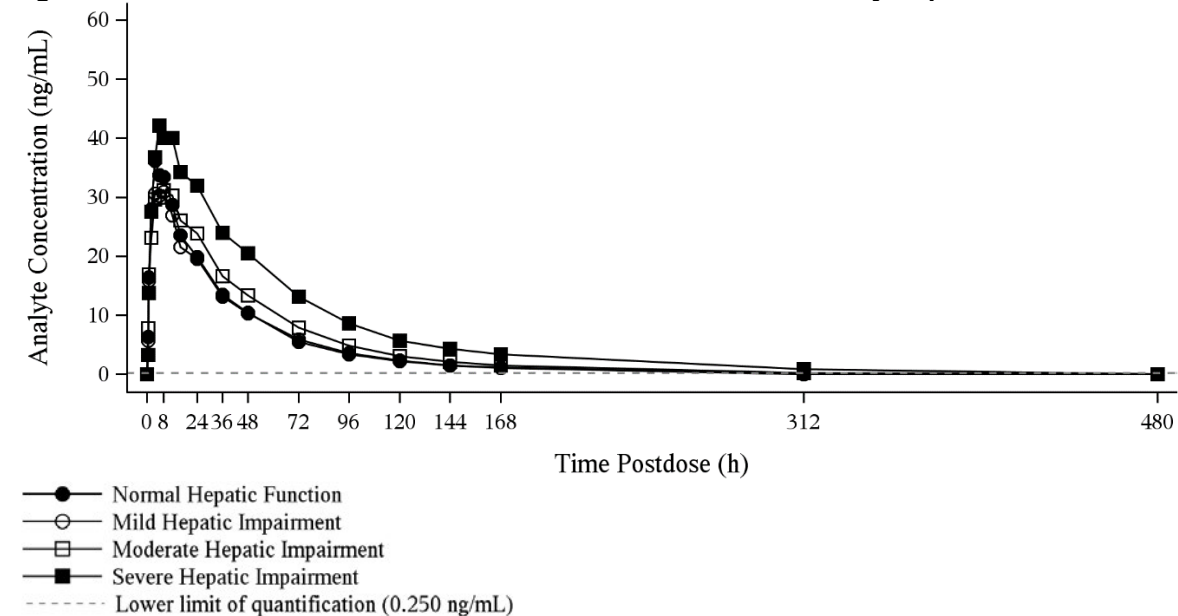
- Group 1 – Normal hepatic function
- Group 2 – Mild hepatic impairment (Child-Pugh Class A)
- Group 3 – Moderate hepatic impairment (Child-Pugh Class B)
- Group 4 – Severe hepatic impairment (Child-Pugh Class C)

Subjects received a single oral dose of 2 mg etrasimod following an overnight fast.

Pharmacokinetic Results

The mean etrasimod plasma concentration-time profiles are graphically displayed by hepatic impairment in Figure 23.

Figure 23. Mean Plasma Concentration-Time Profiles of Etrasimod by Hepatic Function Group



Source: Clinical Study Report APD334-008, Figure 2

The pharmacokinetic parameters of etrasimod are summarized in Table 84.

Table 84. Pharmacokinetic Parameters of a Single Oral Dose of 2 mg Etrasimod in Subjects with Hepatic Impairment and Healthy Control Subjects

Analyte: Etrasimod

Parameter	Mild Hepatic Impairment	Matched Controls	Moderate Hepatic Impairment	Matched Controls	Severe Hepatic Impairment	Matched Controls
	(N=8)	(N=8)	(N=8)	(N=8)	(N=6)	(N=6)
C _{max} (ng/mL)	31.6 (28.7)	33.4 (15.2)	31.5 (30.5)	35.3 (26.3)	42.0 (22.5)	40.5 (29.5)
t _{max} (h)	5.00 (2.00-12.00)	5.00 (1.98-8.08)	8.00 (4.00-12.00)	4.00 (1.98-8.00)	7.00 (4.00-12.00)	5.00 (1.98-8.00)
AUC _{0-t} (h*ng/mL)	1350 (33.7)	1320 (20.5)	1710 (30.9)	1370 (21.3)	2570 (26.9) ^a	1710 (23.5) ^a
AUC ₀₋₂₄ (h*ng/mL)	561 (30.0)	576 (15.9)	614 (29.9)	611 (20.1)	809 (20.7)	703 (22.2)
AUC _{0-∞} (h*ng/mL)	1390 (34.1)	1370 (20.1)	1760 (31.1)	1410 (20.3)	2730 (26.4)	1740 (20.6)
t _{1/2} (h)	55.7 (33.8)	43.9 (14.9)	69.7 (39.2)	49.0 (16.6)	76.5 (15.0)	59.5 (14.8)
CL/F (L/h)	1.44 (34.1)	1.46 (20.1)	1.13 (31.1)	1.42 (20.3)	0.732 (26.4)	1.15 (20.6)
V _z /F (L)	102 (30.8)	88.4 (19.8)	102 (28.6)	95.4 (24.7)	79.3 (35.8)	95.9 (11.7)

^a n = 5

Source: Clinical Study Report APD334-008, Table 9

Point estimates and associated 90% CIs for the C_{max} and AUC ratios of each hepatic impairment group versus healthy control subjects are summarized in Table 85.

Table 85. Statistical Analysis of the Effect of Hepatic Impairment on Etrasimod Pharmacokinetic Parameters

Analyte: Etrasimod

Parameter	Comparison (Test vs. Ref) ^a	Test		Reference		Geo LS Mean Ratio (Test:Ref) ^c		90% CI ^d	P-value
		n ^a	Geo LS Mean (90% CI) ^b	n ^a	Geo LS Mean (90% CI) ^b	(%)	(%)		
C _{max} (ng/mL)	Mild vs Normal	8	33.5 (30.5, 36.9)	8	33.0 (29.9, 36.5)	101.6	88.4, 116.9	0.8395	
	Moderate vs Normal	8	31.3 (28.2, 34.8)	8	34.0 (30.6, 37.6)	92.3	80.0, 106.6	0.3415	
	Severe vs Normal	6	41.7 (35.6, 48.9)	6	40.0 (34.2, 46.8)	104.3	84.4, 128.9	0.7194	
AUC _{0-t} (h*ng/mL)	Mild vs Normal	8	1440 (1260, 1660)	8	1270 (1100, 1470)	113.3	94.9, 135.3	0.2290	
	Moderate vs Normal	8	1720 (1520, 1950)	8	1330 (1180, 1510)	129.1	108.6, 153.3	0.0216	
	Severe vs Normal	4	2310 (1500, 3580)	4	1600 (1040, 2470)	144.7	91.8, 228.1	0.1492	
AUC _{0-∞} (h*ng/mL)	Mild vs Normal	8	1490 (1300, 1710)	8	1320 (1140, 1530)	112.9	94.1, 135.5	0.2523	
	Moderate vs Normal	8	1770 (1570, 2010)	8	1380 (1220, 1550)	128.9	108.6, 153.0	0.0216	
	Severe vs Normal	6	2710 (2160, 3410)	6	1730 (1380, 2160)	157.3	122.0, 202.8	0.0164	

^a n = the number of subjects in each group used in the model.

Source: Clinical Study Report APD334-008, Table 10

The pharmacokinetic parameters of unbound etrasimod are summarized in Table 86.

Table 86. Summary of the Plasma Pharmacokinetic Parameters of Unbound Etrasimod in Subjects with Hepatic Impairment and Healthy Control Subjects

Analyte: Unbound Etrasimod

Parameter	Mild Hepatic Impairment (N = 8)	Matched Controls (N = 8)	Moderate Hepatic Impairment (N = 8)	Matched Controls (N = 8)	Severe Hepatic Impairment (N = 6)	Matched Controls (N = 6)
f_u (%)	4.35 (37.0)	5.02 (27.1)	3.84 (26.6)	5.11 (31.5)	2.59 (45.7)	4.67 (27.2)
$C_{max,u}$ (ng/mL)	1.37 (53.0)	1.68 (26.6)	1.21 (21.3)	1.81 (36.4)	1.09 (40.3)	1.89 (36.8)
$AUC_{0-t,u}$ (h*ng/mL)	58.6 (53.9)	66.5 (38.4)	65.7 (27.0)	70.0 (30.9)	57.6 (33.9) ^a	80.6 (42.6) ^b
$AUC_{0-24,u}$ (h*ng/mL)	24.4 (53.1)	28.9 (33.5)	23.6 (21.1)	31.3 (33.1)	20.9 (40.1)	32.8 (33.6)
$AUC_{0-\infty,u}$ (h*ng/mL)	60.5 (53.5)	68.8 (37.7)	67.7 (27.5)	72.1 (29.8)	70.8 (59.5)	81.1 (37.9)
CL/ F_u (L/h)	33.1 (53.5)	29.1 (37.7)	29.5 (27.5)	27.7 (29.8)	28.3 (59.5)	24.7 (37.9)
V_z/F_u (L)	2350 (23.5)	1760 (31.8)	2650 (33.5)	1860 (42.1)	3060 (83.6)	2060 (27.7)

^a n = 5

^b n = 5

Source: Clinical Study Report APD334-008, Table 12

Statistical analysis of the effect of hepatic impairment on the PK parameters of unbound etrasimod using matched analysis in Table 87.

Table 87. Statistical Analysis of the Effect of Hepatic Impairment on the Pharmacokinetic Parameters of Unbound Etrasimod

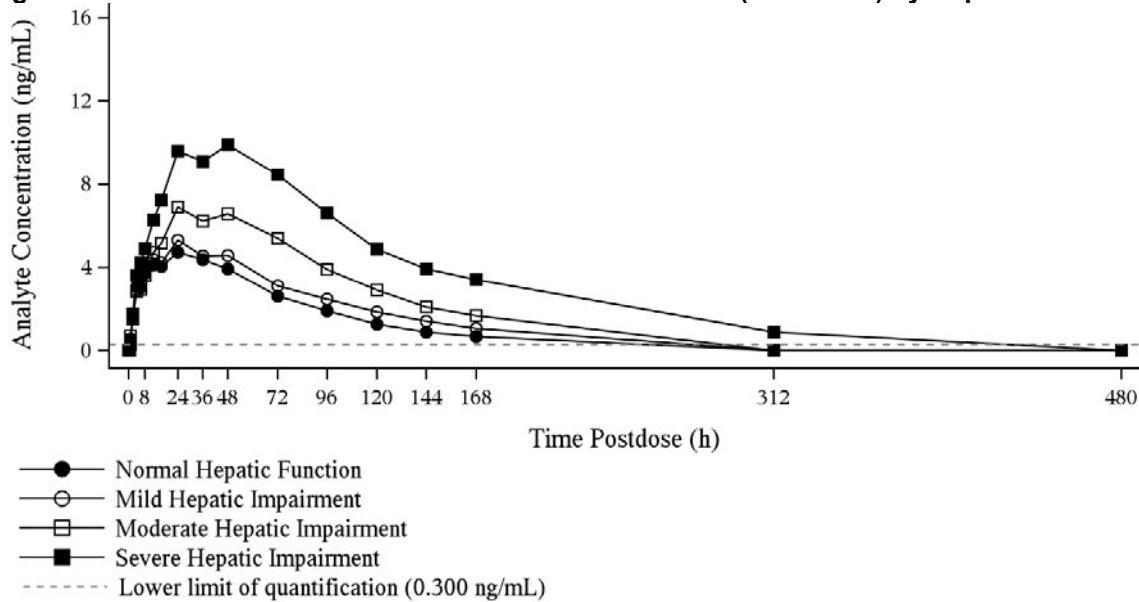
Analyte: Unbound Etrasimod

Parameter	Comparison (Test vs. Ref) ^e	n ^a	Test	Reference	Geo LS Mean Ratio		P-value
			Geo LS Mean (90% CI) ^b	Geo LS Mean (90% CI) ^b	(Test:Ref) ^c (%)	90% CI ^d (%)	
$C_{max,u}$ (ng/mL)	Mild vs Normal	8	1.45 (1.13, 1.87)	8 1.61 (1.24, 2.09)	90.4	62.7, 130.5	0.6340
	Moderate vs Normal	8	1.23 (1.05, 1.44)	8 1.77 (1.52, 2.07)	69.5	56.0, 86.2	0.0109
	Severe vs Normal	6	1.15 (0.849, 1.57)	6 1.99 (1.47, 2.70)	58.0	38.5, 87.3	0.0382
$AUC_{0-t,u}$ (h*ng/mL)	Mild vs Normal	8	62.5 (47.3, 82.5)	8 62.3 (46.5, 83.3)	100.3	66.4, 151.5	0.9895
	Moderate vs Normal	8	67.7 (57.3, 79.9)	8 69.7 (59.1, 82.1)	97.1	77.2, 122.2	0.8240
	Severe vs Normal	4	50.9 (30.6, 84.8)	4 74.0 (44.5, 123)	68.8	35.9, 131.5	0.2857
$AUC_{0-\infty,u}$ (h*ng/mL)	Mild vs Normal	8	64.5 (49.0, 84.8)	8 64.5 (48.4, 86.0)	100.0	66.4, 150.5	0.9997
	Moderate vs Normal	8	69.6 (59.0, 82.2)	8 71.8 (61.0, 84.5)	97.0	77.2, 121.9	0.8165
	Severe vs Normal	6	75.2 (49.8, 113)	6 86.0 (57.4, 129)	87.4	49.6, 154.2	0.6508

Source: Clinical Study Report APD334-008, Table 13

The mean plasma concentration-time profiles for etrasimod's metabolite M3 (AR503641) are displayed by hepatic impairment in Figure 24.

Figure 24. Mean Plasma Concentration-Time Profiles of M3 (AR503641) by Hepatic Function Group



Source: Clinical Study Report APD334-008, Figure 3

The pharmacokinetic parameters of M3 (AR503641) are summarized in Table 88.

Table 88. Summary of the Plasma Pharmacokinetic Parameters of M3 (AR503641) in Subjects with Hepatic Impairment and Healthy Control Subjects
Analyte: M3 (AR503641)

Parameter	Mild Hepatic Impairment (N=8)	Matched Controls (N=8)	Moderate Hepatic Impairment (N=8)	Matched Controls (N=8)	Severe Hepatic Impairment (N=6)	Matched Controls (N=6)
C_{max} (ng/mL)	5.43 (28.9)	4.36 (38.8)	6.71 (28.8)	5.19 (43.1)	9.81 (30.1)	4.79 (42.3)
t_{max} (h)	24.08 (24.00-47.33)	24.00 (12.00-36.00)	24.01 (23.60-48.00)	24.00 (8.00-36.00)	47.58 (24.00-48.00)	30.00 (8.00-36.00)
AUC_{0-t} (h*ng/mL)	472 (35.1)	333 (31.6)	779 (41.1)	401 (59.1)	1350 (52.5) ^a	435 (53.1) ^a
AUC_{0-24} (h*ng/mL)	85.5 (34.3)	74.3 (35.2)	99.0 (30.5)	87.6 (36.1)	131 (41.0)	81.2 (35.3)
$AUC_{0-\infty}$ (h*ng/mL)	562 (32.5)	376 (35.6)	840 (39.2)	466 (60.0)	1330 (46.2)	441 (47.4)
$t_{1/2}$ (h)	64.3 (16.3)	49.0 (13.6)	72.4 (35.4)	58.5 (14.7)	67.3 (18.7)	46.2 (15.2)
MR_{AUC}	0.390 (43.1)	0.265 (36.8)	0.460 (35.3)	0.319 (49.4)	0.469 (32.8)	0.245 (46.7)

^a n = 5

Source: Clinical Study Report APD334-008, Table 14

Statistical analysis of the effect of hepatic impairment on the PK parameters of M3 (AR503641) using matched analysis in Table 89.

Table 89. Statistical Analysis of the Effect of Hepatic Impairment on the Pharmacokinetic Parameters of M3 (AR503641)

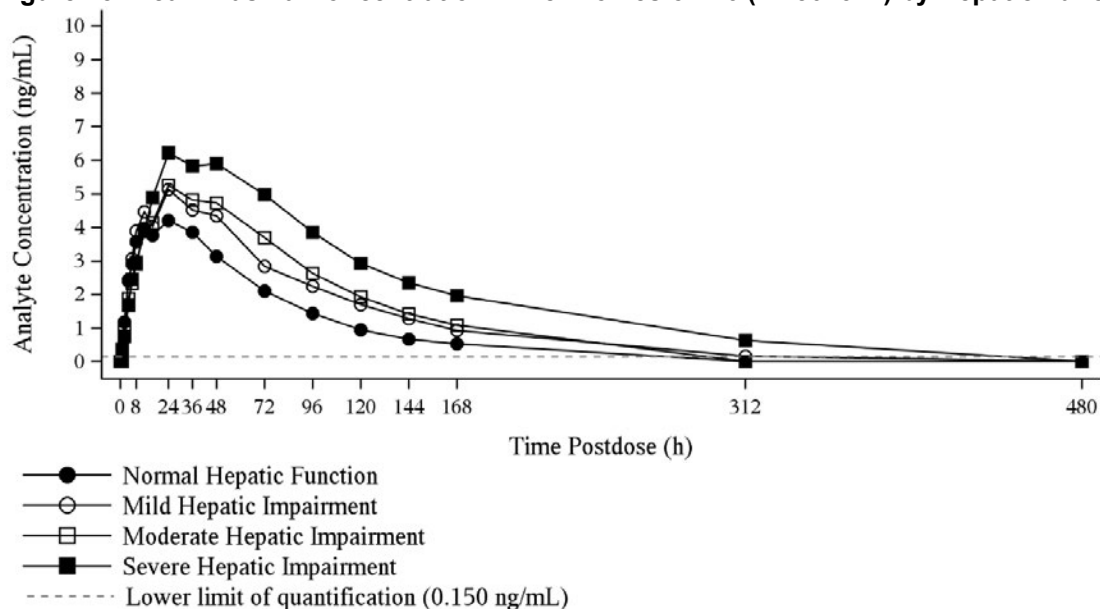
Analyte: M3(AR503641)

Parameter	Comparison (Test vs. Ref) ^e	n ^a	Test	Reference	Geo LS		P-value	
			Geo LS Mean (90% CI) ^b	Geo LS Mean (90% CI) ^b	Mean Ratio (Test:Ref) ^c	90% CI ^d		
C _{max} (ng/mL)	Mild vs Normal	8	5.66 (4.56, 7.02)	8	4.55 (3.61, 5.72)	124.4	96.1, 161.1	0.1564
	Moderate vs Normal	8	6.48 (5.38, 7.82)	8	4.93 (4.10, 5.93)	131.5	101.6, 170.1	0.0829
	Severe vs Normal	6	9.01 (7.18, 11.3)	6	4.41 (3.53, 5.51)	204.5	149.1, 280.3	0.0062
AUC _{0-t} (h*ng/mL)	Mild vs Normal	8	498 (404, 615)	8	328 (263, 410)	151.8	116.8, 197.1	0.0162
	Moderate vs Normal	8	776 (581, 1040)	8	386 (290, 514)	201.0	134.8, 299.7	0.0090
	Severe vs Normal	4	1090 (493, 2390)	4	466 (212, 1030)	232.9	121.4, 447.0	0.0586
AUC _{0-∞} (h*ng/mL)	Mild vs Normal	8	589 (471, 736)	8	377 (297, 478)	156.3	120.0, 203.5	0.0119
	Moderate vs Normal	8	837 (623, 1130)	8	450 (336, 602)	186.0	123.8, 279.6	0.0187
	Severe vs Normal	6	1230 (839, 1800)	6	410 (282, 597)	299.5	176.7, 507.8	0.0088

Source: Clinical Study Report APD334-008, Table 15

The mean plasma concentration-time profiles for etrasimod metabolite M6 (AR504344) are displayed by hepatic impairment in Figure 25.

Figure 25. Mean Plasma Concentration-Time Profiles of M6 (AR504344) by Hepatic Function Group



Source: Clinical Study Report APD334-008, Figure 4

The pharmacokinetic parameters of M6 (AR504344) are summarized in Table 90.

Table 90. Summary of the Plasma Pharmacokinetic Parameters of M6 (AR504344) in Subjects with Hepatic Impairment and Healthy Control Subjects

Analyte: M6 (AR504344)

Parameter	Mild Hepatic Impairment (N=8)	Matched Controls (N=8)	Moderate Hepatic Impairment (N=8)	Matched Controls (N=8)	Severe Hepatic Impairment (N=6)	Matched Controls (N=6)
C _{max} (ng/mL)	5.00 (33.9)	3.54 (36.9)	5.23 (22.0)	4.36 (47.7)	6.04 (46.0)	4.49 (52.1)
t _{max} (h)	24.00 (12.00-36.00)	24.00 (12.00-36.00)	24.03 (23.78-47.93)	18.00 (12.00-36.00)	42.00 (24.00-48.00)	18.00 (8.00-36.00)
AUC _{0-t} (h*ng/mL)	472 (50.6)	266 (32.6)	565 (37.3)	331 (65.2)	777 (60.6) ^a	374 (55.4) ^a
AUC ₀₋₂₄ (h*ng/mL)	81.8 (38.8)	62.9 (33.7)	77.6 (27.2)	74.8 (42.8)	79.7 (52.7)	78.0 (48.1)
AUC _{0-∞} (h*ng/mL)	520 (45.8)	290 (31.6)	605 (35.0)	358 (61.3)	762 (57.8)	392 (45.5)
t _{1/2} (h)	82.0 (29.3)	56.8 (28.2)	79.1 (39.3)	55.2 (12.7)	62.9 (24.7)	64.5 (30.7)
MR _{AUC}	0.363 (42.8)	0.205 (30.5)	0.333 (30.4)	0.246 (47.8)	0.271 (48.9)	0.219 (38.7)

^a n = 5

Source: Clinical Study Report APD334-008, Table 17

Statistical analysis of the effect of hepatic impairment on the PK parameters of M6 (AR504344) using matched analysis in Table 91.

Table 91. Statistical Analysis of the Effect of Hepatic Impairment on the Pharmacokinetic Parameters of M6 (AR504344)

Analyte: M6 (AR504344)

Parameter	Comparison (Test vs. Ref) ^e	Test		Reference		Geo LS Mean Ratio (Test:Ref) ^c		P-value
		n ^a	Geo LS Mean (90% CI) ^b	n ^a	Geo LS Mean (90% CI) ^b	(%)	90% CI ^d (%)	
C _{max} (ng/mL)	Mild vs Normal	8	5.31 (4.29, 6.58)	8	3.57 (2.84, 4.49)	148.6	118.6, 186.2	0.0094
	Moderate vs Normal	8	5.05 (4.22, 6.03)	8	4.13 (3.47, 4.92)	122.2	95.6, 156.2	0.1720
	Severe vs Normal	6	5.37 (4.28, 6.74)	6	3.99 (3.19, 5.00)	134.6	105.8, 171.2	0.0563
AUC _{0-t} (h*ng/mL)	Mild vs Normal	8	511 (400, 652)	8	256 (197, 331)	199.8	144.1, 276.8	0.0035
	Moderate vs Normal	8	556 (416, 742)	8	315 (237, 419)	176.2	118.3, 262.2	0.0261
	Severe vs Normal	4	553 (256, 1200)	4	371 (172, 802)	149.1	105.3, 210.9	0.0771
AUC _{0-∞} (h*ng/mL)	Mild vs Normal	8	562 (447, 707)	8	279 (219, 355)	201.8	152.7, 266.5	0.0011
	Moderate vs Normal	8	597 (451, 789)	8	342 (260, 451)	174.3	118.6, 256.0	0.0243
	Severe vs Normal	6	685 (456, 1030)	6	354 (237, 530)	193.4	120.7, 309.9	0.0386

Source: Clinical Study Report APD334-008, Table 18

Renal Impairment Study APD334-112

Title: A Phase 1, Open-Label, Single-Dose Study to Investigate the Effects of Renal Impairment on the Pharmacokinetics of Etrasimod

Clinical Site: Alliance for Multispecialty Research, Knoxville, TN; Orlando Clinical Research Center, Orlando, FL

Study Date: 22 December 2020 to 26 August 2021

Study Design

This was an open-label, single-dose study to evaluate the PK, safety, and tolerability of etrasimod in subjects with renal impairment.

This study was first conducted using a reduced design. Subjects with severe renal impairment and/or end-stage renal disease (ESRD) (estimated glomerular filtration rate [eGFR] $\leq$ 29 mL/min; either requiring hemodialysis or not; defined as Group C) and matched subjects with normal renal function were initially enrolled. The eGFR was determined by the Modification of Diet in Renal Disease equation. For subjects on dialysis, etrasimod dosing on Day 1 occurred immediately after the approximately 4-hour dialysis procedure completed.

Only if warranted, the study would proceed to the full design (additional evaluation of subjects with mild and moderate renal impairment, and their matched healthy controls) as described in Table 92 below.

Table 92. Renal Impairment Study APD334-112 Full Design

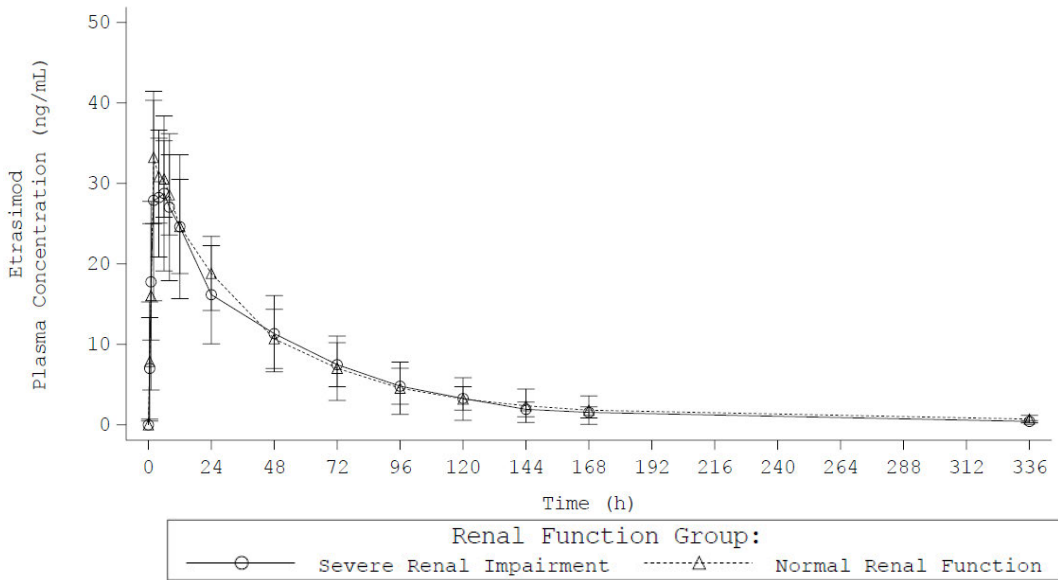
Reduced design:	
Group C	Severe impairment and/or ESRD (eGFR < 29; either requiring hemodialysis or not)
Matched healthy controls	eGFR $\geq$ 90; demographically matched by age ($\pm$ 10 years), body mass index (BMI; $\pm$ 20%), sex, and smoking status to an enrolled renally impaired subject(s)
Full design if warranted:	
Group A	Mild impairment (eGFR $\geq$ 60 to $\leq$ 89)
Group B	Moderate impairment (eGFR $\geq$ 30 to $\leq$ 59)

Of note, the results of the initial investigation did not warrant further investigation, so Group A (mild renal impairment), Group B (moderate renal impairment) and their matched subjects with normal renal function were not studied in this study.

Pharmacokinetic Results

The mean etrasimod plasma concentration-time profiles are graphically displayed by renal impairment in Figure 26.

Figure 26. Mean Plasma Concentration-Time Profiles of Etrasimod by Renal Function Group



Source: Clinical Study Report APD334-112, Figure 3

The pharmacokinetic parameters of etrasimod are summarized in Table 93.

Table 93. Pharmacokinetic Parameters of a Single Oral Dose of 2 mg Etrasimod in Subjects with Renal Impairment and Healthy Control Subjects

Parameter (unit)	Statistics	Severe Renal Impairment (N = 8)	Normal Renal Function (N = 8)
T _{max} (h)	Median	4.00	2.00
	Minimum	2.00	2.00
	Maximum	6.00	12.00
C _{max} (ng/mL)	Mean (SD)	31.5 (10.4)	35.2 (6.53)
	CV%	33.0	18.6
	Geometric Mean	30.0	34.6
	GeoMean CV%	35.2	20.5
AUC _{last} (h*ng/mL)	Mean (SD)	1501 (533)	1606 (697)
	CV%	35.5	43.4
	Geometric Mean	1398	1496
	GeoMean CV%	45.0	40.4
AUC _{inf} (h*ng/mL)	Mean (SD)	1550 (537)	1662 (734)
	CV%	34.7	44.1
	Geometric Mean	1451	1547
	GeoMean CV%	43.1	40.7
t _{1/2} (h)	Mean (SD)	47.8 (14.0)	52.0 (19.4)
	CV%	29.2	37.3
	Geometric Mean	46.2	48.8
	GeoMean CV%	28.5	40.3
CL/F (L/h)	Mean (SD)	1.50 (0.696)	1.38 (0.474)
	CV%	46.6	34.5
	Geometric Mean	1.38	1.29
	GeoMean CV%	43.1	40.7

Source: Adapted from Clinical Study Report APD334-112, Table 8

Statistical analysis of the effect of renal impairment on the PK parameters of etrasimod using matched analysis is presented in Table 94.

Table 94. Statistical Analysis of the Effect of Renal Impairment on Etrasimod Pharmacokinetic Parameters

PK Parameter (Unit)	Comparison	Severe		Normal		GLSM Ratio (%) T:R	90% CI for GLSM Ratio
		n	GLSM (90% CI)	n	GLSM (90% CI)		
C _{max} (ng/mL)	Severe:Normal	8	29.06 (25.71, 32.85)	8	35.67 (31.55, 40.32)	81.49	(68.42, 97.05)
AUC _{last} (h*ng/mL)	Severe:Normal	8	1357 (1067, 1724)	8	1542 (1213, 1959)	88.00	(62.53, 123.84)
AUC _{inf} (h*ng/mL)	Severe:Normal	8	1409 (1111, 1787)	8	1592 (1256, 2019)	88.48	(63.06, 124.14)

Source: Clinical Study Report APD334-112, Table 9

Mass Balance Study APD334-107

Title: A Phase 1, Open-label Study to Evaluate the Absorption, Metabolism, and Excretion of [¹⁴C]Etrasimod (APD334) Following a Single Oral Dose in Healthy Male Subjects

Clinical Site: Covance Clinical Research Unit, Madison, Wisconsin
Study Date: 20 July 2018 to 12 September 2018

Study Design

This was an open-label, non-randomized, single-dose study of radiolabeled etrasimod in eight healthy male subjects. Subjects received a single oral dose of 2 mg [¹⁴C] etrasimod containing approximately 100 µCi radioactivity following an overnight fast.

Blood, urine, and fecal PK samples were collected for determination of total radioactivity concentrations (whole blood, plasma, urine, and feces), for determination of etrasimod concentrations (plasma only), and for metabolite profiling and characterization (plasma, urine, and feces). Beginning on Day 8, subjects were to be discharged if 1 of the following discharge criteria for total radioactivity was met:

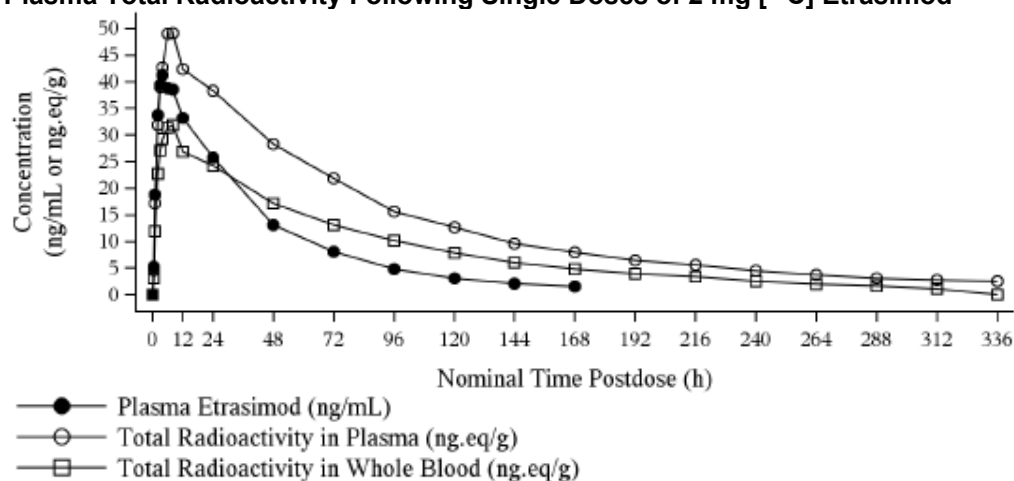
- ≥ 90% of the administered radioactive dose was recovered in the excreta, or
- ≤ 1% of the radioactive dose was recovered in urine and feces for 3 consecutive 24-hour collection intervals where samples were provided.

If neither of these discharge criteria were met by the morning of Day 8, subjects were to remain in the CRU and blood, urine, and feces collections continued at 24-hour intervals until 1 discharge criterion was met or until Day 15.

Pharmacokinetic Results

The mean etrasimod plasma concentrations, and total radioactivity in plasma and whole blood in subjects following single oral administration of 2 mg [¹⁴C] etrasimod are presented in Figure 27.

Figure 27. Mean Concentration-Time Profiles for Plasma Etrasimod, Blood Total Radioactivity, and Plasma Total Radioactivity Following Single Doses of 2 mg [¹⁴C] Etrasimod



Source: Clinical Study Report APD334-107, Figure 3

Etrasimod PK parameters following administration of 2 mg [¹⁴C] etrasimod are summarized in Table 95.

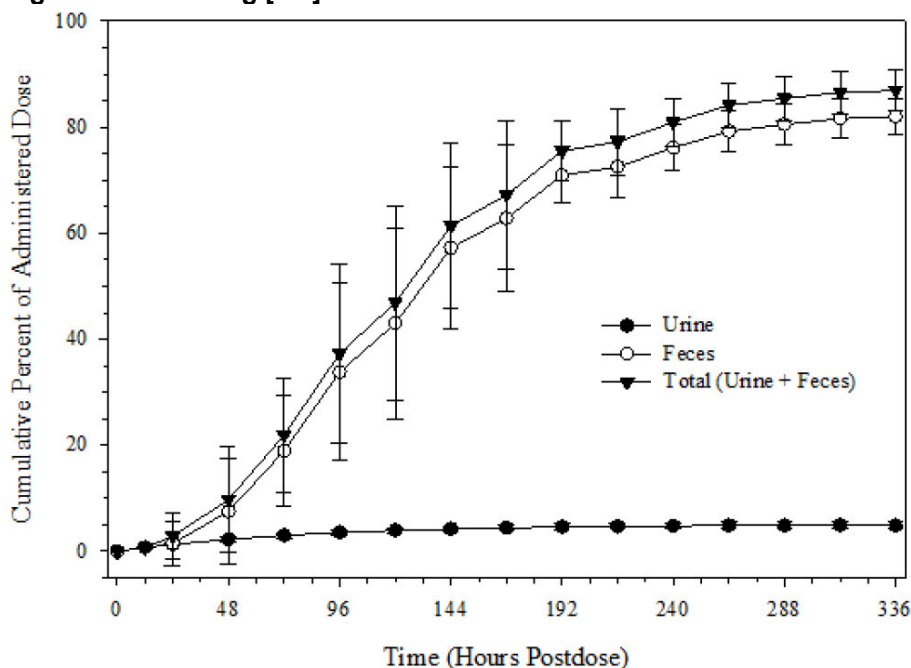
Table 95. Summary of Plasma Etrasimod, Blood Total Radioactivity and Plasma Total Radioactivity Pharmacokinetic Parameters Following Single Doses of 2 mg [¹⁴C] Etrasimod

Parameter	Plasma Etrasimod (N = 8)	Plasma Total Radioactivity (N = 8)	Whole Blood Total Radioactivity (N = 8)
C _{max} (ng/mL or ng.eq/g)	41.5 (22.7)	49.9 (18.9)	33.0 (14.8)
t _{max} (h)	4.00 (3.00, 8.00)	6.01 (6.00, 8.00)	7.01 (6.00, 8.00)
t _{last} (h)	168.00 (168.00, 168.00)	336.00 (312.00, 336.00)	312.00 (216.00, 336.00)
AUC _{0-t} (h*ng/mL or h*ng.eq/g)	1740 (31.4)	4250 (22.5)	2610 (24.2)
AUC ₀₋₁₆₈ (h*ng/mL or h*ng.eq/g)	1740 (31.4)	3550 (21.4)	2220 (20.3)
AUC ₀₋₃₁₂ (h*ng/mL or h*ng.eq/g)	N/A	4210 (22.4)	2620 (22.1)
AUC _{0-∞} (h*ng/mL or h*ng.eq/g)	1820 (32.6)	4580 (22.4)	2810 (23.5)
t _{1/2} (h)	37.8 (3.22)	89.0 (8.52)	78.0 (10.8)
CL/F (L/h)	1.10 (33.3)	N/A	N/A
V _z /F (L)	59.6 (26.0)	N/A	N/A
C _{max} Plasma Etrasimod/ Total Radioactivity Ratio	N/A	0.832 (18.4)	N/A
AUC _{0-t} Plasma Etrasimod/ Total Radioactivity Ratio	N/A	0.409 (11.1)	N/A
AUC _{0-∞} Plasma Etrasimod/ Total Radioactivity Ratio	N/A	0.397 (12.5)	N/A
C _{max} Total [¹⁴ C] Radioactivity Blood/Plasma Ratio	N/A	N/A	0.662 (6.8)
AUC _{0-t} Total [¹⁴ C] Radioactivity Blood/Plasma Ratio	N/A	N/A	0.614 (3.8)
AUC _{0-∞} Total [¹⁴ C] Radioactivity Blood/Plasma Ratio	N/A	N/A	0.612 (3.6)

Source: Clinical Study Report APD334-107, Table 9

The cumulative excretion of total radioactivity in urine and feces up to 336 hours post-dose is summarized in Figure 28.

Figure 28. Mean Cumulative Percent of Radioactive Dose Recovered in Urine and Feces Following Single Doses of 2 mg [¹⁴C] Etrasimod



Source: Clinical Study Report APD334-107, Figure 4

The urinary excretion of etrasimod and total radioactivity, and stool excretion of total radioactivity following administration of 2 mg [¹⁴C] etrasimod are presented in Table 96.

Table 96. Summary of Urinary Excretion of Etrasimod and Total Radioactivity and Stool Excretion of Following Single Doses of 2 mg [¹⁴C] Etrasimod

Analyte (Matrix)	Cumulative A _e	Cumulative F _e
	(mg equivalents)	(%)
Total Radioactivity (Urine)	0.0980 (0.0150)	4.89 (0.647)
Total Radioactivity (Feces)	1.64 (0.0764)	82.0 (3.47)
Total Radioactivity (Total Excreta)	1.74 (0.0878)	86.9 (3.78)

Source: Clinical Study Report APD334-107, Table 10

Metabolite Profiling and Identification Results

Following oral administration of [¹⁴C]etrasimod to healthy male subjects, samples of plasma, urine, and feces were analyzed and a total of 11, 21, and 9 metabolites were observed in plasma, urine, and feces, respectively; of these observed metabolites, 14 were identified.

An overall metabolic pathway for etrasimod in humans is presented in Section 6.2.1 above.

Metabolite profiling was assessed for the 0-to-312-hour cross-subject plasma pool, which represented approximately 92% of the mean AUC_{0-inf} for total radioactivity (Table 97). Etrasimod was identified as the only major radioactive component (49.28%) in the cross-subject extracted pooled plasma. The 2 most abundant metabolites were AR503641 (M3) and AR504344 (M6), with each representing < 10% of total radioactivity, as shown in Table 97.

Table 97. Summary of Radiolabeled Components in Cross-Subject Extracted Plasma Pool (0 to 312 hours)

Component Designation (identification)	Retention Time (mm:ss)	Percent of Total Radioactivity	Estimated AUC₀₋₃₁₂ (ng eq·h/g)^a
M3	40.30	8.27	348.2
M6	43.00	8.54	359.5
APD334	67.30	49.28	2074.7
Region 1 (Unknown)	1.15	1.27	53.5
Region 2 (Unknown)	2.30	0.77	32.4
Region 5 (M17+M43)	27.15	4.58	192.8
Region 8 (M28*)	49.00	6.14	258.5
Region 11 (M29*)	61.45	2.27	95.6
Sum of unknown trace metabolites	NA	18.9	794.8
Total [¹⁴ C]	NA	100	4210

Source: Clinical Study Report APD334-107, Table 14

16.3.6. Bioanalytical Method Report

The Applicant developed six LC-MS/MS (Table 98) and adequately validated. The validation reports were submitted for all methods. The method CA22262-01 was mainly used for etrasimod in most clinical trials and method CA27234-01 was used for metabolites M3 and M6. Five of the methods were developed and validated by (b) (4). One method was transferred, modified, and validated by (b) (4). A method for unbound etrasimod was developed and validated by (b) (4) and used for samples from hepatic and renal impairment studies. The Applicant cross-validated between the methods used by (b) (4). Etrasimod concentrations in human urine samples were measured by a LC-MS/MS method. A summary of the bioanalytical methods by study are shown in Table 98.

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Table 98. Summary of Bioanalytical Methods by Study

Matrix	Analyte(s)	Sample Preparation	Assay Range	Validation Report	Analytical Test Site	Clinical Study
Plasma	Etrasimod	Liquid-liquid extraction	0.05-25 ng/mL	AA99171-01	(b) (4)	APD334-001 APD334-002 APD334-003 APD334-005
Plasma	Etrasimod	Liquid-liquid extraction	0.25-100 ng/mL	CA22262-01		APD334-007 APD334-008 APD334-009 APD334-107 APD334-108 APD334-109 APD334-110 APD334-111 APD334-112 APD334-114 (BE study) APD334-115 APD334-116 APD334-301 APD334-302
Plasma	Etrasimod	Liquid-liquid extraction	0.25-100 ng/mL	ETMDHPP		ES101001
Plasma	Etrasimod	Liquid-liquid extraction/ Protein precipitation	0.25- 100 ng/mL; 0.15-15 ng/mL	CA22262-01 and ETMDHPP; CA27234-01 and ET36HPP		NA
Plasma	Etrasimod AR401967 (opposite enantiomer of Etrasimod)	Liquid-liquid extraction	1.00-100 ng/mL	CA25946-01		APD334-005 (phase 2)
Plasma	M3 and M6	Protein precipitation	0.15-15 ng/mL	CA27234-01		APD334-009 APD334-108 APD334-109 APD334-112 APD334-116 APD334-301 APD334-302
Plasma	Etrasimod (unbound and total)	Ultracentrifugation	100-102,400 pg/mL	ARE-P9986		APD334-108 APD334-112
Urine	Etrasimod	Liquid-liquid extraction	1.00-500 ng/mL	AA99171-02		APD334-001

Source: Module 2.7.1 Summary of Biopharmaceutical Studies and Associated Analytical Methods, Table 3
Note: The method ET36HPP was validated but not applied on any bioanalytical studies in this NDA

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Bioanalytical methods validation performance of CA22262-01, CA27234-01, and CA25946-01 are briefly summarized in Table 99, Table 100, and Table 101. The cross-validation was summarized in Table 102.

Table 99. Summary of Bioanalytical Method Using UPLC-MS for Etrasimod in Plasma (CA22262-01)

Method description	The concentration of etrasimod (APD334) in human plasma (K ₂ EDTA) was determined using UPLC with mass spectrometric detection. In this method, an aliquot of human plasma (K ₂ EDTA) containing the etrasimod and IS was extracted using a liquid-liquid extraction procedure. The samples were analyzed by an UPLC equipped with an AB SCIEX API 4000™ triple quadrupole mass spectrometer using an APCI source. Positive ions were monitored in the MRM mode. Quantitation was determined using weighted linear regression analysis (1/concentration ²) of peak area ratios of the analyte and internal standard.		
Materials used for standard calibration curve and concentration	APD334 L-arginine, 5015-10-02, 21 December 2017; off-white powder		
Validated assay range	0.250 to 100 ng/mL		
Materials used for quality control (QCs) and concentration	APD334 L-arginine, 5015-10-02, 21 December 2017; off-white powder		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	All reagents used are listed in method validation report. d ₉ -APD334, S1048741, 24 February 2019		
Regression model and weighting	Weighted linear regression analysis (1/concentration ²) of peak area ratios of the analyte and IS		
Validation parameters	Method validation summary		Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators	9	CA22262-01 Amendment 4 to Validation Final Report Table 5
	Cumulative accuracy (%Bias)	-5.2 to 3.8%	
	Cumulative precision (%CV)	0.5 to 4.7%	
Performance of QCs during accuracy and precision runs	Intra-batch accuracy (%Bias)	-5.6 to 5.6%	CA22262-01 Amendment 4 to Validation Final Report Table 2
	Intra-batch precision (%CV)	1.5 to 7.7%	
	Inter-batch accuracy (%Bias)	1.2 to 1.8%	
	Inter-batch precision (%CV)	3.4 to 7.7%	

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Selectivity	10 lots: No significant interference at RT and mass transition of etrasimod or IS	CA22262-01 Amendment 4 to Validation Final Report Table 12
Matrix effect	10 lots fortified with etrasimod at LLOQ (0.250 ng/mL): No significant matrix effects observed in any of the 10 lots 10 lots fortified with etrasimod at high QC (75.0 ng/mL): No significant matrix effects observed in any of the 10 lots	CA22262-01 Amendment 4 to Validation Final Report Table 13
Interference and specificity	No quantitative interference was observed in samples fortified with coadministered compounds at the concentrations below: Gemfibrozil (50.0 µg/mL) Fluconazole (10.0 µg/mL) Rifampin (20.0 µg/mL) Itraconazole (2000 ng/mL) Hydroxyitraconazole (4000 ng/mL) Apixaban (100 ng/mL) Minocycline (1000 ng/mL) Azilsartan (10,000 ng/mL) Lacidipine (1500 ng/mL) No quantitative interference was observed in samples fortified with over-the-counter compounds at the concentrations below: Acetaminophen (25.0 µg/mL) Atorvastatin (100 ng/mL) Caffeine (20.0 µg/mL) Cetirizine (500 ng/mL) Dextromethorphan (25.0 ng/mL) Ethinyl Estradiol (0.500 ng/mL) Famotidine (200 ng/mL) Ibuprofen (20.0 µg/mL) Levonorgestrel (5.00 ng/mL) Metformin (2.00 µg/mL) Omeprazole (300 ng/mL) Ondansetron (200 ng/mL) Pseudoephedrine (500 ng/mL)	CA22262-01 Amendment 4 to Validation Final Report Table 16 to Table 19 and Figure 5-8 CA22262-01 Amendment 4 to Validation Final Report Table 20 and Figure 9
	Salicylic Acid (100 µg/mL) No quantitative interference was observed in samples fortified with known metabolites below: M3, AR503641 (15.0 ng/mL) M6, AR504344 (15.0 ng/mL)	CA22262-01 Amendment 4 to Validation Final Report, Table 21 and Figure 10

Source: Module 2.7.1 Summary of Biopharmaceutical Studies and Associated Analytical Methods, Appendix 4
Abbreviations: IS = internal standard; CV = coefficient of variation; QC = quality control; LLOQ = lower limit of quantitation; RT = room temperature

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Table 100. Summary of Bioanalytical Method for Metabolites M3 and M6 (CA27234-01)

Method description	The concentrations of etrasimod metabolites M3 (AR503641) and M6 (AR504344) in human plasma (K ₂ EDTA) was determined using UPLC with mass spectrometric detection. In this method, an aliquot of human plasma (K ₂ EDTA) containing the analytes and IS was extracted using a protein precipitation procedure. The samples were analyzed by UPLC equipped with an AB SCIEX Triple Quad™ 6500 triple quadrupole mass spectrometer using an ESI source. Positive ions were monitored in the MRM mode. Quantitation was determined using weighted linear regression analysis (1/concentration ²) of peak area ratios of the analyte and internal standard.		
Materials used for standard calibration curve and concentration	AR503641, PC00898-95-CI7040588, 02 December 2019; off-white solid AR504344, PC00898-93-CI7040586, 28 November 2019; off-white solid		
Validated assay range	0.150 to 15.0 ng/mL for both M3 (AR503641) and M6 (AR504344)		
Materials used for quality controls (QCs) and concentration	AR503641, PC00898-95-CI7040588, 02 December 2019; off-white solid AR504344, PC00898-93-CI7040586, 28 November 2019; off-white solid		
Minimum required dilutions (MRDs)	NA		
Source and lot of reagents	AR436434 (IS), E01989-17-2, 20 May 2021 All reagents used are listed in method validation report		
Regression model and weighting	Weighted linear regression analysis (1/concentration ²) of peak area ratios of the analyte and IS		
Validation parameters	Method validation summary	Source location	
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators	8	CA27234-01 Amendment 4 to Validation Final Report Tables 8-9
	Cumulative accuracy (%Bias)	M3: -6.3 to 4.4% M6: -4.0 to 4.4%	
	Cumulative precision (%CV)	M3: 0.7 to 3.2% M6: 0.5 to 4.5%	
Performance of QCs during accuracy and precision runs	Intra-batch accuracy (%Bias)	M3: -8.7 to 17.3% M6: -16.7 to 14.7%	CA27234-01 Amendment 4 to Validation Final Report Table 2 to Table 5
	Intra-batch precision (%CV)	M3: 1.2 to 8.6% M6: 0.9 to 10.1%	
	Inter-batch accuracy (%Bias)	M3: 0.0 to 6.0% M6: -4.7 to -0.7%	
	Inter-batch precision (%CV)	M3: 5.0 to 11.8% M6: 3.5 to 15.2%	
Selectivity	10 lots: No significant interference at RT and mass transition of M3, M6 or IS		CA27234-01 Amendment 4 to Validation Final Report Table 22 and Table 23
Matrix effect	10 lots fortified at low QC (0.450 ng/mL): No significant matrix effects observed in any of the 10 lots 10 lots fortified at high QC (12.0 ng/mL): No significant matrix effects observed in any of the 10 lots		CA27234-01 Amendment 4 to Validation Final Report Table 24 and Table 25

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Interference and specificity	No quantitative interference was observed in samples fortified with coadministered compounds below: Sildenafil (500 ng/mL) Itraconazole (1000 ng/mL, 2000 ng/mL) Hydroxyitraconazole (2000 ng/mL, 4000 ng/mL) Aripiprazole (300 ng/mL) Dicloxacillin (200,000 ng/mL) Olmesartan (2000 ng/mL)	CA27234-01 Amendment 4 to Validation Final Report Section 2.4.6 and Table 32 to Table 39
	No quantitative interference was observed in samples fortified with over-the-counter compounds at the concentration below: Acetaminophen (25.0 µg/mL) Atorvastatin (100 ng/mL) Caffeine (20.0 µg/mL) Cetirizine (500 ng/mL) Cotinine (250 ng/mL) Dextropropofol (5000 ng/mL) Ethinyl Estradiol (0.500 ng/mL) Famotidine (200 ng/mL) Ibuprofen (20.0 µg/mL) Metformin (2.00 µg/mL) Norethindrone (10.0 ng/mL) Omeprazole (300 ng/mL) Pseudoephedrine (500 ng/mL) Salicylic Acid (100 µg/mL)	CA27234-01 Amendment 4 to Validation Final Report Section 2.4.7, Table 40 and Table 41
	No significant interference of analytes on the quantification of each other.	CA27234-01 Amendment 4 to Validation Final Report Section 2.4.5, Table 30 and Table 31
	No quantitative interference was observed in samples fortified with 100 ng/mL of etrasimod	CA27234-01 Amendment 4 to Validation Final Report Section 2.4.9, Table 42 and Table 43

Source: Module 2.7.1 Summary of Biopharmaceutical Studies and Associated Analytical Methods, Appendix 4

Abbreviations: IS = internal standard; CV = coefficient of variation; QC = quality control; LLOQ = lower limit of quantitation; RT = room temperature

Table 101. Summary of Bioanalytical Method for Etrasimod and Its Enantiomer (CA25946-01)

Method description	The assay was developed and qualified as a reliable Chiral LC-MS/MS separation and quantitation method for APD334 and its enantiomer AR401967 in human plasma. The assay involves a liquid-liquid plasma extraction followed by analysis/ detection by chiral LC-MS/MS method. An AB SCIEX Triple Quad™ 6500 triple quadrupole mass spectrometer equipped with an APCI source was used as the detector. Positive ions were monitored in the MRM mode.	
Materials used for standard calibration curve and concentration	Etrasimod (APD334), 5015-10-02, 31 December 2018; off-white powder AR401967 (enantiomer), SJ-PD00005-22-03, 31December 2019; off-white powder	
Validated assay range	1.00 to 100 ng/mL	
Materials used for quality control samples (QCs) and concentration	APD334 L-arginine, 5015-10-02, 31 December 2018; off-white powder	
Minimum required dilutions (MRDs)	NR	
Source and lot of reagents	Etrasimod IS (d ₉ -APD334), S1048741 All reagents used are listed in the method qualification report	
Regression model and weighting	Weighted linear regression analysis (1/concentration ²) of peak area ratios of the analyte and IS	
Validation parameters	Method validation summary	Source location
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators	8
	Cumulative accuracy (%Bias)	APD334: -2.50 to 3.00% AR401967: -2.50 to 1.00%
	Cumulative precision (%CV)	APD334: 0.600 to 3.60% AR401967: 0.800 to 3.70%

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Performance of QCs during accuracy and precision runs	Intra-batch accuracy (%Bias)	APD334: -6.00 to 10.0% AR401967: -1.60 to 13.0%	CA25946-01 Table 2 and Table 3
	Intra-batch precision (%CV)	APD334: 0.700 to 6.70% AR401967: 0.700 to 4.6%	
	Inter-batch accuracy (%Bias)	APD334: -0.800 to 2.00% AR401967: 4.00 to 5.00%	
	Inter-batch precision (%CV)	APD334: 3.60 to 7.30% AR401967: 3.20 to 7.40%	
Selectivity and Matrix Effects	No significant interference or matrix effect of APD334, AR401967 and IS were observed from endogenous components in any of the 10 human plasma (EDTA) lots screened or of d ₅ -APD334 (IS) in any of the 10 human plasma (EDTA) lots screened.		CA25946-01 Section 3.3, Table 8, Table 9, Table 10, and Table 11
Interference and specificity	The selectivity of the method in the presence of APD334-glucuronide (a metabolite of APD334 not quantified by the method) was evaluated. APD334/AR401967 QC samples at low and high concentrations were fortified with APD334-glucuronide at 40.0 ng/mL and analyzed after incubation on the bench (in an ice water bath) for 1 hour and 2 hours, respectively. The samples were assessed against calibration standards in control matrix without APD334-glucuronide. The selectivity of APD334 and AR401967 from APD334-glucuronide was demonstrated.		CA25946-01 Section 3.3.2, Table 14, and Table 15

Source: Module 2.7.1 Summary of Biopharmaceutical Studies and Associated Analytical Methods, Appendix 4
Abbreviations: IS = internal standard; CV = coefficient of variation; QC = quality control; LLOQ = lower limit of quantitation; RT = room temperature

Table 102. Summary of Bioanalytical Method Modifications and Cross-Validation Results

Changes in method	A cross-validation of performance consistency between validated bioanalytical methods to quantitate APD334 (etrasimod) and metabolites AR503641 (M3) and AR504344 (M6) in human plasma was conducted at (b) (4) CA29122-01/CA29122-02 and (b) (4) 8420-496 by Arena Pharmaceuticals, Inc. (San Diego, CA). Both analytical labs applied their fully validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods to analyze APD334 and AR503641 and AR504344 metabolite concentrations from the same pooled post study human plasma sample replicates from a clinical study. ISR acceptance criteria were used to compare the bioanalytical results and to check the performance consistency between (b) (4) validation reports CA22262-01 (APD334) and CA27234-01 (AR503641 and AR504344) and (b) (4) validation reports 8392-178 (APD334) and 8410-598 (AR503641 and AR504344) ^a were used to support the cross-validation		
New validated assay range if any	The calibration range was 0.250 to 100 ng/mL using 9 non-zero calibrators		
Validation parameters	Cross-validation performance		Source location
Standard calibration curve performance during accuracy and precision runs	Cumulative accuracy (% bias) in standard calibrators from LLOQ (0.250 ng/mL) to ULOQ (100 ng/mL)	(b) (4) APD334: -3.3 to 4.0% AR503641: -3.3 to 3.3% AR504344: -2.0 to 2.7% APD334: -3.0 to 3.0% AR503641: -1.3 to 2.0% AR504344: -1.1 to 0.7%	APD334.MV.004, Rev.01 Appendix 1 and Appendix 2 CA29122-01/CA29122-02 Table 3 8420-496 Table 7.2, Table 7.3, and Table 7.4
	Cumulative precision (% CV) from LLOQ to ULOQ	APD334: NA AR503641: NA AR504344: NA APD334: NA AR503641: ≤ 3.2% AR504344: ≤ 4.0%	APD334.MV.004, Rev.01 Appendix 1 and Appendix 2 CA29122-01/CA29122-02, Table 3 8420-496, Table 7.2, Table 7.3 and Table 7.4
Performance of QCs during accuracy and precision runs	Cumulative accuracy (% bias)	APD334: -6.5 to -0.9% AR503641: 0.7 to 4.7% AR504344: 4.2 to 7.3% APD334: 0.1 to 3.5% AR503641: -6.0 to 3.3% AR504344: -3.3 to 4.2%	

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	Cumulative precision (%CV)	(b) (4) APD334: NA AR503641: ≤ 3.8% AR504344: ≤ 3.0% APD334: ≤ 5.6 AR503641: ≤ 6.9% AR504344: ≤ 8.2%	APD334.MV.004, Rev.01 Appendix 1 and Appendix 2 CA29122-01/CA29122-02 Table 2 8420-496 Table 7.8 , Table 7.9 , and Table 7.10
Cross-validation	The plasma concentration data obtained at (b) (4) from the 34 pooled human plasma replicates showed an ISR passing rate for the three analytes listed below: APD334: 34 of 34 passed or 100% AR503641: 32 of 34 passed or 94.1% AR504344: 33 of 34 passed or 97.1% The results of this cross-validation indicates that the bioanalytical methods as well as laboratory performance at (b) (4) are robust, reproducible, and reliable. The bioanalytical laboratories of (b) (4) herefore successfully passed inter-laboratory cross validations for the determination of APD334 and metabolites AR503641 and AR504344 in human plasma samples.	(b) (4)	APD334.MV.004, Rev.01 Table 1 , Table 2 , and Table 3

Source: Module 2.7.1 Summary of Biopharmaceutic Studies and Associated Analytical Methods, Appendix 4
Abbreviations: CV = coefficient of variation; QC = quality control; LLOQ = lower limit of quantitation; ULOQ = upper limit of quantitation; ISR = incurred sample reanalysis

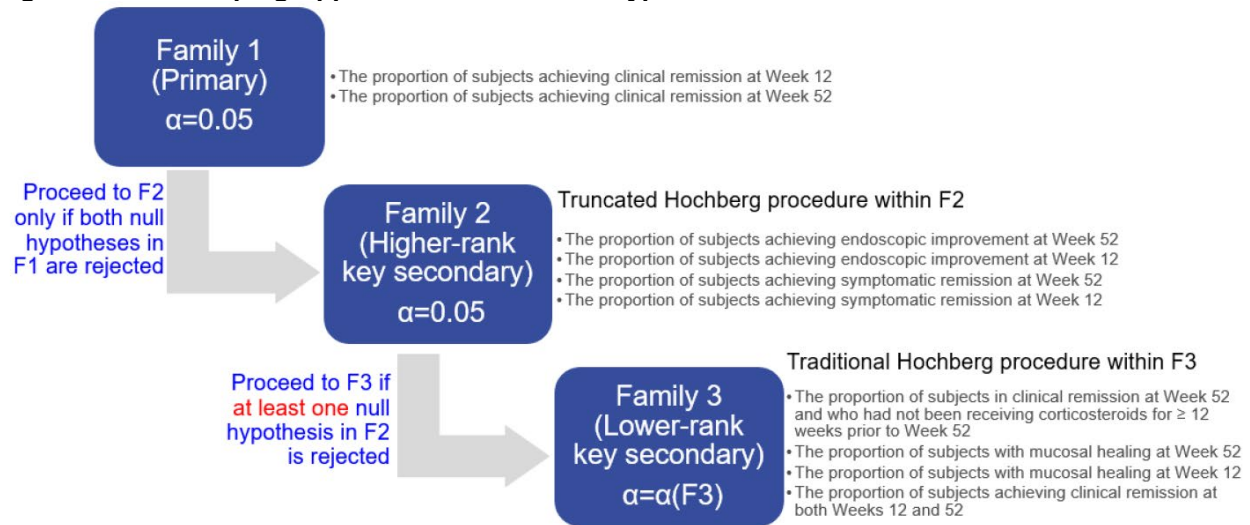
In addition, plasma concentrations for moxifloxacin and the two components of a monophasic oral contraceptive (ethinyl estradiol/levonorgestrel) were also quantified by validated LC-MS/MS methods, respectively.

16.4. Additional Efficacy Information

16.4.1. Multiple Testing Procedure

A prespecified gatekeeping multiple testing approach was implemented to control the overall Type I error rate at 2-sided alpha of 0.05 for all co-primary and multiplicity-controlled secondary endpoints for APD334-301 and APD334-302, as shown in Figure 29 and Figure 30, respectively. First, the whole alpha was spent on testing family 1 (F1) consisting of the co-primary endpoints. If both null hypotheses in F1 were rejected, the testing with the whole alpha proceeded to F2. Truncated Hochberg procedure with a truncation fraction 0.8 was implemented for testing hypotheses in F2. Since all null hypotheses in F2 were rejected, the whole alpha was spent on testing F3 based on a traditional Hochberg procedure. The Hochberg method may not control type I error when endpoints within a family are negatively correlated; however, given the efficacy results for these studies, the review team is not concerned with potential type I error inflation in this instance.

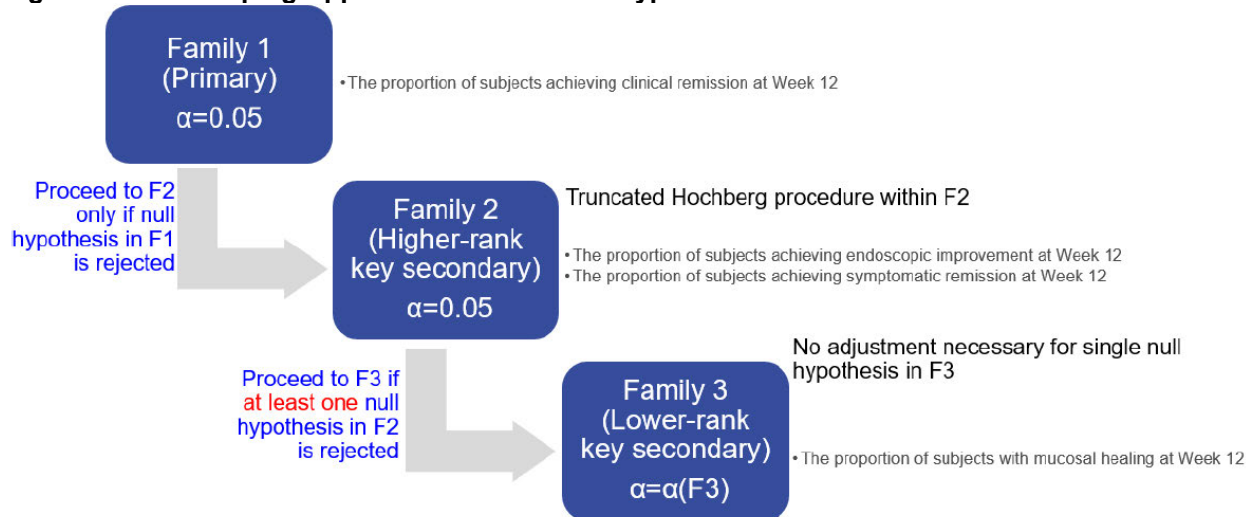
Figure 29. Gatekeeping Approach to Control the Type I Error Rate for APD334-301



F1, family 1; F2, family 2, F3, family 3

Source: Statistical Analysis Plan (Version 1.0) for APD334-301, Figure 2 (page 26)

Figure 30. Gatekeeping Approach to Control the Type I Error Rate for APD334-302



F1, family 1; F2, family 2, F3, family 3

Source: Statistical Analysis Plan (Version 1.0) for APD334-302, Figure 2 (page 22)

16.4.2. Applicant's Original Efficacy Analyses

Table 103 and Table 104 display the Applicant's analysis results for the primary and multiplicity-controlled secondary endpoints in APD334-301 using the Applicant's prespecified protocol analyses (i.e., assessed within subjects with baseline mMS of 5 or more in the FAS and using the Applicant's definition of clinical remission). All endpoints were statistically significant, and the results are consistent with the results of the Agency's preferred analyses as shown in Section 8.1.2.

Table 103. Results for Co-Primary Endpoints, Subjects With mMS of 5 to 9 in the FAS, APD334-301

Parameter	Etrasimod 2 mg QD (N=274)	Placebo (N=135)
Clinical Remission ¹ at Week 12, n (%)	74 (27.0)	10 (7.5)
Treatment Difference ² , (95% CI)		19.8 (12.9, 26.6)
P-value ³		< 0.001
Clinical Remission ¹ at Week 52, n (%)	88 (32.1)	9 (6.7)
Treatment Difference ² , (95% CI)		25.4 (18.4, 32.4)
P-value ³		< 0.001

Source: clinical study report, verified by the review team

¹ Clinical remission was defined as SFS = 0 (or = 1 with a ≥ 1-point decrease from baseline), RBS = 0 and ES ≤ 1 (excluding friability).

² Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

³ The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

Table 104. Results for Multiplicity-Controlled Secondary Endpoints, Subjects With mMS of 5 to 9 in the FAS, APD334-301

Endpoints	Etrasimod 2 mg QD n (%) (N=274)	Placebo n (%) (N=135)	Treatment Difference ¹ vs. Placebo (95% CI)	P- value ²
Endoscopic improvement at Week 12	96 (35.0)	19 (14.1)	21.18 (13.03, 29.32)	<0.001
Endoscopic improvement at Week 52	102 (37.2)	14 (10.4)	26.69 (18.99, 34.39)	<0.001
Symptomatic remission at Week 12	126 (46.0)	29 (21.5)	24.55 (15.46, 33.63)	<0.001
Symptomatic remission at Week 52	119 (43.4)	25 (18.5)	24.89 (16.17, 33.60)	<0.001
Corticosteroid-free clinical remission ³ at Week 52	88 (32.1)	9 (6.7)	25.39 (18.42, 32.36)	<0.001
Mucosal healing at Week 12	58 (21.2)	6 (4.4)	16.88 (10.78, 22.98)	<0.001
Mucosal healing at Week 52	73 (26.6)	11 (8.1)	18.39 (11.39, 25.39)	<0.001
Maintenance of clinical remission ⁴	49 (17.9)	3 (2.2)	15.84 (10.66, 21.03)	<0.001

Source: clinical study report, verified by the review team

¹ Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

² The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

³ Corticosteroid-free clinical remission is defined as clinical remission at Week 52 without receiving corticosteroids for at least 12 weeks prior to Week 52.

⁴ Maintenance of clinical remission is defined as achievement of clinical remission at both Week 12 and Week 52.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

For APD334-302, all multiplicity-controlled endpoints were statistically significant according to the Applicant's original analyses, as summarized in Table 105 and Table 106. The results align with the results of the Agency's preferred analyses presented in Section 8.1.4.

Table 105. Results for the Primary Endpoint, FDA Preferred Analysis Population, APD334-302

Parameter	Etrasimod 2 mg QD (N=222)	Placebo (N=112)
Clinical Remission at Week 12, n (%)	55 (24.8)	17 (15.2)
Treatment Difference ¹ , (95% CI)		9.7 (1.1, 18.2)
P-value ²		0.026

Source: clinical study report, verified by the review team

¹ Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

² The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

Table 106. Results for Multiplicity-Controlled Secondary Endpoints, FDA Preferred Analysis Population, APD334-302

Endpoints	Etrasimod 2 mg QD n (%) (N=222)	Placebo n (%) (N=112)	Treatment Difference ¹ vs. Placebo (95% CI)	P-value ²
Endoscopic improvement at Week 12	68 (30.6)	21 (18.8)	12.11 (3.00, 21.23)	0.009
Symptomatic remission at Week 12	104 (46.8)	33 (29.5)	17.48 (6.81, 28.15)	0.001
Mucosal healing at Week 12	36 (16.2)	10 (8.9)	7.44 (0.50, 14.39)	0.036

Source: clinical study report, verified by the review team

¹ Difference (%) is for etrasimod minus placebo and is based on estimated common risk difference using the Mantel-Haenszel weights, adjusted for the following stratification factors: naïve to biologic or Janus kinase inhibitor therapy at trial entry (yes/no), baseline corticosteroid use (yes/no), and baseline disease activity (modified Mayo score: [4-6] or [7-9]).

² The 2-sided p-value is to test the hypothesis of the treatment difference being 0.

Abbreviations: N, number of subjects in treatment group; QD, once daily; n, number of events; CI, confidence interval

16.5. Supplemental Safety Information

Grouping Strategy for Adverse Events

Preferred terms that were similar were grouped by the review team to avoid splitting of TEAEs due to minor differences in nomenclature. Table 107 describes the preferred term grouping strategy by the review team.

Table 107. Reviewer’s Preferred Term (PT) Grouping Strategy for Assessment for Adverse Events

Group Term	Preferred Terms Included in Each Grouping
Bradycardia	Bradycardia, sinus bradycardia, and heart rate decreased
Atrioventricular (AV) conduction delay	AV block first degree, AV block second degree
Hypertension (HTN)	HTN, blood pressure increased, essential hypertension, and hypertensive crisis
Herpes viral infection	Herpes simplex, herpes zoster, and oral herpes
Elevated liver tests	Alanine aminotransferase increased, aspartate aminotransferase increased, blood alkaline phosphatase increased, hyperbilirubinemia, blood bilirubin increased, cholestasis, gamma glutamyltransferase increased, hepatic enzyme increased, liver function test increased, liver function test abnormal, hepatic function abnormal, and transaminases increased
Headache	Headache, migraine, sinus headache, and tension headache
Dizziness	Dizziness, dizziness exertional, and dizziness postural
Urinary tract infection	Urinary tract infection and cystitis
Hypercholesterolemia	Hypercholesterolemia and blood cholesterol increased
Diarrhea	Diarrhea and frequent bowel movements

Source: Reviewer generated table
Coded as MedDRA 24.1 preferred term

Clinical Laboratory Tests

The laboratory tests conducted by the Applicant during the etrasimod development program are listed in Table 108.

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Table 108. Laboratory Tests Conducted by The Applicant

SCREENING Only		
Virology		
HIV, HBsAg, HCV (RIBA), QuantiFERON		
Stool Sample		
Bacterial culture, ova and parasites, <i>C. difficile</i>		
Drugs of Abuse		
Amphetamine, barbiturates, benzodiazepines, cocaine, methadone, methamphetamine, methylenedioxymethamphetamine, opiate, oxycodone, phencyclidine		
Others		
Hemoglobin A1C		
PREGNANCY TESTING		
Pregnancy Testing		
Serum pregnancy test human beta-chorionic gonadotropin (β-hCG) - Screening		
Urine β-hCG (only for women of childbearing potential)		
CLINICAL CHEMISTRY, HEMATOLOGY, AND COAGULATION		
Hematology	Serum Chemistry	
Hematocrit	Albumin	Potassium
Hemoglobin	Alkaline phosphatase	Sodium
Mean corpuscular hemoglobin	Alanine aminotransferase	Thyroid-stimulating hormone
Mean corpuscular hemoglobin concentration	Aspartate aminotransferase	Thyroxine free
Mean corpuscular volume	Bicarbonate	Total bilirubin
Platelet count	Blood urea nitrogen	Triiodothyronine free
Red blood cell count	C-reactive protein	Total cholesterol
White blood cell count with differential ^a	Calcium	Total protein
CD4 T-cell count ^a	Chloride	Triglycerides
Coagulation	Creatinine	Uric acid
Prothrombin time	Creatine kinase	
Activated partial thromboplastin time	Direct bilirubin	
International normalized ratio	Glucose	
	Gamma-glutamyl transferase	
	Lactate dehydrogenase	
	Phosphorus	
URINALYSIS		
Appearance	Nitrite	
Bilirubin	Occult blood	
Color	pH	
Glucose	Protein	
Ketones	Specific gravity	
Microscopic examination of sediment	Urobilinogen	
BIOMARKERS		
Lymphocytes ^{a,b}		
hs-CRP ^b		
Fecal calprotectin ^b		
Immunophenotyping		
Proteomics		
RNA Transcriptomics		
Fecal microbiome		
GENETICS		
Genomic DNA (optional)		
Stool Sample ^c		
Ova and parasites, <i>C. difficile</i>		

Schedule of Assessments

The schedules of assessments were similar for APD334-301 and APD334-302 through Week 12 and are described in Table 115. The schedule of assessments for APD334-301 through Week 40 are provided in Table 116.

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Table 109. Schedule of Assessments Through Week 12, APD334-301 and APD334-302

Evaluation	Screening Period	12-Week Treatment Period					2-Week Follow-Up Visit ^b ± 3 Days	4-Week Follow-Up Visit ^b ± 3 Days
	-28 to -1	W0/D1	W2/D15 ± 3 Days	W4/D29 ± 3 Days	W8/D57 ± 3 Days	W12/D85 / Early Termination ^a ± 3 Days		
Informed consent	X							
Inclusion/exclusion criteria	X	X						
Demographics	X							
Medical and social history ^c	X							
Ulcerative colitis history	X							
Chest X-ray ^d	X							
Tuberculosis test ^e	X							
Tuberculosis questionnaire ^e	X	X	X	X	X	X	X	X
Virology screen (HIV, HBV, HCV) ^f	X							
Randomization		X						
eDiary instruction ^g	X							
eDiary review		X	X	X	X	X		
MMS ^h		X				X		
Stool frequency and rectal bleeding subscore ⁱ		X	X	X	X	X		
PGA for total Mayo Clinic Score		X				X		
IBDQ, UC-PRO/SS, SF-36, WPAI-UC, and abdominal pain and urgency NRS		X				X		
Adverse event assessment	X	X	X	X	X	X	X	X
Vital signs ^j	X ^l	X ^h	X	X	X	X	X	X
12-lead ECG ^j	X ^l	X ^h				X		
Physical examination ^k	X	X	X	X	X	X	X	X
Extraintestinal manifestations ^o	X					X	X	X
Pulmonary function test ^o	X ^h					X ^o	X ^o	
Ophthalmoscopy with OCT	X ^l					X ^o	X ^o	
Urine drug screen ⁿ	X							
Pregnancy test ⁿ	X	X		X	X	X		X
Genomic DNA (optional)		X						
CBC with differential and platelets	X	X	X	X	X	X	X	X
TBNE	X	X	X	X	X	X	X	X
Laboratory tests including hs-CRP ^o	X	X	X	X	X	X	X	
Stool sample/fecal calprotectin ^o	X		X	X	X	X		
Stool sample for microbiome	X					X		
Flexible proctosigmoidoscopy/colonoscopy and biopsy ^m	X					X		
PK assessments ^a		X	X	X	X	X	X ^a	X ^a
Biomarkers blood sample ^v		X				X		
Concomitant medications and procedures ^d		X	X	X	X	X	X	X
Drug dispensation/ accountability		X	X	X	X	X ^{aa}		
Study treatment administration ^{bb}						X – Once daily		

Table 110. Schedule of Assessments Through Week 52, APD334-301

Evaluation	40-Week Treatment Period							2-Week Follow-Up Visit ^b ± 3 Days	4-Week Follow-Up Visit ^b ± 3 Days
	W16/ D113 ± 7 Days	W20/ D141 ± 7 Days	W24/ D169 ± 7 Days	W32/ D225 ± 7 Days	W40/ D281 ± 7 Days	W48/ D337 ± 7 Days	W52/D365 ± 14 Days/ Early Termination ^a ± 7 Days		
eDiary review	X	X	X	X	X	X	X		
MMS ^e							X		
Stool frequency and rectal bleeding subscore ^d	X	X	X	X	X	X	X		
PGA for total Mayo Clinic Score							X		
IBDQ, UC-PRO/SS, SF-36, WPAI-UC, and pain and urgency NRS							X		
Adverse event assessment	X	X	X	X	X	X	X	X	X
Vital signs ^e	X	X	X	X	X	X	X	X	X
12-lead ECG							X		
Physical examination ^f	X	X	X	X	X	X	X	X	X
Extraintestinal manifestations ^g							X	X	X
Pulmonary function test ^h				X ^h			X	X ^h	
Ophthalmoscopy with OCT ⁱ							X	X	
Pregnancy test ^j	X	X	X	X	X	X	X		X
CBC with differential and platelets	X	X	X	X	X	X	X	X	X
TBNK	X	X	X	X	X	X	X	X	X
Laboratory tests including hs-CRP ^k	X	X	X	X	X	X	X	X	
Stool sample/fecal calprotectin ^l			X				X		
Stool sample for microbiome							X		
Flexible proctosigmoidoscopy/colonoscopy and biopsy ^m							X		
PK assessments ⁿ	X	X	X	X	X	X	X	X ⁿ	X ⁿ
Biomarkers blood sample ^o							X		
Concomitant medications and procedures ^p	X	X	X	X	X	X	X	X	X
Tuberculosis questionnaire ^q	X	X	X	X	X	X	X	X	X
Tuberculosis test ^r							X		
Drug dispensation/accountability ^s	X	X	X	X	X	X	X		
Study treatment administration ^t	Once daily								

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Velsipity (etrasimod)

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Velsipity (etrasimod)

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Velsipity (etrasimod)

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA ASSESSMENT AND EVALUATION

Application Number*: NDA 216956
Supporting Document Number/s: SDN 1
CDER Receipt Date: October 14, 2022
Applicant: Arena Pharmaceuticals, Inc.
Product: Etrasimod L-arginine
Pharmacologic Class: Sphingosine 1-phosphate subtype 1,4,5
(S1P_{1,4,5}) receptor agonist
Indication: Moderately to severely active ulcerative
colitis
Therapeutic area: Gastroenterology
Clinical Review Division: Division of Gastroenterology (DG)
Pharm/Tox Division: Division of Pharm/Tox for Immunology and
Inflammation (DPT-II)
Reviewer: Sarah J. Morgan, PhD
Supervisor/Team Leader: Jackye Peretz, PhD
Project Manager: Anum Shami, PharmD
Purpose of Review: Study Report Review
Reviewer Completion Date: May 1, 2023

Template Version: Sep 11, 2020

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Executive Summary (Clinical Relevance)

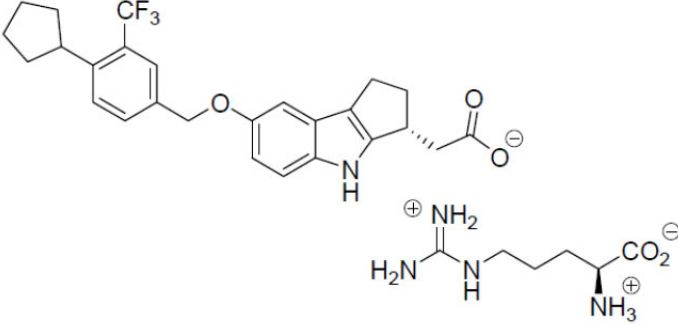
As part of the NDA package, the Applicant submitted three in vitro pharmacology studies and a pre- and postnatal development (PPND) study in rats with etrasimod, as well as genetic toxicology studies with two process-related impurities. These studies have not previously been reviewed under any IND for etrasimod and are reviewed here.

Etrasimod is a sphingosine-1-phosphate receptor subtype 1,4, and 5 (S1P_{1,4,5}) agonist. Etrasimod inhibited generation of cAMP, rapidly induced S1P₁ internalization, and induced β -arrestin recruitment to hS1P₁ and hS1P₅ with similar potency and efficacy to other S1P receptor modulators (i.e., fingolimod, ozanimod, and siponimod). However, etrasimod was generally a less potent agonist of GTP γ S binding to S1P receptors, including S1P₁, compared to other S1P receptor modulators. Etrasimod also induced a smaller increase in the I_{KACH} channel current in vitro using isolated human atrial cardiomyocytes compared to ozanimod or fingolimod under the same assay conditions.

In the PPND study, the NOAEL for maternal toxicity was considered the low dose of 0.4 mg/kg/day (approximately 2 times exposure at the recommended clinical dose of 2 mg, based on AUC) due to observations of mortality during delivery, increased post-implantation loss, increased number of females with stillborn pups, increased number of stillborn pups per litter, decreased viability index, and decreased lactation index at higher doses. A developmental NOAEL could not be established based on the findings of increased perinatal mortalities (≥ 2 mg/kg/day), adverse clinical signs and decreased body weight/body weight gain in offspring during the preweaning period (all doses), as well as a reduced number of implantations, increased rate of pre-implantation loss, and corresponding decrease in the number of live embryos per litter observed (4 mg/kg/day); these effects occurred at exposures less than that expected at the clinical dose.

Nonclinical Summary

Drug Information⁺

Type of Product:	Small molecule, oral tablet
Code/ Generic Name:	Etrasimod L-arginine (APD 334)
Chemical Name (optional):	L-Arginine salt of (R)-2-(7-(4-cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl) acetic acid
CAS# or UNII (if available):	CAS: 1206123-97-8
Structure or Biochemical Description:	
Molecular Formula/ Molecular Weight:	L-arginine salt: C ₃₂ H ₄₀ F ₃ N ₅ O ₅ , 631.69 g/mol Free acid: C ₂₆ H ₂₅ F ₃ NO ₃ , 456.17 g/mol
Comment on Excipients:	None
Comment on Impurities:	None
Proposed 505(b)(2):	N
Biosimilar:	N

Pharmacology/ Toxicology Summary

Pharmacology

Key Findings:

Etrasimod is sphingosine-1-phosphate receptor subtype 1,4, and 5 (S1P_{1,4,5}) agonist. Etrasimod concentration-dependently inhibited generation of cAMP in vitro with similar efficacy and potency compared to the endogenous ligand, S1P, as well as other S1P receptor modulators (i.e., fingolimod phosphate, ozanimod, siponimod). Similar to other S1P receptor modulators but in contrast to S1P, etrasimod rapidly and concentration-dependently induced S1P₁ internalization in S1P₁-overexpressing CHO cells, with the maximum effect occurring by 2 hours and persisting through at least 6 hours post-treatment.

Etrasimod potently induced GTPγS binding to human S1P₁ (hS1P₁) and hS1P₅. Etrasimod was also a partial agonist for GTPγS binding at hS1P₃ but not hS1P₂ or hS1P₄. Etrasimod was generally a less potent agonist of GTPγS binding to S1P receptors, including S1P₁, compared to other S1P receptor modulators.

Etrasimod potently induced β -arrestin recruitment to hS1P₁ and hS1P₅. Etrasimod was a partial agonist at hS1P₄ but did not induce β -arrestin recruitment to hS1P₂ or hS1P₃. Compared to ozanimod and siponimod, etrasimod had similar efficacy and potency for β -arrestin recruitment to hS1P₁. Etrasimod also had similar efficacy but lower potency at this receptor compared to fingolimod phosphate.

While etrasimod concentration-dependently induced increases in the I_{KACH} channel current, the maximum increase of 39% at 1000 nM (4 times higher than the mean steady state C_{max} in healthy volunteers following 21 days of dosing with etrasimod at 2 mg) was less than that of ozanimod or fingolimod under the same assay conditions.

Genotoxicity

Key Findings:

Bacterial reverse mutation assays were conducted with process-related impurities (b) (4) was considered positive for mutagenicity, while (b) (4) was considered negative for mutagenicity under the tested conditions.

(b) (4) is a starting material used in the preparation of an (b) (4) manufacturing product. As (b) (4) is considered a potentially genotoxic impurity, it is controlled in the (b) (4) manufacturing product at below (b) (4) $\mu\text{g/day}$ ((b) (4) ppm for the clinical dose of etrasimod of 2 mg/day, given a purge factor of at least (b) (4)), in accordance with ICH M7 option 2.

Reproductive Toxicology

Key Findings:

Pregnant Sprague Dawley rats were administered vehicle or etrasimod at 0.4, 2, or 4 mg/kg/day from GD 6 through LD 20. There were no adverse effects on maternal clinical signs, or body weights at any dose of etrasimod, although decreased feed consumption was observed in all treated groups during gestation.

One female at 4 mg/kg/day was prematurely terminated on GD 22 due to severe adverse clinical signs during delivery. A significant increase in post-implantation loss was observed at 4 mg/kg/day and significantly increased gestation length was observed at 2 and 4 mg/kg/day compared to controls. A significant increase in the number of females with stillborn pups at 2 and 4 mg/kg/day and increased number of stillborn pups per litter at 2 mg/kg/day compared to controls were also observed. The viability index was decreased at 2 and 4 mg/kg/day while the lactation index was decreased at 4 mg/kg/day compared to controls.

Etrasimod-related clinical signs in F1 offspring included cold to touch, suspected dehydration (based on skin turgor), discolored skin, skin pallor, swelling, and thin appearance at 4 mg/kg/day and no milk band in the stomach at 2 and 4 mg/kg/day. Decreased maternal feed consumption during lactation corresponded to decreased pup body weights and body weight gain during the preweaning period at 2 and 4 mg/kg/day. No etrasimod-related macroscopic findings were observed in F1 offspring at the

preweaning terminal necropsy; however, drug-related findings in prematurely terminated or stillborn pups included full body edema at 4 mg/kg/day and no milk in the stomach at 2 and 4 mg/kg/day. No etrasimod-related findings, including behavioral assessments and sexual maturation, were observed in pups during the postweaning period, and the previously observed differences in body weights normalized during this time in all treated groups compared to controls.

There were no etrasimod-related effects on pre-coital intervals, mating index, or fertility index in the F1 generation. A reduced number of implantations and increased rate of pre-implantation loss were observed at 4 mg/kg/day compared to controls and correlated with a decreased number of live embryos per litter at 4 mg/kg/day compared to controls.

The NOAEL for maternal toxicity was considered the low dose of 0.4 mg/kg/day (approximately 2 times exposure at the recommended clinical dose of 2 mg, based on AUC), due to observations of mortality during delivery, increased post-implantation loss, increased number of females with stillborn pups, increased number of stillborn pups per litter, decreased viability index, and decreased lactation index at higher doses. Based on increased perinatal mortalities at ≥ 2 mg/kg/day, adverse clinical signs and decreased body weight/body weight gain in offspring prior to weaning at all doses, as well as the reduced number of implantations, increased rate of pre-implantation loss, and corresponding decrease in the number of live embryos per litter in F1 animals observed at 4 mg/kg/day during the postweaning period, a developmental NOAEL could not be determined.

1 Background

1.1 Regulatory History

The Applicant, Pfizer, Inc. (Arena Pharmaceuticals was acquired by Pfizer Inc. in 2022), submitted a Type B pre-IND meeting request on January 29, 2015 (IND 125154, SDN 2). No nonclinical questions were included in this meeting; however, preliminary comments from the Agency included recommendations to conduct a study in an animal model of inflammatory bowel disease (IBD) to show the potential effectiveness of etrasimod in the intended study population and to conduct a fertility and early embryonic development (FEED) study prior to the initiation of the proposed phase 2 clinical trial.¹ The Applicant addressed these comments in a subsequent submission (SDN 6; May 4, 2015). The Applicant stated that a study in an animal model of IBD would be conducted concurrently with clinical development. Additionally, the Applicant referenced previously submitted 3-month toxicology studies in rats and dogs (b) (4) and indicated that these data would be supplemented with a study of sperm morphology and motility to adequately address all FEED study endpoints prior to the initiation of the phase 2 clinical study. The final report of a 28-day study of the effects of etrasimod on spermatogenesis in male rats was submitted (b) (4).

The Applicant submitted IND 125154 to the Division of Gastroenterology and Inborn Errors Products (DGIEP, now the Division of Gastroenterology [DG]) on May 4, 2015 (SDN 5) to develop etrasimod as a treatment for subjects with moderately to severely active ulcerative colitis. No nonclinical studies were submitted to IND 125154 (b) (4).

On June 4, 2015, no safety concerns were identified with the proposed phase 2 clinical protocol in subjects with ulcerative colitis under IND 125154 (Nonclinical Review; Dr. Sruthi King).

(b) (4)
Most nonclinical studies submitted (b) (4) were reviewed by Dr. John Dougherty under IND 142182.³

A Type B, End of Phase 2 (EOP2) meeting was held between the Applicant and the Agency on October 30, 2018. During this meeting, the Agency noted that the completed, ongoing, and planned nonclinical studies appeared adequate to support the proposed phase 3 clinical; however, a pre- and postnatal development study in rats

¹ IND 125154: [Type B PIND Meeting – Preliminary Comments](#). K. Richards (DGIEP); March 20, 2015.
IND 125154: [Type B PIND Meeting – Final Meeting Minutes](#). K. Richards (DGIEP); April 2, 2015.
(b) (4)

³ IND 142182: [30-Day IND Full Review](#). J. Dougherty (DDDP); June 10, 2019.

should be conducted prior to the NDA submission. Final meeting minutes were issued following the meeting.⁴

NDA 216956 for etrasimod for the treatment of moderately to severely active ulcerative colitis was submitted to DG on October 14, 2022 (SDN 1). The purpose of the current review is to review studies submitted to the NDA that were not previously submitted to any IND for etrasimod.

1.2 Relevant INDs, NDAs, BLAs or DMFs

(b) (4)

IND 125154: Moderately to severely active ulcerative colitis (DG); active
 IND 125509: Moderately to severely active Crohn's disease (DG); active
 (b) (4)

IND 142182: Atopic dermatitis (DDD); active
 IND 146360: Moderate to severe alopecia areata (DDDP); active
 IND 146975: Eosinophilic Esophagitis (DG); active
 (b) (4)

DMF (b) (4)

1.3 Previous Reviews Referenced

Application	Reviewer	Date in DARRTS	Notes
IND 142182	John Dougherty, PhD	June 10, 2019	30-day IND review (most studies submitted, but not reviewed, (b) (4))
(b) (4)			

⁴ IND 125154: [End of Phase 2 Final Meeting Minutes](#). K. Richards (DGIEP); October 30, 2018.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology – Primary	4.2.1.1
Activity of Etrasimod and Other S1P Modulators in S1P1 Cyclic AMP and Internalization Assays in Recombinant CHO Cells	APD334.TS.012
Pharmacological Profiling of Etrasimod, Its M3 and M6 Metabolites, and Other S1P Receptor Modulators in β -Arrestin and GTP γ S Assays for S1P ₁₋₅ Receptors	APD334.TS.020
Pharmacology – Secondary	4.2.1.2
Analysis of Effects of AR401959, Fingolimod and Ozanimod on GIRK (IKAch currents) in voltage clamp studies in human atrial myocytes	APD334.TS.011
Toxicology – Genotoxicity <i>In Vitro</i>	4.2.3.3.1
^{(b) (4)} Bacterial Reverse Mutation Assay	APD334.TOX.006
Bacterial Reverse Mutation Assay	APD334.TOX.010
Toxicology – Reproductive and Developmental	4.2.3.5
A Dose Range-Finding Prenatal/Postnatal Development Study of APD334 Administered by Oral Gavage to Sprague Dawley Rats	APD334.TOX.012
An Oral Pre- and Postnatal Development Study of APD334 in Rats, Including Toxicokinetics and a Postnatal Neurobehavioral and Reproductive Functional Evaluation	APD334.TOX.011

4 Pharmacology

4.1 Primary Pharmacology

Activity of Etrasimod and Other S1P Modulators in S1P1 Cyclic AMP and Internalization Assays in Recombinant CHO Cells
[APD334.TS.012](#)

The ability of etrasimod and other sphingosine 1-phosphate (S1P) receptor modulators, fingolimod phosphate, ozanimod, and siponimod, to induce S1P₁ receptor internalization and inhibit cyclic adenosine monophosphate (cAMP) generation was evaluated using two Chinese hamster ovary (CHO) cell lines engineered to express human S1P₁ (one high-expressing line, CHO-21BWc1, and one moderate-expressing line, CHO-21BWc2) and primary human umbilical vein endothelial cells (HUVEC), which endogenously express S1P₁.

Etrasimod concentration-dependently inhibited cAMP generation in both CHO-21BWc1 and CHO-21BWc2 cells, with IC₅₀ values of 453.9 and 464.5 pM, respectively, and 103% efficacy relative to that of the endogenous ligand S1P in both cell lines. These findings were similar to those observed with fingolimod phosphate (IC₅₀ = 7.2 and 7.9 pM, 101% and 103% efficacy, respectively), ozanimod (IC₅₀ = 29.5 and 38.9 pM, 104% and 100% efficacy), siponimod (IC₅₀ = 28.2 and 27.5 pM, 101% and 103% efficacy), and S1P itself (IC₅₀ = 28.2 and 27.5 pM).

Table 1. Inhibition of cAMP Generation by S1P Receptor Modulators in Recombinant hS1P1-Expressing Cell Line CHO-21BWc1

	Etrasimod	Fingolimod(P)	Ozanimod	Siponimod	S1P
pIC ₅₀	9.34	11.14	10.53	10.55	10.55
SD	0.26	0.31	0.25	0.28	0.25
IC ₅₀ (pM)	453.9	7.2	29.5	28.2	28.2
Lower 95% CI of mean IC ₅₀ , pM	325.8	4.9	20.4	19.1	19.5
Upper 95% CI of mean IC ₅₀ , pM	632.4	10.7	41.7	40.7	39.8
Efficacy, % of S1P	103	101	104	101	101
SD, % of S1P	4	6	5	4	5
N	15	15	13	14	13

cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; CI, confidence interval; IC₅₀, concentration required for 50% of the maximum effect; fingolimod(P), fingolimod-phosphate; hS1P₁, human sphingosine 1-phosphate receptor 1; pIC₅₀, negative log(IC₅₀); S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-012).

Table 2. Inhibition of cAMP Generation by S1P Receptor Modulators in Recombinant hS1P1-Expressing Cell Line CHO-21BWc2

	Etrasimod	Fingolimod(P)	Ozanimod	Siponimod	S1P
pIC ₅₀	9.33	11.10	10.41	10.56	10.56
SD	0.23	0.27	0.21	0.37	0.24
IC ₅₀ (pM)	464.5	7.9	38.9	27.5	27.5
Lower 95% CI of mean IC ₅₀ , pM	335.7	5.2	29.5	16.6	19.5
Upper 95% CI of mean IC ₅₀ , pM	642.7	12.0	52.5	46.8	38.9
Efficacy, % of S1P	103	103	100	103	101
SD, % of S1P	6	5	4	4	5
N	13	11	13	13	12

cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; CI, confidence interval; IC₅₀, concentration required for 50% of the maximum effect; Fingolimod(p), fingolimod phosphate; hS1P₁, human sphingosine 1-phosphate receptor 1; pIC₅₀, negative log(IC₅₀); S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-012).

In HUVEC, etrasimod concentration-dependently inhibited cAMP generation with an IC₅₀ of 30.5 nM and an efficacy of 101% relative to S1P. These findings were similar to those observed with fingolimod phosphate (IC₅₀ = 1.6 nM, 94% efficacy), ozanimod (IC₅₀ = 2.0 nM, 102% efficacy), siponimod (IC₅₀ = 1.6 nM, 101% efficacy), and S1P itself (IC₅₀ = 19.1 nM).

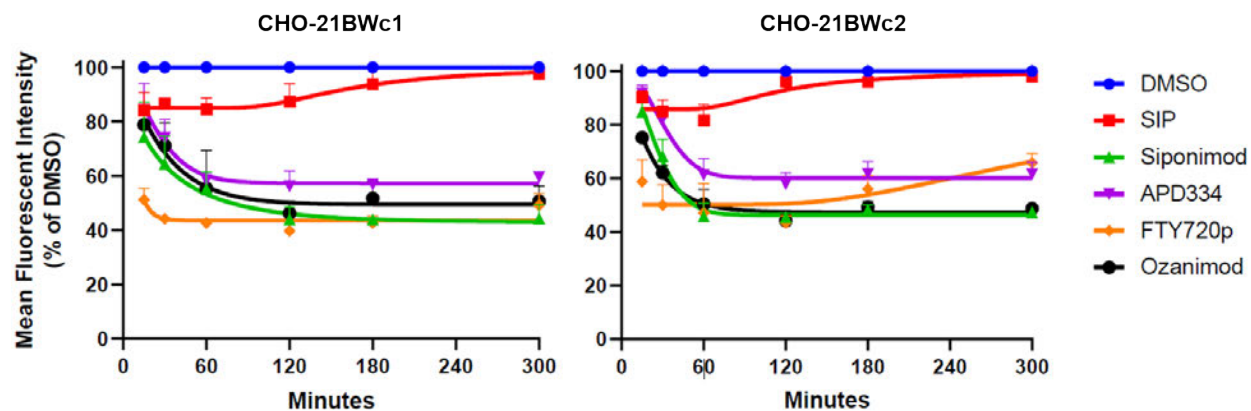
Table 3. Inhibition of cAMP Generation by S1P Receptor Modulators in Primary HUVEC

	Etrasimod	Fingolimod(p)	Ozanimod	Siponimod	S1P
pIC ₅₀	7.52	8.79	8.71	8.81	7.72
SD	0.20	0.14	0.30	0.23	0.36
IC ₅₀ (nM)	30.5	1.6	2.0	1.6	19.1
Lower 95% CI of mean IC ₅₀ , nM	19.9	1.2	1.2	1.0	9.5
Upper 95% CI of mean IC ₅₀ , nM	46.7	2.3	3.1	2.4	38.2
Efficacy, % of S1P	101	94	102	101	96
SD, % of S1P	5	4	9	7	3
N	7	6	11	9	8

cAMP, cyclic adenosine monophosphate; CI, confidence interval; IC₅₀, concentration required for 50% of the maximum effect; Fingolimod(p), fingolimod phosphate; pIC₅₀, negative log(IC₅₀); S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-012).

Following incubation of hS1P₁-expressing CHO cells with 100 nM of the various S1P receptor modulators, all compounds induced rapid internalization in both CHO-21BWc1 and CHO-21BWc2 cells that reached a maximum level within 2 hours and persisted through the end of the assay (6 hours). In contrast, S1P induced minimal internalization, and surface expression of S1P₁ returned to baseline levels within approximately 3 hours.

Figure 1. Time Course of S1P₁ Receptor Internalization in Recombinant CHO Cells

APD334: etrasimod; FTY720p: fingolimod phosphate.

Figure adapted from study report (Study APD334-TS-012).

As S1P at 100 nM induced minimal and more transient internalization in the previous assay, internalization of S1P₁ with S1P receptor modulators at 10x the concentration (1 μ M) was quantified in the recombinant CHO cell lines with an incubation time of 2 hours. Fingolimod phosphate was used as the control to determine the relative efficacy of the other compounds.

Etrasimod concentration-dependently induced S1P₁ internalization in both CHO-21BWc1 and CHO-21BWc2 cells, with IC₅₀ values of 8.9 and 8.3 nM, respectively. The efficacy of etrasimod for internalization relative to fingolimod phosphate was 90% and 99% in the two cell lines, respectively. These findings were similar to those observed with ozanimod (IC₅₀ = 4.2 and 3.5 nM, 103% and 111% efficacy, respectively), siponimod (IC₅₀ = 4.2 and 1.8 nM, 107% and 103% efficacy) and fingolimod phosphate (IC₅₀ = 3.7 and 1.4 nM). Under the tested conditions, S1P dose-dependently induced internalization with IC₅₀ values of 5211.9 and 608.1 nM and efficacy of 103% and 94%, respectively, relative to fingolimod phosphate.

Table 4. Internalization of hS1P₁ by S1P Receptor Modulators in Recombinant hS1P1-Expressing Cell Line CHO-21BWc1

	Etrasimod	Fingolimod(p)	Ozanimod	Siponimod	S1P
pIC ₅₀	8.05	8.43	8.38	8.37	5.28
SD	0.29	0.24	0.31	0.46	0.52
IC ₅₀ (nM)	8.9	3.7	4.2	4.2	5211.9
Lower 95% CI of mean IC ₅₀ , nM	3.9	1.9	2.4	0.8	1482.5
Upper 95% CI of mean IC ₅₀ , nM	20.0	7.3	7.3	22.6	18,323.1
Efficacy, % of S1P*	90	103	103	107	103
SD, % of S1P	10	8	5	4	23
N	5	5	9	4	6

CHO, Chinese hamster ovary; CI, confidence interval; hS1P₁, human sphingosine 1-phosphate receptor 1; IC₅₀, concentration required for 50% of the maximum effect; Fingolimod(p), fingolimod phosphate; pIC₅₀, negative log(IC₅₀); S1P, sphingosine 1-phosphate; SD, standard deviation

*Although efficacy is labeled as % of S1P, fingolimod phosphate was used as the scaling control in this experiment, per the study report.

Table adapted slightly from study report (Study APD334-TS-012).

Table 5. Internalization of hS1P₁ by S1P Receptor Modulators in Recombinant hS1P1-Expressing Cell Line CHO-21BwC2

	Etrasimod	Fingolimod(p)	Ozanimod	Siponimod	S1P
pIC ₅₀	8.08	8.85	8.46	8.74	6.22
SD	0.19	0.43	0.25	0.21	0.78
IC ₅₀ (nM)	8.3	1.4	3.5	1.8	608.1
Lower 95% CI of mean IC ₅₀ , nM	4.1	0.3	2.1	0.9	35.4
Upper 95% CI of mean IC ₅₀ , nM	16.6	6.8	5.6	3.9	10,447.2
Efficacy, % of S1P*	99	99	111	103	94
SD, % of S1P	5	8	13	6	11
N	4	4	8	4	4

CHO, Chinese hamster ovary; CI, confidence interval; hS1P₁, human sphingosine 1-phosphate receptor 1; IC₅₀, concentration required for 50% of the maximum effect; Fingolimod(p), fingolimod phosphate; pIC₅₀, negative log(IC₅₀); S1P, sphingosine 1-phosphate; SD, standard deviation

*Although efficacy is labeled as % of S1P, fingolimod phosphate was used as the scaling control in this experiment, per the study report.

Table adapted slightly from study report (Study APD334-TS-012).

Pharmacological Profiling of Etrasimod, Its M3 and M6 Metabolites, and Other S1P Receptor Modulators in β -Arrestin and GTP γ S Assays for S1P1-5 Receptors [APD334.TS.020](#)

The ability of etrasimod, its M3 and M6 metabolites, and other S1P receptor modulators ozanimod, ozanimod ketone metabolite, fingolimod phosphate, and siponimod, to induce the binding of guanosine 5'-O-(gamma-thio)-triphosphate (GTP γ S) and recruit β -arrestin to human S1P₁₋₅ were evaluated using in vitro assays. GTP γ S binding was evaluated in S1P receptor-expressing CHO-K1 cells using radiolabeled GTP. β -arrestin recruitment was evaluated using the relevant DiscoverX assay (a modified chemiluminescent assay using HEK cells). Efficacy values for experiments with hS1P_{1,2,3,5} were normalized to the endogenous ligand, S1P, while experiments with hS1P₄ were normalized to amiselimod phosphate.

GTP γ S binding

Etrasimod potently induced GTP γ S binding to hS1P₁ and hS1P₅ (EC_{50s} = 57 nM and 63.5 nM, respectively, and efficacy 99% and 72% of S1P, respectively). Etrasimod was also a partial agonist at hS1P₃ (EC₅₀ = 6050 nM, efficacy 42%); however, the clinical relevance of this effect is expected to be limited, as the EC₅₀ of 6050 nM is approximately 25 times higher than the mean steady state C_{max} in healthy volunteers following 21 days of dosing with etrasimod at 2 mg (113 ng/mL or 247 nM; Study APD334-002). Etrasimod did not induce GTP γ S binding to hS1P₂ or hS1P₄.

Etrasimod metabolite M3 had similar efficacy with approximately 10-fold lower potency for GTP γ S binding at hS1P₁ and hS1P₅ as well as similar efficacy and potency at hS1P₃

compared to the parent drug. Metabolite M6 had similar efficacy with approximately 3-fold lower potency at hS1P₁ as well as similar efficacy and potency at hS1P₃ and hS1P₅ compared to the parent drug.

At hS1P₁, etrasimod had similar efficacy but 15- to 48-fold lower potency compared to ozanimod, fingolimod phosphate, and siponimod. At hS1P₅, etrasimod had similar efficacy but 577-fold lower potency compared to fingolimod phosphate but lower efficacy and 52- to 1270-fold lower potency compared to ozanimod and siponimod. Overall, etrasimod is a less potent agonist of GTPγS binding to S1P receptors, including S1P₁, compared to other S1P receptor modulators. This finding may have clinical relevance, as GTPγS binding to S1P₁ has been proposed to contribute to the transient heart rate reduction observed after the first dose of other S1P receptor modulators.^{5,6,7}

Table 6. Activities of S1P Receptor Modulators on GTPγS Binding to hS1P₁

Result	Etrasimod	Etrasimod Met M3	Etrasimod Met M6	Ozanimod	Ozanimod Ketone	Fingolimod(P)	Siponimod	S1P
pEC ₅₀ (nM), mean	7.24	6.26	6.82	8.93	8.14	8.91	8.43	6.81
SD (nM)	0.31	0.13	0.17	0.14	0.27	0.29	0.20	0.10
EC ₅₀ (nM), mean	57	545	153	1.2	7.3	1.3	3.7	156
Lower 95% CI of EC ₅₀ (nM)	18	333	57	0.7	2.7	0.4	1.8	108
Upper 95% CI of EC ₅₀ (nM)	180	892	408	2	19.9	3.6	7.7	226
Efficacy, % of S1P	99	89	101	103	97	86	93	101
N	4	4	3	4	4	4	4	4

CI, confidence interval; EC₅₀, concentration required for 50% of the maximum effect; hS1P₁, human sphingosine 1-phosphate receptor 1; Met, metabolite; M3, AR503641; M6, AR504344; N, number; pEC₅₀, negative logarithm of EC₅₀; S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-020).

Table 7. Activities of S1P Receptor Modulators on GTPγS Binding to hS1P₂

Result	Etrasimod	Etrasimod Met M3	Etrasimod Met M6	Ozanimod	Ozanimod Ketone	Fingolimod(P)	Siponimod	S1P
pEC ₅₀ (nM), mean	NR	NR	NR	NR	NR	5.77	NR	6.94
SD (nM)	NA	NA	NA	NA	NA	0.26	NA	0.34
EC ₅₀ (nM), mean	NR	NR	NR	NR	NR	1680	NR	115
Lower 95% CI of EC ₅₀ (nM)	NA	NA	NA	NA	NA	660	NA	32.6
Upper 95% CI of EC ₅₀ (nM)	NA	NA	NA	NA	NA	4290	NA	405
Efficacy, % of S1P	NR	NR	NR	NR	NR	35	NR	108
N	4	4	4	4	4	4	4	4

CI, confidence interval; EC₅₀, concentration required for 50% of the maximum effect; hS1P₂, human sphingosine 1-phosphate receptor 2; Met, metabolite; M3, AR503641; M6, AR504344; N, number; NA, not applicable; NR, no response; pEC₅₀, negative logarithm of EC₅₀; S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-020).

⁵ Camm J, Hla T, Bakshi R, Brinkmann V. Cardiac and vascular effects of fingolimod: Mechanistic basis and clinical implications. Am Heart J. 2014;168(5):632-644.

⁶ Sykes DA, Riddy DM, Stamp C, et al. Investigating the molecular mechanisms through which FTY720-P causes persistent S1P1 receptor internalization. Br J Pharmacol. 2014;171(21):4797-4807.

⁷ Taylor S, Gray JRJ, Willis R, et al. The utility of pharmacokinetic-pharmacodynamic modeling in the discovery and optimization of selective S1P1 agonists. Xenobiotica. 2012;42(7):671-686.

Table 8. Activities of S1P Receptor Modulators on GTPyS Binding to hS1P₃

Result	Etrasimod	Etrasimod Met M3	Etrasimod Met M6	Ozanimod	Ozanimod Ketone	Fingolimod(P)	Siponimod	S1P
pEC ₅₀ (nM), mean	5.22	5.15	5.27	5.17	NR	7.86	NR	6.49
SD (nM)	0.24	0.12	0.13	0.19	NA	0.17	NA	0.28
EC ₅₀ (nM), mean	6050	7020	5350	6820	NR	13.8	NR	323
Lower 95% CI of EC ₅₀ , nM	2540	4590	3370	3430	NA	7.5	NA	116
Upper 95% CI of EC ₅₀ , nM	14400	10700	8480	13500	NA	25.4	NA	900
Efficacy, % of S1P	42	22	26	52	NR	39	NR	113
N	4	4	4	4	4	4	3	4

CI, confidence interval; EC₅₀, concentration required for 50% of the maximum effect; hS1P₃, human sphingosine 1-phosphate receptor 3; Met, metabolite; M3, AR503641; M6, AR504344; N, number; NA, not applicable; NR, no response; pEC₅₀, negative logarithm of EC₅₀; S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-020).

Table 9. Activities of S1P Receptor Modulators on GTPyS Binding to hS1P₄

Result	Etrasimod	Etrasimod Met M3	Etrasimod Met M6	Ozanimod	Ozanimod Ketone	Fingolimod(P)	Siponimod	S1P
pEC ₅₀ (nM), mean	NR	NR	NR	NR	NR	8.22	6.29	6.86
SD (nM)	NA	NA	NA	NA	NA	0.23	0.15	0.17
EC ₅₀ (nM), mean	NR	NR	NR	NR	NR	6.1	510	140
Lower 95% CI of EC ₅₀ (nM)	NA	NA	NA	NA	NA	2.6	293	74.1
Upper 95% CI of EC ₅₀ (nM)	NA	NA	NA	NA	NA	14.2	887	263
Efficacy, % of amiselimod(P)	NR	NR	NR	NR	NR	38	42	31
N	4	4	4	4	4	4	4	4

CI, confidence interval; EC₅₀, concentration required for 50% of the maximum effect; hS1P₄, human sphingosine 1-phosphate receptor 4; Met, metabolite; M3, AR503641; M6, AR504344; N, number; NA, not applicable; NR, no response; pEC₅₀, negative logarithm of EC₅₀; S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-020).

Table 10. Activities of S1P Receptor Modulators on GTPyS Binding to hS1P₅

Result	Etrasimod	Etrasimod Met M3	Etrasimod Met M6	Ozanimod	Ozanimod Ketone	Fingolimod(P)	Siponimod	S1P
pEC ₅₀ (nM), mean	7.20	5.24	7.01	7.91	7.02	8.97	9.27	7.30
SD (nM)	0.36	0.07	0.35	0.16	0.25	0.17	0.28	0.28
EC ₅₀ (nM), mean	63.5	5804	97	12.3	95.8	1.1	0.5	49.7
Lower 95% CI of EC ₅₀ (nM)	17	3824	26.6	6.8	38.4	0.6	0.2	17.7
Upper 95% CI of EC ₅₀ (nM)	237.7	8808	354	22	239	2	1.5	139.4
Efficacy, % of S1P	72	51	64	110	105	69	118	100
N	4	3	4	4	4	4	4	4

CI, confidence interval; EC₅₀, concentration required for 50% of the maximum effect; hS1P₅, human sphingosine 1-phosphate receptor 5; Met, metabolite; M3, AR503641; M6, AR504344; N, number; pEC₅₀, negative logarithm of EC₅₀; S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-020).

β-arrestin recruitment

Etrasimod potently induced β-arrestin recruitment to hS1P₁ and hS1P₅ (EC₅₀s = 44 nM and 63.4 nM, respectively, and efficacy 120% and 86% of S1P, respectively). Etrasimod was a partial agonist of β-arrestin recruitment at hS1P₄ (EC₅₀ = 141 nM, efficacy 54% of amiselimod phosphate); the physiological or clinical relevance of this finding is unknown. Etrasimod did not induce β-arrestin recruitment to hS1P₂ or hS1P₃.

Etrasimod metabolites M3 and M6 had similar efficacy at hS1P₁ and hS1P₅ but approximately 3-fold (M6) or 10-fold (M3) lower potency compared to the parent drug. M6 was also a partial agonist at hS1P₄, with similar efficacy and approximately 10-fold lower potency compared to the parent drug.

Compared to ozanimod and siponimod, etrasimod had similar efficacy and potency (EC₅₀) in the β -arrestin recruitment assay at hS1P₁. Etrasimod also had similar efficacy but approximately 4-fold lower potency at this receptor compared to fingolimod phosphate. Notably, the various S1P receptor modulators differed in efficacy and potency at hS1P₄ and hS1P₅ (see tables below). Etrasimod consistently recruited β -arrestin more potently in this assay compared to other S1P receptor modulators, though with a lower efficacy.

Table 11. Activities of S1P Receptor Modulators on β -Arrestin Recruitment to hS1P₁

Result	Etrasimod	Etrasimod Met M3	Etrasimod Met M6	Ozanimod	Ozanimod Ketone	Fingolimod(P)	Siponimod	S1P
pEC ₅₀ (nM), mean	7.36	6.25	6.87	7.48	7.13	7.96	7.27	7.68
SD (nM)	0.02	0.07	0.09	0.14	0.14	0.09	0.39	0.21
EC ₅₀ (nM), mean	44	568	136	33.2	73.9	11	53.9	21
Lower 95% CI of EC ₅₀ (nM)	38.3	433	82.8	19.8	43.8	7.9	12.7	9.7
Upper 95% CI of EC ₅₀ (nM)	50.5	744	224	55.7	124	15.3	229	45.6
Efficacy, % of S1P	120	118	122	114	123	109	111	111
N	3	4	3	4	4	4	4	4

CI, confidence interval; EC₅₀, concentration required for 50% of the maximum effect; hS1P₁, human sphingosine 1-phosphate receptor 1; Met, metabolite; M3, AR503641; M6, AR504344; N, number; pEC₅₀, negative logarithm of EC₅₀; S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-020).

Table 12. Activities of S1P Receptor Modulators on β -Arrestin Recruitment to hS1P₄

Result	Etrasimod	Etrasimod Met M3	Etrasimod Met M6	Ozanimod	Ozanimod Ketone	Fingolimod(P)	Siponimod	S1P
pEC ₅₀ (nM), mean	6.85	5.85	6.37	6.12	6.23	8.16	6.25	8.26
SD (nM)	0.06	0.23	0.16	0.06	0.10	0.18	0.33	0.31
EC ₅₀ (nM), mean	141	1410	428	766	588	6.9	563	5.4
Lower 95% CI of EC ₅₀ (nM)	97.5	614	240	604	404	3.6	170	1.7
Upper 95% CI of EC ₅₀ (nM)	204	3230	763	971	855	13.5	1870	17
Efficacy, % of S1P	54	84	74	105	89	78	93	101
N	3	4	4	4	4	4	4	4

CI, confidence interval; EC₅₀, concentration required for 50% of the maximum effect; hS1P₄, human sphingosine 1-phosphate receptor 4; Met, metabolite; M3, AR503641; M6, AR504344; N, number; pEC₅₀, negative logarithm of EC₅₀; S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-020).

Table 13. Activities of S1P Receptor Modulators on β -Arrestin Recruitment to hS1P₅

Result	Etrasimod	Etrasimod Met M3	Etrasimod Met M6	Ozanimod	Ozanimod Ketone	Fingolimod(P)	Siponimod	S1P
pEC ₅₀ (nM), mean	7.20	NR	6.16	7.54	7.23	8.23	7.96	8.84
SD (nM)	0.08	NA	0.12	0.22	0.19	0.08	0.38	0.09
EC ₅₀ (nM), mean	63.4	NR	698	28.8	58.5	5.8	11	1.4
Lower 95% CI of EC ₅₀ (nM)	40	NA	356	12.6	28.7	4.4	2.7	0.9
Upper 95% CI of EC ₅₀ (nM)	101	NA	1370	65.6	119	7.7	44.6	2.4
Efficacy, % of S1P	86	NR	91	119	115	48	107	104
N	3	4	3	4	4	4	4	3

CI, confidence interval; EC₅₀, concentration required for 50% of the maximum effect; hS1P₅, human sphingosine 1-phosphate receptor 5; Met, metabolite; M3, AR503641; M6, AR504344; N, number; NA, not applicable; NR, no response; pEC₅₀, negative logarithm of EC₅₀; S1P, sphingosine 1-phosphate; SD, standard deviation

Table copied directly from study report (Study APD334-TS-020).

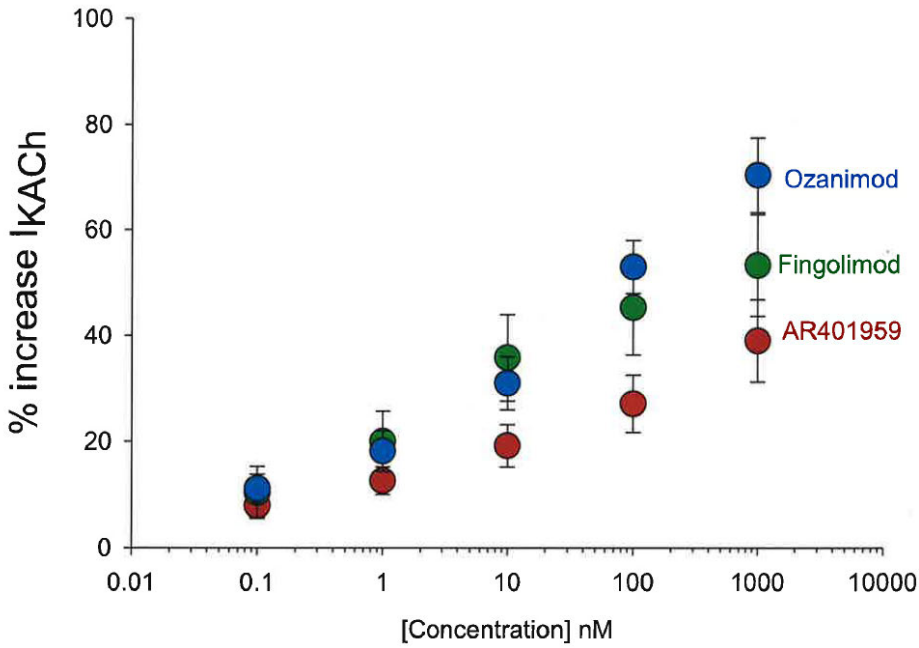
4.2 Secondary Pharmacology

Analysis of Effects of AR401959, Fingolimod and Ozanimod on GIRK (IK_{ACh} currents) in voltage clamp studies in human atrial myocytes [APD334.TS.011](#)

The ability of etrasimod (named AR401959 in this report), ozanimod, and fingolimod to affect I_{K_{ACh}} channel current was evaluated using patch clamping of isolated human atrial cardiomyocytes (n = 6 donors). Compounds were tested over the range of 0.1-1000 nM. Changes in the I_{K_{ACh}} channel current were normalized the increased observed with 10 μ M carbachol.

Overall, etrasimod increased I_{K_{ACh}} channel current in human atrial cardiomyocytes less than ozanimod and fingolimod. Etrasimod concentration-dependently induced increases in the I_{K_{ACh}} channel current, with a maximum increase of 39% at 1000 nM, (4 times higher than the mean steady state C_{max} in healthy volunteers following 21 days of dosing with etrasimod at 2 mg: 113 ng/mL or 247 nM; Study APD334-002). Ozanimod and fingolimod also concentration-dependently induced increases in the I_{K_{ACh}} channel current, with maximum increases of 70.4% and 53.3%, respectively, at 1000 nM.

Figure 2. Effects of S1P Receptor Modulators on I_{KACH} Channel Current in Human Atrial Cardiomyocytes



AR401959: etrasimod.

Figure copied directly from study report (Study APD334-TS-011).

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

7.1.1 ^{(b) (4)} Bacterial Reverse Mutation Assay

Study no.: [APD334.TOX.006](#)
 Study report location: EDR 4.2.3.7.6
 Conducting laboratory and location: ^{(b) (4)}
 GLP compliance: Y
 Drug, lot #, and % purity: ^{(b) (4)} (process-related impurity)
 Lot # A0384033
 Purity 98.2%

Methods

Strains: *S. typhimurium* TA98, TA100, TA1535 and TA1537
E. coli WP2 *uvrA*
 Concentrations in definitive study: See table below
 Basis of concentration selection: Cytotoxicity –S9 at ≥ 66.7 $\mu\text{g}/\text{plate}$ in strains TA98 and TA 100, ≥ 33 $\mu\text{g}/\text{plate}$ in strain TA1535, and ≥ 100 $\mu\text{g}/\text{plate}$ in strain TA 1537; +S9 at ≥ 1000 $\mu\text{g}/\text{plate}$ in strains TA100 and TA1535 and ≥ 3333 $\mu\text{g}/\text{plate}$ in strains TA98 and TA1537 in the preliminary assay.
 Negative control: DMSO
 Positive control: 2-aminoanthracene (1.0, 2.0, 15 $\mu\text{g}/\text{plate}$)
 2-nitrofluorene (1.0 $\mu\text{g}/\text{plate}$)
 Sodium azide (1.0 $\mu\text{g}/\text{plate}$)
 9-aminoacridine (75 $\mu\text{g}/\text{plate}$)
 Methyl methanesulfonate (1000 $\mu\text{g}/\text{plate}$)
 Formulation/Vehicle: DMSO
 Incubation & sampling time: 48-72 hours at 37°C
 Comment on Study Validity: Valid

Table 14. Concentrations of (b) (4) Used in Definitive Assay

Tester Strains	Activation Condition	Dose Levels (µg per plate)
TA98	Without	3.33, 10.0, 25.0, 33.3, 50.0, 66.7, 100 and 333
	With	6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000, 3333 and 5000
TA100	Without	0.333, 1.00, 3.33, 10.0, 33.3, 100 and 333
	With	10.0, 33.3, 100, 333, 500, 667, 1000 and 3333
TA1535	Without	0.333, 1.00, 3.33, 10.0, 33.3, 100 and 333
	With	10.0, 33.3, 100, 333, 500, 667, 1000 and 3333
TA1537	Without	0.333, 1.00, 3.33, 10.0, 33.3, 100 and 333
	With	10.0, 33.3, 100, 333, 500, 667, 1000 and 3333
WP2 <i>uvrA</i>	Without	6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1000,
	With	3333 and 5000

Table copied directly from study report (Study APD334-TOX-006).

Results

Positive mutagenic responses (maximum increases (b) (4)-fold control) were observed with (b) (4) in strains TA98 and WP2 *uvrA* in the presence and absence of S9 activation and with TA1537 in the presence of S9 activation.

Equivocal responses (maximum increases (b) (4)-fold control, respectively) were observed with TA100 in the presence of S9 activation and TA1537 in the absence of S9 activation. Although the increases in the number of revertant colonies for these conditions were outside of the respective historical control limits, they did not completely meet all protocol acceptance criteria for evaluation as positive (i.e., not concentration-dependent and/or not meeting the prespecified threshold).

Based on these findings, (b) (4) was considered positive for mutagenicity.

Table 15. Revertant Colonies in *S. typharium* and *E. coli* Strains Treated with (b) (4) ± S9 Mix

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	DMSO (b) (4)	50.0 µL/plate	17 ± 3	102 ± 4	15 ± 5	10 ± 4	32 ± 3
		0.333		89 ± 5	21 ± 8	7 ± 4	
		1.00		100 ± 10	18 ± 3	12 ± 2	
		3.33	24 ± 4	90 ± 10	20 ± 5	9 ± 3	
		6.67					
		10.0	45 ± 9	103 ± 5	19 ± 4	14 ± 1	34 ± 1
		25.0	56 ± 9				33 ± 1
		33.3	56 ± 5	113 ± 9	14 ± 7	18 ± 2	61 ± 13
		50.0	59 ± 1				
		66.7	58 ± 11				130 ± 2
		100	44 ± 7	115 ± 13	0 ± 0	23 ± 3	181 ± 12
		333	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
		667					0 ± 0
		1000					0 ± 0
		3333					0 ± 0 ^P
		5000					0 ± 0 ^P
		2NF	1.00	82 ± 5			
SA	1.00		541 ± 43	686 ± 93			
9AAD	75.0				196 ± 67		
MMS	1000					435 ± 16	

Key to Positive Controls

SA	sodium azide	^P	precipitate
9AAD	9-Aminoacridine		
2NF	2-nitrofluorene		
MMS	methyl methanesulfonate		

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)					
			TA98	TA100	TA1535	TA1537	WP2uvrA	
With Activation	DMSO (b) (4)	50.0 µL/plate	27 ± 3	107 ± 9	11 ± 3	11 ± 2	36 ± 5	
		6.67	30 ± 11				44 ± 3	
		10.0	28 ± 4	115 ± 10	16 ± 7	13 ± 3	46 ± 9	
		33.3	34 ± 4	99 ± 10	14 ± 6	13 ± 2	89 ± 2	
		66.7	33 ± 4				121 ± 4	
		100	37 ± 5	117 ± 13	15 ± 1	23 ± 1	151 ± 11	
		333	85 ± 16	152 ± 3	15 ± 2	30 ± 1	429 ± 32	
		500		161 ± 9	9 ± 3	41 ± 11		
		667	178 ± 20	134 ± 20	13 ± 4	36 ± 5	1047 ± 7	
		1000	185 ± 7	134 ± 12	0 ± 0	58 ± 4	1329 ± 188	
		3333 ^P	63 ± 5	0 ± 0	0 ± 0	34 ± 11	212 ± 41	
		5000 ^P	0 ± 0				0 ± 0	
		2AA	1.00	204 ± 20		67 ± 13		
		2AA	2.00		651 ± 19		52 ± 14	
		2AA	15.0					273 ± 26

Key to Positive Controls

2AA	2-aminoanthracene	^P	precipitate

Table copied directly from study report (Study APD334-TOX-006).

7.1.2 ^{(b) (4)} **Bacterial Reverse Mutation Assay**

Study no.: [APD334.TOX.010](#)
 Study report location: EDR 4.2.3.7.6
 Conducting laboratory and location: ^{(b) (4)}
 GLP compliance: Yes
 Drug, lot #, and % purity: ^{(b) (4)} (process-related impurity)
 Lot # 2280-20170103
 Purity 99.5%

Methods

Strains: *S. typhimurium* TA98, TA100, TA1535 and TA1537
E. coli WP2 uvrA
 Concentrations in definitive study: 50.0, 150, 500, 1500 and 5000 µg/plate
 Basis of concentration selection: Lack of cytotoxicity in the preliminary assay up to 5000 µg/plate
 Negative control: DMSO
 Positive control: 2-aminoanthracene (1.0, 2.0, 15 µg/plate)
 2-nitrofluorene (1.0 µg/plate)
 Sodium azide (1.0 µg/plate)
 9-aminoacridine (75 µg/plate)
 Methyl methanesulfonate (1000 µg/plate)
 Formulation/Vehicle: DMSO
 Incubation & sampling time: 48 to 72 hours at 37°C
 Comment on Study Validity: Valid

Results

No increase in the number of revertant colonies was observed with ^{(b) (4)} up to 5000 µg/plate under any condition tested. Thus, ^{(b) (4)} was considered negative for mutagenicity under the conditions of the assay.

9 Reproductive and Developmental Toxicology

9.3 Prenatal and Postnatal Development (PPND)⁺

9.3.1 A Dose Range-Finding Prenatal/Postnatal Development Study of APD334 Administered by Oral Gavage to Sprague Dawley Rats

Study no.: [APD334.TOX.012](#)
 Study report location: EDR 4 2 3 5 4
 Conducting laboratory and location: (b) (4)
 GLP compliance: No
 Drug, lot #, and % purity: Etrasimod (APD334)
 Lot # 18-534-003
 Purity 99.83%
 Enhanced PPND Study: N

Methods

Doses: 0.05, 0.2, 1, 2 mg/kg/day
 Frequency of dosing: Once daily
 Number/Sex/Group: 8/group (females only)
 Dose volume: 5 mL/kg
 Formulation/Vehicle: L-arginine monohydrochloride at 0.189 mg/mL in 0.5% (v/v) Tween 20, 10 mM Tris, pH 8.5-9.0
 Route of administration: ORAL GAVAGE
 Species: Rat
 Strain: Sprague Dawley
 Comment on Study Design and Conduct: Pregnant females were dosed from gestation day (GD) 6 through lactation day (LD) 20. Offspring were not directly dosed.
 Dosing Solution Analysis: Acceptable ($\pm$ (b) (4) % of nominal)

Summary of Findings

In this preliminary dose range-finding study, pregnant Sprague Dawley rats were administered vehicle or etrasimod (named APD334 in this report) at 0.05, 0.2, 1, or 2 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 20. There were no maternal deaths, clinical signs of toxicity, or drug-related effects on body weight/body weight gain, feed consumption, or macroscopic examinations during gestation or lactation. No effects of etrasimod administration were observed on parturition. An increased number of stillborn pups and decreased viability index were observed at ≥ 1 mg/kg/day compared to controls. Lower pup body weights were observed at ≥ 0.2 mg/kg/day compared to controls on PNDs 20 and 21, but these effects were not dose-dependent. Based on these findings, a high dose of at least 2 mg/kg/day was considered appropriate for use in the pivotal PPND study.

9.3.2 An Oral Pre- and Postnatal Development Study of APD334 in Rats, Including Toxicokinetics and a Postnatal Neurobehavioral and Reproductive Functional Evaluation

Study no.: [APD334.TOX.011](#)
 Study report location: EDR 4.2.3.5.4
 Conducting laboratory and location:  (b) (4)
 GLP compliance: Yes
 Drug, lot #, and % purity: Etrasimod (APD334)
 Lot # 18-534-003
 Purity 99.83%
 Enhanced PPND Study: N

Methods

Doses: 0.4, 2, 4 mg/kg/day
 Frequency of dosing: Once daily
 Number/Sex/Group: Main groups: 22/group (females only)
 TK groups: control = 4, treated = 8/group
 Dose volume: 5 mL/kg
 Formulation/Vehicle: L-arginine monohydrochloride at 0.189 mg/mL in 0.5% (v/v) Tween 20, 10 mM Tris, pH 8.5-9.0
 Route of administration: ORAL GAVAGE
 Species: Rat
 Strain: Sprague Dawley
 Comment on Study Design and Conduct: Pregnant females were dosed from gestation day (GD) 6 through lactation day (LD) 20. Offspring were not directly dosed and were maintained until approximately PND 127 for males and PND 92 for females. Male and female offspring were cohabitated starting at PND 92-95 for evaluation of reproductive capacity.
 Dosing Solution Analysis: Acceptable ($\pm$  (b) (4) % of nominal)

Table 16. Design of Pre- and Postnatal Development Study with Etrasimod (APD334) in Sprague Dawley Rats

Group No.	Test Material	Dose Level (free acid) (mg/kg/day) ^b	Dose Concentration (mg/mL)	Dose Volume ^a (mL/kg)	No. Main Study Females (F0 generation)	No. TK Females (F0 generation)
1	Control Article ^c	0	0	5	22	4
2	APD334	0.4	0.08	5	22	8
3	APD334	2	0.4	5	22	8
4	APD334	4	0.8	5	22	8

TK = Toxicokinetic.

^a Based on the most recent body weight measurement. With the exception of using GD 18 body weight measurements for dose administration on GD 19 through 24.

^b Dose levels represented active moiety (APD334 free acid).

^c Vehicle containing L-arginine monohydrochloride at 0.372 mg/mL.

Table copied directly from study report (Study APD334-TOX-011).

Summary of Findings

Pregnant Sprague Dawley rats were administered vehicle or etrasimod (named APD334 in this report) at 0.4, 2, or 4 mg/kg/day from GD 6 through LD 20. There were no adverse effects on maternal clinical signs, or body weights at any dose of etrasimod, although decreased feed consumption was observed in all treated groups during gestation.

The number of pregnant females was similar in all groups. One female at 4 mg/kg/day was prematurely terminated on GD 22 due to severe, adverse clinical signs during delivery. A significant increase in post-implantation loss was observed at 4 mg/kg/day. There was also a significant increase in the number of females with stillborn pups at 2 and 4 mg/kg/day and increased number of stillborn pups per litter at 2 mg/kg/day compared to controls. The viability index was decreased at 2 and 4 mg/kg/day while the lactation index was decreased at 4 mg/kg/day compared to controls.

An increased number of F1 offspring were found dead or found missing and presumed to be cannibalized, predominantly between PND 0-4, in the 2 and 4 mg/kg/day groups compared to controls. Etrasimod-related clinical signs in F1 offspring from birth through PND 4 included cold to touch, suspected dehydration (based on skin turgor), discolored skin, skin pallor, and swelling at 4 mg/kg/day and no milk band in the stomach at 2 and 4 mg/kg/day. After litters were culled on PND 4, clinical signs related to etrasimod administration at 4 mg/kg/day included suspected dehydration (based on skin turgor) and thin appearance. Decreased maternal feed consumption during lactation corresponded to decreased pup body weights and body weight gain during the preweaning period at 2 and 4 mg/kg/day. Pup body weights at the end of the preweaning period (PND 21) were 10%, 12%, and 20% lower than controls at 0.4, 2, and 4 mg/kg/day, respectively, while total body weight gains were 11%, 14%, and 23% lower than controls, respectively. No etrasimod-related macroscopic findings were

observed in F1 offspring at the preweaning terminal necropsy; however, drug-related findings in prematurely terminated or stillborn pups included full body edema at 4 mg/kg/day and no milk in the stomach at 2 and 4 mg/kg/day. No etrasimod-related findings were observed in the F1 generation during the postweaning period, including behavioral assessments and sexual maturation, and the previously observed differences in body weights normalized during this time in all treated groups compared to controls.

There were no etrasimod-related effects on pre-coital intervals, mating index, or fertility index in the F1 generation. A reduced number of implantations and increased rate of pre-implantation loss were observed at 4 mg/kg/day compared to controls. These findings correlated with a decrease in the number of live embryos per litter at 4 mg/kg/day compared to controls.

Based on the premature termination of one female during delivery (4 mg/kg/day), increased post-implantation loss (4 mg/kg/day), increased number of females with stillborn pups (2 and 4 mg/kg/day), increased number of stillborn pups per litter (2 mg/kg/day), decreased viability index (2 and 4 mg/kg/day), and decreased lactation index (4 mg/kg/day), the NOAEL for maternal toxicity was considered the low dose of 0.4 mg/kg/day (approximately 2 times exposure at the recommended clinical dose of 2 mg, based on AUC). Based on the increased perinatal mortalities at ≥ 2 mg/kg/day, adverse clinical signs, and findings of decreased body weight/body weight gain at all doses during the preweaning period as well as a reduced number of implantations, increased rate of pre-implantation loss, and corresponding decrease in the number of live embryos per litter observed at 4 mg/kg/day, a developmental NOAEL could not be determined.

Observations and Results

F0 Dams

Mortality

One control female and three females at 4 mg/kg/day were prematurely terminated during the study.

The control female and two females at 4 mg/kg/day were prematurely terminated on lactation day (LD) 1 due to a lack of surviving pups. No clinical signs of toxicity or adverse changes in body weight or feed consumption were observed in these animals prior to termination. Upon necropsy, one of the 4 mg/kg/day females was observed to have pup tissue in the stomach.

A third female at 4 mg/kg/day was prematurely terminated on GD 22 due to severe, adverse clinical signs during delivery. No clinical signs of toxicity were observed during the gestation period. However, this animal lost 25 g (7% of body weight) from GD 18-22. There were no macroscopic abnormalities observed during necropsy. This rat was pregnant and had a litter consisting of 14 dead fetuses.

All other F0 animals survived to scheduled necropsy.

Clinical Signs

No clinical signs related to etrasimod administration were observed.

Body Weight

Although isolated differences in body weight and/or body weight gain were observed between treated and untreated groups (or in individual animals), no clear effects of etrasimod on these parameters were observed during gestation or lactation.

Feed Consumption

Decreased feed consumption was observed in all treated groups during gestation and lactation.

During gestation, significantly decreased feed consumption exceeding 10% below controls was observed at 2 and 4 mg/kg/day from GD 6-9. Significantly decreased feed consumption (less than 10% different from controls) was also observed at 4 mg/kg/day from GD 12-15. These differences resulted in an overall reduction in feed consumption over the gestation period (GD 6-18) of -6.6% at 2 mg/kg/day and -8.5% at 4 mg/kg/day compared to controls. Decreased feed consumption did not correlate with decreased body weight or decreased body weight gain during any interval in any group.

During the lactation period, significantly decreased feed consumption was consistently observed at 4 mg/kg/day, such that overall feed consumption during this period was 27% lower than the control group. Feed consumption was also decreased by approximately 10% at 2 mg/kg/day during the periods of PND 1-4 and PND 17-21 compared to controls. Decreased feed consumption did not correlate with decreased body weight or decreased body weight gain during any interval of the lactation period in any treated group.

Table 17. Feed Consumption in Female Rats Administered Etrasimod from Gestation Day 6 through Postnatal Day 20

Etrasimod (mg/kg/day)		0	0.4	2	4
Gestation					
GD 6-9	g/day	23.3	21.7	20.7*	19.5*
	% from controls	--	-7.0	-11	-16
GD 9-12	g/day	24.1	23.4	22.5	23.9
	% from controls	--	-3.3	-6.6	-1.0
GD 12-15	g/day	24.9	24.7	23.8	22.7*
	% from controls	--	-1.1	-4.7	-9.1
GD 15-18	g/day	26.9	26.5	25.7	24.8
	% from controls	--	-1.6	-4.6	-7.8
GD 6-18	g/day	24.8	24.0	23.2*	22.7*
	% from controls	--	-3.1	-6.6	-8.5
Lactation					
PND 1-4	g/day	33.59	31.98	30.17	24.25*
	% from controls	--	-4.8	-10	-28
PND 4-7	g/day	43.54	42.48	39.82	32.02*
	% from controls	--	-2.4	-8.6	-26
PND 7-10	g/day	53.54	51.79	50.53	38.23*
	% from controls	--	-3.3	-5.6	-29
PND 10-14	g/day	64.41	64.58	60.64	44.01*
	% from controls	--	+0.3	-5.9	-29
PND 14-17	g/day	67.28	68.30	62.35	44.16*
	% from controls	--	+1.5	-7.3	-34
PND 17-21	g/day	67.20	68.31	60.58	42.43*
	% from controls	--	+1.7	-9.9	-37
PND 1-21	g/day	52.31	54.17	51.59	38.09*
	% from controls	--	+3.6	-1.4	-27
*Significantly different from control group, p < 0.05. GD: gestation day; PND: postnatal day.					

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

Maternal Performance

The number of pregnant females was similar in all groups (22, 21, 22, and 22 in the control, low, mid, and high dose groups, respectively). One female at 4 mg/kg/day was prematurely terminated on GD 22 due to severe, adverse clinical signs during delivery.

A significant increase in post-implantation loss was observed at 4 mg/kg/day compared to controls (26.15% versus 12.29%).

Although gestation length significantly increased at 2 and 4 mg/kg/day compared to controls (21.9 and 21.8 days, respectively, versus 21.4 days), these values were all within the historical control range (mean: 21.9 days, range: 21.3-22.7 days). There was also a significant increase in the number of females at 2 and 4 mg/kg/day with stillborn pups (seven and four, respectively, compared to zero in the control group). This corresponded with a significant increase in the number of stillborn pups per litter at 2 mg/kg/day (3.11) but not at 4 mg/kg/day (2.24) compared to controls (0); the number of stillborn pups at both 2 and 4 mg/kg/day exceeded the historical control range (0.4-1.7). The viability index (percentage of liveborn pups that survived to PND 4) was decreased at 2 mg/kg/day (87.36%) and significantly decreased at 4 mg/kg/day (42.84%) compared to controls (92.45%). Lactation index (percentage of liveborn pups that survived to PND 21) was decreased at 4 mg/kg/day compared to controls (88.95% versus 99.40%).

Although not significant, the percentage of live male pups was lower at 4 mg/kg/day compared to controls (44.71% versus 51.30%).

No effects of etrasimod were observed on maternal-litter interactions (e.g., pup grooming, nesting, nursing).

No etrasimod-related macroscopic abnormalities were observed in F0 females following necropsy.

Table 18. Pregnancy Status of Female Rats Administered Etrasimod from Gestation Day 6 through Postnatal Day 20

Etrasimod (mg/kg/day)	0	0.4	2	4
No. females	22	22	22	22
Non-pregnant	0	1	0	0
Pregnant	22	21	22	22
Gestation Length (days)	21.4	21.3	21.9*	21.8*
With live pups at birth	22	21	22	21
No. live pups at birth	9.9	11.7	10.0	8.5
With stillborn pups	0	1	7*	4*
No. stillborn pups (total)	0	1	8	4
Stillborn pups/litter	0	0.53	3.11*	2.24
Live birth index (%)	100	99.47	96.89*	97.76
Live male pups/litter (%)	51.30	53.91	51.96	44.71
Viability index (%)	92.45	97.14	87.36	42.84*
Lactation index (%)	99.40	98.21	97.73	88.95
Implantation sites (total)	10.9	13.0*	11.9	11.8
Post-implantation loss/litter (%)	12.29	9.85	15.65	26.15*

*Statistically significant compared to respective control group, $p < 0.05$.

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

Toxicokinetics

Following the first dose of etrasimod on GD 6, plasma concentrations for all post-dose samples (i.e., 1, 4, and 24 hours post-dose) were below the lower limit of quantification (< 5.00 ng/mL) for all control animals as well as for one animal at 0.4 mg/kg/day and one animal at 4 mg/kg/day. All other treated animals had measurable concentrations of drug. There were no deviations or documented events noted in the dosing records; thus, no explanation was provided for the observed lack of drug exposure in these animals. Toxicokinetic analyses were reported both with and without the apparently anomalous data from these animals, but only the analysis excluding the anomalous values is presented below. No unexplained abnormalities were observed in the toxicokinetic data collected on GD 17 or PND 20.

Following a single dose (GD 6), C_{max} and AUC of etrasimod increased approximately proportionally with increasing dose. T_{max} of etrasimod occurred between 1-4 hours and tended to occur later with increasing dose.

On GD 17, C_{max} and AUC of etrasimod increased approximately proportionally with increasing dose. T_{max} of etrasimod occurred at 4 hours for all dose levels. No significant accumulation of etrasimod was observed between GD 6 and 20.

On PND 20, C_{max} and AUC of etrasimod increased approximately proportionally with increasing dose. T_{max} of etrasimod occurred between 1-2 hours with no clear effect of dose. A slight loss of exposure to etrasimod was observed at 0.4 and 2 mg/kg/day (PND 20 AUC values 64% and 69% those observed on GD 6, respectively) but not at 4 mg/kg/day.

Table 19. Toxicokinetic Parameters of Etrasimod in F0 Female Sprague Dawley Rats Treated from Gestation Day 6 through Postpartum Day 20

Etrasimod (mg/kg/day)	Day	T_{max} (h)	C_{max} (ng/mL)	AUC _{0-24h} (ng·h/mL)
0.4	GD 6	1	339	3730
	GD 17	4	290	4320
	PND 20	2	296	2390
2	GD 6	2	1400	16600
	GD 17	4	1640	21600
	PND 20	1	668	11400
4	GD 6	4	4000	43100
	GD 17	4	3520	51200
	PND 20	1	2960	36700

GD: gestation day; PND: postnatal day.

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

F1 Generation**Prewaning**

An increased number of mortalities was observed in pups from the 2 and 4 mg/kg/day groups compared to controls, either found dead or missing/cannibalized. Specifically, 12 and 27 pups were found dead at 2 and 4 mg/kg/day, respectively, compared to 3 pups in the control group (5.3% and 15% of liveborn pups, respectively, versus 1.4% of liveborn control pups). Most pups were found dead between PND 0-4. Additionally, 18 and 60 pups were found missing and presumed cannibalized at 2 and 4 mg/kg/day, respectively, compared to 6 pups in the control group (7.9% and 33% of liveborn pups, respectively, versus 2.8% of liveborn control pups). Most presumptive cannibalizations occurred between PND 0-4. The mortality rates at 2 and 4 mg/kg/day greatly exceed the historical control rates (maximum 1.3% of liveborn pups through PND 21 in studies with culling).

Table 20. Mortalities in Liveborn F1 Offspring

Etrasimod (mg/kg/day)	0	0.4	2	4
Liveborn Pups	217	247	228	183
Found Dead	3 (1.4%)	1 (0.4%)	12 (5.3%)	27 (15%)
Missing, Presumed Cannibalized	6 (2.8%)	7 (2.8%)	18 (7.9%)	60 (33%)

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

Etrasimod-related clinical signs in F1 offspring from birth through PND 4 included cold to touch, suspected dehydration (based on skin turgor), discolored skin, skin pallor, swelling, and no milk band in the stomach. No milk band in the stomach was also observed at 2 mg/kg/day.

Table 21. Clinical Signs in F1 Offspring – Birth Through Postnatal Day 4

Etrasimod (mg/kg/day)	0	0.4	2	4
Litters	21	21	22	19
Cold to touch				
No. times recorded		2		6
No. litters affected		1		3
Suspected dehydration				
No. times recorded				5
No. litters affected				3
Discolored skin				
No. times recorded	2	1		5
No. litters affected	1	1		2
Skin pallor				
No. times recorded		2	1	7
No. litters affected		1	1	2
Swelling				
No. times recorded				5
No. litters affected				3
No milk band in stomach				
No. times recorded			9	10
No. litters affected			3	3

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

After litters were culled on PND 4, clinical signs related to etrasimod administration at 4 mg/kg/day included suspected dehydration (based on skin turgor) and thin appearance. No clinical signs were observed in pups from the 0.4 or 2 mg/kg/day groups during this period.

Table 22. Clinical Signs in F1 Offspring – Postnatal Days 5-21

Etrasimod (mg/kg/day)	0	0.4	2	4
Litters	21	21	22	19
Suspected dehydration				
No. times recorded	4			34
No. pups affected	1			9
No. litters affected	1			5
Thin appearance				
No. times recorded	4			12
No. pups affected	1			4
No. litters affected	1			4

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

Decreased pup body weight was observed in all treated groups compared to controls. Based on sex-combined data, significantly decreased body weight (> 10% difference from controls) was observed starting at PND 7 and persisted throughout the study. Final body weight at 4 mg/kg/day was 20% lower compared to controls, and total body weight gain over the study was 23% lower than controls. At 2 mg/kg/day, final body weight was 12% lower than controls, and body weight gain from PND 1-21 was 14% lower than controls. Finally, at 0.4 mg/kg/day, final body weight was 10% lower than controls and total body weight gain was 11% lower than controls. The observed effects on body weight were similar between male and female pups.

Table 23. Body Weights of F1 Offspring

Parameter	PND	Etrasimod (mg/kg/day)			
		0	0.4	2	4
Body Weight (g)	1	7.57	7.19	8.02	7.8
	4	11.26	10.49	11.26	11.21
	7	17.85	16.41*	16.61	15.57*
	10	24.86	22.92	23.03	21.36*
	14	35.79	32.87*	33.33	30.40*
	17	43.36	39.84*	40.41	36.13*
	21	58.85	52.91*	51.96*	47.04*
Body Weight Gain (g)	1-21	51.28	45.72	43.94	39.24

*Statistically significant compared to respective control group, $p < 0.05$.
Statistical testing was not performed on body weight gain.

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

Pups at 2 and 4 mg/kg/day had significantly earlier eye opening compared to control animals (13.7 and 13.5 days, respectively, versus 14.3 days). These findings in treated animals were outside of the historical control range (13.9-14.4 days); however, these differences were not considered adverse due to the small magnitude and the slightly longer gestation length in these groups relative to controls.

No difference in the day of pinna detachment was observed with etrasimod compared to controls.

Table 24. Reflex and Development of F1 Offspring

Parameter	Etrasimod (mg/kg/day)			
	0	0.4	2	4
Pinna Detached (days)	2.8	3.1	2.6	2.7
Eyes Opened (days)	14.3	14.3	13.7*	13.5*

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

Drug-related macroscopic findings were observed in pups examined prior to PND 21 (i.e., pups found dead, stillbirths, unscheduled terminations). One pup at 4 mg/kg/day had full body edema, fluid-filled body, and edema of the subcutis resulting in malformation. Three pups at 2 mg/kg/day and four pups at 4 mg/kg/day had no milk in the stomach. Additionally, four pups at 2 mg/kg/day and seven pups at 4 mg/kg/day were autolyzed and unable to be examined. No drug-related macroscopic findings were observed at 0.4 mg/kg/day. No etrasimod-related macroscopic findings were observed in F1 offspring at the end of the preweaning period.

Table 25. Macroscopic Findings in F1 Offspring of Female Rats Administered Etrasimod from Gestation Day 6 through Postnatal Day 20 – Birth Through Postnatal Day 4

Etrasimod (mg/kg/day)	0	0.4	2	4
No. Pups Examined	3	3	20	54
No. Litters Examined	2	3	13	18
Entire body, edema				
Pups, N(%)				1(1.9)
Litters, N(%)				1(5.6)
Entire body, fluid filled				
Pups, N(%)				1(1.9)
Litters, N(%)				1(5.6)
Entire subcutis, edema - malformation				
Pups, N(%)				1(1.9)
Litters, N(%)				1(5.6)
Stomach content, absent				
Pups, N(%)			3(18.8)	4(8.9)
Litters, N(%)			2(20.0)	4(22.2)

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

Postweaning

No etrasimod-related mortality or clinical signs of toxicity were observed in F1 offspring during the postweaning period.

In males, though the observed decreased body weight during the preweaning period resolved during the postweaning period in all treated groups, reduced body weight gain (> 10% difference) was observed at 4 mg/kg/day compared to controls between PND 22-57; these findings correlated with reduced feed consumption during this period (-10% to -15% compared to controls). However, body weight gains were similar after this point. Final body weights were not significantly different between treated and control animals (-0.3%, -4.6%, and -7.6% in the low, mid, and high dose groups, respectively, compared to controls).

Figure 3. Body Weights of F1 Offspring Postweaning – Males

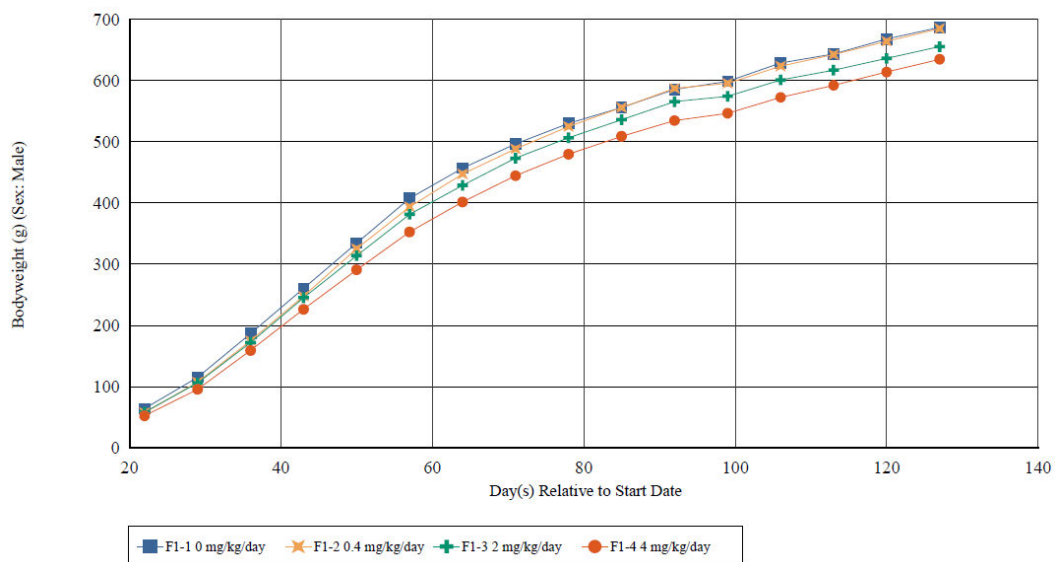


Figure copied directly from study report (APD334-TOX-011).

In females, though the observed decreased body weight during the preweaning period resolved during the postweaning period in all treated groups, reduced body weight gain (> 10% difference) was observed at 4 mg/kg/day compared to controls between PND 22-29 with no corresponding difference in feed consumption. However, body weight gains were similar after this point. Final body weights were not significantly different between treated and control animals (-3.5%, -2.8%, and -3.6% in the low, mid, and high dose groups, respectively, compared to controls).

Figure 4. Body Weights of F1 Offspring Postweaning – Females

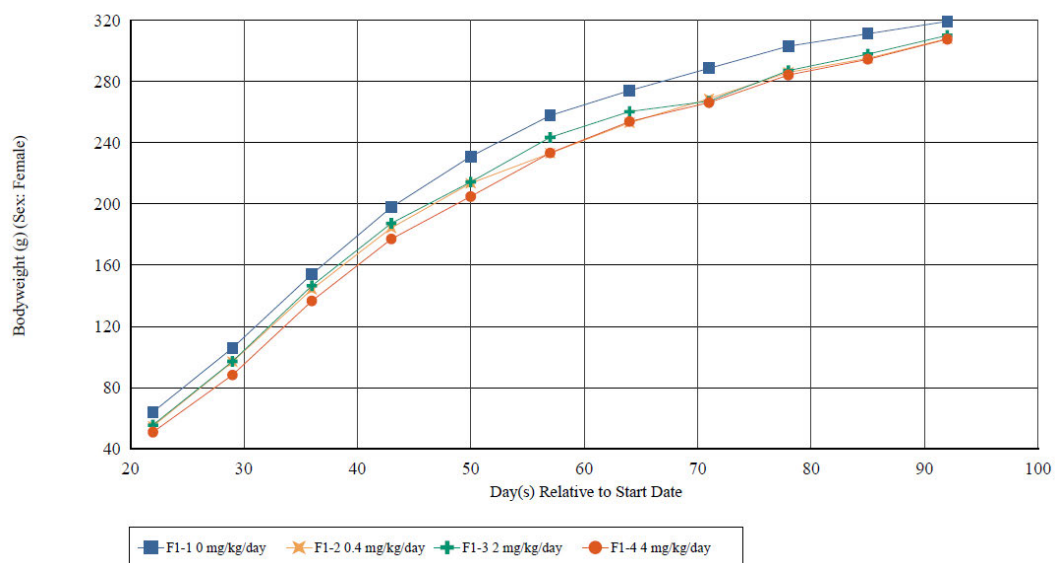


Figure copied directly from study report (APD334-TOX-011).

No effects of etrasimod were observed on vaginal patency in females. In male offspring at 2 and 4 mg/kg/day, preputial separation occurred significantly later than controls (46.0 and 49.2 days, respectively, compared to 44.4 days), and the number of days at 4 mg/kg/day exceeded the historical control range of 40.6-46.7 days. These findings were considered a consequence of the reduced pup body weight in these groups and not a direct effect of etrasimod administration.

Table 26. Sexual Maturation in F1 Males

Parameter	Etrasimod (mg/kg/day)			
	0	0.4	2	4
Preputial Separation (days)	44.4	45.3	46.0*	49.2*
Body Weight at Maturity (g)	277.6	270.4	277.1	284.5

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

No effects of etrasimod were observed on behavioral assessments (i.e., motor activity, acoustic startle habituation, Morris water maze), the number or length of estrous cycles in females, or macroscopic findings.

Reproductive Performance

There were no etrasimod-related effects on pre-coital intervals, mating index, or fertility index. Pregnancy was confirmed in 16, 17, 19, and 14 F1 females in the control, 0.4, 2, and 4 mg/kg/day groups, respectively. All values for mating, fertility, and pregnancy index were within the historical controls range.

In pregnant F1 females, no differences in body weight, body weight gain, or feed consumption were observed over the period GD 0-13.

The number of implantations was highly variable in all groups, including controls, and ranged from 3-21 per animal. The mean number of implantations was reduced at 4 mg/kg/day compared to controls (11.1 versus 14.8). Pre-implantation loss was also increased at 4 mg/kg/day compared to controls (23.76% versus 3.56%). The number of implantations and pre-implantation loss at 4 mg/kg/day were outside the respective historical control ranges. The decrease in implantations correlated with a decrease in the number of live embryos per litter at 4 mg/kg/day compared to controls (10.5 versus 14.3). No effects of etrasimod were observed at 0.4 or 2 mg/kg/day.

Table 27. Ovarian, Uterine, and Litter Observations in F1 Females

Etrasimod (mg/kg/day)	0	0.4	2	4
No. females	21	21	21	17
Non-pregnant	5	4	2	3
Pregnant	16	17	19	14
No. with live embryos	15	17	19	14
No. with any nonviable embryos	3	6	4	7
No. corpora lutea	15.5	15.5	15.2	14.9
No. implantations	14.8	15.1	14.8	11.1*
Pre-implantation loss (%)	3.56	1.94	2.42	23.76*
Total no. of embryos	14.8	15.1	14.8	11.1
No. live embryos	14.3	14.5	14.5	10.5*
No. dead embryos	0.5	0.6	0.3	0.6
Post-implantation loss (%)	2.81	3.44	1.85	5.19

*Statistically significant compared to respective control group, $p < 0.05$.

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

Toxicokinetics

A full toxicokinetic analysis was not performed in F1 offspring.

On PND 4, the plasma concentration of etrasimod increased approximately proportionally with dose in male offspring at all doses and in females at 0.4 and 4 mg/kg/day but not 2 mg/kg/day. The pup to maternal concentration ratio in male pups decreased with increasing dose, while no clear effect of dose was observed on the pup to maternal concentration ratio in female pups. Plasma concentrations were consistently higher in dams compared to pups.

Table 28. Absolute and Relative-to-Maternal Etrasimod Concentration in F1 Offspring on Postnatal Day 4

Etrasimod (mg/kg/day)	Maternal Concentration (ng/mL)	Male Pup Concentration (ng/mL)	Male Pup/Maternal Ratio (%)	Female Pup Concentration (ng/mL)	Female Pup/Maternal Ratio (%)
0.4	319	119	40.2	127	39.8
2	1530	455	29.2	339	23.4
4	3070	865	26.7	1330	43.0

Concentrations are reported at 2 hours post-dose.

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

On PND 20, the plasma concentration of etrasimod at both 2 and 4 hours post-dose increased approximately proportionally with dose in male offspring at 0.4 and 4 mg/kg/day but not 2 mg/kg/day. The plasma concentration of etrasimod at both 2 and 4 hours post-dose increased approximately proportionally with dose in female offspring at 0.4 and 2 mg/kg/day but greater than proportionally at 4 mg/kg/day. The pup to maternal concentration ratio in male pups was similar at 0.4 and 4 mg/kg/day but lower

at 2 mg/kg/day. In female pups, the pup to maternal concentration ratio at 2 hours post-dose was similar at 0.4 and 2 mg/kg/day but higher at 4 mg/kg/day, while the ratio at 4 hours post-dose was similar at 0.4 and 4 mg/kg/day but lower at 2 mg/kg/day. Plasma concentrations were consistently higher in dams compared to pups. Maternal and pup plasma concentrations were generally similar to those observed on PND 4, except for male pups at 0.4 mg/kg/day (1.5-fold higher) and 4 mg/kg/day (2-fold higher) as well as female pups at 2 mg/kg/day (1.7-fold higher).

Table 29. Absolute and Relative-to-Maternal Etrasimod Concentration in F1 Offspring on Postnatal Day 20

Etrasimod (mg/kg/day)	Time Post-Dose (hours)	Maternal Concentration (ng/mL)	Male Pup Concentration (ng/mL)	Male Pup/Maternal Ratio (%)	Female Pup Concentration (ng/mL)	Female Pup/Maternal Ratio (%)
0.4	2	296	176	59.5	129	43.6
	4	179	117	65.4	118	65.9
2	2	1330	416	31.3	580	43.6
	4	1130	363	32.1	451	39.9
4	2	2790	1710	61.3	1740	62.4
	4	2380	1370	57.6	1740	73.1

Table created by reviewer, based on Applicant's provided data (Study APD334-TOX-011).

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA ASSESSMENT AND EVALUATION

Application Number*: 216956
Supporting Document Number/s: SDN 1
CDER Receipt Date: October 14, 2022
Applicant: Pfizer, Inc.
Product: Etrasimod L-arginine
Pharmacologic Class: Sphingosine 1-phosphate subtype 1,4,5
(S1P_{1,4,5}) receptor agonist
Indication: Moderately to severely active ulcerative
colitis
Therapeutic area: Gastroenterology
Clinical Review Division: Division of Gastroenterology (DG)
Pharm/Tox Division: Division of Pharm/Tox for Immunology and
Inflammation (DPT-II)
Reviewer: Sarah J. Morgan, PhD
Supervisor/Team Leader: Jackye Peretz, PhD
Project Manager: Anum Shami, PharmD
Purpose of Review: Study Report Review
Reviewer Completion Date: April 13, 2023

Template Version: Sep 11, 2020

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Executive Summary (Clinical Relevance)

To support clinical development of etrasimod in subjects with ulcerative colitis, the Applicant submitted a nonclinical study with etrasimod using the CD4⁺CD45RB^{high} T cell transfer mouse model of ulcerative colitis. In this model, etrasimod ameliorated the effects of colitis induction including body weight loss, increased colon weight to length ratio, increased mucosal thickness, increased histopathology score, increased expression of immune cell markers, and increased expression of pro-inflammatory cytokines. The observed effects of etrasimod in this model were not dose-dependent, but generally occurred at ≥ 3 mg/kg/day (approximately 2.4-fold the clinical dose of 2 mg/day, based on HED).

Nonclinical Summary

Drug Information⁺

Type of Product:	Small molecule, oral tablet
Code/ Generic Name:	Etrasimod L-arginine (APD 334)
Chemical Name (optional):	L-Arginine salt of (R)-2-(7-(4-cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl) acetic acid
CAS# or UNII (if available):	CAS: 1206123-97-8
Structure or Biochemical Description:	
Molecular Formula/ Molecular Weight:	L-arginine salt: C ₃₂ H ₄₀ F ₃ N ₅ O ₅ , 631.69 g/mol Free acid: C ₂₆ H ₂₅ F ₃ NO ₃ , 456.17 g/mol
Comment on Excipients:	None
Comment on Impurities:	None
Proposed 505(b)(2):	N
Biosimilar:	N

Pharmacology/ Toxicology Summary

Pharmacology

Key Findings:

The pharmacodynamic effects of etrasimod were investigated in the CD4⁺CD45RB^{high} T cell transfer mouse model of ulcerative colitis. Etrasimod ameliorated the effects of colitis induction including body weight loss, increased colon weight to length ratio, increased mucosal thickness, increased histopathology score, increased expression of immune cell markers, and increased expression of pro-inflammatory cytokines. The observed effects of etrasimod in this model were not dose-dependent, but generally occurred at ≥ 3 mg/kg/day.

1 Background

1.1 Regulatory History

The Applicant, Pfizer, Inc. (Arena Pharmaceuticals was acquired by Pfizer Inc. in 2022), submitted a Type B pre-IND meeting request on January 29, 2015 (IND 125154, SDN 2). No nonclinical questions were included in this meeting; however, preliminary comments from the Agency included recommendations to conduct a study in an animal model of inflammatory bowel disease (IBD) to show the potential effectiveness of etrasimod in the intended study population and to conduct a fertility and early embryonic development (FEED) study prior to the initiation of the proposed phase 2 clinical trial.¹ The Applicant addressed these comments in a subsequent submission (SDN 6; May 4, 2015). The Applicant stated that a study in an animal model of IBD would be conducted concurrently with clinical development. Additionally, the Applicant referenced previously submitted 3-month toxicology studies in rats and dogs (b) (4) and indicated that these data would be supplemented with a study of sperm morphology and motility to adequately address all FEED study endpoints prior to the initiation of the phase 2 clinical study. The final report of a 28-day study of the effects of etrasimod on spermatogenesis in male rats was submitted (b) (4)

The Applicant submitted IND 125154 to the Division of Gastroenterology and Inborn Errors Products (DGIEP, now the Division of Gastroenterology [DG]) on May 4, 2015 (SDN 5) to develop etrasimod as a treatment for subjects with moderately to severely active ulcerative colitis. No nonclinical studies were submitted to IND 125154. (b) (4)

On June 4, 2015, no safety concerns were identified with the proposed phase 2 clinical protocol in subjects with ulcerative colitis under IND 125154 (Nonclinical Review; Dr. Sruthi King).

(b) (4)
Most nonclinical studies submitted (b) (4) were reviewed by Dr. John Dougherty under IND 142182.³

A Type B, End of Phase 2 (EOP2) meeting was held between the Applicant and the Agency on October 30, 2018. During this meeting, the Agency noted that the completed, ongoing, and planned nonclinical studies appeared adequate to support the proposed phase 3 clinical; however, a pre- and postnatal development study in rats should be conducted prior to the NDA submission. Final meeting minutes were issued following the meeting.⁴

¹ IND 125154: [Type B PIND Meeting – Preliminary Comments](#). K. Richards (DGIEP); March 20, 2015.
IND 125154: [Type B PIND Meeting – Final Meeting Minutes](#). K. Richards (DGIEP); April 2, 2015. (b) (4)

³ IND 142182: [30-Day IND Full Review](#). J. Dougherty (DDDP); June 10, 2019.

⁴ IND 125154: [End of Phase 2 Final Meeting Minutes](#). K. Richards (DGIEP); October 30, 2018.

NDA 216956 for etrasimod for the treatment of moderately to severely active ulcerative colitis was submitted to DG on October 14, 2022 (SDN 1). The purpose of the current review is to review a nonclinical pharmacology study submitted to IND [REDACTED] (b) (4) for etrasimod that is relevant to the NDA but was not previously reviewed.

1.2 Relevant INDs, NDAs, BLAs or DMFs

[REDACTED] (b) (4)
 IND 125154: Moderately to severely active ulcerative colitis (DG); active
 IND 125509: Moderately to severely active Crohn's disease (DG); active
 [REDACTED] (b) (4)
 IND 142182: Atopic dermatitis (DDD); active
 IND 146360: Moderate to severe alopecia areata (DDDP); active
 IND 146975: Eosinophilic Esophagitis (DG); active
 [REDACTED] (b) (4)
 DMF [REDACTED] (b) (4)

1.3 Previous Reviews Referenced

Application	Reviewer	Date in DARRTS	Notes
IND 142182	John Dougherty, PhD	June 10, 2019	30 day IND review (most studies submitted [REDACTED] (b) (4))
[REDACTED] (b) (4)			

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology – Primary	4.2.1.1
Effect of Treatment with 1 & 3 mg/kg APD334 on Experimental Colitis in Mice	APD334.TS.001

4 Pharmacology

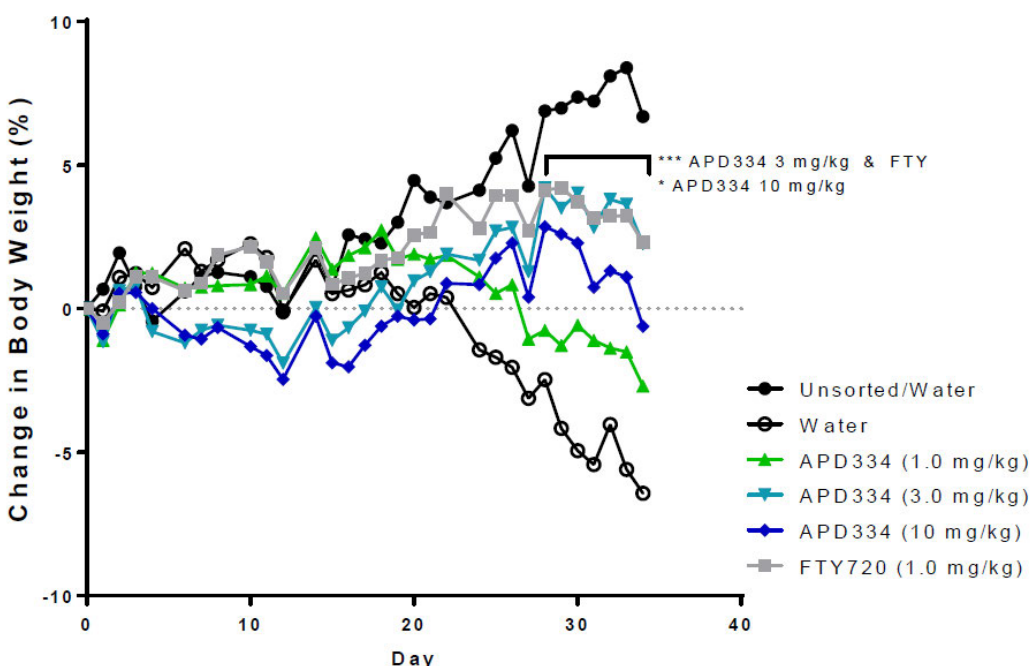
4.1 Primary Pharmacology

Effect of Treatment with 1 & 3 mg/kg APD334 on Experimental Colitis in Mice APD334.TS.001

The pharmacodynamic effects of etrasimod (named APD334 in this report) were investigated in a mouse model of ulcerative colitis. In this model, CD4⁺CD45RB^{high} T cells are isolated from wild type mice and transferred to SCID mice (“naïve”) wherein they induce colon inflammation and pathology reminiscent of human ulcerative colitis (e.g., loose, mucinous stool, body weight loss). These mice were administered etrasimod at 1, 3, or 10 mg/kg/day from the day of T cell transfer (day 1) through day 32.

While healthy mice (labeled “unsorted/water” in the figures below) gained weight during the course of the study, vehicle-treated colitis mice (“water”) lost body weight during the course of the study, particularly after day 20. Etrasimod significantly inhibited colitis-associated body weight loss at 3 and 10 mg/kg/day compared to vehicle, though the effects were independent of dose. Fingolimod (named FTY720 in this report) also significantly inhibited colitis-associated body weight loss compared to vehicle.

Figure 1. Effects of Etrasimod on Body Weight Change in Colitis Mice



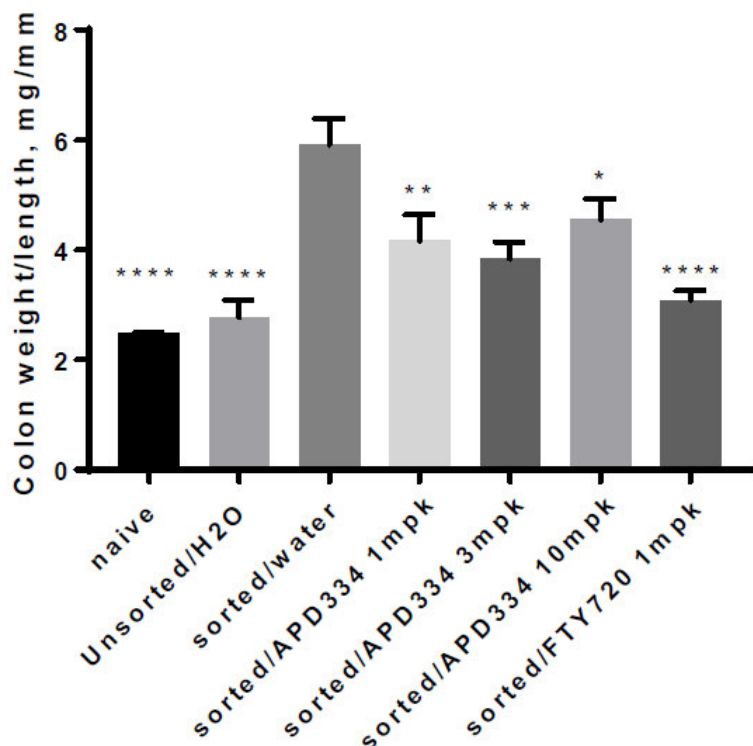
* $p < 0.05$, *** $p < 0.001$ compared to vehicle.

APD334: etrasimod; FTY720: fingolimod.

Figure copied directly from study report (Study APD334-TS-001).

The induction of colitis resulted in increased colon weight to length ratio compared to healthy animals (“naïve” or “unsorted/H₂O”). Etrasimod significantly reduced the colitis-associated increase in colon weight to length ratio at all tested doses compared to vehicle (“sorted/water”). Fingolimod also significantly reduced the colitis-associated increase in colon weight to length ratio compared to vehicle.

Figure 2. Effects of Etrasimod on Colon Weight to Length Ratio in Colitis Mice

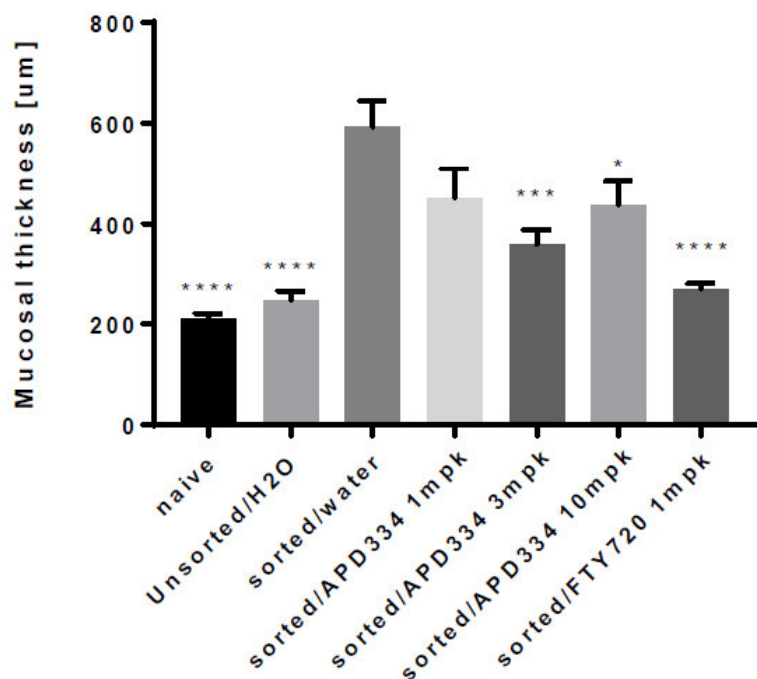


* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to vehicle.

APD334: etrasimod; FTY720: fingolimod.

Figure copied directly from study report (Study APD334-TS-001).

The induction of colitis also resulted in increased mucosal thickness compared to healthy animals. Etrasimod significantly reduced the colitis-associated increase in mucosal thickness at 3 and 10 mg/kg/day compared to vehicle. Fingolimod also significantly reduced the colitis-associated increase in mucosal thickness compared to vehicle.

Figure 3. Effects of Etrasimod on Mucosal Thickness in Colitis Mice

* $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$ compared to vehicle.

APD334: etrasimod; FTY720: fingolimod

Figure copied directly from study report (Study APD334-TS-001).

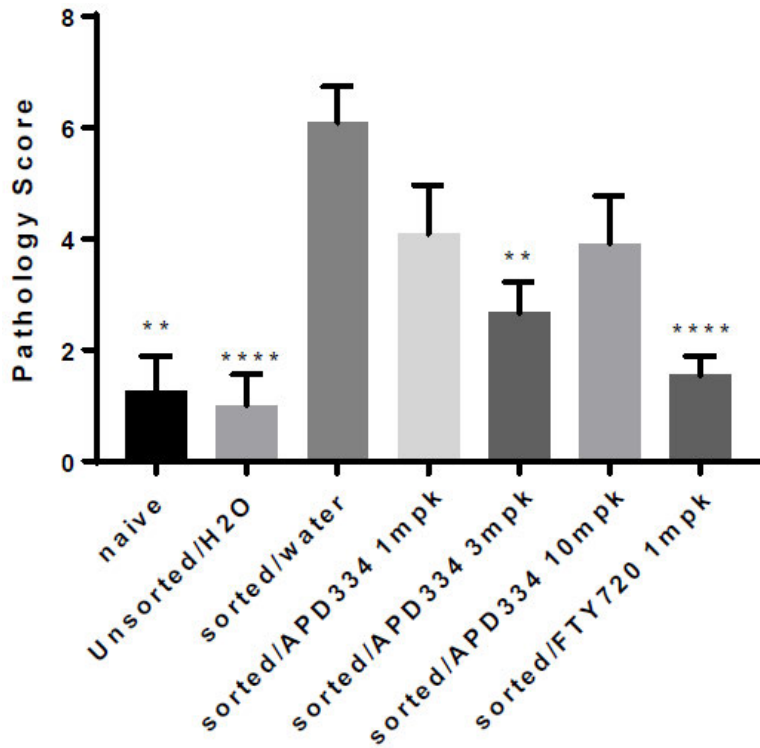
Histopathology was performed on the distal colon. The tissue was scored based on the following criteria:

- Crypt score based on abnormal crypt architecture including distortion, branching, atrophy and crypt loss (range 0-3)
- goblet cell loss (range 0-2)
- mucosal erosion and ulceration (range 0-1)
- immune cell infiltration (range 0-2)

Total histopathological score was calculated by combining the scores for each of the four parameters for a maximum score of 8. However, the Applicant did not clearly report which criteria contributed to the observed drug-related effects on this parameter.

The histopathology score was significantly increased in colitis animals compared to healthy controls. Etrasimod treatment significantly reduced the histopathology score at 3 mg/kg/day only compared to vehicle control. Fingolimod also significantly reduced the histopathology score compared to vehicle control

Figure 4. Effects of Etrasimod on Distal Colon Histopathology Score in Colitis Mice



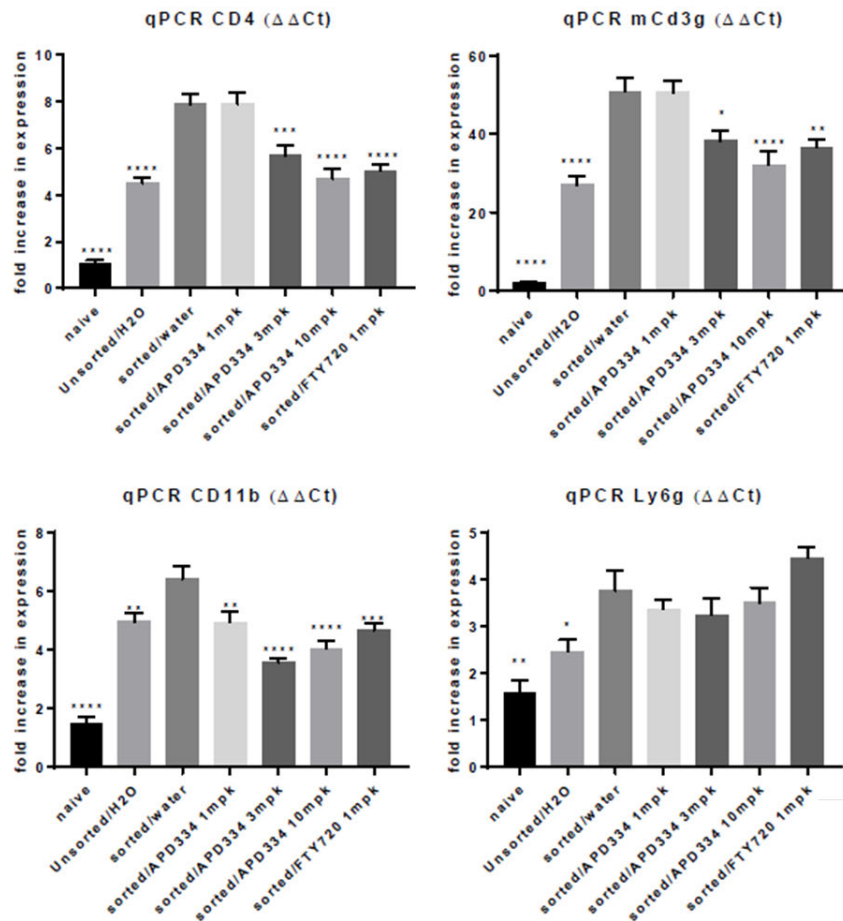
** p < 0.01, **** p < 0.0001 compared to vehicle.

APD334: etrasimod; FTY720: fingolimod

Figure copied directly from study report (Study APD334-TS-001).

Compared to healthy mice, colitis mice demonstrated increased mRNA expression of immune cell markers and cytokines in colonic tissue. Specifically, colitis mice had higher levels of CD4 (indicative of T cells, natural killer cells, and/or dendritic cells), CD3 γ (T cells), and CD11b (monocytes, natural killer cells, and/or dendritic cells), while levels of Ly6G (neutrophils and/or granulocytes) were unchanged. Etrasimod treatment resulted in decreased levels of CD4 (≥ 3 mg/kg/day), CD3 γ (≥ 3 mg/kg/day), and CD11b (all doses) compared to vehicle controls, while no differences were observed on Ly6G.

Figure 5. Effects of Etrasimod on Inflammatory Cell Recruitment as Determined by mRNA Expression in Distal Colon Tissue of Colitis Mice

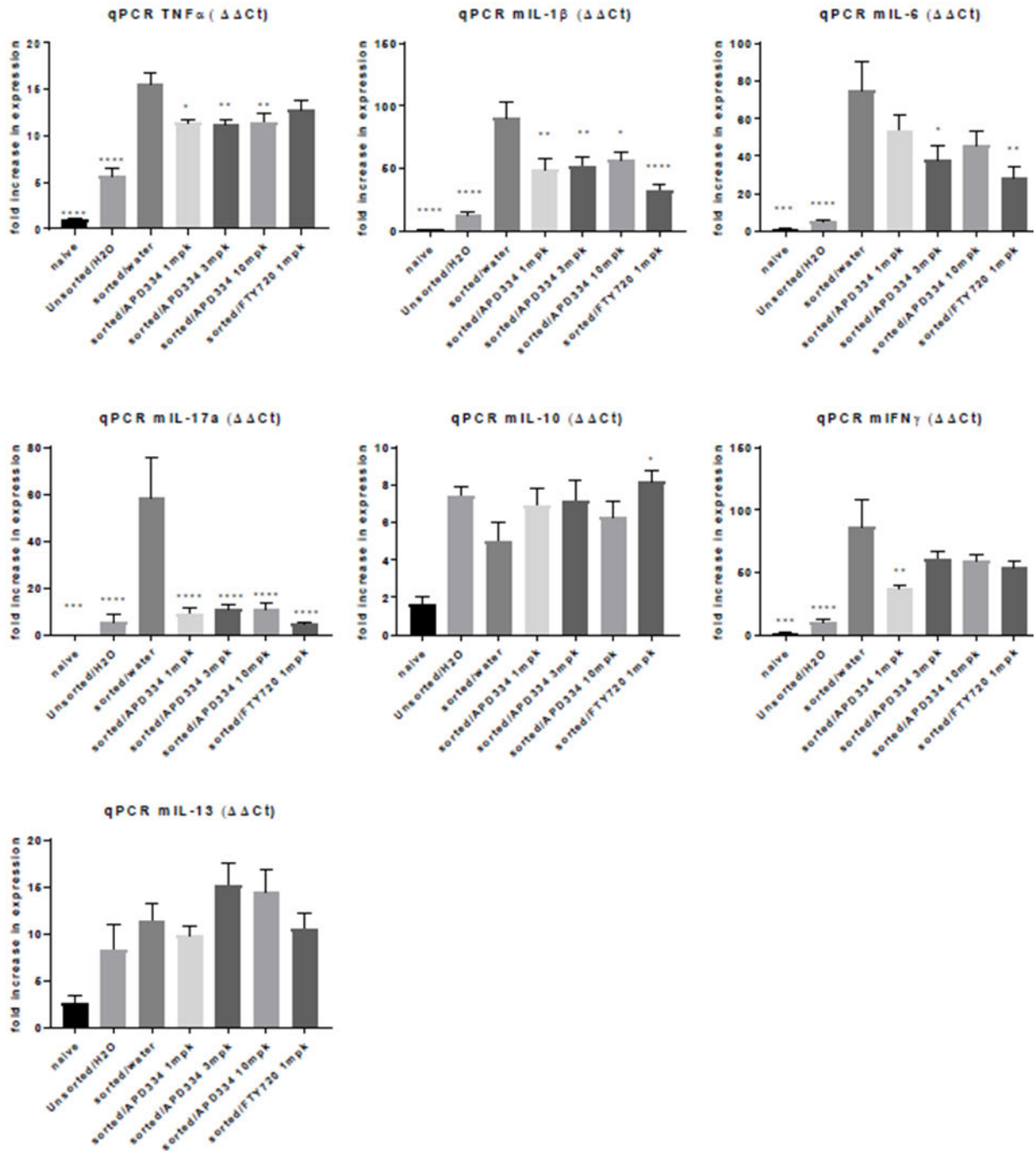


* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 compared to vehicle.
APD334: etrasimod; FTY720: fingolimod.

Figure copied directly from study report (Study APD334-TS-001).

Similarly, mRNA expression of T cell and/or monocyte-derived pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interleukin (IL)-1 β , IL-6, IL-17A, and interferon gamma (IFN- γ), was higher in colitis mice compared to healthy animals (“naïve” or “unsorted/H₂O”), while no differences were observed in the expression of IL-10 or IL-13 compared to vehicle control mice (“unsorted/H₂O”). Etrasimod treatment significantly decreased mRNA expression of TNF- α (all doses), IL-1 β (all doses), IL-6 (3 mg/kg/day only), IL-17A (all doses), and IFN- γ (1 mg/kg/day only) compared to vehicle controls.

Figure 6. Effects of Etrasimod on mRNA Expression of Pro-Inflammatory Cytokines in Distal Colon Tissue of Colitis Mice



* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 compared to vehicle.
 APD334: etrasimod; FTY720: fingolimod.

Figure copied directly from study report (Study APD334-TS-001).

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA ASSESSMENT AND EVALUATION

Application Number*: 216956

Supporting Document Number/s: 1

CDER Receipt Date: October 14, 2022

Applicant: Pfizer, Inc.

Product: Etrasimod L-arginine

Pharmacologic Class: Sphingosine 1-phosphate subtype 1,4,5
(S1P_{1,4,5}) receptor agonist

Indication: Moderate to severe ulcerative colitis

Therapeutic area: Gastroenterology

Clinical Review Division: Division of Gastroenterology (DG)

Pharm/Tox Division: Division of Pharm/Tox for Immunology and
Inflammation (DPT-II)

Reviewer: Sarah J. Morgan, PhD

Supervisor/Team Leader: Jackye Peretz, PhD

Project Manager: Anum Shami, PharmD

Purpose of Review: Study Report Review
Final mouse and rat carcinogenicity study
reports

Reviewer Completion Date: April 4, 2023

Template Version: Sep 11, 2020

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Figure 8. Body Weights in Female Rats Administered Etrasimod 29

Executive Summary (Clinical Relevance)

Etrasimod was positive for carcinogenicity in mice but negative for carcinogenicity in rats.

In a two-year carcinogenicity study in mice, a statistically significant positive dose-response in the incidences of hemangiosarcoma malignant tumor (multicentric neoplasm) and combined hemangioma/hemangiosarcoma were observed at 6 and 20 mg/kg/day males and females compared to controls. These doses correspond to 46- and 134-fold, respectively, the MRHD of 2 mg/day etrasimod (based on AUC comparison).

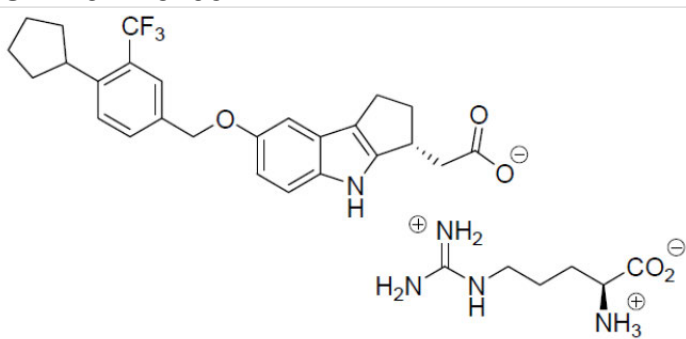
In a two-year carcinogenicity study in rats, no carcinogenic findings were observed in males or females administered up to 20 mg/kg/day for 86 to 89 weeks, after which mortalities precluded continued conduct of the study. This dose corresponds to 88- and 198-fold the MRHD of 2 mg/day etrasimod in males and females, respectively (based on AUC comparison).

The Executive Carcinogenicity Assessment Committee concurred that the two-year mouse carcinogenicity study was adequately conducted, and there was a statistically significant, drug-related increase in the incidences of hemangiosarcoma malignant tumor (multicentric neoplasm) and combined hemangioma/hemangiosarcoma at the mid and high doses (6 and 20 mg/kg/day) in male and female mice. The Committee also concurred that the two-year rat carcinogenicity study was adequately conducted, and there were no drug-related neoplasms in either males or females.¹

¹ NDA 216956: [ECAC Meeting Minutes](#). R. Aziz (OND IO); April 4, 2023.

Nonclinical Summary

Drug Information⁺

Type of Product:	Small molecule (oral tablet)
Code/ Generic Name:	Etrasimod L-arginine (APD 334, AR401959)
Chemical Name (optional):	L-Arginine salt of (R)-2-(7-(4-cyclopentyl-3-(trifluoromethyl)benzyloxy)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl) acetic acid
CAS# or UNII (if available):	CAS: 1206123-97-8 UNII: 6WH8495MMH
Structure or Biochemical Description:	
Molecular Formula/ Molecular Weight:	L-arginine salt: C ₃₂ H ₄₀ F ₃ N ₅ O ₅ , 631.69 g/mol Free acid: C ₂₆ H ₂₅ F ₃ NO ₃ , 456.17 g/mol
Comment on Excipients:	None
Comment on Impurities:	None
Proposed 505(b)(2):	N
Biosimilar:	N

Pharmacology/ Toxicology Summary

Genotoxicity

Key Findings:

Etrasimod was not mutagenic or clastogenic in a standard battery of genotoxicity studies.²

(b) (4), a starting material and potential drug substance impurity, was negative for genotoxicity in a bacterial reverse mutation assay and is therefore being controlled as a non-mutagenic impurity in accordance with ICH Q3A.

(b) (4) a raw/starting material and potential process impurity, was positive for the ability to induce reverse mutations at selected loci of selected strains of *S. typhimurium* and at the tryptophan locus of *E. coli* WP2uvrA in the presence and absence of an exogenous metabolic activation system. This substance is therefore considered a class

² IND 142182: [Nonclinical Review](#) (most studies submitted (b) (4) J. Dougherty (DDDP); June 10, 2019.

2 impurity and is being controlled in the (b) (4) product (b) (4) at a maximum level of (b) (4) ppm (i.e., below the toxic threshold of concern of (b) (4) µg/day based on a daily dose of etrasimod of 2 mg) in accordance with ICH M7(R1) option 2.

Other potentially genotoxic impurities (class 2 and 3) include (b) (4). These impurities are being controlled in accordance with ICH M7(R1).

Carcinogenicity

Key Findings:

In a 2-year carcinogenicity study in mice, male and female animals were orally administered etrasimod at 2, 6, or 20 mg/kg/day. Etrasimod dose-dependently and significantly increased the mortality rate in high dose males and females compared to vehicle controls. Drug-related non-neoplastic lesions were observed in the lungs (minimal to marked alveolar histiocytosis, minimal to moderate alveolar eosinophilic accumulation, minimal to mild multifocal fibrosis, minimal to mild osseous metaplasia, and minimal fibrin deposition) in all treated groups and in the thymus (minimally to moderately increased lymphocytes in the medulla) in the mid and high dose groups. Additionally, angiomatous hyperplasia was observed in various tissues (bone marrow, epididymides, heart, liver, subcutis, femorotibial joint) in high dose males and in the uterus of mid and high dose females with increased incidence compared to control animals. Etrasimod-related neoplastic lesions included a dose-dependent increase in the incidence of hemangiosarcomas (multicentric neoplasm) and combined hemangiomas/hemangiosarcomas in both male and female mice compared to control animals.

In a 2-year carcinogenicity study in rats, male and female animals were orally administered etrasimod at 2, 6, or 20 mg/kg/day. Etrasimod did not significantly affect the mortality rate. Drug-related non-neoplastic lesions were observed in the lymphoid tissues (increased medullary lymphocytes in the thymus; decreased marginal zone lymphocytes and increased incidence of extramedullary hematopoiesis in the spleen; sinus dilation, sinus histiocytosis and/or sinus erythrocytosis/erythrophagocytosis in the mandibular, mesenteric, iliac, renal and/or mediastinal lymph nodes) and brain (minimal to mild focal thalamic mineralization) of males and females at all doses as well as in the liver (basophilic, clear, and/or eosinophilic foci of cellular alteration and minimal to mild bile duct hyperplasia) of all treated females and males in the mid and high dose groups. No statistically significant increases in tumor incidences occurred in this study.

1 Background

1.1 Regulatory History

The original IND for etrasimod, IND (b) (4) was submitted to the Division of Neurology Products (DNP) (b) (4). The Applicant has subsequently submitted INDs 125154, 125509, (b) (4), 142182, 146360, and 146975 (b) (4) for etrasimod to various divisions. To support the development of etrasimod for use in subjects with ulcerative colitis (IND 125154), (b) (4)

(b) (4) II nonclinical studies (including carcinogenicity protocols and discussion of early termination criteria) were submitted (b) (4)

Etrasimod has been evaluated in 2-year oral carcinogenicity studies in rat and mouse. Special protocol assessment (SPA) requests were submitted (b) (4)

(b) (4) The SPAs were reviewed by the Executive CAC (ECAC), and their agreement and recommendations were communicated to the Applicant (b) (4)

Rat Study:

- On August 7, 2020 (SDN 44), the Applicant requested advice on their proposed modification plans for the 2-year carcinogenicity study in rats, based on decreased survival at Week 78. The Division of Pharmacology/Toxicology for Neurology (DPT-N) advised that no actions be taken at that time but recommended standard stopping and termination criteria in the event that survival decreased further.⁵
- On September 17, 2020, the Applicant notified DPT-N by email that survival in the female water control group had declined to 25, and the Applicant planned to terminate all females when survival in this group reached 20; the Division agreed with this plan.⁶ On October 21, 2020 (SDN 47), the Applicant notified the Division that survival in the female water control group had reached 20 at Week 90, and all surviving females were terminated at that time.
- In October 2020, the Applicant notified DPT-N by email that survival in the male high dose group had declined to 20. The Applicant stopped dosing in this group and planned to terminate all surviving high dose males when survival in this group reached 15 prior to Week 100 or to terminate all males in the study should survival in the high dose male group reach 15 at Week 100 or later; the Division agreed with this plan.⁷ On October 27, 2020, the Applicant notified the Division

(b) (4)

that survival in the control males had reached 20 at Week 91, and all surviving males were terminated at that time (SDN 48).

Mouse Study:

- On October 2, 2020 (SDN 46), the Applicant requested advice from DPT-N on their proposed modification plans for the 2-year carcinogenicity study in mice, based on decreased survival at Week 79. ECAC recommended that no actions be taken at that time but provided recommended stopping and termination criteria should survival decrease further.⁸
- On December 30, 2020 (SDN 53), the Applicant notified DPT-N that survival in the high dose male group had reached 20 at Week 91, and dose administration was suspended at that time. This group reached 15 survivors at Week 99, triggering termination of surviving males. Survival in the mid dose male group reached 20 at Week 99, and dose administration was suspended at that time. However, mid dose males did not reach 15 survivors prior to scheduled necropsy at the end of the study period. Water control, vehicle control, and low-dose male groups were administered control or etrasimod daily, as appropriate, until the end of the scheduled dosing period (Week 104).
- On November 17, 2020 (SDN 51), the Applicant notified DPT-N that survival in the high dose female group had reached 20 at week 84, and dose administration was suspended at that time. On December 30, 2020 (SDN 53), the Applicant notified the Division that survival in the high dose female group had reached 15 at Week 91, triggering termination of surviving females in that group. Survival in mid and low dose females reached 20 at Weeks 92 and 98, respectively, resulting in suspension of dose administration. The mid dose female group reached 15 survivors at Week 96, triggering termination of surviving females in this group. Survival in the female water control group reached 20 at Week 100, triggering termination of all surviving females at this time.

On October 14, 2022 (SDN 1), the Applicant submitted NDA 216956 to the Division of Gastroenterology (DG) for etrasimod as a treatment of moderately to severely active ulcerative colitis. The NDA submission included the final study reports and corresponding SEND datasets for the 2-year carcinogenicity studies in mice and rats. The adequacy and findings of these studies are the subject of this review.

(b) (4)

1.2 Relevant INDs, NDAs, BLAs or DMFs

(b) (4)
IND 125154: Inflammatory bowel disease - ulcerative colitis (DG); active
IND 125509: Moderately to severely active Crohn's disease (DG); active
(b) (4)

IND 142182: Atopic dermatitis (DDD); active
IND 146360: Moderate to severe alopecia areata (DDDP); active
IND 146975: Eosinophilic esophagitis (DG); active
(b) (4)

DMF (b) (4)

8 Carcinogenicity

8.1 APD334 L-Arginine: A 104-Week Oral Oncogenicity Study in Mice

Study no.:	APD334.TOX.003
Study report location:	EDR 4.2.3.4
Study initiation date:	March 5, 2019
Conducting laboratory and location:	 (b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	Etrasimod (APD334 L-arginine) Lot # 5015-12-16 Purity 99.95%
Prior Exec CAC Dose Concurrence:	Y
Basis for Dose Selection:	The high dose of 20 mg/kg/day was based on findings of mortality at 200 mg/kg/day and adverse histopathology findings (lung and thyroid) at ≥ 60 mg/kg/day in the 91-day preliminary study (Study 900-169). The mid and low doses were selected to provide an appropriate dose-response.

Reviewer Carcinogenicity Conclusion (negative/ positive): Positive

ECAC Carcinogenicity Conclusion (negative/ positive): Positive

Key Study Findings

- Etrasimod had a dose-dependent effect on the mortality rate with significantly increased mortalities in high dose males and females compared to vehicle controls. Mortality rates were 72%, 64%, 66%, 72%, and 77% for water control, vehicle control, low, mid, and high dose males, respectively. Mortality rates were 73%, 69%, 69%, 77%, and 77% in female mice, respectively.
- Etrasimod-related non-neoplastic lesions were observed in the lungs in all treated groups and in the thymus in the mid and high dose groups. Additionally, angiomatous hyperplasia was observed in various tissues (bone marrow, epididymides, heart, liver, subcutis, femorotibial joint) in high dose males and in the uterus of mid and high dose females with increased incidence compared to control animals.
- Etrasimod-related neoplastic lesions included a dose-dependent increase in the incidence of combined hemangioma and hemangiosarcomas (multicentric neoplasm) in both male and female mice compared to control animals.

Tumor Findings:

Independent analyses of the tumor findings were conducted by FDA statistical reviewer, Feng Zhou, M.S.^{9,10} Statistically significant positive dose-responses in the incidences of hemangiosarcoma malignant tumor (multicentric neoplasm) and combined hemangioma/hemangiosarcoma were observed in both male and female mice ($P < 0.005$) when compared with the vehicle control group. The incidences of hemangiosarcoma malignant tumor and combined hemangioma/hemangiosarcoma in the mid and high dose groups were statistically significantly higher compared to the vehicle control group ($P < 0.01$). Tumor incidences were similar between the vehicle control and water control groups.

Methods

Doses: 2, 6, 20 mg/kg/day
Frequency of dosing: Once daily
Number/Sex/Group: Control groups: 75/sex/group
Treated groups: 65/sex/group
TK control groups: 6/sex/group
TK treated groups: 27/sex/group (including 3 potential replacement animals/group)
Dose volume: Initially, the low, mid, and high dose groups were dosed at 1, 3, and 10 mL/kg, respectively. Beginning on day 92, all groups were administered 10 mL/kg.
Formulation/Vehicle: L-arginine monohydrochloride 0.92 mg/mL in deionized water
Route of administration: ORAL GAVAGE
Species: MOUSE
Strain: CD1(ICR)
Age: 10 weeks
Comment on Study Design and Conduct: The carcinogenicity study was adequately conducted.

⁹ NDA 216956: Email correspondence from F. Zhou to S. Morgan; RE: REVIEW REQUESTED: NDA 216956 General Consult Request (FRM-CONSULT-01); November 17, 2022.

¹⁰ NDA 216956: [Statistical Review and Evaluation](#). F. Zhou (DB VI); April 3, 2023.

Dosing Comments (Dose Adjustments or Early Termination): Dose administration was suspended due to survival in the indicated group reaching 20:

- High dose females: Week 84
- High dose males: Week 91
- Mid dose females: Week 92
- Low dose females: Week 98
- Mid dose males: Week 99

All surviving animals in the group were terminated due to survival in the group reaching 15:

- High dose females: Week 91
- Mid dose females: Week 96
- High dose males: Week 99

All surviving females from the water control, vehicle control, and low dose groups were terminated at week 100 due to survival in the water control female group reaching 20.

Dosing Solution Analysis: During weeks 1-3 of the study, the 2 mg/mL formulation concentrations were 80.5%, 73.5%, and 85% of nominal, respectively. Following optimization of the formulation preparation procedures and batch sizes, all further concentrations (week 4 onward) were within the acceptable range ($\pm$ (b)₍₄₎ % of nominal). As all treated groups were equally affected and the deviations were not large, these findings do not appear to have affected the validity of the study.

Table 1. Design of 2-Year Carcinogenicity Study with Etrasimod in Mice

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dose Volume (mL/kg) ^a	Animal Numbers	
					Male	Female
1	Water control	0	0	10	1001 to 1075	1501 to 1575
2	Control article ^b	0	0	10	2001 to 2075	2501 to 2575
3	APD334 L-arginine	2	2/0.2 ^c	1/10 ^c	3001 to 3065	3501 to 3565
4	APD334 L-arginine	6	2/0.6 ^c	3/10 ^c	4001 to 4065	4501 to 4565
5	APD334 L-arginine	20	2	10	5001 to 5065	5501 to 5565
Group No.	Test Material	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Dose Volume (mL/kg) ^a	Animal Numbers	
					Toxicokinetic	
					Male	Female
6	Water control	0	0	10	6001 to 6006 ^d	6501 to 6506 ^d
7	Control article ^b	0	0	10	7001 to 7006 ^d	7501 to 7506 ^d
8	APD334 L-arginine	2	2/0.2 ^c	1/10 ^c	8001 to 8027 ^d	8501 to 8527 ^d
9	APD334 L-arginine	6	2/0.6 ^c	3/10 ^c	9001 to 9027 ^d	9501 to 9527 ^d
10	APD334 L-arginine	20	2	10	10001 to 10027 ^d	10501 to 10527 ^d

^a Based on the most recent body weight measurement.

^b The control article was an aqueous solution of L-arginine hydrochloride at the concentration of L-arginine (0.92 mg/mL) in the high dose of the formulation (20 mg/kg/day).

^c Beginning on Day 92, the dose concentrations for the low- and mid-dose groups were changed to 0.2 mg/mL and 0.6 mg/mL, respectively, using dose volumes of 10 mL/kg for both groups, so as to allow for equal dose volumes across all groups.

^d 3 additional animals/sex/group were included as possible replacement animals.

Table adapted from study report (Study APD334-TOX-003).

Observations and Results

Mortality

Animals were observed at least twice daily for morbidity and mortality. Increased early terminations related to hemangiomas/hemangiosarcomas were reported at ≥ 6 mg/kg/day and were attributed to etrasimod administration. Other causes of morbidity/moribundity were observed with similar incidence in all groups, including controls, and therefore were considered unrelated to etrasimod administration.

Due to mortality, dosing was suspended and/or groups were terminated prior to completion of the planned 104-week study. the timeline of dosing cessation and/or termination for this study is provided in Table 3.

Table 2. Unscheduled Deaths Related to Administration of Etrasimod in Mice

Sex	Males					Females				
	0 (WC)	0 (VC)	2	6	20	0 (WC)	0 (VC)	2	6	20
Etrasimod (mg/kg/day)	0 (WC)	0 (VC)	2	6	20	0 (WC)	0 (VC)	2	6	20
No. Unscheduled Deaths	54	48	43	47	50	55	52	45	50	50
No. with hemangiosarcoma or hemangioma as cause of death	7	3	5	13	26	2	3	2	10	15

VC: vehicle control (20 mg/kg/day L-arginine hydrochloride in water); WC: water control.

Table created by reviewer, based on Applicant's raw data (Study APD334-TOX-003).

Table 3. Dosing Cessation and Termination Timetable in Mice

Sex	Males					Females				
Etrasimod (mg/kg/day)	0 (WC)	0 (VC)	2	6	20	0 (WC)	0 (VC)	2	6	20
No. Unscheduled Deaths	54	48	43	47	50	55	52	45	50	50
Week of Dosing Cessation	104	104	104	99	91	100	100	92	98	84
Week of Termination	104	104	104	104	99	100	100	100	96	91

VC: vehicle control (20 mg/kg/day L-arginine hydrochloride in water); WC: water control.

Table created by reviewer, based on Applicant's raw data (Study APD334-TOX-003).

Independent analyses of survival were conducted by FDA statistical reviewer, Feng Zhou, M.S.^{9,10} The survival distributions of mice in all treatment groups were estimated using the Kaplan-Meier product limit method. For control, low, medium, and high dose groups, the dose-response relationship was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test.

The statistical reviewer's analysis showed the numbers (percent) of deaths that occurred prior to scheduled termination were 54 (72%), 48 (64%), 43 (66%), 47 (72%), or 50 (77%) in male mice and 55 (73%), 52 (69%), 45 (69%), 50 (77%), or 50 (77%) in female mice in the WC, VC, LD, MD, and HD groups, respectively. Survival analyses showed a statistically significant dose-response relationship in mortality in both males and females when compared with the VC group ($P_s < 0.05$). Mortality rates in HD groups were statistically significantly higher than VC group for both male and female mice ($P_s < 0.05$). Death rates for the WC group were similar as that of the VC group for both male and female mice.

Table 4. Intercurrent Mortality Rate in Male Mice

Week / Type of Death	Water Control		Vehicle Control		Low Dose		Mid Dose		High Dose	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	1	1.33	3	4.00	7	10.77	3	4.62	9	13.85
53 - 78	16	22.67	16	25.33	15	33.85	15	27.69	22	47.69
79 - 96	20	49.33	22	54.67	16	58.46	26	67.69	17	73.85
97 - 104	17	72.00	7	64.00	5	66.15	3	72.31	2	76.92
Terminal sacrifice	21	28.00	27	36.00	22	33.85	18	27.69	15	23.08
Total	75	.	75	.	65	.	65	.	65	.
Test			All Dose Groups	Vehicle C vs. Low	Vehicle C vs. Mid	Vehicle C vs. High	Vehicle C vs. Water C			
Dose-Response (Likelihood Ratio)			0.0041	0.6168	0.3004	0.0055	0.6568			
Homogeneity (Log-Rank)			0.0280	0.6132	0.2929	0.0044	0.6535			

Table copied from the FDA statistical review by Feng Zhou, M.S (Tables 2A and 4A).

Table 5. Intercurrent Mortality Rate in Female Mice

Week / Type of Death	Water Control		Vehicle Control		Low Dose		Mid Dose		High Dose	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	4	5.33	8	10.67	5	7.69	7	10.77	7	10.77
53 - 78	20	32.00	20	37.33	17	33.85	24	47.69	27	52.31
79 - 96	27	68.00	23	68.00	21	66.15	19	76.92	16	76.92
97 - 104	4	73.33	1	69.33	2	69.23	.	.	.	.
Terminal sacrifice	20	26.67	23	30.67	20	30.77	15	23.08	15	23.08
Total	75	.	75	.	65	.	65	.	65	.

Test	All Dose Groups	Vehicle C vs. Low	Vehicle C vs. Mid	Vehicle C vs. High	Vehicle C vs. Water C
Dose-Response (Likelihood Ratio)	0.0069	0.9484	0.1668	0.0124	0.8028
Homogeneity (Log-Rank)	0.0344	0.9478	0.1591	0.0107	0.8002

Table copied from the FDA statistical review by Feng Zhou, M.S (Tables 2B and 4B).

Figure 1. Kaplan-Meier Survival Functions for Male Mice

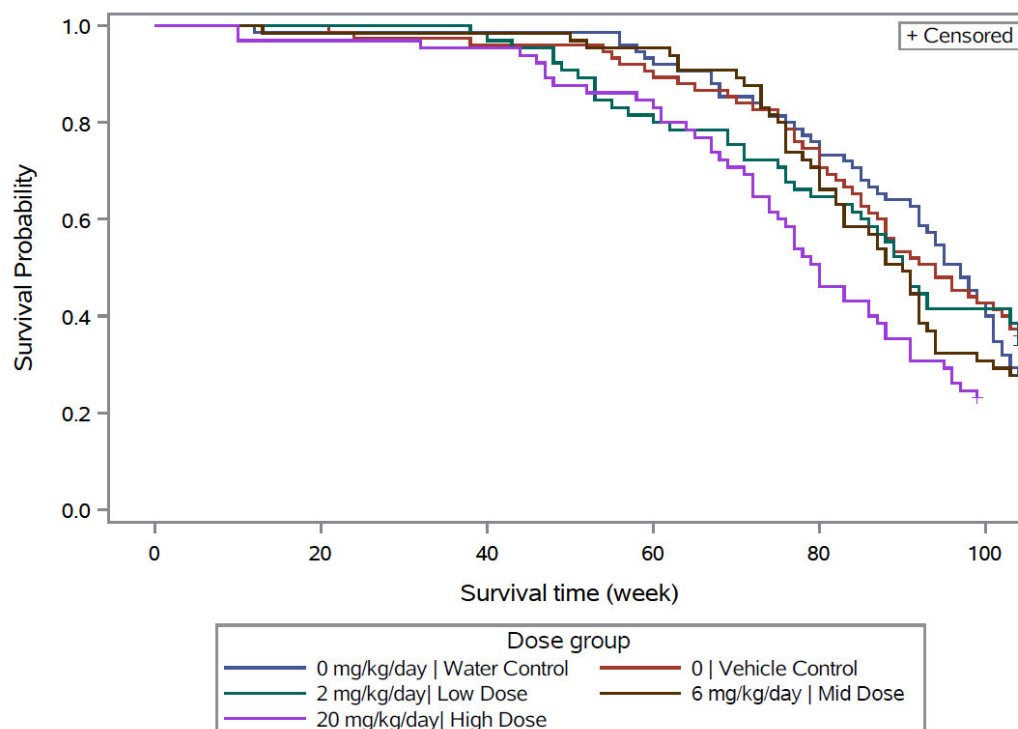


Figure copied from the FDA statistical review by Feng Zhou, M.S (Figure 2A).

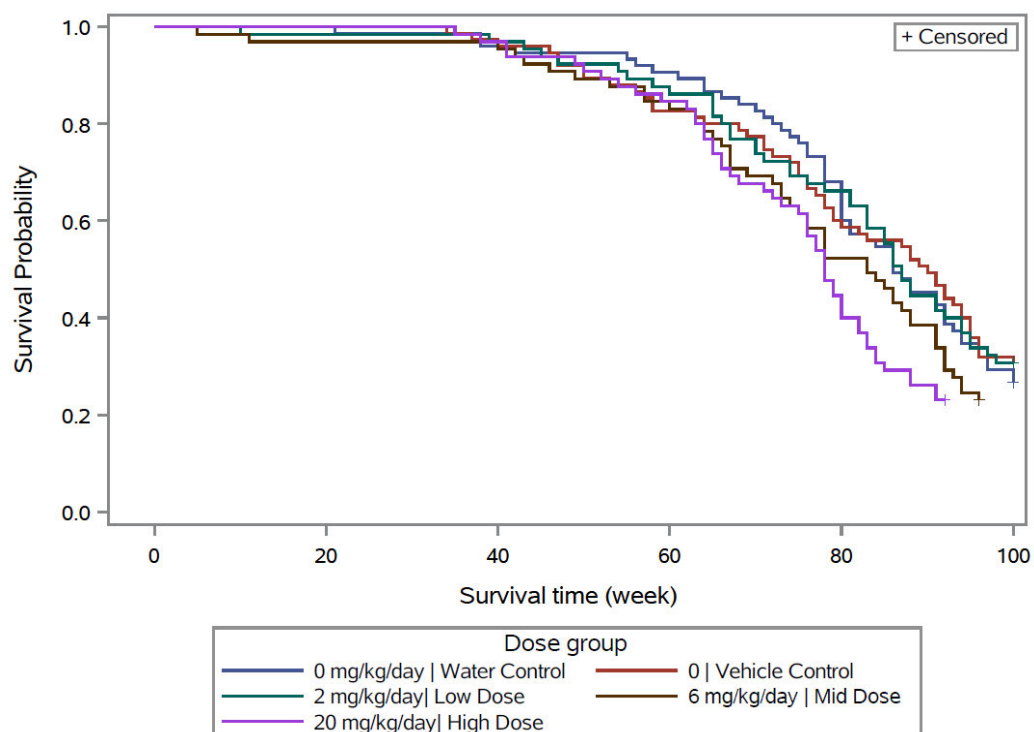
Figure 2. Kaplan-Meier Survival Functions for Female Mice

Figure copied from the FDA statistical review by Feng Zhou, M.S (Figure 2B).

Clinical Signs

Detailed clinical examinations were conducted weekly throughout the study. No etrasimod-related clinical signs were observed, including no trends in the incidence or frequency of palpable masses/nodules.

Body Weights

Decreased absolute body weight was observed in all treated groups compared to the WC throughout the study, though differences rarely exceeded 10%. Decreased body weight was also observed in all treated groups compared to the VC throughout the study, with differences exceeding 10% on some occasions from Week 76 onward. Similarly, dose-independent decreased body weight gain was observed in all treated males compared to the WC (-10% to -25%) and VC groups (-16% to -30%) and in LD an HD females compared to the WC (-31% and -28%, respectively) and VC groups (-30% and -27%).

Figure 3. Body Weights in Male Mice Administered Etrasimod

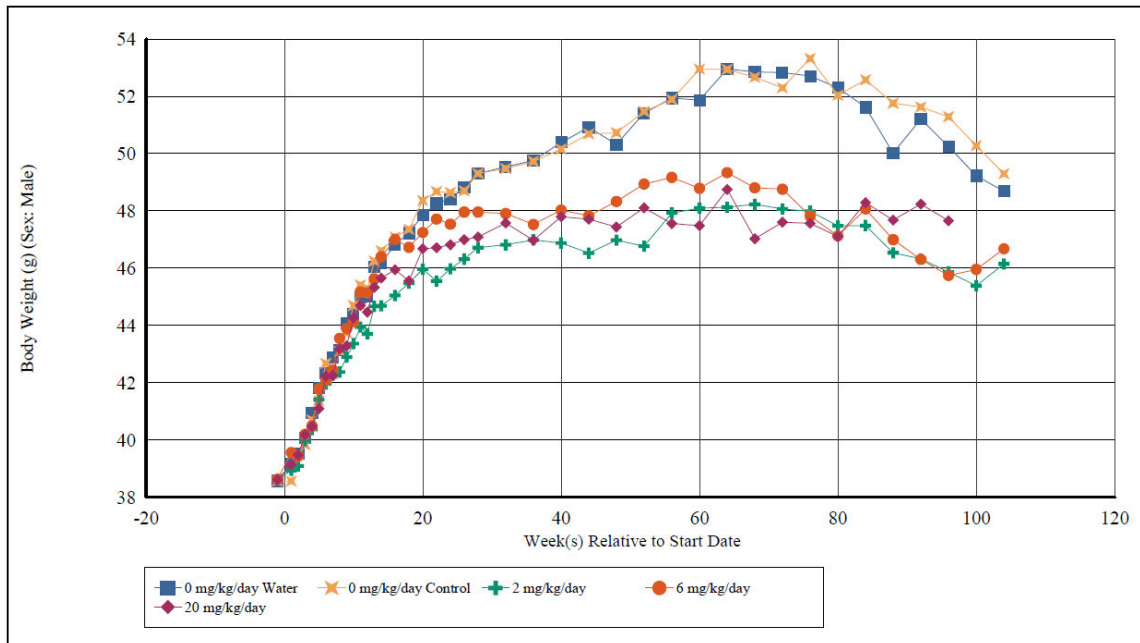


Figure copied directly from study report (Study APD334-TOX-003).

Figure 4. Body Weights in Female Mice Administered Etrasimod

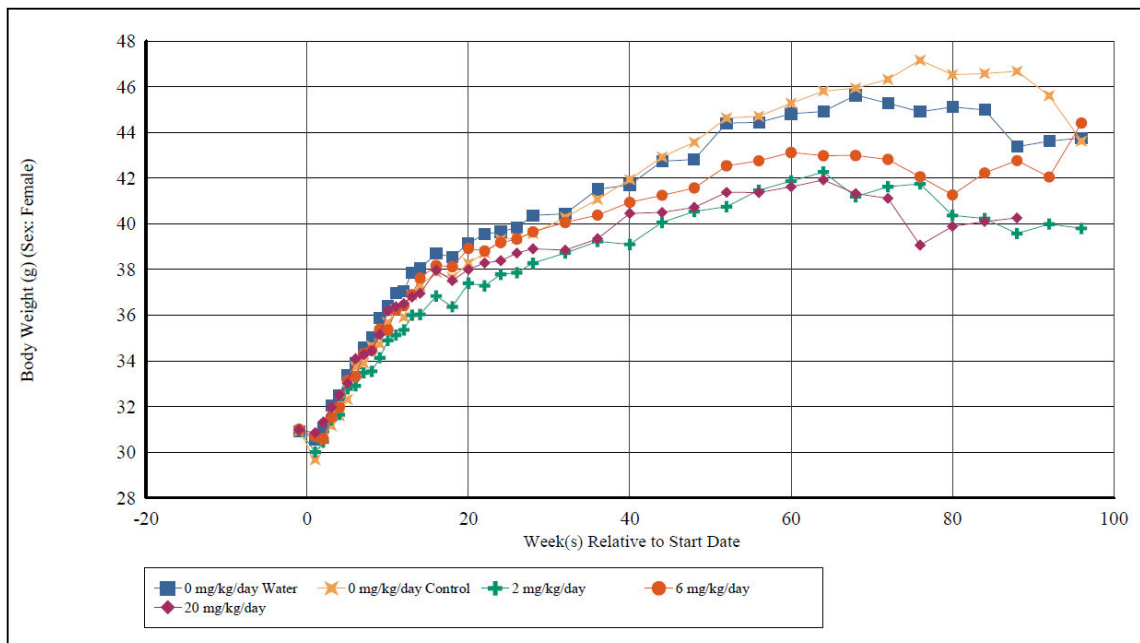


Figure copied directly from study report (Study APD334-TOX-003).

Feed Consumption

Decreased feed consumption was observed in all treated groups compared to WC and VC groups at various points during the study with no dose-dependence. Decreased feed consumption generally correlated with decreased body weights in treated compared to control animals.

Gross Pathology

Etrasimod-related macroscopic findings were observed in the liver and subcutis of a few HD males. In most cases, cysts in the liver and edema in the subcutis of these animals correlated with histopathological findings of hemangiosarcoma.

No treatment-related macroscopic findings were reported in LD or MD males or in treated females from any group.

Table 6. Macroscopic Findings in Mice Administered Etrasimod

Sex	Males					Females				
Etrasimod (mg/kg/day)	0 (WC)	0 (VC)	2	6	20	0 (WC)	0 (VC)	2	6	20
No. Examined	75	75	65	65	65	75	75	65	65	65
Liver										
Cyst	2			1	5		1		1	
Skin, subcutis										
Edema	1	2	3	2	6	1			4	4

VC: vehicle control (20 mg/kg/day L-arginine hydrochloride in water); WC: water control.

Table created by reviewer, based on Applicant's raw data (Study APD334-TOX-003).

Histopathology

Peer Review Conducted: Yes (Theodore Slone, Jr., DVM, DACVP)

Historical Control Provided for Tumor Incidence: No

Neoplastic

Neoplastic findings attributed by the Applicant to treatment with etrasimod were limited to an increased incidence of hemangiosarcomas and hemangiomas in males and females in the MD and HD groups. Commonly affected tissues included the liver, subcutis, heart, bone marrow, and spleen. A few hemangiosarcomas/hemangiomas were also observed in the uterus, intestine, skeletal muscle, lung, thymus, adrenal glands, kidneys, eyes, mesenteric lymph node, mesentery/peritoneum, spinal cord, and epididymides. These neoplasms were observed earlier in the study in the MD and HD groups compared to control animals. Additionally, the number of animals with two or more tissues with hemangiosarcomas/hemangiomas was higher in MD and HD males as well as HD females compared to controls.

Independent analyses of survival were conducted by FDA statistical reviewer, Feng Zhou, M.S., using the SAS data sets received on October 14, 2022, via NDA 216956/0001.^{9,10} The tumor data were analyzed for dose-response relationships and

pairwise comparisons of the WC and VC groups separately with each of the treated groups using the Poly-k method (see Statistical Review for further details).

Statistically significant positive dose-responses in the incidences of hemangiosarcoma malignant tumor (multicentric neoplasm) and combined hemangioma/hemangiosarcoma were observed in both male and female mice ($P_s < 0.005$) when compared with the VC group. The incidences of hemangiosarcoma malignant tumor and combined hemangioma/hemangiosarcoma in the MD and HD groups were statistically significantly higher compared to the VC group ($P_s < 0.01$). Tumor incidences were similar between the VC and WC groups.

Table 7. Tumor Types with Statistically Significant (at 0.05 significant level) Dose-response Relationships or Pairwise Comparisons of Treated Groups and Vehicle Control in Mice

SEX	Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
Male	Whole Body	Hemangioma, benign	2/75 (74) 0.1137	2/65 (65) 0.6398	1/65 (64) 0.8487	4/65 (63) 0.2673	2/75 (74) 0.6901
		Hemangiosarcoma, malignant	4/75 (74) 0.0000 *	9/65 (65) 0.0785	17/65 (64) 0.0005 **	31/65 (63) 0.0000 **	8/75 (74) 0.1836
		Comb_Hemangiosarcoma/ Hemangioma	6/75 (74) 0.0000 *	11/65 (65) 0.0928	18/65 (64) 0.0019 **	32/65 (63) 0.0000 **	10/75 (74) 0.2140
		Female	Whole Body	Hemangioma, benign	1/75 (75) 0.0060	0/65 (64) 1.0000	1/65 (63) 0.7064
Hemangiosarcoma, malignant	4/75 (75) 0.0001 *	5/65 (64) 0.4006		14/65 (63) 0.0034 **	18/65 (65) 0.0003 **	2/75 (75) 0.8954	
Comb_Hemangiosarcoma/ Hemangioma	5/75 (75) 0.0001 *	5/65 (64) 0.5241		14/65 (63) 0.0081 **	19/65 (65) 0.0004 **	3/75 (75) 0.8622	
& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NS = Not significant. Note: The p-values marked with an asterisk * indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.							

Table copied from the FDA statistical review by Feng Zhou, M.S. (page 10).

Non Neoplastic

Non-neoplastic findings related to etrasimod (based on increased incidence and/or severity compared to controls) included angiomatous hyperplasia in various tissues as well as findings in the lungs and thymus.

Angiomatous hyperplasia, consisting of focal to multifocal localized areas of increased capillaries and small vessels lined by flattened endothelium with no distortion of the adjacent normal tissue, was observed in various tissues (bone marrow, epididymides, heart, liver, subcutis, femorotibial joint) in HD males with increased overall incidence compared to control animals. Angiomatous hyperplasia was observed in various tissues in females (control or treated) as well, but only considered drug-related in the uterus, (

thick-walled blood vessel within the myometrium that was more prominent near the broad ligament); this finding was observed with increased incidence and severity in MD and HD females compared to controls.

Etrasimod-related changes in the lungs, including minimal to marked alveolar histiocytosis, minimal to moderate alveolar eosinophilic accumulation, minimal to mild multifocal fibrosis, minimal to mild osseous metaplasia, and minimal fibrin deposition, were observed in all treated groups compared to controls; two or more of these findings were often observed in affected animals.

Finally, minimally to moderately increased lymphocytes were observed in the medulla of the thymus with increased incidence and/or severity in MD and HD males and females compared to controls.

Table 8. Non-Neoplastic Histopathology Findings in Mice Administered Etrasimod

Sex	Males					Females				
Etrasimod (mg/kg/day)	0 (WC)	0 (VC)	2	6	20	0 (WC)	0 (VC)	2	6	20
No. Examined	75	75	65	65	65	75	75	65	65	65
Various tissues										
Angiomatous hyperplasia	2	1		1	10	5	6	7	16	16
Lungs										
Histiocytosis, alveolar										
Minimal	7	4	6	7	16	6	17	7	20	14
Mild	3	6	8	20	24	8	6	16	20	31
Moderate	1	5	7	12	10	2	3	9	7	7
Marked	4	1	4	5	3	1		4	2	4
Accumulation, eosinophilic, alveolar										
Minimal			3	16	11	1		9	20	17
Mild				3	9			6	6	4
Moderate									2	
Fibrosis										
Minimal			3	13	11	1	1	11	13	21
Mild		1		1	1	1		5	4	8
Moderate										1
Metaplasia, osseous										
Minimal		1	7	10	16		2	8	12	15
Mild			1	1	4		1			
Fibrin deposition										
Minimal					1	1	2	5	3	6
Thymus										
Increased lymphocytes, medulla										
Minimal	10	12	14	10	12	10	11	8	2	7
Mild	6	6	6	14	18	19	14	24	30	31
Moderate	1	1				1		2	2	4

VC: vehicle control (20 mg/kg/day L-arginine hydrochloride in water); WC: water control.

Table created by reviewer, based on Applicant's raw data (Study APD334-TOX-003).

Toxicokinetics

Etrasimod was not detected in any control group samples (limit of quantitation = 500 ng/mL).

Etrasimod was quantifiable in plasma samples from all treated animals through 24 hours post-dose on day 364 of dosing. $C_{\max}$ and AUC for etrasimod increased approximately proportionally with increasing dose over the tested range. $T_{\max}$ occurred between 2-12 hours with no clear effect of dose. No sex differences in toxicokinetic parameters were observed.

Table 9. Toxicokinetic Parameters for Etrasimod in Mice

Etrasimod (mg/kg/day)	$C_{\max}$ (ng/mL)	$T_{\max}$ (h)	AUC_{0-24h} (h·ng/mL)
2	2310	12	42100
6	5710	2	90600
20	16800	4	261000

Table created by reviewer, based on Applicant's raw data (Study APD334-TOX-003).

8.2 APD334 L-Arginine: A 104-Week Oral Oncogenicity Study in Rats

Study no.: [APD334.TOX.002](#)
Study report location: EDR 4.2.3.4
Study initiation date: January 7, 2019
Conducting laboratory and location:  (b) (4)

GLP compliance: Yes
Drug, lot #, and % purity: APD334 L-arginine
Lot # 5015-12-16
Purity 99.95%

Prior Exec CAC Dose Concurrence: Y
Basis for Dose Selection: Based on the findings in a previous 6-month study in rats (Study TX14009), the high dose of 20 mg/kg/day was expected to exceed 25 times the clinical exposure (AUC) for both etrasimod and the metabolite M3. The low and mid doses were selected to allow for adequate AUC spacing between doses.

Reviewer Carcinogenicity Conclusion (negative/ positive): Negative
ECAC Carcinogenicity Conclusion (negative/ positive): Negative

Key Study Findings

- Etrasimod did not have a dose-dependent effect on the mortality rate or significantly increased mortalities in treated animals compared to vehicle controls. The statistical reviewer's analysis showed the numbers (percent) of deaths that occurred prior to scheduled termination of the group were 73%, 67%, 57%, 62%, and 72% in male rats and 73%, 65%, 55%, 58%, and 58% in female mice in the water control, vehicle control, low dose, mid dose, and high dose groups, respectively.
- Etrasimod-related non-neoplastic lesions were observed in the lymphoid tissues and brain of males and females at all tested doses as well as in the liver of all treated females and males in the MD and HD groups.
- No statistically significant increases in tumor incidences occurred in this study.

Tumor Findings:

Independent analyses of the tumor findings were conducted by FDA statistical reviewer, Feng Zhou, M.S.^{9,10} The tumor analysis showed no statistically significant positive dose-response relationship between the treated groups and vehicle control group for tumors of any type.

Methods

Doses: 0 (vehicle), 0 (water), 2, 6, and 20 mg/kg/day

Frequency of dosing: Once daily

Number/Sex/Group: Main study: controls = 75/sex/group, treated = 65/sex/group
TK: controls = 5/sex/group, treated = 9/sex/group

Dose volume: 10 mL/kg (with deviations)

Formulation/Vehicle: L-arginine monohydrochloride 0.92 mg/mL in deionized water

Route of administration: ORAL GAVAGE

Species: RAT

Strain: SPRAGUE-DAWLEY

Age: 8 weeks

Comment on Study Design and Conduct: Although metabolites were not assessed in this study, other PK studies (i.e., Study APD334-DMPK-002) have demonstrated that rats produce the major human metabolites, M3 and M6, at levels that exceed those in humans when tested at the dose levels used in this study. Overall, the carcinogenic potential of the major human metabolites was adequately assessed, and the carcinogenicity study was adequately conducted.

Dosing Comments (Dose Adjustments or Early Termination): Week 86: Survival in the male HD group (20 mg/kg/day) reached 20, and dose administration was suspended.

Week 89: All surviving females were terminated due to survival in the water control group reaching 20.

Week 90: All surviving males were terminated due to survival in the water control group reaching 20.

Dosing Solution Analysis: At Weeks 4 and 8, the observed concentrations for the 0.2 mg/mL formulation were 63% and 68.5% of nominal, respectively. At Week 8, the observed concentration for the 0.6 mg/mL formulation was 53.9% of nominal. The 2 mg/mL formulation was not affected. Following review of the preparation methodology, beginning on Day 54, all dose formulations were prepared at 2 mg/mL. All formulations following this time point were acceptable

(± ^(b)₍₄₎ % of nominal). These deviations did not appear to have affected the study results.

Table 10. Design of 2-Year Carcinogenicity Study with Etrasimod in Rats

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume ^a (mL/kg)	Dose Concentration (mg/mL)	Animal Numbers	
					Main Study	
					Male	Female
1	Water control	0	10	0	1001-1075	1501-1575
2	Control article ^b	0	10	0	2001-2075	2501-2518, 2521-2575, 2619, 2620
3	APD334 L-arginine	2	10/15 ^c /1 ^d	0.2 ^c /2 ^d	3001-3065	3501-3554, 3556-3565, 3655
4	APD334 L-arginine	6	10/20.6 ^c /3 ^d	0.6 ^c /2 ^d	4001-4065	4501-4565
5	APD334 L-arginine	20	10	2	5001-5065	5501-5565

^a Based on the most recent body weight measurement.

^b The formulated control article was an aqueous solution of L-arginine hydrochloride at the concentration of L-arginine (0.92 mg/mL) in the high dose of the test article (20 mg/kg/day).

^c Due to low recovery in the Week 8 low and mid-dose formulation samples, animals in Groups 3 and 4 were dosed on Days 52 and 53 with dose concentrations of 0.2 and 0.6 mg/mL, respectively, using dose volumes of 15.0 mL/kg and 20.6 mL/kg, respectively, to ensure administration of the proper dosages.

^d Beginning on Day 54 and for the remainder of the dosing phase, a dose concentration of 2 mg/mL was used for Groups 3 and 4, with dose volumes of 1 mL/kg and 3 mL/kg, respectively.

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume ^a (mL/kg)	Dose Concentration (mg/mL)	Animal Numbers	
					Toxicokinetic	
					Male	Female
6	Water control	0	10	0	6001-6005 ^b	6501-6505 ^b
7	Control article ^c	0	10	0	7001-7005 ^b	7501-7505 ^b
8	APD334 L-arginine	2	10/15 ^d /1 ^e	0.2 ^d /2 ^e	8001-8009 ^b	8501-8509 ^b
9	APD334 L-arginine	6	10/20.6 ^d /3 ^e	0.6 ^d /2 ^e	9001-9009 ^b	9501-9509 ^b
10	APD334 L-arginine	20	10	2	10001-10009 ^b	10501-10509 ^b

^a Based on the most recent body weight measurement.

^b Additional animals per sex per group were included as possible replacement animals.

^c The formulated control article was an aqueous solution of L-arginine hydrochloride at the concentration of L-arginine (0.92 mg/mL) in the high dose of the test article (20 mg/kg/day).

^d Due to low recovery in the Week 8 low and mid-dose formulation samples, animals in Groups 3 and 4 were dosed on Days 52 and 53 with dose concentrations of 0.2 and 0.6 mg/mL, respectively, using dose volumes of 15.0 mL/kg and 20.6 mL/kg, respectively, to ensure administration of the proper dosages.

^e Beginning on Day 54 and for the remainder of the dosing phase, a dose concentration of 2 mg/mL was used for Groups 8 and 9, with dose volumes of 1 mL/kg and 3 mL/kg, respectively.

Table adapted from study report (Study APD334-TOX-002).

Observations and Results

Mortality

Animals were observed at least twice daily for morbidity and mortality. No morbidity or mortality was attributed to administration of etrasimod (named APD334 in this report).

Survival in the male HD group (20 mg/kg/day) reached 20 at Week 86, and dose administration was suspended at that time. Additionally, survival in the male and female WC groups reached 20 at Week 90 and Week 89, respectively. As such, all surviving

males were necropsied during Weeks 90 and 91 and all surviving females were necropsied during Weeks 89 and 90.

Independent analyses of survival were conducted by FDA statistical reviewer, Feng Zhou, M.S.^{9,10} The survival distributions of rats in all treatment groups were estimated using the Kaplan-Meier product limit method. For control, LD, MD, and HD groups, the dose-response relationship was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test.

The statistical reviewer's analysis showed the numbers (percent) of deaths that occurred prior to scheduled termination of the group were 73%, 67%, 57%, 62%, and 72% in male rats and 73%, 65%, 55%, 58%, and 58% in female mice in the WC, VC, LD, MD, and HD groups, respectively. No statistically significant dose-response relationship in mortality was observed in treated males and females compared to the VC group. Death rates for the WC group were similar to that of the VC group for both male and female mice.

Table 11. Intercurrent Mortality Rate in Male Rats

Week / Type of Death	Water Control		Vehicle Control		Low Dose		Mid Dose		High Dose	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	5	6.67	8	10.67	3	4.62	7	10.77	9	13.85
53 - 78	31	48.00	26	45.33	19	33.85	19	40.00	24	50.77
79 - 96	19	73.33	16	66.67	14	55.38	14	61.54	14	72.31
Accidental Death	.	.	.	.	1	1.54	.	.	.	.
Terminal sacrifice	20	26.67	25	33.33	28	43.08	25	38.46	18	27.69
Total	75	.	75	.	65	.	65	.	65	.
Test			All Dose Groups	Vehicle C vs. Low	Vehicle C vs. Mid	Vehicle C vs. High	Vehicle C vs. Water C			
Dose-Response (Likelihood Ratio)			0.1362	0.1920	0.3747	0.4339	0.5255			
Homogeneity (Log-Rank)			0.1618	0.1877	0.3691	0.4261	0.5195			

Table copied from the FDA statistical review by Feng Zhou, M.S. (Tables 1A and 3A).

Table 12. Intercurrent Mortality Rate in Female Rats

Week / Type of Death	Water Control		Vehicle Control		Low Dose		Mid Dose		High Dose	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	5	6.67	6	8.00	5	7.69	2	3.08	2	3.08
53 - 78	34	52.00	28	45.33	15	30.77	20	33.85	18	30.77
79 - 96	16	73.33	15	65.33	16	55.38	16	58.46	18	58.46
Terminal sacrifice	20	26.67	26	34.67	29	44.62	27	41.54	27	41.54
Total	75	.	75	.	65	.	65	.	65	.

Test	All Dose Groups	Vehicle C vs. Low	Vehicle C vs. Mid	Vehicle C vs. High	Vehicle C vs. Water C
Dose-Response (Likelihood Ratio)	0.4690	0.2181	0.3603	0.2499	0.2616
Homogeneity (Log-Rank)	0.5426	0.2126	0.3547	0.2440	0.2536

Table copied from the FDA statistical review by Feng Zhou, M.S. (Tables 1B and 3B).

Figure 5. Kaplan-Meier Survival Functions for Male Rats

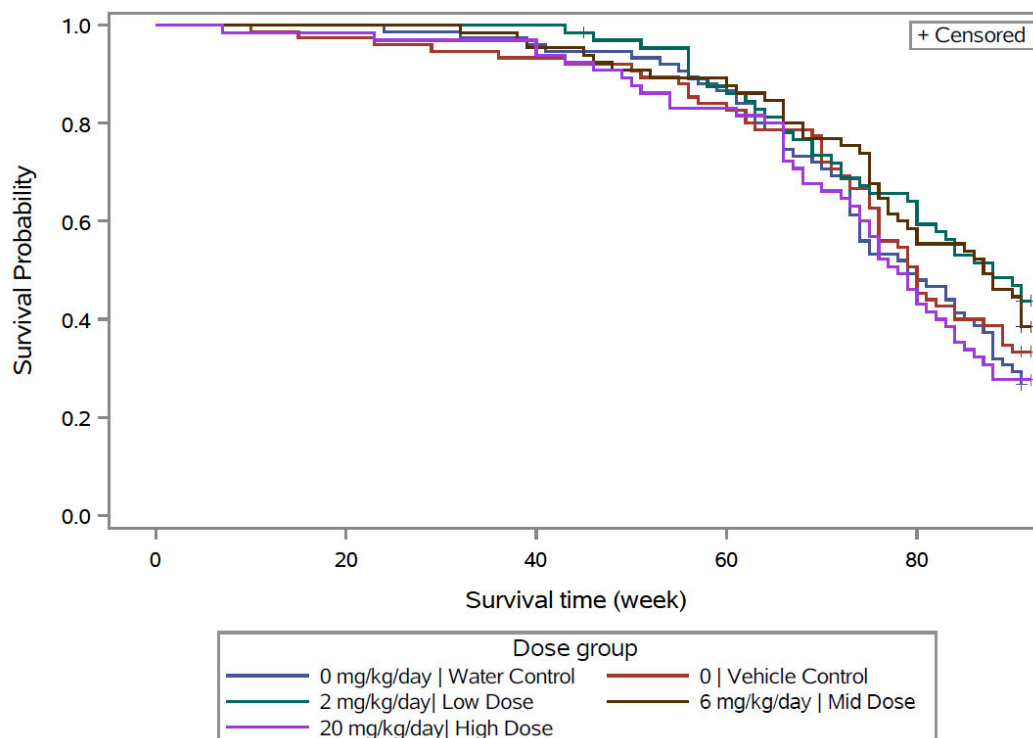


Figure copied from the FDA statistical review by Feng Zhou, M.S. (Figure 1A).

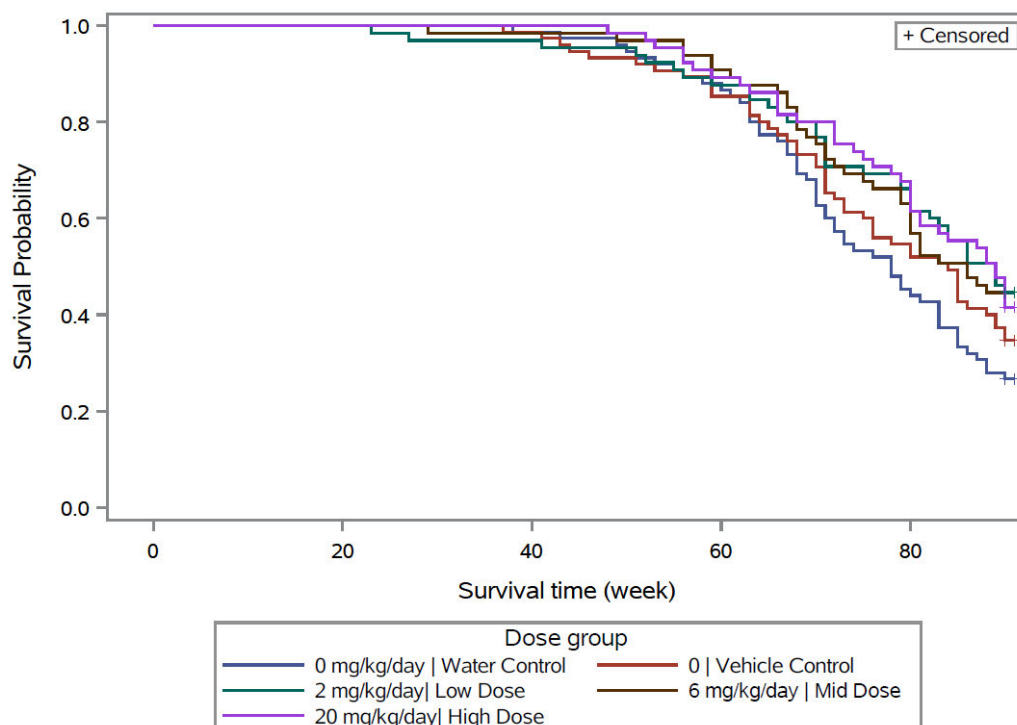
Figure 6. Kaplan-Meier Survival Functions for Female Rats

Figure copied from the FDA statistical review by Feng Zhou, M.S. (Figure 1B).

Clinical Signs

Detailed clinical examinations were conducted weekly throughout the study. No etrasimod-related clinical signs were observed, including no trends in the incidence or frequency of palpable masses/nodules.

Body Weights

Decreased absolute body weight was observed in all treated groups compared to WC or VC throughout the study, though differences rarely exceeded 10%. Similarly, dose-independent decreased body weight gain was observed in LD and HD males compared to the WC and VC groups (-10% and -14%, respectively) and in all treated females compared to the WC (-16% to -18%) and VC groups (-14% to -16%).

Figure 7. Body Weights in Male Rats Administered Etrasimod

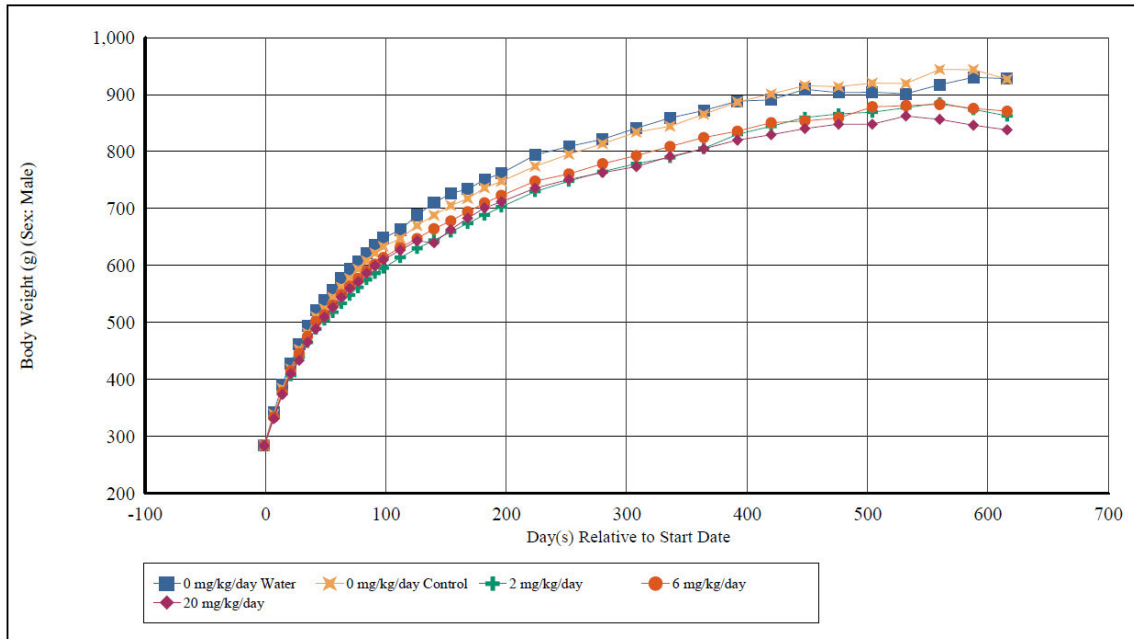


Figure copied directly from study report (Study APD334-TOX-002).

Figure 8. Body Weights in Female Rats Administered Etrasimod

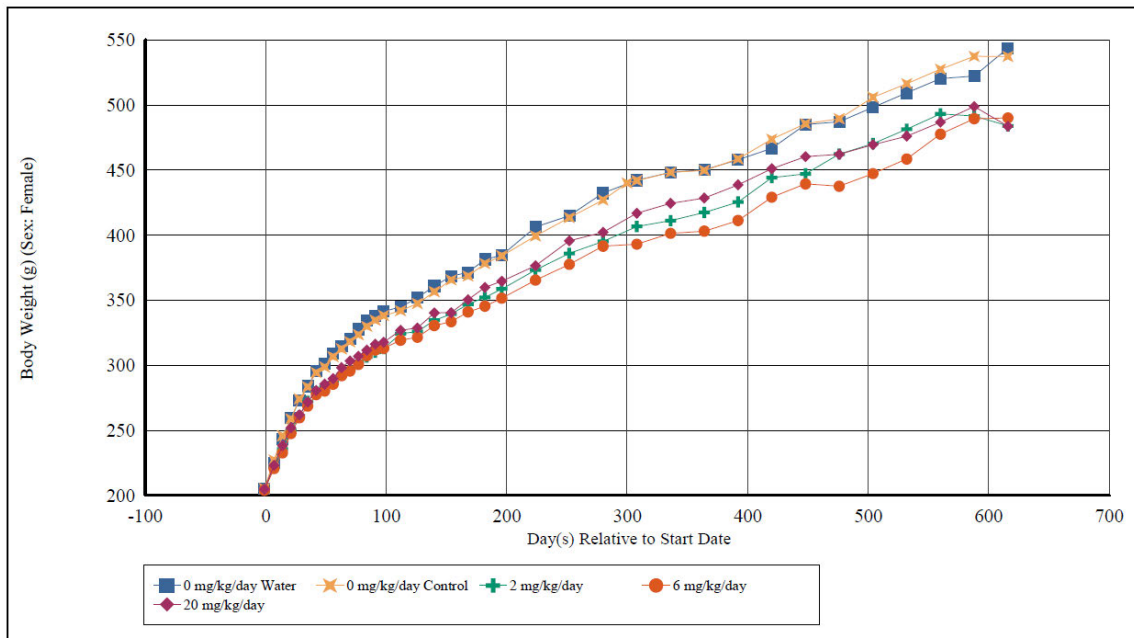


Figure copied directly from study report (Study APD334-TOX-002).

Feed Consumption

Decreased feed consumption was observed in all treated groups compared to the WC and VC groups at various points during the study with no dose-dependence. Decreased feed consumption generally correlated with decreased body weights in treated compared to control animals.

Gross Pathology

No macroscopic findings related to etrasimod administration were reported.

Histopathology

Peer Review Conducted: Yes (Joelle Ibanes, DVM, DACVP)

Historical Control Provided for Tumor Incidence: No

Neoplastic

According to the Applicant, there were no neoplastic findings related to etrasimod administration. A statistically significant incidence of carcinoma of the Zymbal gland was reported in female mice treated with etrasimod compared to controls ($p = 0.0244$ by the Poly-3 trend test). However, the absolute incidences of this tumor were low in all groups (0/74, 1/74, 0/64, 0/65, and 2/63 in the WC, VC, LD, MD, and HD groups, respectively), no statistically significant differences were observed using other tests (Fisher exact test, Cochran-Armitage trend test, and Peto test), and a similar trend was not observed in male animals. The incidence in females at the high dose (2/63 or 3.2%) was slightly above the top of the historical control range for females (1.7%). Based on these factors, this finding was considered incidental and not related to treatment with etrasimod.

Independent analyses of survival were conducted by FDA statistical reviewer, Feng Zhou, M.S., using the SAS data sets received on October 14, 2022, via NDA 216956/0001.^{9,10} The tumor data were analyzed for dose-response relationships and pairwise comparisons of the WC and VC groups separately with each of the treated groups using the Poly-k method (see Statistical Review for further details).

Based on the criteria of adjustment for multiple testing, there were no statistically significant positive dose-response relationships between the treated groups and VC group for male and female rats. The tumor incidences were similar between the vehicle control group and water control group.

Non Neoplastic

Non-neoplastic findings related to etrasimod administration (based on increased incidence and/or severity compared to controls) were observed in the lymphoid system (lymph nodes, spleen, thymus) and brain of males and females at all tested doses as well as in the liver of all treated females and males in the MD and HD groups.

Etrasimod-related effects in the lymphoid organs of males and females at all doses included minimally to moderately increased lymphocytes in the thymus medulla, minimally to markedly decreased lymphocytes in the marginal zone of the spleen,

minimally to markedly increased extramedullary hematopoiesis in the spleen, as well as variably increased incidence and/or severity of sinus dilation, sinus histiocytosis, and/or sinus erythrocytosis/erythrophagocytosis in the iliac, mandibular, mediastinal, mesenteric, and/or renal lymph nodes. Findings in the thymus were generally not dose-dependent in incidence or severity, although increased severity (moderate) was observed in HD males. Decreased marginal zone lymphocytes and increased extramedullary hematopoiesis in the spleen were observed without dose-dependent incidence or severity. Lymph node findings also did not show clear or consistent dose-dependence.

An increased incidence of minimal to mild focal thalamic mineralization, frequently bilateral, was observed in the brain in all etrasimod-treated animals with no dose-dependence. The mineralization was characterized by focal accumulations of circular, densely basophilic concretions within the thalamus.

In the liver, increased incidence and/or severity of basophilic foci of cellular alteration were observed in females at all doses compared to controls. Increased incidence and/or severity of clear or eosinophilic foci of cellular alteration were observed in MD and HD males and females. Increased incidence of minimal or mild bile duct hyperplasia was observed in MD and HD males and females compared to controls. Findings in the liver were generally not dose-dependent in incidence or severity.

Table 13. Non-Neoplastic Histopathology Findings in Rats Administered Etrasimod

Sex	Males					Females				
Etrasimod (mg/kg/day)	0 (WC)	0 (VC)	2	6	20	0 (WC)	0 (VC)	2	6	20
No. Examined*	75	75	65	65	65	75	75	65	65	65
Thymus										
Increased lymphocytes, medulla										
Minimal			18	16	4			31	10	11
Mild			25	30	31			18	36	30
Moderate			3	1	11			6	15	6
Spleen										
Decreased lymphocytes, marginal zone										
Minimal	8	5	21	26	22	4	2	17	22	20
Mild	4	1	19	19	20	2	2	8	21	19
Moderate	1	2	1	2	1	1		1		
Marked						1				
Hematopoiesis, extramedullary, increased										
Minimal		1	20	10	12	16	14	28	37	34
Mild		1	1	2		7	6	11	5	8
Moderate			1				5		3	1
Marked		1							1	
Lymph node, iliac										
Dilation, sinus										
Moderate	--	--	--	1/1	1/1	--	--	--	--	--
Lymph node, mandibular										
Dilation, sinus										
Minimal	1	1	2	5	6	1		3	3	8
Mild	2		7	7	9			1	1	5
Moderate		1	4	3	6					
Histiocytosis, sinus										
Minimal	1								1	2
Erythrocytosis/erythrophagocytosis, sinus										
Minimal									1	
Mild						1	1	1		
Moderate					1					
Marked			1							

Sex	Males					Females				
Etrasimod (mg/kg/day)	0 (WC)	0 (VC)	2	6	20	0 (WC)	0 (VC)	2	6	20
No. Examined*	75	75	65	65	65	75	75	65	65	65
Lymph node, mediastinal Dilation, sinus Mild	--	--	--	1/1	--	--	--	--	--	--
Lymph node, mesenteric Dilation, sinus Minimal Mild Moderate				5	3	2	1	2		2
Histiocytosis, sinus Minimal Mild	3	1	11	16	13	13	16	28	22	22
Erythrocytosis/ erythrophagocytosis, sinus Minimal Mild Moderate	1	1	5	9	4	1		8	7	7
	2	2	1	5	5	4	1	2	3	3
		1		3		1	1	2	2	3
		1	1		0		1		1	1
Lymph node, renal Dilation, sinus Moderate	--	--	--	1/1	--	--	--	--	--	--
Brain Mineralization, focal Minimal Mild	1	1	28	35	36			24	42	36
			23	22	12			23	9	11

Sex	Males					Females				
Etrasimod (mg/kg/day)	0 (WC)	0 (VC)	2	6	20	0 (WC)	0 (VC)	2	6	20
No. Examined*	75	75	65	65	65	75	75	65	65	65
Liver										
Focus of cellular alteration, basophilic										
Minimal	14	9	8	8	8	29	33	23	27	23
Mild	2					4	9	117	11	13
Moderate						2	1	2	1	6
Marked							1			
Focus of cellular alteration, clear										
Minimal	16	17	16	23	19	12	19	12	13	15
Mild	7	10	6	6	7		2	1	4	5
Moderate				3	3					3
Focus of cellular alteration, eosinophilic										
Minimal	6	5	5	8	4	3	5	6	9	18
Mild	2	3		1		1			6	4
Moderate				1				1		1
Hyperplasia, bile duct										
Minimal	30	27	31	31	22	42	28	27	33	26
Mild	19	24	23	26	28	14	14	29	19	30
Moderate	3			1	3		2		1	

VC: vehicle control (20 mg/kg/day L-arginine hydrochloride in water); WC: water control.
* Unless otherwise noted. --: not examined.

Table created by reviewer, based on Applicant's raw data (Study APD334-TOX-002).

Toxicokinetics

Etrasimod was not detected in any control group samples (limit of quantitation = 5.00 ng/mL).

Etrasimod was quantifiable in plasma samples from all treated animals through 24 hours post-dose on day 364 of dosing. C_{max} and AUC for etrasimod increased approximately proportionally with increasing dose over the tested range. T_{max} occurred between 2-12 hours with no clear effect of dose. AUC and C_{max} values for etrasimod were approximately 2-fold higher in females compared to males at all tested doses.

Table 14. Toxicokinetic Parameters for Etrasimod in Rats

Etrasimod (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24h} (h·ng/mL)
2	M	1990	6	27600
	F	4550	2	65200
6	M	5190	4	68200
	F	8440	4	147000
20	M	12100	6	172000
	F	24200	6	387000

Table created by reviewer, based on Applicant's raw data (Study APD334-TOX-002).

Appendix 1: Summary of Deaths from Applicant – Mice

Table 15. Probable Cause of Death in Male Mice

Removal Reason: UNSCHEDULED	----- MALES -----				
	0 mg/kg/day (54)	0 mg/kg/day (48)	2 mg/kg/day (43)	6 mg/kg/day (47)	20 mg/kg/day (50)
Number of Dead Animals on Study:					
ACCESSORY SEX GLAND TUMOR	1	1	0	0	0
ACCIDENTAL INJURY	0	0	0	1	0
AMYLOIDOSIS	1	0	3	0	2
BRAIN HEMORRHAGE/NECROSIS	0	0	1	0	0
CHRONIC PROGRESSIVE NEPHROPATHY/UREMIA	1	1	0	0	1
DOSING INJURY	1	1	1	0	1
EYES INFLAMMATION/NECROSIS	0	0	1	0	0
FIBROSARCOMA/FIBROMA	1	1	1	0	0
GASTROINTESTINAL ULCERATION	0	0	1	0	1
HARDERIAN GLAND TUMOR	1	3	3	0	0
HEART FAILURE/ATRIAL THROMBUS	1	0	1	1	0
HEMANGIOSARCOMA/HEMANGIOMA	7	3	5	13	26
HISTIOCYTIC SARCOMA	3	2	0	0	0
INFLAMMATION/SEPTICEMIA	1	0	0	0	1
KIDNEY INFLAMMATION/NECROSIS	0	0	1	0	0
LEIOMYOMA/LEIOMYOSARCOMA	0	0	1	0	0
LEUKEMIA	0	1	0	0	0
LIVER TUMOR	5	4	1	0	0
LUNG TUMOR	6	3	5	8	3
LYMPHOID TUMOR	4	7	5	5	2
PITUITARY TUMOR	1	1	0	0	0
POLYARTERITIS	2	0	1	0	0
SKIN INFLAMMATION/NECROSIS	0	2	1	2	1
SKIN TUMOR	0	1	0	0	0
SPINAL CORD HEMORRHAGE/NECROSIS/DEGENERATION	0	0	1	0	0
UNDETERMINED	9	9	7	11	10
UROGENITAL INFLAMMATION/OBSTRUCTION/CALCULI	7	8	2	6	1
Gastrointestinal inflammation	0	0	1	0	0
nerve, sciatic degeneration, axonal/myelin; mild	1	0	0	0	0
penis inflammation; marked	0	0	0	0	1
testes carcinoma, leydig cell; malignant; primary	1	0	0	0	0

Table copied directly from study report (Study APD334-TOX-003).

Table 16. Probable Cause of Death in Female Mice

Removal Reason: UNSCHEDULED	FEMALES				
	0 mg/kg/day (55)	0 mg/kg/day (52)	2 mg/kg/day (45)	6 mg/kg/day (50)	20 mg/kg/day (50)
Number of Dead Animals on Study:					
AMYLOIDOSIS	2	1	0	3	0
BONE TUMOR	0	0	1	0	1
BRAIN TUMOR	0	0	1	0	0
CHRONIC PROGRESSIVE NEPHROPATHY/UREMIA	0	1	0	0	0
DOSING INJURY	0	0	0	1	0
EYES INFLAMMATION/NECROSIS	0	1	0	1	1
FIBROSARCOMA/FIBROMA	1	0	0	1	0
HARDERIAN GLAND TUMOR	0	2	0	1	0
HEMANGIOSARCOMA/HEMANGIOMA	2	3	2	10	15
HISTIOCYTIC SARCOMA	1	0	1	0	1
LEUKEMIA	0	1	0	0	0
LUNG INFLAMMATION/NECROSIS	0	2	0	0	0
LUNG TUMOR	4	1	1	0	3
LYMPHOID TUMOR	15	18	14	11	12
MAMMARY TUMOR	5	3	1	2	0
MESOTHELIOMA	0	0	0	2	0
NOSE/ORAL INFLAMMATION/ULCERATION	0	0	0	0	1
OVARIAN CYST/HEMORRHAGE	4	5	4	3	5
OVARY TUMOR	1	0	0	0	0
PITUITARY TUMOR	0	0	1	0	0
POLYARTERITIS	1	0	0	0	1
SCHWANNOMA	0	0	1	0	0
SKIN INFLAMMATION/NECROSIS	3	0	0	0	1
SKIN TUMOR	0	1	0	0	0
UNDETERMINED	10	8	14	9	5
UTERUS HEMORRHAGE	2	3	0	2	1
UTERUS HEMORRHAGE/INFLAMMATION/NECROSIS	0	0	1	0	0
UTERUS INFLAMMATION/NECROSIS	1	0	1	0	1
UTERUS PROLAPSE	0	1	0	1	0
UTERUS TUMOR	0	1	1	0	1
brain					
inflammation, perivascular: mild	0	0	0	0	1
liver					
vacuolation, periportal: marked	0	0	0	1	0
lung					
accumulation, eosinophilic alveolar: moderate	0	0	0	1	0
histiocytosis, alveolar: marked	0	0	0	1	0
nerve, sciatic					
degeneration, axonal/myelin: moderate	1	0	0	0	0
skin, subcutis					
osteosarcoma: malignant: primary	1	0	0	0	0
spinal cord					
degeneration, axonal/myelin: mild	0	0	1	0	0
thymus					
hyperplasia, lymphoid, generalized: severe	1	0	0	0	0

Table copied directly from study report (Study APD334-TOX-003).

Appendix 2: Tumor Findings in Mice

Table 17. Neoplastic Findings in Male Mice

Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
Artery, aorta	Carcinoma, bronchioloalveolar, malignant	1/75 (74)	0/65 (65)	1/65 (64)	0/65 (63)	0/75 (74)
		0.7279	1.0000	0.7143	1.0000	1.0000
Bone, sternum	Fibrosarcoma, malignant	1/75 (74)	0/65 (65)	0/65 (64)	0/65 (63)	0/75 (74)
		1.0000	1.0000	1.0000	1.0000	1.0000
Epididymis	Leiomyosarcoma, malignant	0/75 (74)	1/65 (65)	0/65 (64)	0/65 (63)	0/74 (73)
		0.7218	0.4676	NC	NC	NC
Gland, adrenal	Adenoma, adrenocortical, benign	0/74 (73)	0/65 (65)	1/64 (63)	0/65 (63)	2/75 (74)
		0.4773	NC	0.4632	NC	0.2517
	Adrenal tumor, subcapsular, benign	10/74 (73)	2/65 (65)	5/64 (63)	1/65 (63)	9/75 (74)
		0.9893	0.9959	0.9120	0.9993	0.6993
Gland, adrenal	Pheochromocytoma, benign	0/74 (73)	1/65 (65)	0/64 (63)	0/65 (63)	0/75 (74)
		0.7235	0.4710	NC	NC	NC
Gland, coagulating	Carcinoma, malignant	1/75 (74)	0/65 (65)	0/65 (64)	0/65 (63)	0/75 (74)
		1.0000	1.0000	1.0000	1.0000	1.0000
Gland, harderian	Adenocarcinoma, malignant	1/75 (74)	1/65 (65)	0/65 (64)	0/65 (63)	1/75 (74)
		0.9234	0.7184	1.0000	1.0000	0.7517
	Adenoma, benign	11/75 (74)	9/65 (65)	13/65 (64)	2/65 (63)	11/75 (74)
		0.9924	0.6586	0.2683	0.9972	0.5911
Gland, pituitary	Adenoma, pars distalis, benign	2/74 (73)	1/64 (64)	0/64 (63)	0/62 (61)	1/73 (72)
		0.9788	0.8516	1.0000	1.0000	0.8750
Gland, prostate	Adenocarcinoma, malignant	0/75 (74)	1/65 (65)	0/65 (64)	0/65 (63)	0/75 (74)
		0.7218	0.4676	NC	NC	NC
Gland, seminal vesicle	Adenocarcinoma, malignant	2/75 (74)	0/65 (65)	1/65 (64)	0/65 (63)	1/75 (74)
		0.8729	1.0000	0.8487	1.0000	0.8776
	Adenoma, benign	1/75 (74)	0/65 (65)	0/65 (64)	0/65 (63)	0/75 (74)
		1.0000	1.0000	1.0000	1.0000	1.0000
Gland, seminal vesicle	Leiomyosarcoma, malignant	0/75 (74)	1/65 (65)	0/65 (64)	0/65 (63)	0/75 (74)
		0.7218	0.4676	NC	NC	NC
Gland, thyroid	Adenoma, follicular cell, benign	0/75 (74)	1/65 (65)	1/64 (63)	0/65 (63)	0/75 (74)
		0.5925	0.4676	0.4599	NC	NC
	Carcinoma, follicular cell, malignant	0/75 (74)	1/65 (65)	0/64 (63)	0/65 (63)	0/75 (74)
		0.7208	0.4676	NC	NC	NC
Heart	Carcinoma, bronchioloalveolar, malignant	0/75 (74)	0/65 (65)	3/65 (64)	0/65 (63)	1/75 (74)
		0.5705	NC	0.0972	NC	0.5000

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Kidney	Adenoma, renal cell, benign	0/75 (74) 0.2661	1/65 (65) 0.4676	1/65 (64) 0.4638	1/65 (63) 0.4599	2/75 (74) 0.2483
	Carcinoma, bronchioloalveolar, malignant	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	1/75 (74) 0.5000
	Carcinoma, malignant	1/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
Large intestine, cecum	Adenocarcinoma, malignant	0/75 (74) 0.4774	0/65 (65) NC	1/65 (64) 0.4638	0/65 (63) NC	0/75 (74) NC
Liver	Adenoma, hepatocellular, benign	16/75 (74) 0.8913	4/65 (65) 0.9984	6/65 (64) 0.9872	6/65 (63) 0.9859	15/75 (74) 0.6567
	Carcinoma, hepatocellular, malignant	5/75 (74) 0.9992	2/65 (65) 0.9189	0/65 (64) 1.0000	0/65 (63) 1.0000	5/75 (74) 0.6274
	Hepatoblastoma, malignant	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	1/75 (74) 0.5000
Lung	Adenocarcinoma, malignant	1/75 (74) 0.9228	1/65 (65) 0.7184	0/64 (63) 1.0000	0/65 (63) 1.0000	1/75 (74) 0.7517
	Adenoma, bronchioloalveolar, benign	14/75 (74) 0.9646	13/65 (65) 0.5204	8/64 (63) 0.8898	6/65 (63) 0.9655	15/75 (74) 0.5000
	Carcinoma, bronchioloalveolar, malignant	14/75 (74) 0.9465	11/65 (65) 0.6998	16/64 (63) 0.2397	6/65 (63) 0.9655	14/75 (74) 0.5830
	Carcinoma, hepatocellular, malignant	3/75 (74) 0.9943	1/65 (65) 0.9227	0/64 (63) 1.0000	0/65 (63) 1.0000	1/75 (74) 0.9400
	Leydig cell tumor, malignant	0/75 (74) NC	0/65 (65) NC	0/64 (63) NC	0/65 (63) NC	1/75 (74) 0.5000
	Lymph node, mandibular	Leiomyosarcoma, malignant	0/73 (73) 0.7192	1/64 (64) 0.4672	0/64 (63) NC	0/62 (60) NC
Lymph node, mediastinal	Carcinoma, bronchioloalveolar, malignant	0/75 (74) 0.4774	0/65 (65) NC	1/65 (64) 0.4638	0/65 (63) NC	0/75 (74) NC
Mediastinum/Pleura	Carcinoma, bronchioloalveolar, malignant	0/75 (74) 0.4755	0/65 (65) NC	2/65 (64) 0.2133	0/65 (63) NC	0/75 (74) NC
Mesentery /Peritoneum	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Multicentric neoplasm	Hemangioma, benign	2/75 (74) 0.1137	2/65 (65) 0.6398	1/65 (64) 0.8487	4/65 (63) 0.2673	2/75 (74) 0.6901
	Hemangiosarcoma, malignant	4/75 (74) 0.0000 *	9/65 (65) 0.0785	17/65 (64) 0.0005 **	31/65 (63) 0.0000 **	8/75 (74) 0.1836
	Leukemia, malignant	1/75 (75) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
	Lymphoma, malignant	11/75 (74) 0.9563	9/65 (65) 0.6586	8/65 (64) 0.7408	4/65 (63) 0.9715	5/75 (74) 0.9694

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Pancreas	Sarcoma, histiocytic, malignant	2/75 (74) 0.5414	0/65 (65) 1.0000	1/65 (64) 0.8487	1/65 (63) 0.8454	3/75 (74) 0.5000
	Adenoma, islet cell, benign	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	3/75 (74) 0.1224
	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Skin	Adenoma, sebaceous, benign	0/75 (74) 0.7869	2/65 (65) 0.2169	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
	Basosquamous tumor, malignant	1/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
	Keratoacanthoma, benign	1/75 (74) 0.7279	0/65 (65) 1.0000	1/65 (64) 0.7143	0/65 (63) 1.0000	0/75 (74) 1.0000
Skin/Subcutis	Fibrosarcoma, malignant	1/75 (74) 0.8966	3/65 (65) 0.2625	1/65 (64) 0.7143	0/65 (63) 1.0000	2/75 (74) 0.5000
	Melanoma, amelanotic, malignant	2/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
	Schwannoma, benign	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	1/75 (74) 0.5000
Small intestine, duodenum	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Small intestine, ileum	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Testis	Leydig cell tumor, benign	3/75 (74) 0.7546	1/65 (65) 0.9227	1/65 (64) 0.9204	1/65 (63) 0.9181	5/75 (74) 0.3594
	Leydig cell tumor, malignant	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	2/75 (74) 0.2483
Thymus	Carcinoma, bronchioloalveolar, malignant	0/68 (67) 0.5877	0/58 (58) NC	3/59 (58) 0.0971	0/62 (60) NC	2/71 (70) 0.2592

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.
 Note: The p-values marked with an asterisk * indicate statistically significant dose-responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Table copied from the FDA statistical review by Feng Zhou, M.S. (Table 6A).

Table 18. Neoplastic Findings in Female Mice

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Bone marrow, sternum	Osteosarcoma, malignant	0/74 (74) NC	0/64 (63) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5034
Brain	Meningioma, malignant	0/75 (75) 0.7191	1/65 (64) 0.4604	0/65 (63) NC	0/65 (65) NC	0/75 (75) NC
Gland, adrenal	Adenoma, adrenocortical, benign	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Adrenal tumor, subcapsular, benign	2/75 (75) 0.5685	0/65 (64) 1.0000	0/65 (63) 1.0000	1/65 (65) 0.8491	1/75 (75) 0.8775
	Adrenal tumor, subcapsular, malignant	0/75 (75) 0.7191	1/65 (64) 0.4604	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Pheochromocytoma, benign	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
Gland, clitoral	Adenocarcinoma, malignant	0/75 (75) 0.2434	0/65 (64) NC	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC
Gland, harderian	Adenocarcinoma, malignant	3/75 (75) 0.9462	0/65 (64) 1.0000	1/65 (63) 0.9160	0/65 (65) 1.0000	0/75 (75) 1.0000
	Adenoma, benign	3/75 (75) 0.9177	5/65 (64) 0.2749	1/65 (63) 0.9160	1/65 (65) 0.9207	1/75 (75) 0.9400
Gland, mammary	Adenocarcinoma, malignant	2/75 (75) 0.5419	3/65 (64) 0.4250	2/64 (62) 0.6153	2/65 (65) 0.6344	6/75 (75) 0.1378
	Adenoma, benign	0/75 (75) NC	0/65 (64) NC	0/64 (62) NC	0/65 (65) NC	1/75 (75) 0.5000
	Carcinoma, adeno squamous, malignant	4/75 (75) 0.9836	1/65 (64) 0.9571	1/64 (62) 0.9538	0/65 (65) 1.0000	2/75 (75) 0.8954
	Carcinosarcoma, malignant	2/75 (75) 1.0000	0/65 (64) 1.0000	0/64 (62) 1.0000	0/65 (65) 1.0000	1/75 (75) 0.8775
Gland, pituitary	Adenoma, pars distalis, benign	7/75 (75) 0.9988	6/64 (63) 0.5973	3/64 (62) 0.9116	0/65 (65) 1.0000	2/75 (75) 0.9832
	Adenoma, pars intermedia, benign	0/75 (75) 0.2453	0/64 (63) NC	0/64 (62) NC	1/65 (65) 0.4643	0/75 (75) NC
Hindlimb	Osteosarcoma, malignant	0/75 (75) 0.2434	0/65 (64) NC	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC
Kidney	Adenoma, renal cell, benign	0/75 (75) 0.2434	0/65 (64) NC	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC
Liver	Adenocarcinoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Adenoma, hepatocellular, benign	1/75 (75) 0.3698	0/65 (64) 1.0000	1/65 (63) 0.7064	1/65 (65) 0.7148	2/75 (75) 0.5000
	Carcinoma, hepatocellular, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	1/75 (75) 0.7517
	Osteosarcoma, malignant	0/75 (75) 0.7191	1/65 (64) 0.4604	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000

Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC	
Lung	Adenocarcinoma, malignant	3/75 (75) 0.9699	2/65 (64) 0.7636	1/65 (63) 0.9160	0/65 (65) 1.0000	2/75 (75) 0.8167	
	Adenoma, bronchioloalveolar, benign	11/75 (75) 0.1130	10/65 (64) 0.5301	7/65 (63) 0.8076	14/65 (65) 0.2010	12/75 (75) 0.5000	
	Carcinoma, adeno squamous, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000	
	Carcinoma, bronchioloalveolar, malignant	6/75 (75) 0.1888	4/65 (64) 0.7643	3/65 (63) 0.8678	7/65 (65) 0.3916	8/75 (75) 0.3900	
	Carcinoma, hepatocellular, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000	
	Carcinosarcoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000	
	Choriocarcinoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000	
	Fibrosarcoma, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000	
	Mesothelioma, malignant	0/75 (75) 0.4794	0/65 (64) NC	1/65 (63) 0.4565	0/65 (65) NC	0/75 (75) NC	
	Osteosarcoma, malignant	0/75 (75) 0.2910	1/65 (64) 0.4604	0/65 (63) NC	1/65 (65) 0.4643	2/75 (75) 0.2483	
	Lymph node, iliac	Adenocarcinoma, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/64 (64) 1.0000	0/75 (75) 1.0000
		Adenocarcinoma, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/64 (64) 1.0000	0/74 (74) 1.0000
	Lymph node, mandibular	Adenocarcinoma, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/64 (64) 1.0000	0/74 (74) 1.0000
		Mediastinum/Pleura	Carcinosarcoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC
Mesothelioma, malignant			0/75 (75) 0.4833	0/65 (64) NC	2/65 (63) 0.2066	0/65 (65) NC	0/75 (75) NC
Osteosarcoma, malignant	Osteosarcoma, malignant	0/75 (75) 0.2434	0/65 (64) NC	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC	
	Mesentery /Peritoneum	Osteosarcoma, malignant	0/75 (75) 0.7191	1/65 (64) 0.4604	0/65 (63) NC	0/65 (65) NC	0/75 (75) NC
Multicentric neoplasm	Hemangioma, benign	1/75 (75) 0.0060	0/65 (64) 1.0000	1/65 (63) 0.7064	5/65 (65) 0.0748	1/75 (75) 0.7517	
	Hemangiosarcoma, malignant	4/75 (75) 0.0001 *	5/65 (64) 0.4006	14/65 (63) 0.0034 **	18/65 (65) 0.0003 **	2/75 (75) 0.8954	
	Leukemia, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000	
	Lymphoma, malignant	26/75 (75) 0.9239	25/65 (64) 0.3593	19/65 (63) 0.7715	17/65 (65) 0.8987	19/75 (75) 0.9232	
	Sarcoma, histiocytic, malignant	0/75 (75) 0.4116	2/65 (64) 0.2102	0/65 (63) NC	1/65 (65) 0.4643	1/75 (75) 0.5000	

Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
Nerve, sciatic	Fibrosarcoma, malignant	0/75 (75) 0.4794	0/65 (64) NC	1/65 (63) 0.4565	0/65 (65) NC	0/75 (75) NC
Nose	Adenocarcinoma, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
Ovary	Choriocarcinoma, malignant	0/73 (73) NC	0/65 (64) NC	0/63 (61) NC	0/64 (64) NC	1/75 (75) 0.5068
	Cystadenoma, benign	4/73 (73) 0.8445	1/65 (64) 0.9598	0/63 (61) 1.0000	1/64 (64) 0.9598	0/75 (75) 1.0000
	Ovarian sex cord-Stromal tumor, benign	1/73 (73) 0.7321	4/65 (64) 0.1446	2/63 (61) 0.4325	1/64 (64) 0.7179	2/75 (75) 0.5102
	Stromal tumor, gonadal, malignant	1/73 (73) 1.0000	0/65 (64) 1.0000	0/63 (61) 1.0000	0/64 (64) 1.0000	0/75 (75) 1.0000
	Adenoma, islet cell, benign	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
Pancreas	Carcinoma, islet cell, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Carcinoma, basal cell, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
	Papilloma, squamous cell, benign	0/75 (75) 0.4116	2/65 (64) 0.2102	0/65 (63) NC	1/65 (65) 0.4643	1/75 (75) 0.5000
Skin	Carcinoma, basal cell, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
	Papilloma, squamous cell, benign	0/75 (75) 0.4116	2/65 (64) 0.2102	0/65 (63) NC	1/65 (65) 0.4643	1/75 (75) 0.5000
Skin/Subcutis	Fibrosarcoma, malignant	1/75 (75) 0.6677	0/65 (64) 1.0000	2/65 (63) 0.4345	0/65 (65) 1.0000	1/75 (75) 0.7517
	Osteosarcoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	2/75 (75) 0.2483
Small intestine, duodenum	Polyp, glandular, benign	0/75 (75) 0.2434	0/65 (64) NC	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC
Spleen	Adenocarcinoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/64 (64) NC	1/75 (75) 0.5000
Stomach	Papilloma, squamous cell, benign	0/75 (75) 0.4794	0/65 (64) NC	1/65 (63) 0.4565	0/65 (65) NC	0/75 (75) NC
Thymus	Adenocarcinoma, malignant	0/75 (75) NC	0/63 (62) NC	0/60 (58) NC	0/61 (61) NC	1/73 (73) 0.4932
	Carcinoma, bronchioloalveolar, malignant	0/75 (75) 0.2383	0/63 (62) NC	0/60 (58) NC	1/61 (61) 0.4485	1/73 (73) 0.4932
Urinary bladder	Adenocarcinoma, malignant	1/75 (75) 1.0000	0/64 (63) 1.0000	0/65 (63) 1.0000	0/64 (64) 1.0000	0/75 (75) 1.0000

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Uterus/Cervix	Adenocarcinoma, malignant	1/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	2/75 (75)
		1.0000	1.0000	1.0000	1.0000	0.5000
	Granular cell tumor, benign	1/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	0/75 (75)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Leiomyoma, benign	3/75 (75)	0/65 (64)	1/65 (63)	0/65 (65)	1/75 (75)
		0.9462	1.0000	0.9160	1.0000	0.9400
	Polyp, endometrial stromal, benign	1/75 (75)	7/65 (64)	3/65 (63)	1/65 (65)	3/75 (75)
		0.8723	0.0181	0.2471	0.7148	0.3100
	Polyp, glandular, benign	0/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	1/75 (75)
		NC	NC	NC	NC	0.5000
Schwannoma, malignant	0/75 (75)	1/65 (64)	0/65 (63)	1/65 (65)	0/75 (75)	
	0.2910	0.4604	NC	0.4643	NC	
Stromal sarcoma, endometrial, malignant	3/75 (75)	0/65 (64)	0/65 (63)	2/65 (65)	2/75 (75)	
	0.3720	1.0000	1.0000	0.7691	0.8167	
Vagina	Stromal sarcoma, endometrial, malignant	1/74 (74)	0/65 (64)	0/65 (63)	0/65 (65)	0/75 (75)
		1.0000	1.0000	1.0000	1.0000	1.0000

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.
 Note: The p-values marked with an asterisk * indicate statistically significant dose-responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Table copied from the FDA statistical review by Feng Zhou, M.S. (Table 6B).

Appendix 3: Summary of Deaths from Applicant – Rats

Table 19. Probable Cause of Death in Male Rats

Removal Reason: UNSCHEDULED	----- MALES -----				
	0 mg/kg/day Water (55)	0 mg/kg/day Control (50)	2 mg/kg/day (37)	6 mg/kg/day (41)	20 mg/kg/day (47)
Number of Dead Animals on Study:					
BONE TUMOR	0	0	1	0	0
BRAIN TUMOR	0	0	0	1	3
CHRONIC PROGRESSIVE NEPHROPATHY/UREMIA	1	0	0	1	0
DOSING INJURY	0	0	1	0	0
FIBROSARCOMA/FIBROMA	3	0	1	3	0
HEART TUMOR	0	0	0	1	0
LEUKEMIA	0	0	0	0	1
LIVER INFLAMMATION/NECROSIS	1	0	0	0	0
LIVER TUMOR	0	1	0	0	0
LYMPHOID TUMOR	1	1	2	1	0
PANCREAS TUMOR	1	0	0	0	0
PITUITARY TUMOR	23	23	16	15	18
SARCOMA, TYPE UNDETERMINED	0	0	0	1	0
SCHWANNOMA	0	0	0	0	1
SKIN INFLAMMATION/NECROSIS	0	1	0	0	0
SKIN TUMOR	2	2	0	0	0
UNDETERMINED	16	13	12	10	17
URINARY BLADDER TUMOR	0	1	0	0	0
UROGENITAL INFLAMMATION/OBSTRUCTION/CALCULI	0	1	0	2	0
ZYMBALS GLAND TUMOR	2	1	1	0	1
Exsanguination via abdominal vena cava.	0	0	0	1	0
INTESTINAL OSTEOSARCOMA	0	0	0	0	1
NASAL INFLAMMATION	2	0	0	0	0
NASAL INFLAMMATION/FUNGUS	2	0	2	3	3
SKIN INFLAMMATION	0	1	0	0	0
UROGENITAL INFLAMMATION	0	0	0	1	0
cavity, abdominal					
sarcoma (primary site unknown); malignant; primary	0	0	0	0	1
foot/feet					
erosion/ulcer; marked	0	2	0	0	0
inflammation, subacute/chronic; marked	0	1	0	1	0
large intestine, colon					
intussusception; marked	0	1	0	0	0
skin					
laceration; marked	0	0	0	0	1
skin, subcutis					
abscess; marked	1	0	0	0	0
neural crest tumor; benign; primary	0	0	1	0	0
tooth/teeth					
fracture, tooth; marked	0	1	0	0	0

Table copied directly from study report (Study APD334-TOX-002).

Table 20. Probable Cause of Death in Female Rats

Removal Reason: UNSCHEDULED

	FEMALES				
	0 mg/kg/day Water (55)	0 mg/kg/day Control (49)	2 mg/kg/day (36)	6 mg/kg/day (38)	20 mg/kg/day (38)
Number of Dead Animals on Study:					
ADRENAL GLAND TUMOR	1	1	1	0	1
BRAIN TUMOR	2	0	1	0	0
FIBROSARCOMA/FIBROMA	1	0	1	1	4
GASTROINTESTINAL ULCERATION	0	0	0	1	0
HISTIOCYTIC SARCOMA	0	1	0	2	2
LEIOMYOMA/LEIOMYOSARCOMA	0	0	0	2	0
LIVER TUMOR	0	0	0	0	1
LYMPHOID TUMOR	0	1	1	0	1
MAMMARY TUMOR	17	18	8	13	10
PITUITARY TUMOR	28	20	15	12	9
SCHWANNOMA	2	0	0	0	1
UNDETERMINED	1	4	6	4	6
UTERUS HEMORRHAGE	0	0	0	1	1
UTERUS TUMOR	0	1	2	0	1
VAGINA TUMOR	0	1	0	0	0
ZYMBALS GLAND TUMOR	1	0	0	0	0
Exsanguination via abdominal vena cava.	1	0	0	0	0
EYE INFLAMMATION	0	1	0	0	1
UTERUS HEMORRHAGE/INFLAMMATION	0	0	1	0	0
lung					
granuloma, foreign body; moderate	0	0	0	1	0
spleen					
inflammation, chronic-active; marked	0	0	0	1	0
tail					
erosion/ulcer; marked	1	0	0	0	0
urinary bladder					
leiomyosarcoma; malignant; primary	0	1	0	0	0

Table copied directly from study report (Study APD334-TOX-002).

Appendix 4: Tumor Findings in Rats

Table 21. Neoplastic Findings in Male Rats

Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
Body cavity, abdominal	Lipoma, benign	0/75 (48) 0.4749	0/65 (46) NC	1/65 (46) 0.4894	0/65 (39) NC	0/75 (48) NC
	Sarcoma, malignant	0/75 (48) 0.1626	0/65 (46) NC	1/65 (46) 0.4894	1/65 (40) 0.4545	0/75 (48) NC
Bone	Osteoma, benign	0/75 (48) 0.6477	1/65 (47) 0.4947	3/65 (46) 0.1132	0/65 (39) NC	0/75 (48) NC
	Osteosarcoma, malignant	0/75 (48) 0.7318	1/65 (46) 0.4894	0/65 (46) NC	0/65 (39) NC	0/75 (48) NC
Brain	Glioma, mixed, malignant	1/75 (48) 0.0496	0/65 (46) 1.0000	1/65 (46) 0.7419	3/65 (42) 0.2595	1/75 (48) 0.7526
	Granular cell tumor, malignant	1/75 (49) 0.3458	0/65 (46) 1.0000	1/65 (46) 0.7366	1/65 (40) 0.6997	1/75 (48) 0.7474
	Oligodendroglioma, malignant	0/75 (48) 0.2179	0/65 (46) NC	0/65 (46) NC	1/65 (39) 0.4483	0/75 (48) NC
	Osteosarcoma, malignant	0/75 (48) 0.7318	1/65 (46) 0.4894	0/65 (46) NC	0/65 (39) NC	0/75 (48) NC
Gland, adrenal	Adenoma, adrenocortical, benign	0/75 (48) 0.2179	0/65 (46) NC	0/65 (46) NC	1/65 (39) 0.4483	0/75 (48) NC
	Pheochromocytoma, benign	5/75 (49) 0.2251	9/65 (47) 0.1707	5/65 (47) 0.6029	8/65 (43) 0.1966	12/75 (50) 0.0592
	Pheochromocytoma, malignant	1/75 (48) 0.7256	0/65 (46) 1.0000	1/65 (46) 0.7419	0/65 (39) 1.0000	1/75 (48) 0.7526
Gland, mammary	Adenoma, benign	0/75 (48) NC	0/65 (46) NC	0/65 (46) NC	0/65 (39) NC	1/75 (48) 0.5000
	Fibroadenoma, benign	0/75 (48) 0.2717	1/65 (46) 0.4894	0/65 (46) NC	1/65 (39) 0.4483	1/75 (48) 0.5000
Gland, parathyroid	Adenoma, benign	0/71 (46) 0.5302	0/61 (43) NC	3/64 (45) 0.1168	0/60 (36) NC	3/72 (47) 0.1250
Gland, pituitary	Adenoma, pars distalis, benign	39/75 (62) 0.0618	34/65 (56) 0.6680	35/65 (57) 0.6398	40/65 (54) 0.1382	44/75 (61) 0.1842
	Adenoma, pars intermedia, benign	0/75 (48) 0.5870	1/65 (46) 0.4894	1/65 (46) 0.4894	0/65 (39) NC	1/75 (48) 0.5000
	Carcinoma, pars distalis, malignant	0/75 (48) 0.2222	0/65 (46) NC	0/65 (46) NC	1/65 (40) 0.4545	0/75 (48) NC
	Adenoma, benign	3/75 (50) 0.1344	1/65 (46) 0.9307	3/65 (46) 0.6206	4/65 (41) 0.3892	3/75 (48) 0.6415

Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
Gland, salivary, submandibular	Myoepithelioma, malignant	1/75 (48) 1.0000	0/64 (46) 1.0000	0/65 (46) 1.0000	0/64 (39) 1.0000	0/73 (46) 1.0000
Gland, thyroid	Adenoma, c-Cell, benign	8/75 (50) 0.7673	13/65 (48) 0.1377	6/65 (47) 0.7701	6/64 (40) 0.6608	8/75 (49) 0.5900
	Adenoma, follicular cell, benign	0/75 (48) 0.0854	1/65 (46) 0.4894	0/65 (46) NC	2/64 (40) 0.2038	2/75 (48) 0.2474
	Carcinoma, c-Cell, malignant	2/75 (49) 0.8711	0/65 (46) 1.0000	1/65 (46) 0.8669	0/64 (39) 1.0000	1/75 (48) 0.8750
	Carcinoma, follicular cell, malignant	1/75 (49) 0.1271	0/65 (46) 1.0000	1/65 (46) 0.7366	2/64 (39) 0.4142	1/75 (48) 0.7474
Gland, zymbal	Carcinoma, malignant	1/74 (48) 0.4770	1/64 (46) 0.7419	0/65 (46) 1.0000	1/64 (39) 0.6985	2/75 (49) 0.5078
	Papilloma, squamous cell, benign	1/74 (47) 0.7313	0/64 (45) 1.0000	1/65 (46) 0.7473	0/64 (39) 1.0000	0/75 (48) 1.0000
Heart	Mesothelioma, atriocaval, benign	1/75 (48) 1.0000	0/65 (46) 1.0000	0/65 (46) 1.0000	0/65 (39) 1.0000	0/75 (48) 1.0000
	Schwannoma, endocardial, malignant	0/75 (48) 0.4778	0/65 (46) NC	1/65 (47) 0.4947	0/65 (39) NC	0/75 (48) NC
Liver	Adenoma, hepatocellular, benign	2/75 (49) 0.2980	1/65 (47) 0.8711	0/65 (46) 1.0000	2/65 (40) 0.6114	0/75 (48) 1.0000
Lung	Adenoma, bronchioloalveolar, benign	0/75 (48) NC	0/65 (46) NC	0/65 (46) NC	0/65 (39) NC	1/75 (48) 0.5000
	Carcinoma, pars distalis, malignant	0/75 (48) 0.2222	0/65 (46) NC	0/65 (46) NC	1/65 (40) 0.4545	0/75 (48) NC
Lymph node, mesenteric	Lymphangioma, benign	0/75 (48) 0.4749	0/65 (46) NC	1/65 (46) 0.4894	0/64 (39) NC	0/75 (48) NC
Multicentric neoplasm	Hemangiosarcoma, malignant	1/75 (49) 0.3941	0/65 (46) 1.0000	0/65 (46) 1.0000	1/65 (40) 0.6997	1/75 (48) 0.7474
	Leukemia, malignant	0/75 (48) 0.2768	1/65 (46) 0.4894	0/65 (46) NC	1/65 (40) 0.4545	0/75 (48) NC
	Lymphoma, malignant	1/75 (49) 0.8466	2/65 (47) 0.4842	1/65 (47) 0.7421	0/65 (39) 1.0000	1/75 (48) 0.7474
Nose	Adenoma, benign	0/75 (48) 0.4749	0/65 (46) NC	1/65 (46) 0.4894	0/65 (39) NC	0/75 (48) NC
Pancreas	Adenoma, acinar cell, benign	1/75 (48) 0.8022	1/65 (46) 0.7419	1/65 (46) 0.7419	0/65 (39) 1.0000	0/75 (48) 1.0000
	Adenoma, islet cell, benign	2/75 (49) 0.8232	5/65 (46) 0.1923	3/65 (46) 0.4698	1/65 (39) 0.8321	1/75 (48) 0.8750
	Carcinoma, islet cell, malignant	2/75 (48) 0.8580	1/65 (46) 0.8710	2/65 (46) 0.6753	0/65 (39) 1.0000	3/75 (48) 0.5000

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Skin	Adenoma, sebaceous, benign	1/75 (49)	1/65 (46)	0/65 (46)	1/65 (40)	0/75 (48)
		0.4811	0.7366	1.0000	0.6997	1.0000
	Basal cell tumor, benign	1/75 (49)	0/65 (46)	0/65 (46)	0/65 (39)	0/75 (48)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Basosquamous tumor, malignant	0/75 (48)	1/65 (46)	0/65 (46)	0/65 (39)	1/75 (48)
		0.7318	0.4894	NC	NC	0.5000
	Carcinoma, squamous cell, malignant	0/75 (48)	0/65 (46)	0/65 (46)	2/65 (40)	1/75 (48)
		0.0484	NC	NC	0.2038	0.5000
	Hair follicle neoplasm, benign	0/75 (48)	0/65 (46)	0/65 (46)	1/65 (39)	1/75 (48)
		0.2179	NC	NC	0.4483	0.5000
	Keratoacanthoma, benign	5/75 (49)	1/65 (46)	5/65 (46)	2/65 (40)	2/75 (48)
		0.6709	0.9839	0.5888	0.9064	0.9411
Papilloma, benign	0/75 (48)	0/65 (46)	0/65 (46)	1/65 (40)	0/75 (48)	
	0.2222	NC	NC	0.4545	NC	
Papilloma, squamous cell, benign	2/75 (49)	0/65 (46)	0/65 (46)	0/65 (39)	0/75 (48)	
	1.0000	1.0000	1.0000	1.0000	1.0000	
Skin/Subcutis	Fibroma, benign	0/75 (48)	0/65 (46)	3/65 (46)	2/65 (39)	2/75 (49)
		0.0776	NC	0.1132	0.1981	0.2526
	Fibrosarcoma, malignant	0/75 (48)	2/65 (46)	3/65 (48)	1/65 (40)	2/75 (48)
		0.4232	0.2368	0.1211	0.4545	0.2474
	Ganglioneuroma, benign	0/75 (48)	2/65 (47)	0/65 (46)	0/65 (39)	0/75 (48)
		0.7899	0.2421	NC	NC	NC
Lipoma, benign	0/75 (48)	0/65 (46)	1/65 (46)	0/65 (39)	2/75 (48)	
	0.4749	NC	0.4894	NC	0.2474	
Schwannoma, malignant	0/75 (48)	0/65 (46)	0/65 (46)	1/65 (40)	1/75 (48)	
	0.2222	NC	NC	0.4545	0.5000	
Small intestine, jejunum	Osteosarcoma, malignant	0/75 (48)	0/65 (46)	0/65 (46)	1/64 (39)	0/75 (48)
		0.2179	NC	NC	0.4483	NC
Spinal cord	Glioma, malignant	1/75 (49)	0/65 (46)	0/65 (46)	0/65 (39)	0/75 (48)
		1.0000	1.0000	1.0000	1.0000	1.0000
Testis	Leydig cell tumor, benign	3/75 (48)	0/65 (46)	2/65 (46)	1/65 (40)	4/75 (48)
		0.6436	1.0000	0.8057	0.9166	0.5000
Thymus	Thymoma, benign	0/75 (48)	0/63 (44)	1/61 (43)	0/64 (39)	0/74 (47)
		0.4713	NC	0.4725	NC	NC
Urinary bladder	Carcinoma, urothelial, malignant	1/75 (49)	0/65 (46)	0/65 (46)	0/65 (39)	0/75 (48)
		1.0000	1.0000	1.0000	1.0000	1.0000

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.
 Note: The p-values marked with an asterisk * indicate statistically significant dose-responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Table copied from the FDA statistical review by Feng Zhou, M.S. (Table 5A).

Table 22. Neoplastic Findings in Female Rats

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Body cavity, abdominal	Leiomyosarcoma, malignant	0/75 (51)	0/65 (48)	1/65 (48)	0/65 (49)	0/75 (47)
		0.4949	NC	0.4848	NC	NC
Body cavity, thoracic	Hibernoma, malignant	0/75 (51)	0/65 (48)	1/65 (48)	0/65 (49)	0/75 (47)
		0.4949	NC	0.4848	NC	NC
Bone, skull	Carcinoma, pars distalis, malignant	0/75 (51)	1/65 (48)	0/65 (47)	0/65 (49)	0/75 (47)
		0.7385	0.4848	NC	NC	NC
Brain	Carcinoma, pars distalis, malignant	0/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)
		NC	NC	NC	NC	0.4796
	Glioma, mixed, malignant	2/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)
		1.0000	1.0000	1.0000	1.0000	0.8631
	Granular cell tumor, malignant	0/75 (51)	0/65 (48)	1/65 (47)	0/65 (49)	0/75 (47)
		0.4923	NC	0.4796	NC	NC
	Oligodendroglioma, malignant	0/75 (51)	1/65 (48)	0/65 (47)	0/65 (49)	2/75 (49)
0.7385		0.4848	NC	NC	0.2376	
Reticulosis, malignant	0/75 (51)	1/65 (49)	0/65 (47)	0/65 (49)	0/75 (47)	
	0.7398	0.4900	NC	NC	NC	
Eye	Melanoma, amelanotic, malignant	0/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)
		NC	NC	NC	NC	0.4796
Gland, adrenal	Adenoma, adrenocortical, benign	1/75 (51)	3/65 (49)	4/65 (48)	1/65 (49)	2/75 (48)
		0.7081	0.2937	0.1627	0.7424	0.4770
	Carcinoma, adrenocortical, malignant	1/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)
		1.0000	1.0000	1.0000	1.0000	0.7317
	Pheochromocytoma, benign	2/75 (52)	2/65 (48)	0/65 (47)	2/65 (49)	1/75 (47)
		0.4591	0.6604	1.0000	0.6685	0.8591
Pheochromocytoma, malignant	0/75 (51)	1/65 (48)	0/65 (47)	1/65 (49)	0/75 (47)	
		0.3083	0.4848	NC	0.4900	NC
Gland, mammary	Adenocarcinoma, malignant	23/75 (60)	10/65 (51)	12/65 (51)	5/65 (50)	19/74 (53)
		0.9990	0.9915	0.9706	0.9999	0.6796
	Adenoma, benign	1/75 (51)	2/65 (48)	0/65 (47)	1/65 (49)	2/74 (47)
		0.6233	0.4770	1.0000	0.7424	0.4691
	Carcinosarcoma, malignant	1/75 (51)	0/65 (48)	0/65 (47)	1/65 (49)	0/74 (46)
		0.4404	1.0000	1.0000	0.7424	1.0000
	Fibroadenoma, benign	28/75 (57)	25/65 (53)	23/65 (53)	23/65 (55)	27/74 (55)
0.7813		0.6537	0.7861	0.8329	0.5762	
Gland, parathyroid	Adenoma, benign	2/70 (47)	1/60 (43)	3/61 (45)	1/59 (43)	1/68 (42)
		0.6352	0.8620	0.4792	0.8620	0.8572

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day	
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC	
Gland, pituitary	Adenoma, pars distalis, benign	61/74 (68)	38/65 (56)	47/65 (59)	43/65 (57)	57/75 (68)	
		0.8336	0.9995	0.9668	0.9912	0.8975	
	Carcinoma, pars distalis, malignant	0/74 (50)	1/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)	
		0.7423	0.4898	NC	NC	0.4845	
	Schwannoma, malignant	0/74 (50)	1/65 (48)	0/65 (47)	0/65 (49)	0/75 (47)	
		0.7423	0.4898	NC	NC	NC	
	Gland, thyroid	Adenoma, c-Cell, benign	8/75 (53)	11/65 (50)	9/65 (50)	9/65 (51)	7/74 (49)
			0.5164	0.2583	0.4472	0.4651	0.6522
Adenoma, follicular cell, benign		0/75 (51)	1/65 (48)	0/65 (47)	1/65 (49)	0/74 (46)	
		0.3083	0.4848	NC	0.4900	NC	
	Carcinoma, c-Cell, malignant	1/75 (51)	3/65 (48)	1/65 (47)	2/65 (49)	3/74 (46)	
		0.4417	0.2860	0.7317	0.4848	0.2705	
	Carcinoma, follicular cell, malignant	0/75 (51)	0/65 (48)	0/65 (47)	1/65 (49)	0/74 (46)	
		0.2513	NC	NC	0.4900	NC	
Gland, zymbal	Carcinoma, malignant	0/74 (50)	0/64 (47)	0/65 (47)	2/63 (48)	1/74 (47)	
		0.0615	NC	NC	0.2373	0.4845	
	Papilloma, squamous cell, benign	0/74 (50)	0/64 (47)	0/65 (47)	0/63 (48)	1/74 (46)	
	NC	NC	NC	NC	0.4792		
Kidney	Adenoma, renal cell, benign	0/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)	
		NC	NC	NC	NC	0.4796	
Large intestine, cecum	Leiomyoma, benign	0/75 (51)	0/65 (48)	1/65 (47)	0/65 (49)	0/75 (47)	
		0.4923	NC	0.4796	NC	NC	
Large intestine, rectum	Schwannoma, malignant	0/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)	
		NC	NC	NC	NC	0.4796	
Liver	Adenoma, hepatocellular, benign	1/75 (51)	0/65 (48)	1/65 (47)	1/65 (49)	0/75 (47)	
		0.3871	1.0000	0.7317	0.7424	1.0000	
	Cholangiocarcinoma, malignant	0/75 (51)	0/65 (48)	0/65 (47)	1/65 (49)	0/75 (47)	
		0.2513	NC	NC	0.4900	NC	
	Hepatoblastoma, malignant	0/75 (51)	0/65 (48)	1/65 (47)	0/65 (49)	0/75 (47)	
		0.4923	NC	0.4796	NC	NC	
Lung	Pheochromocytoma, malignant	0/75 (51)	0/65 (48)	0/65 (47)	1/65 (49)	0/75 (47)	
		0.2513	NC	NC	0.4900	NC	
	Adenocarcinoma, malignant	0/75 (51)	2/65 (49)	0/65 (47)	0/65 (49)	0/74 (46)	
		0.8025	0.2376	NC	NC	NC	
	Carcinoma, adrenocortical, malignant	1/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/74 (46)	
		1.0000	1.0000	1.0000	1.0000	0.7262	
	Carcinoma, squamous cell, malignant	0/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/74 (46)	
		NC	NC	NC	NC	0.4742	
	Pheochromocytoma, malignant	0/75 (51)	1/65 (48)	0/65 (47)	1/65 (49)	0/74 (46)	
		0.3083	0.4848	NC	0.4900	NC	

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Lymph node, mesenteric	Thymoma, malignant	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/64 (48) 1.0000	0/75 (47) 1.0000
Multicentric	Hemangioma, benign	0/75 (51) 0.7385	1/65 (48) 0.4848	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
	Lymphoma, malignant	1/75 (51) 0.0942	1/65 (49) 0.7424	0/65 (47) 1.0000	3/65 (50) 0.3012	0/75 (47) 1.0000
	Sarcoma, histiocytic, malignant	1/75 (51) 0.2690	1/65 (48) 0.7372	2/65 (49) 0.4848	2/65 (50) 0.4925	0/75 (47) 1.0000
Muscle, skeletal	Sarcoma, malignant	0/75 (51) 0.4923	0/65 (48) NC	1/65 (47) 0.4796	0/65 (49) NC	0/75 (47) NC
	Schwannoma, malignant	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/75 (47) 0.4796
Nerve, optic	Schwannoma, malignant	0/75 (51) 0.7385	1/65 (48) 0.4848	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
Nerve, sciatic	Schwannoma, malignant	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/75 (47) 0.4796
Pancreas	Adenoma, islet cell, benign	0/75 (51) 0.2906	1/65 (48) 0.4848	1/65 (47) 0.4796	1/65 (49) 0.4900	0/75 (47) NC
	Carcinoma, islet cell, malignant	0/75 (51) 0.7385	1/65 (48) 0.4848	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
	Leiomyosarcoma, malignant	0/75 (51) 0.4949	0/65 (48) NC	1/65 (48) 0.4848	0/65 (49) NC	0/75 (47) NC
Skin	Basal cell tumor, benign	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/74 (47) 0.4796
	Carcinoma, squamous cell, malignant	0/75 (51) 0.7385	1/65 (48) 0.4848	0/65 (47) NC	0/65 (49) NC	0/74 (46) NC
	Papilloma, benign	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/74 (47) 0.4796
	Papilloma, squamous cell, benign	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/74 (46) NC
Skin/Subcutis	Fibroma, benign	0/75 (51) 0.1846	0/65 (48) NC	1/65 (48) 0.4848	1/65 (49) 0.4900	0/75 (47) NC
	Fibrosarcoma, malignant	2/75 (51) 0.1487	2/65 (48) 0.6681	1/65 (48) 0.8672	4/65 (51) 0.3390	2/75 (48) 0.6681
	Lipoma, benign	2/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Osteosarcoma, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
Stomach	Leiomyosarcoma, malignant	0/75 (51) 0.4949	0/65 (48) NC	1/65 (48) 0.4848	0/65 (49) NC	0/75 (47) NC
Thymus	Thymoma, malignant	1/75 (51) 1.0000	0/64 (47) 1.0000	0/65 (47) 1.0000	0/61 (46) 1.0000	0/75 (47) 1.0000

Organ name	Tumor name	0	2	6 mg/kg/day	20	0 mg/kg/day
		mg/kg/day Vehicle C P - Trend	mg/kg/day Low (L) P - VC vs. L	Mid (M) P - VC vs. M	mg/kg/day High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Urinary bladder	Leiomyosarcoma, malignant	1/75 (51) 1.0000	0/65 (48) 1.0000	0/64 (46) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Papilloma, transitional cell, benign	1/75 (51) 1.0000	0/65 (48) 1.0000	0/64 (46) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Schwannoma, malignant	0/75 (51) NC	0/65 (48) NC	0/64 (46) NC	0/65 (49) NC	1/75 (47) 0.4796
Uterus/Cervix	Adenocarcinoma, malignant	0/75 (51) 0.8032	2/65 (48) 0.2325	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
	Adenoma, benign	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Carcinoma, squamous cell, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
	Granular cell tumor, benign	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Leiomyoma, benign	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
	Polyp, endometrial stromal, benign	9/75 (53) 0.9439	5/65 (48) 0.8936	5/65 (48) 0.8936	3/65 (50) 0.9817	0/75 (47) 1.0000
	Schwannoma, malignant	0/75 (51) 0.0622	0/65 (48) NC	0/65 (47) NC	2/65 (49) 0.2376	2/75 (48) 0.2325
	Stromal sarcoma, endometrial,	0/75 (51)	1/65 (49)	0/65 (47)	0/65 (49)	0/75 (47)
	Stromal sarcoma, malignant	0/75 (51) 0.4923	0/65 (48) NC	1/65 (47) 0.4796	0/65 (49) NC	0/75 (47) NC
	Vagina	Carcinoma, squamous cell, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900
Granular cell tumor, benign		1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
Polyp, benign		1/75 (51) 0.3871	0/65 (48) 1.0000	1/65 (47) 0.7317	1/65 (49) 0.7424	0/75 (47) 1.0000
Schwannoma, malignant		0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	2/75 (48) 0.2325

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.

Note: The p-values marked with an asterisk * indicate statistically significant dose-responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Table copied from the FDA statistical review by Feng Zhou, M.S. (Table 5B).

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Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION CARCINOGENICITY STUDIES

NDA/BLA #: NDA216956

Drug Name: Etrasimod tablet

Indication(s): The treatment of moderately to severely active ulcerative colitis (UC) (b) (4)
(b) (4)

Applicant: Arena Pharmaceuticals, Inc.
6154 Nancy Ridge Drive • San Diego, CA 92121, USA
Laboratory: (b) (4)
(b) (4)

Consult Received Date(s): Received 11/1/2022

Documents Reviewed: Studies apd334-tox-003 (mice) apd334-tox-002 (rats) and the electronic tumor data (SEND) were submitted on 10/14/2022 (via NDA 216956/S0001).

Review Priority: Priority Review

Biometrics Division: Division of Biometrics VI

Statistical Reviewer: Feng Zhou, MS

Concurring Reviewers: Karl Lin, Ph. D., Team Leader

Medical Division: CDER/OND/OII/DG

Nonclinical Team: Sarah Morgan, Ph.D.; Jackye Peretz, Ph.D.

Project Manager: Anum Shami, PharmD.

Keywords: Carcinogenicity, Dose response

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1 Summary

This review evaluates statistically the data of the 2-year oral carcinogenicity study of Etrasimod (referred to as APD334 hereafter) in rats and the 2-year oral carcinogenicity study of APD334 in mice. The review analyzes the dose-response relationship of tumor incidences and mortality (including tumor-related mortality).

For the rat study:

The reviewer's survival data analysis results showed that the survival analyses didn't show any statistically significant dose response relationship in mortality in males and females when compared with vehicle control. The death rates for the water control group were similar with that of vehicle control group for both male and female rats.

The reviewer's tumor data analysis results showed that there were no statistically significant positive dose response relationships between the treated groups and vehicle control group for male and female rats. The tumor incidences were similar between the vehicle control group and water control group.

For the mouse study:

The reviewer's survival data analyses show a statistically significant dose response relationship in mortality in both males and females when compared with vehicle control group ($P_s < 0.05$). The mortality rates in high dose treated groups were statistical significantly higher than vehicle control group for both male and female mice ($P_s < 0.05$). The death rates for the water control group were similar as that of vehicle control group for both male and female mice.

The reviewer's tumor data analysis results showed that APD334 cause a statistically significant positive dose response in the combined incidence of hemangiosarcoma malignant tumor for all organs in whole body in male and female mice. For this tumor type, the pairwise comparisons between the vehicle control group and APD334 mid dose (6 mg/kg/day) group and APD334 high dose (20 mg/kg/day) group were statistically significant. See Table 1 below for tumor incidences and p-values of the tumor types mentioned above. The tumor incidences were similar between the vehicle control group and water control group.

APD334 also cause a statistically significant positive dose response in incidence of the combination of hemangiosarcoma malignant tumor with hemangioma, benign tumor for all organs in whole body in male and female mice. For this tumor type, the pairwise comparisons between the vehicle control group and APD334 mid dose (6 mg/kg/day) group and APD334 high dose (20 mg/kg/day) group were statistically significant. See Table 1 below for tumor incidences and p-values of the tumor types mentioned above. The tumor incidences were similar between the vehicle control group and water control group.

Table 1: Tumor Types with Statistically Significant Dose Response Relationships and Pairwise Comparisons of Treated Groups and Controls in Mice

SEX	Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
			Vehicle C	Low (L)	Mid (M)	High (H)	Water C (WC)
			P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. WC
Male	Whole Body	Hemangioma, benign	2/75 (74)	2/65 (65)	1/65 (64)	4/65 (63)	2/75 (74)
			0.1137	0.6398	0.8487	0.2673	0.6901

		Hemangiosarcoma, malignant	4/75 (74) 0.0000 *	9/65 (65) 0.0785	17/65 (64) 0.0005 **	31/65 (63) 0.0000 **	8/75 (74) 0.1836
		Comb_Hemangiosarcoma/ Hemangioma	6/75 (74) 0.0000 *	11/65 (65) 0.0928	18/65 (64) 0.0019 **	32/65 (63) 0.0000 **	10/75 (74) 0.2140
Female	Whole Body	Hemangioma, benign	1/75 (75) 0.0060	0/65 (64) 1.0000	1/65 (63) 0.7064	5/65 (65) 0.0748	1/75 (75) 0.7517
		Hemangiosarcoma, malignant	4/75 (75) 0.0001 *	5/65 (64) 0.4006	14/65 (63) 0.0034 **	18/65 (65) 0.0003 **	2/75 (75) 0.8954
		Comb_Hemangiosarcoma/ Hemangioma	5/75 (75) 0.0001 *	5/65 (64) 0.5241	14/65 (63) 0.0081 **	19/65 (65) 0.0004 **	3/75 (75) 0.8622

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NS = Not significant.

Note: The p-values marked with an asterisk * indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

2 Background

The sponsor, Arena Pharmaceuticals, Inc, is submitting, under section 505(b)(1) of the Federal Food, Drug, and Cosmetic Act and 21 CFR 314.50, a New Drug Application (NDA) for etrasimod for the treatment of moderately to severely active ulcerative colitis (UC) (b) (4)

The sponsor submitted two carcinogenicity study reports: Study apd334-tox-003: "A 104-Week Oral Oncogenicity Study in Mice" and Study apd334-tox-002: "A 104-Week Oral Oncogenicity Study in Rats". The study reports and the electronic SEND datasets were submitted on 10/14/2022 (via NDA 216956/S0001).

The phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidences rate as dose increases. Results of this review have been discussed with the nonclinical team.

3 Rat Study- apd334-tox-002

Study Report: apd334-tox-002.pdf (statistical report on page 2784)

SAS data: SEND

The objective of this study was to evaluate the oncogenic potential of a test article, Etrasimod (referred to as APD334 hereafter), in male and female Sprague Dawley CD® [CrI:CD®(SD)] rats when administered daily for up to approximately 91 weeks (104 weeks originally intended) via oral gavage. In addition, steady-state systemic exposure to APD334 was evaluated on Day 364. The study design was as follows:

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume ^a (mL/kg)	Dose Concentration (mg/mL)	Main Study	
					No. of Males	No. of Females
1	Water control	0	10	0	75	75
2	Control article ^b	0	10	0	75	75
3	APD334 L-arginine	2	10/15.0 ^c /1 ^d	0.2 ^c /2 ^d	65	65
4	APD334 L-arginine	6	10/20.6 ^c /3 ^d	0.6 ^c /2 ^d	65	65
5	APD334 L-arginine	20	10	2	65	65

^a Based on the most recent body weight measurement.

^b The formulated control article was an aqueous solution of L-arginine hydrochloride at the concentration of L-arginine (0.92 mg/mL) in the high-dose formulation (20 mg/kg/day).

^c Due to low recovery in the Week 8 low and mid-dose formulation samples, animals in Groups 3 and 4 were dosed on Days 52 and 53 with dose concentrations of 0.2 and 0.6 mg/mL, respectively, using dose volumes of 15.0 mL/kg and 20.6 mL/kg, respectively, to ensure the administration of the proper dosages.

^d Beginning on Day 54 and for the remainder of the dosing phase, a dose concentration of 2 mg/mL was used for Groups 3 and 4, with dose volumes of 1 mL/kg and 3 mL/kg, respectively.

The following parameters and endpoints were evaluated in this study: mortality/survival, clinical signs and masses, body weight, body weight gains, food consumption, toxicokinetic parameters, and macroscopic and microscopic examinations.

3.1 Sponsor's Analyses

3.1.1 Survival Analysis

Intercurrent mortality data were analyzed using the Kaplan-Meier product-limit method. An overall test comparing all groups was conducted using a log-rank test (Allison, PD, 1995). If this overall test was significant ($p < 0.05$) and there were more than two groups, then a follow up analysis was done where each treatment group was compared to the control group using a log-rank test.

Sponsor's concluded results: No definitive APD334-related effects on survival were observed.

3.1.2 Tumor Data Analysis

Tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test (Agresti, A. 2002) were calculated, and Fisher's exact test (Zar JH, 1999) was used to compare each treatment group with the control group. Trend test was applied for all control and treatment groups that were able to be arranged in a strictly increasing dose level. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto et al. (Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, Pete J, Richards S, Wahrendorf J. 1980).

The Poly-3 method (Bailer AJ, Portier CJ. 1988) (Bieler GS, Williams RL.1993) was used to assess prevalence of tumors. The survival-adjusted rates based on the risk weights were displayed. The tests of significance included both an overall trend and pair-wise comparisons of each treatment group with the control. All p-values were reported using upper-tailed tests, unless otherwise indicated.

Evaluation criteria (p-values of significance) was applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%) (Haseman JK, 1983). The evaluation criteria (from the FDA) (CDER, 2001) are given in the following table.

Evaluation Criteria for Common and Rare Tumors

Test for Positive Trends	Control-High Pair-wise Comparisons
Common and rare tumors were tested at 0.005 and 0.025 significance levels, respectively using upper-tailed tests	Common and rare tumors were tested at 0.01 and 0.05 significance levels, respectively using upper-tailed tests

Sponsor's concluded results: There were no APD334-related oncogenic findings.

3.2 Reviewer's Analyses

To verify the sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this review analyzed the SAS data sets of these studies received on 10/14/2022 via NDA 216956/S0001. The vehicle control group was compared to the treated groups (pairwise and trend) and vehicle control group was compared to water control (pairwise only) for the survival analyses and the tumor analyses.

3.2.1 Data Quality

The sponsor only submitted SEND datasets on 10/14/2022 via NDA 216956/S0001. The data quality was acceptable.

3.2.2 Survival Analysis

The survival distributions of rates in all treatment groups were estimated using the Kaplan-Meier product limit method. For control, low, medium, and high dose groups, the dose response relationship was tested using the likelihood ratio test and the homogeneity of survival distributions were tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 1A and 1B in the appendix for male and female rats, respectively. The intercurrent mortality data are given in Tables 1A and 1B in the appendix for male and female rats, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 3A and 3B in the appendix for male and female rats, respectively.

Reviewer's findings: The termination started at Week 91 for male rats and 90 for female rats. This reviewer's analysis showed the numbers (percent) of death that occurred prior to termination of the group were 55 (73%), 50 (67%), 37 (57%), 40 (62%), or 47 (72%) in male rats and 55 (73%), 49 (65%), 36 (55%), 38 (58%), or 38 (58%) in female rats in the WC, VC, LD, MD, or HD groups, respectively. The survival analyses didn't show any statistically significant dose response relationship in mortality in males and females when compared with vehicle control. The death rates for the water control group were similar with that of vehicle control group for both male and female rats.

3.2.3 Tumor Data Analysis

The tumor data were analyzed for dose response relationships and pairwise comparisons of control group with each of the treated groups. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-k method described in the papers of Bailer and Portier [2] and Bieler and Williams [3]. In this method an animal that lives the full study period ($w_{\max}$) or dies before the terminal sacrifice but develops the tumor type being tested gets a score of $s_h = 1$. An animal that dies at week w_h without developing the tumor before the end of the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}}\right)^k < 1$. The adjusted group size is defined as $\sum s_h$. As an interpretation, an animal with score $s_h = 1$ can be considered as a whole animal

while an animal with score $s_h < 1$ can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor of the tumor type being tested, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidences pattern with the increased dose. For long term 104-week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used.

The adjusted levels of significance for testing a positive dose response in the 2-year rat study are 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The adjusted levels of significance for the pairwise comparison in the 2-year rat study are 0.01 and 0.05 for a common tumor and a rare tumor, respectively. A rare tumor is defined as one in which the tumor rate is less than 1% in the vehicle control group. The tumor rates and the p-values of the tested tumor types are listed in Tables 5A, and 5B in the appendix for male and female rats, respectively.

Reviewer's findings: Following table displays the tumor types showing p-values less than or equal to 0.05 either for dose response relationships or for pairwise comparisons of treated groups and control for the injection sites.

Tumor Types with Statistically Significant (at 0.05 significant level) Dose Response Relationships or Pairwise Comparisons of Treated Groups and Vehicle Control in Rats

SEX	Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
			Vehicle C	Low (L)	Mid (M)	High (H)	Water C (WC)
			P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. WC
Male Rats	Brain	Glioma, mixed, malignant	1/75 (48)	0/65 (46)	1/65 (46)	3/65 (42)	1/75 (48)
			0.0496	1.0000	0.7419	0.2595	0.7526
	Skin	Carcinoma, squamous cell, malignant	0/75 (48)	0/65 (46)	0/65 (46)	2/65 (40)	1/75 (48)
			0.0484	NC	NC	0.2038	0.5000

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NS = Not significant.

Note: The p-values marked with an asterisk * indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Based on the criteria of adjustment for multiple testing discussed above, there were no statistically significant positive dose response relationships between the treated groups and vehicle control group for male and female rats. The tumor incidences were similar between the vehicle control group and water control group.

4 Mouse Study- apd334-tox-003

Study Report: apd334-tox-003.pdf (statistical report on page 3518)

SAS data: SEND database

The objective of this study was to evaluate the oncogenic potential of the test article, Etrasimod (referred to as APD334 hereafter), in male and female Crl:CD1® (ICR) mice when administered via daily oral gavage for up to 104 weeks. In addition, steady-state systemic exposure to APD334 was evaluated on Day 364. The study design was as follows:

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume ^a (mL/kg)	Dose Concentration (mg/mL)	Main Study	
					No. of Males	No. of Females
1	Water control	0	10	0	75	75
2	Control article ^b	0	10	0	75	75
3	APD334 L-arginine	2	1/10 ^c	2/0.2 ^c	65	65
4	APD334 L-arginine	6	3/10 ^c	2/0.6 ^c	65	65
5	APD334 L-arginine	20	10	2	65	65

^a Based on the most recent body weight measurement.

^b The control article was an aqueous solution of L-arginine hydrochloride at the concentration of L-arginine (0.92 mg/mL) in the high dose of the formulation (20 mg/kg/day).

^c Beginning on Day 92, the dose concentrations for the low- and mid-dose groups were changed to 0.2 mg/mL and 0.6 mg/mL, respectively, using dose volumes of 10 mL/kg for both groups, so as to allow for equal dose volumes across all groups.

The following parameters and endpoints were evaluated in this study: mortality/survival, clinical signs, body weights, body weight gains, food consumption, toxicokinetic parameters, and macroscopic and microscopic examinations.

4.1 Sponsor's Analyses

4.1.1 Survival Analysis

The sponsor used the same survival analysis methods for the rat study in this mouse study.

Sponsor's concluded results: Statistically significant decreases in survival were noted in males at 20 mg/kg/day when compared to both the water control and control article groups, but not in any of the other groups on study.

4.1.2 Tumor Data Analysis

The sponsor used the same tumor data analysis methods for the rat study in this mouse study.

Sponsor's findings: Administration of with APD334 was associated with an increase in incidence of hemangiosarcomas and/or hemangiomas in males and females at ≥ 6 mg/kg/day which resulted in increased mortality at these dose levels and early dosing cessation/termination of males at 20 mg/kg/day and females at all dose levels. The hemangiosarcomas were associated with the macroscopic findings of cysts in the liver and edema in the subcutis in males at 20 mg/kg/day and in red discoloration of some of the masses in the subcutis of the females at 20 mg/kg/day. Commonly affected tissues were the liver, the subcutis, the heart, the bone marrow, and the spleen. A few hemangiosarcomas /hemangiomas were also noted in the uterus, intestine, skeletal muscle, lung, thymus, adrenal gland, kidney, eye, mesenteric lymph node, mesentery/peritoneum, spinal cord, and

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epididymides. The hemangiosarcomas and hemangiomas in males and females at ≥ 6 mg/kg/day occurred earlier in the study compared to those present in water control and control article groups. The number of hemangiosarcomas/hemangiomas present per affected animals was also increased in males at ≥ 6 mg/kg/day and in females at 20 mg/kg/day.

4.2 Reviewer's Analyses

To verify the sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this review analyzed the SAS data sets of these studies received on 10/14/2022 via NDA 216956/S0001. The vehicle control group was compared to the treated groups (pairwise and trend) and vehicle control group was compared to water control (pairwise only) for the survival analyses and the tumor analyses.

4.2.1 Data Quality

The sponsor only submitted SEND datasets on 10/14/2022 via NDA 216956/S0001. The data quality was acceptable.

4.2.2 Survival Analysis

The Kaplan-Meier curves for survival rates of all treatment groups are given in Figures 2A and 2B in the appendix for male and female mice, respectively. The intercurrent mortality data of all treatment groups are given in Tables 2A and 2B in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals for control, low, medium, and high dose groups are given in Tables 4A and 4B in the appendix for male and female mice, respectively.

Reviewer's findings: The termination started at Week 99 for male mice and 92 for female mice. This reviewer's analysis showed the numbers (percent) of death that occurred prior to termination of the group were 54 (72%), 48 (64%), 43 (66%), 47 (72%), or 50 (77%) in male mice and 55 (73%), 52 (69%), 45 (69%), 50 (77%), or 50 (77%) in female mice in the WC, VC, LD, MD, or HD groups, respectively. The survival analyses show a statistically significant dose response relationship in mortality in both males and females when compared with vehicle control group ($P < 0.05$). The mortality rates in high dose treated groups were statistically significantly higher than vehicle control group for both male and female mice ($P < 0.05$).

The death rates for the water control group were similar as that of vehicle control group for both male and female mice.

4.2.3 Tumor Data Analysis

The tumor data were analyzed for dose response relationships and pairwise comparisons of the water control group and the vehicle control group separately with each of the treated groups

using the same method that was used for the rat study. The tumor rates and the p-values of the tested tumor types are listed in Tables 6A, and 6B in the appendix for male and female mice, respectively.

Reviewer's findings: Based on the criteria of adjustment for multiple testing discussed above, the positive dose response was statistically significant in the combined incidence of hemangiosarcoma malignant tumor for all organs in whole body in male and female mice ($P_s < 0.005$) when compared with vehicle control group. For this tumor type, the incidences rates of mid dose group and high dose group were statistically significantly higher than vehicle ($P_s < 0.01$). The tumor incidences were similar between the vehicle control group and water control group.

There was a statistically significant positive dose response in incidence of the combination of hemangiosarcoma malignant tumor with hemangioma, benign tumor for all organs in whole body in male and female mice. mice ($P_s < 0.005$) when compared with vehicle control group. For this tumor type, the incidences rates of mid dose group and high dose group were statistically significantly higher than vehicle ($P_s < 0.01$). The tumor incidences were similar between the vehicle control group and water control group.

Tumor Types with Statistically Significant (at 0.05 significant level) Dose Response Relationships or Pairwise Comparisons of Treated Groups and Vehicle Control in Mice

SEX	Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day		
			Vehicle C	Low (L)	Mid (M)	High (H)	Water C (WC)		
			P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. WC		
Male	Whole Body	Hemangioma, benign	2/75 (74)	2/65 (65)	1/65 (64)	4/65 (63)	2/75 (74)		
			0.1137	0.6398	0.8487	0.2673	0.6901		
		Hemangiosarcoma, malignant	4/75 (74)	9/65 (65)	17/65 (64)	31/65 (63)	8/75 (74)		
			0.0000 *	0.0785	0.0005 **	0.0000 **	0.1836		
Male	Whole Body	Comb_Hemangiosarcoma/Hemangioma	6/75 (74)	11/65 (65)	18/65 (64)	32/65 (63)	10/75 (74)		
			0.0000 *	0.0928	0.0019 **	0.0000 **	0.2140		
		Female	Whole Body	Hemangioma, benign	1/75 (75)	0/65 (64)	1/65 (63)	5/65 (65)	1/75 (75)
					0.0060	1.0000	0.7064	0.0748	0.7517
Hemangiosarcoma, malignant	4/75 (75)			5/65 (64)	14/65 (63)	18/65 (65)	2/75 (75)		
	0.0001 *			0.4006	0.0034 **	0.0003 **	0.8954		
Female	Whole Body	Comb_Hemangiosarcoma/Hemangioma	5/75 (75)	5/65 (64)	14/65 (63)	19/65 (65)	3/75 (75)		
			0.0001 *	0.5241	0.0081 **	0.0004 **	0.8622		

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NS = Not significant.

Note: The p-values marked with an asterisk * indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Feng Zhou
Mathematical Statistician

Concurring Reviewer: Karl Lin, Ph.D., Team Leader, Biometrics-6

cc:

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5 Appendix

Table 1A: Intercurrent Mortality Rate in Male Rats

Week / Type of Death	Water Control		Vehicle Control		Low Dose		Mid Dose		High Dose	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	5	6.67	8	10.67	3	4.62	7	10.77	9	13.85
53 - 78	31	48.00	26	45.33	19	33.85	19	40.00	24	50.77
79 - 96	19	73.33	16	66.67	14	55.38	14	61.54	14	72.31
Accidental Death	.	.	.	.	1	1.54	.	.	.	.
Terminal sacrifice	20	26.67	25	33.33	28	43.08	25	38.46	18	27.69
Total	75	.	75	.	65	.	65	.	65	.

Table 1B: Intercurrent Mortality Rate in Female Rats

Week / Type of Death	Water Control		Vehicle Control		Low Dose		Mid Dose		High Dose	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	5	6.67	6	8.00	5	7.69	2	3.08	2	3.08
53 - 78	34	52.00	28	45.33	15	30.77	20	33.85	18	30.77
79 - 96	16	73.33	15	65.33	16	55.38	16	58.46	18	58.46
Terminal sacrifice	20	26.67	26	34.67	29	44.62	27	41.54	27	41.54
Total	75	.	75	.	65	.	65	.	65	.

Table 2A: Intercurrent Mortality Rate in Male Mice

Week / Type of Death	Water Control		Vehicle Control		Low Dose		Mid Dose		High Dose	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	1	1.33	3	4.00	7	10.77	3	4.62	9	13.85
53 - 78	16	22.67	16	25.33	15	33.85	15	27.69	22	47.69
79 - 96	20	49.33	22	54.67	16	58.46	26	67.69	17	73.85
97 - 104	17	72.00	7	64.00	5	66.15	3	72.31	2	76.92
Terminal sacrifice	21	28.00	27	36.00	22	33.85	18	27.69	15	23.08
Total	75	.	75	.	65	.	65	.	65	.

Table 2B: Intercurrent Mortality Rate in Female Mice

Week / Type of Death	Water Control		Vehicle Control		Low Dose		Mid Dose		High Dose	
	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %	No. of Death	Cum %
0 - 52	4	5.33	8	10.67	5	7.69	7	10.77	7	10.77
53 - 78	20	32.00	20	37.33	17	33.85	24	47.69	27	52.31
79 - 96	27	68.00	23	68.00	21	66.15	19	76.92	16	76.92
97 - 104	4	73.33	1	69.33	2	69.23	.	.	.	.
Terminal sacrifice	20	26.67	23	30.67	20	30.77	15	23.08	15	23.08
Total	75	.	75	.	65	.	65	.	65	.

Table 3A: Intercurrent Mortality Comparison in Male Rats

Test	All Dose Groups	Vehicle C vs. Low	Vehicle C vs. Mid	Vehicle C vs. High	Vehicle C vs. Water C
Dose-Response (Likelihood Ratio)	0.1362	0.1920	0.3747	0.4339	0.5255
Homogeneity (Log-Rank)	0.1618	0.1877	0.3691	0.4261	0.5195

Table 3B: Intercurrent Mortality Comparison in Female Rats

Test	All Dose Groups	Vehicle C vs. Low	Vehicle C vs. Mid	Vehicle C vs. High	Vehicle C vs. Water C
Dose-Response (Likelihood Ratio)	0.4690	0.2181	0.3603	0.2499	0.2616
Homogeneity (Log-Rank)	0.5426	0.2126	0.3547	0.2440	0.2536

Table 4A: Intercurrent Mortality Comparison in Male Mice

Test	All Dose Groups	Vehicle C vs. Low	Vehicle C vs. Mid	Vehicle C vs. High	Vehicle C vs. Water C
Dose-Response (Likelihood Ratio)	0.0041	0.6168	0.3004	0.0055	0.6568
Homogeneity (Log-Rank)	0.0280	0.6132	0.2929	0.0044	0.6535

Table 4B: Intercurrent Mortality Comparison in Female Mice

Test	All Dose Groups	Vehicle C vs. Low	Vehicle C vs. Mid	Vehicle C vs. High	Vehicle C vs. Water C
Dose-Response (Likelihood Ratio)	0.0069	0.9484	0.1668	0.0124	0.8028
Homogeneity (Log-Rank)	0.0344	0.9478	0.1591	0.0107	0.8002

Table 5A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Rats Compared with Vehicle Control

Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
		Vehicle C	Low (L)	Mid (M)	High (H)	Water C (WC)
		P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. WC

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Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
Body cavity, abdominal	Lipoma, benign	0/75 (48) 0.4749	0/65 (46) NC	1/65 (46) 0.4894	0/65 (39) NC	0/75 (48) NC
	Sarcoma, malignant	0/75 (48) 0.1626	0/65 (46) NC	1/65 (46) 0.4894	1/65 (40) 0.4545	0/75 (48) NC
Bone	Osteoma, benign	0/75 (48) 0.6477	1/65 (47) 0.4947	3/65 (46) 0.1132	0/65 (39) NC	0/75 (48) NC
	Osteosarcoma, malignant	0/75 (48) 0.7318	1/65 (46) 0.4894	0/65 (46) NC	0/65 (39) NC	0/75 (48) NC
Brain	Glioma, mixed, malignant	1/75 (48) 0.0496	0/65 (46) 1.0000	1/65 (46) 0.7419	3/65 (42) 0.2595	1/75 (48) 0.7526
	Granular cell tumor, malignant	1/75 (49) 0.3458	0/65 (46) 1.0000	1/65 (46) 0.7366	1/65 (40) 0.6997	1/75 (48) 0.7474
	Oligodendroglioma, malignant	0/75 (48) 0.2179	0/65 (46) NC	0/65 (46) NC	1/65 (39) 0.4483	0/75 (48) NC
	Osteosarcoma, malignant	0/75 (48) 0.7318	1/65 (46) 0.4894	0/65 (46) NC	0/65 (39) NC	0/75 (48) NC
Gland, adrenal	Adenoma, adrenocortical, benign	0/75 (48) 0.2179	0/65 (46) NC	0/65 (46) NC	1/65 (39) 0.4483	0/75 (48) NC
	Pheochromocytoma, benign	5/75 (49) 0.2251	9/65 (47) 0.1707	5/65 (47) 0.6029	8/65 (43) 0.1966	12/75 (50) 0.0592
	Pheochromocytoma, malignant	1/75 (48) 0.7256	0/65 (46) 1.0000	1/65 (46) 0.7419	0/65 (39) 1.0000	1/75 (48) 0.7526
Gland, mammary	Adenoma, benign	0/75 (48) NC	0/65 (46) NC	0/65 (46) NC	0/65 (39) NC	1/75 (48) 0.5000
	Fibroadenoma, benign	0/75 (48) 0.2717	1/65 (46) 0.4894	0/65 (46) NC	1/65 (39) 0.4483	1/75 (48) 0.5000
Gland, parathyroid	Adenoma, benign	0/71 (46) 0.5302	0/61 (43) NC	3/64 (45) 0.1168	0/60 (36) NC	3/72 (47) 0.1250
Gland, pituitary	Adenoma, pars distalis, benign	39/75 (62) 0.0618	34/65 (56) 0.6680	35/65 (57) 0.6398	40/65 (54) 0.1382	44/75 (61) 0.1842
	Adenoma, pars intermedia, benign	0/75 (48) 0.5870	1/65 (46) 0.4894	1/65 (46) 0.4894	0/65 (39) NC	1/75 (48) 0.5000
	Carcinoma, pars distalis, malignant	0/75 (48) 0.2222	0/65 (46) NC	0/65 (46) NC	1/65 (40) 0.4545	0/75 (48) NC
	Gland, prostate	Adenoma, benign	3/75 (50) 0.1344	1/65 (46) 0.9307	3/65 (46) 0.6206	4/65 (41) 0.3892
Gland, salivary, submandibular	Myoepithelioma, malignant	1/75 (48) 1.0000	0/64 (46) 1.0000	0/65 (46) 1.0000	0/64 (39) 1.0000	0/73 (46) 1.0000
Gland, thyroid	Adenoma, c-Cell, benign	8/75 (50) 0.7673	13/65 (48) 0.1377	6/65 (47) 0.7701	6/64 (40) 0.6608	8/75 (49) 0.5900
	Adenoma, follicular cell, benign	0/75 (48) 0.0854	1/65 (46) 0.4894	0/65 (46) NC	2/64 (40) 0.2038	2/75 (48) 0.2474
	Carcinoma, c-Cell, malignant	2/75 (49) 0.8711	0/65 (46) 1.0000	1/65 (46) 0.8669	0/64 (39) 1.0000	1/75 (48) 0.8750
	Carcinoma, follicular cell, malignant	1/75 (49) 0.1271	0/65 (46) 1.0000	1/65 (46) 0.7366	2/64 (39) 0.4142	1/75 (48) 0.7474

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Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
		Vehicle C	Low (L)	Mid (M)	High (H)	Water C (WC)
		P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. WC
Gland, zymbal	Carcinoma, malignant	1/74 (48) 0.4770	1/64 (46) 0.7419	0/65 (46) 1.0000	1/64 (39) 0.6985	2/75 (49) 0.5078
	Papilloma, squamous cell, benign	1/74 (47) 0.7313	0/64 (45) 1.0000	1/65 (46) 0.7473	0/64 (39) 1.0000	0/75 (48) 1.0000
Heart	Mesothelioma, atriocaval, benign	1/75 (48) 1.0000	0/65 (46) 1.0000	0/65 (46) 1.0000	0/65 (39) 1.0000	0/75 (48) 1.0000
	Schwannoma, endocardial, malignant	0/75 (48) 0.4778	0/65 (46) NC	1/65 (47) 0.4947	0/65 (39) NC	0/75 (48) NC
Liver	Adenoma, hepatocellular, benign	2/75 (49) 0.2980	1/65 (47) 0.8711	0/65 (46) 1.0000	2/65 (40) 0.6114	0/75 (48) 1.0000
Lung	Adenoma, bronchioloalveolar, benign	0/75 (48) NC	0/65 (46) NC	0/65 (46) NC	0/65 (39) NC	1/75 (48) 0.5000
	Carcinoma, pars distalis, malignant	0/75 (48) 0.2222	0/65 (46) NC	0/65 (46) NC	1/65 (40) 0.4545	0/75 (48) NC
Lymph node, mesenteric	Lymphangioma, benign	0/75 (48) 0.4749	0/65 (46) NC	1/65 (46) 0.4894	0/64 (39) NC	0/75 (48) NC
Nose	Adenoma, benign	0/75 (48) 0.4749	0/65 (46) NC	1/65 (46) 0.4894	0/65 (39) NC	0/75 (48) NC
Pancreas	Adenoma, acinar cell, benign	1/75 (48) 0.8022	1/65 (46) 0.7419	1/65 (46) 0.7419	0/65 (39) 1.0000	0/75 (48) 1.0000
	Adenoma, islet cell, benign	2/75 (49) 0.8232	5/65 (46) 0.1923	3/65 (46) 0.4698	1/65 (39) 0.8321	1/75 (48) 0.8750
	Carcinoma, islet cell, malignant	2/75 (48) 0.8580	1/65 (46) 0.8710	2/65 (46) 0.6753	0/65 (39) 1.0000	3/75 (48) 0.5000
Skin	Adenoma, sebaceous, benign	1/75 (49) 0.4811	1/65 (46) 0.7366	0/65 (46) 1.0000	1/65 (40) 0.6997	0/75 (48) 1.0000
	Basal cell tumor, benign	1/75 (49) 1.0000	0/65 (46) 1.0000	0/65 (46) 1.0000	0/65 (39) 1.0000	0/75 (48) 1.0000
	Basosquamous tumor, malignant	0/75 (48) 0.7318	1/65 (46) 0.4894	0/65 (46) NC	0/65 (39) NC	1/75 (48) 0.5000
	Carcinoma, squamous cell, malignant	0/75 (48) 0.0484	0/65 (46) NC	0/65 (46) NC	2/65 (40) 0.2038	1/75 (48) 0.5000
	Hair follicle neoplasm, benign	0/75 (48) 0.2179	0/65 (46) NC	0/65 (46) NC	1/65 (39) 0.4483	1/75 (48) 0.5000
	Keratoacanthoma, benign	5/75 (49) 0.6709	1/65 (46) 0.9839	5/65 (46) 0.5888	2/65 (40) 0.9064	2/75 (48) 0.9411
	Papilloma, benign	0/75 (48) 0.2222	0/65 (46) NC	0/65 (46) NC	1/65 (40) 0.4545	0/75 (48) NC
	Papilloma, squamous cell, benign	2/75 (49) 1.0000	0/65 (46) 1.0000	0/65 (46) 1.0000	0/65 (39) 1.0000	0/75 (48) 1.0000
	Skin/Subcutis	Fibroma, benign	0/75 (48) 0.0776	0/65 (46) NC	3/65 (46) 0.1132	2/65 (39) 0.1981
Fibrosarcoma, malignant		0/75 (48) 0.4232	2/65 (46) 0.2368	3/65 (48) 0.1211	1/65 (40) 0.4545	2/75 (48) 0.2474
Ganglioneuroma, benign		0/75 (48)	2/65 (47)	0/65 (46)	0/65 (39)	0/75 (48)

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Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
		0.7899	0.2421	NC	NC	NC
	Lipoma, benign	0/75 (48)	0/65 (46)	1/65 (46)	0/65 (39)	2/75 (48)
		0.4749	NC	0.4894	NC	0.2474
	Schwannoma, malignant	0/75 (48)	0/65 (46)	0/65 (46)	1/65 (40)	1/75 (48)
		0.2222	NC	NC	0.4545	0.5000
Small intestine, jejunum	Osteosarcoma, malignant	0/75 (48)	0/65 (46)	0/65 (46)	1/64 (39)	0/75 (48)
		0.2179	NC	NC	0.4483	NC
Spinal cord	Glioma, malignant	1/75 (49)	0/65 (46)	0/65 (46)	0/65 (39)	0/75 (48)
		1.0000	1.0000	1.0000	1.0000	1.0000
Testis	Leydig cell tumor, benign	3/75 (48)	0/65 (46)	2/65 (46)	1/65 (40)	4/75 (48)
		0.6436	1.0000	0.8057	0.9166	0.5000
Thymus	Thymoma, benign	0/75 (48)	0/63 (44)	1/61 (43)	0/64 (39)	0/74 (47)
		0.4713	NC	0.4725	NC	NC
Urinary bladder	Carcinoma, urothelial, malignant	1/75 (49)	0/65 (46)	0/65 (46)	0/65 (39)	0/75 (48)
		1.0000	1.0000	1.0000	1.0000	1.0000
Whole Body	Hemangiosarcoma, malignant	1/75 (49)	0/65 (46)	0/65 (46)	1/65 (40)	1/75 (48)
		0.3941	1.0000	1.0000	0.6997	0.7474
	Leukemia, malignant	0/75 (48)	1/65 (46)	0/65 (46)	1/65 (40)	0/75 (48)
		0.2768	0.4894	NC	0.4545	NC
	Lymphoma, malignant	1/75 (49)	2/65 (47)	1/65 (47)	0/65 (39)	1/75 (48)
		0.8466	0.4842	0.7421	1.0000	0.7474

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.

Note: The p-values marked with an asterisk * indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Table 5B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Rats Compared with Vehicle Control

Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
Body cavity, abdominal	Leiomyosarcoma, malignant	0/75 (51)	0/65 (48)	1/65 (48)	0/65 (49)	0/75 (47)
		0.4949	NC	0.4848	NC	NC
Body cavity, thoracic	Hibernoma, malignant	0/75 (51)	0/65 (48)	1/65 (48)	0/65 (49)	0/75 (47)
		0.4949	NC	0.4848	NC	NC
Bone, skull	Carcinoma, pars distalis, malignant	0/75 (51)	1/65 (48)	0/65 (47)	0/65 (49)	0/75 (47)
		0.7385	0.4848	NC	NC	NC
Brain	Carcinoma, pars distalis, malignant	0/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)
		NC	NC	NC	NC	0.4796
	Glioma, mixed, malignant	2/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)
		1.0000	1.0000	1.0000	1.0000	0.8631
	Granular cell tumor, malignant	0/75 (51)	0/65 (48)	1/65 (47)	0/65 (49)	0/75 (47)
		0.4923	NC	0.4796	NC	NC
	Oligodendroglioma, malignant	0/75 (51)	1/65 (48)	0/65 (47)	0/65 (49)	2/75 (49)
		0.7385	0.4848	NC	NC	0.2376

Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
	Reticulosis, malignant	0/75 (51) 0.7398	1/65 (49) 0.4900	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
Eye	Melanoma, amelanotic, malignant	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/75 (47) 0.4796
Gland, adrenal	Adenoma, adrenocortical, benign	1/75 (51) 0.7081	3/65 (49) 0.2937	4/65 (48) 0.1627	1/65 (49) 0.7424	2/75 (48) 0.4770
	Carcinoma, adrenocortical, malignant	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	1/75 (47) 0.7317
	Pheochromocytoma, benign	2/75 (52) 0.4591	2/65 (48) 0.6604	0/65 (47) 1.0000	2/65 (49) 0.6685	1/75 (47) 0.8591
	Pheochromocytoma, malignant	0/75 (51) 0.3083	1/65 (48) 0.4848	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
Gland, mammary	Adenocarcinoma, malignant	23/75 (60) 0.9990	10/65 (51) 0.9915	12/65 (51) 0.9706	5/65 (50) 0.9999	19/74 (53) 0.6796
	Adenoma, benign	1/75 (51) 0.6233	2/65 (48) 0.4770	0/65 (47) 1.0000	1/65 (49) 0.7424	2/74 (47) 0.4691
	Carcinosarcoma, malignant	1/75 (51) 0.4404	0/65 (48) 1.0000	0/65 (47) 1.0000	1/65 (49) 0.7424	0/74 (46) 1.0000
	Fibroadenoma, benign	28/75 (57) 0.7813	25/65 (53) 0.6537	23/65 (53) 0.7861	23/65 (55) 0.8329	27/74 (55) 0.5762
Gland, parathyroid	Adenoma, benign	2/70 (47) 0.6352	1/60 (43) 0.8620	3/61 (45) 0.4792	1/59 (43) 0.8620	1/68 (42) 0.8572
Gland, pituitary	Adenoma, pars distalis, benign	61/74 (68) 0.8336	38/65 (56) 0.9995	47/65 (59) 0.9668	43/65 (57) 0.9912	57/75 (68) 0.8975
	Carcinoma, pars distalis, malignant	0/74 (50) 0.7423	1/65 (48) 0.4898	0/65 (47) NC	0/65 (49) NC	1/75 (47) 0.4845
	Schwannoma, malignant	0/74 (50) 0.7423	1/65 (48) 0.4898	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
Gland, thyroid	Adenoma, c-Cell, benign	8/75 (53) 0.5164	11/65 (50) 0.2583	9/65 (50) 0.4472	9/65 (51) 0.4651	7/74 (49) 0.6522
	Adenoma, follicular cell, benign	0/75 (51) 0.3083	1/65 (48) 0.4848	0/65 (47) NC	1/65 (49) 0.4900	0/74 (46) NC
	Carcinoma, c-Cell, malignant	1/75 (51) 0.4417	3/65 (48) 0.2860	1/65 (47) 0.7317	2/65 (49) 0.4848	3/74 (46) 0.2705
	Carcinoma, follicular cell, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/74 (46) NC
Gland, zymbal	Carcinoma, malignant	0/74 (50) 0.0615	0/64 (47) NC	0/65 (47) NC	2/63 (48) 0.2373	1/74 (47) 0.4845
	Papilloma, squamous cell, benign	0/74 (50) NC	0/64 (47) NC	0/65 (47) NC	0/63 (48) NC	1/74 (46) 0.4792
Kidney	Adenoma, renal cell, benign	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/75 (47) 0.4796
Large intestine, cecum	Leiomyoma, benign	0/75 (51) 0.4923	0/65 (48) NC	1/65 (47) 0.4796	0/65 (49) NC	0/75 (47) NC
Large intestine,	Schwannoma, malignant	0/75 (51)	0/65 (48)	0/65 (47)	0/65 (49)	1/75 (47)

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Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
rectum		NC	NC	NC	NC	0.4796
Liver	Adenoma, hepatocellular, benign	1/75 (51) 0.3871	0/65 (48) 1.0000	1/65 (47) 0.7317	1/65 (49) 0.7424	0/75 (47) 1.0000
	Cholangiocarcinoma, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
	Hepatoblastoma, malignant	0/75 (51) 0.4923	0/65 (48) NC	1/65 (47) 0.4796	0/65 (49) NC	0/75 (47) NC
	Pheochromocytoma, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
Lung	Adenocarcinoma, malignant	0/75 (51) 0.8025	2/65 (49) 0.2376	0/65 (47) NC	0/65 (49) NC	0/74 (46) NC
	Carcinoma, adrenocortical, malignant	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	1/74 (46) 0.7262
	Carcinoma, squamous cell, malignant	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/74 (46) 0.4742
	Pheochromocytoma, malignant	0/75 (51) 0.3083	1/65 (48) 0.4848	0/65 (47) NC	1/65 (49) 0.4900	0/74 (46) NC
Lymph node, mesenteric	Thymoma, malignant	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/64 (48) 1.0000	0/75 (47) 1.0000
Muscle, skeletal	Sarcoma, malignant	0/75 (51) 0.4923	0/65 (48) NC	1/65 (47) 0.4796	0/65 (49) NC	0/75 (47) NC
	Schwannoma, malignant	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/75 (47) 0.4796
Nerve, optic	Schwannoma, malignant	0/75 (51) 0.7385	1/65 (48) 0.4848	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
Nerve, sciatic	Schwannoma, malignant	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/75 (47) 0.4796
Pancreas	Adenoma, islet cell, benign	0/75 (51) 0.2906	1/65 (48) 0.4848	1/65 (47) 0.4796	1/65 (49) 0.4900	0/75 (47) NC
	Carcinoma, islet cell, malignant	0/75 (51) 0.7385	1/65 (48) 0.4848	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
	Leiomyosarcoma, malignant	0/75 (51) 0.4949	0/65 (48) NC	1/65 (48) 0.4848	0/65 (49) NC	0/75 (47) NC
Skin	Basal cell tumor, benign	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/74 (47) 0.4796
	Carcinoma, squamous cell, malignant	0/75 (51) 0.7385	1/65 (48) 0.4848	0/65 (47) NC	0/65 (49) NC	0/74 (46) NC
	Papilloma, benign	0/75 (51) NC	0/65 (48) NC	0/65 (47) NC	0/65 (49) NC	1/74 (47) 0.4796
	Papilloma, squamous cell, benign	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/74 (46) NC
Skin/Subcutis	Fibroma, benign	0/75 (51) 0.1846	0/65 (48) NC	1/65 (48) 0.4848	1/65 (49) 0.4900	0/75 (47) NC
	Fibrosarcoma, malignant	2/75 (51) 0.1487	2/65 (48) 0.6681	1/65 (48) 0.8672	4/65 (51) 0.3390	2/75 (48) 0.6681
	Lipoma, benign	2/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000

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Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
	Osteosarcoma, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
Stomach	Leiomyosarcoma, malignant	0/75 (51) 0.4949	0/65 (48) NC	1/65 (48) 0.4848	0/65 (49) NC	0/75 (47) NC
Thymus	Thymoma, malignant	1/75 (51) 1.0000	0/64 (47) 1.0000	0/65 (47) 1.0000	0/61 (46) 1.0000	0/75 (47) 1.0000
Urinary bladder	Leiomyosarcoma, malignant	1/75 (51) 1.0000	0/65 (48) 1.0000	0/64 (46) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Papilloma, transitional cell, benign	1/75 (51) 1.0000	0/65 (48) 1.0000	0/64 (46) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Schwannoma, malignant	0/75 (51) NC	0/65 (48) NC	0/64 (46) NC	0/65 (49) NC	1/75 (47) 0.4796
Uterus/Cervix	Adenocarcinoma, malignant	0/75 (51) 0.8032	2/65 (48) 0.2325	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
	Adenoma, benign	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Carcinoma, squamous cell, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
	Granular cell tumor, benign	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Leiomyoma, benign	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
	Polyp, endometrial stromal, benign	9/75 (53) 0.9439	5/65 (48) 0.8936	5/65 (48) 0.8936	3/65 (50) 0.9817	0/75 (47) 1.0000
	Schwannoma, malignant	0/75 (51) 0.0622	0/65 (48) NC	0/65 (47) NC	2/65 (49) 0.2376	2/75 (48) 0.2325
	Stromal sarcoma, endometrial,	0/75 (51) 0.7398	1/65 (49) 0.4900	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
	Stromal sarcoma, malignant	0/75 (51) 0.4923	0/65 (48) NC	1/65 (47) 0.4796	0/65 (49) NC	0/75 (47) NC
Vagina	Carcinoma, squamous cell, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	0/75 (47) NC
	Granular cell tumor, benign	1/75 (51) 1.0000	0/65 (48) 1.0000	0/65 (47) 1.0000	0/65 (49) 1.0000	0/75 (47) 1.0000
	Polyp, benign	1/75 (51) 0.3871	0/65 (48) 1.0000	1/65 (47) 0.7317	1/65 (49) 0.7424	0/75 (47) 1.0000
	Schwannoma, malignant	0/75 (51) 0.2513	0/65 (48) NC	0/65 (47) NC	1/65 (49) 0.4900	2/75 (48) 0.2325
Whole Body	Hemangioma, benign	0/75 (51) 0.7385	1/65 (48) 0.4848	0/65 (47) NC	0/65 (49) NC	0/75 (47) NC
	Lymphoma, malignant	1/75 (51) 0.0942	1/65 (49) 0.7424	0/65 (47) 1.0000	3/65 (50) 0.3012	0/75 (47) 1.0000
	Sarcoma, histiocytic, malignant	1/75 (51) 0.2690	1/65 (48) 0.7372	2/65 (49) 0.4848	2/65 (50) 0.4925	0/75 (47) 1.0000

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.

Note: The p-values marked with an asterisk * indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Table 6A: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Male Mice Compared with Vehicle Control

Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
		Vehicle C P - Trend	Low (L) P - VC vs. L	Mid (M) P - VC vs. M	High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
Artery, aorta	Carcinoma, bronchioloalveolar, malignant	1/75 (74) 0.7279	0/65 (65) 1.0000	1/65 (64) 0.7143	0/65 (63) 1.0000	0/75 (74) 1.0000
Bone, sternum	Fibrosarcoma, malignant	1/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
Epididymis	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/74 (73) NC
Gland, adrenal	Adenoma, adrenocortical, benign	0/74 (73) 0.4773	0/65 (65) NC	1/64 (63) 0.4632	0/65 (63) NC	2/75 (74) 0.2517
	Adrenal tumor, subcapsular, benign	10/74 (73) 0.9893	2/65 (65) 0.9959	5/64 (63) 0.9120	1/65 (63) 0.9993	9/75 (74) 0.6993
	Pheochromocytoma, benign	0/74 (73) 0.7235	1/65 (65) 0.4710	0/64 (63) NC	0/65 (63) NC	0/75 (74) NC
Gland, coagulating	Carcinoma, malignant	1/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
Gland, harderian	Adenocarcinoma, malignant	1/75 (74) 0.9234	1/65 (65) 0.7184	0/65 (64) 1.0000	0/65 (63) 1.0000	1/75 (74) 0.7517
	Adenoma, benign	11/75 (74) 0.9924	9/65 (65) 0.6586	13/65 (64) 0.2683	2/65 (63) 0.9972	11/75 (74) 0.5911
Gland, pituitary	Adenoma, pars distalis, benign	2/74 (73) 0.9788	1/64 (64) 0.8516	0/64 (63) 1.0000	0/62 (61) 1.0000	1/73 (72) 0.8750
Gland, prostate	Adenocarcinoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Gland, seminal vesicle	Adenocarcinoma, malignant	2/75 (74) 0.8729	0/65 (65) 1.0000	1/65 (64) 0.8487	0/65 (63) 1.0000	1/75 (74) 0.8776
	Adenoma, benign	1/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Gland, thyroid	Adenoma, follicular cell, benign	0/75 (74) 0.5925	1/65 (65) 0.4676	1/64 (63) 0.4599	0/65 (63) NC	0/75 (74) NC
	Carcinoma, follicular cell, malignant	0/75 (74) 0.7208	1/65 (65) 0.4676	0/64 (63) NC	0/65 (63) NC	0/75 (74) NC
Heart	Carcinoma, bronchioloalveolar, malignant	0/75 (74) 0.5705	0/65 (65) NC	3/65 (64) 0.0972	0/65 (63) NC	1/75 (74) 0.5000
Kidney	Adenoma, renal cell, benign	0/75 (74) 0.2661	1/65 (65) 0.4676	1/65 (64) 0.4638	1/65 (63) 0.4599	2/75 (74) 0.2483
	Carcinoma, bronchioloalveolar, malignant	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	1/75 (74) 0.5000
	Carcinoma, malignant	1/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
Large intestine,	Adenocarcinoma, malignant	0/75 (74)	0/65 (65)	1/65 (64)	0/65 (63)	0/75 (74)

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Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
		Vehicle C P - Trend	Low (L) P - VC vs. L	Mid (M) P - VC vs. M	High (H) P - VC vs. H	Water C (WC) P - VC vs. WC
cecum		0.4774	NC	0.4638	NC	NC
Liver	Adenoma, hepatocellular, benign	16/75 (74) 0.8913	4/65 (65) 0.9984	6/65 (64) 0.9872	6/65 (63) 0.9859	15/75 (74) 0.6567
	Carcinoma, hepatocellular, malignant	5/75 (74) 0.9992	2/65 (65) 0.9189	0/65 (64) 1.0000	0/65 (63) 1.0000	5/75 (74) 0.6274
	Hepatoblastoma, malignant	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	1/75 (74) 0.5000
Lung	Adenocarcinoma, malignant	1/75 (74) 0.9228	1/65 (65) 0.7184	0/64 (63) 1.0000	0/65 (63) 1.0000	1/75 (74) 0.7517
	Adenoma, bronchioloalveolar, benign	14/75 (74) 0.9646	13/65 (65) 0.5204	8/64 (63) 0.8898	6/65 (63) 0.9655	15/75 (74) 0.5000
	Carcinoma, bronchioloalveolar, malignant	14/75 (74) 0.9465	11/65 (65) 0.6998	16/64 (63) 0.2397	6/65 (63) 0.9655	14/75 (74) 0.5830
	Carcinoma, hepatocellular, malignant	3/75 (74) 0.9943	1/65 (65) 0.9227	0/64 (63) 1.0000	0/65 (63) 1.0000	1/75 (74) 0.9400
	Leydig cell tumor, malignant	0/75 (74) NC	0/65 (65) NC	0/64 (63) NC	0/65 (63) NC	1/75 (74) 0.5000
Lymph node, mandibular	Leiomyosarcoma, malignant	0/73 (73) 0.7192	1/64 (64) 0.4672	0/64 (63) NC	0/62 (60) NC	0/73 (73) NC
Lymph node, mediastinal	Carcinoma, bronchioloalveolar, malignant	0/75 (74) 0.4774	0/65 (65) NC	1/65 (64) 0.4638	0/65 (63) NC	0/75 (74) NC
Mediastinum/Pleura	Carcinoma, bronchioloalveolar, malignant	0/75 (74) 0.4755	0/65 (65) NC	2/65 (64) 0.2133	0/65 (63) NC	0/75 (74) NC
Mesentery /Peritoneum	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Pancreas	Adenoma, islet cell, benign	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	3/75 (74) 0.1224
	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Skin	Adenoma, sebaceous, benign	0/75 (74) 0.7869	2/65 (65) 0.2169	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
	Basosquamous tumor, malignant	1/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
	Keratoacanthoma, benign	1/75 (74) 0.7279	0/65 (65) 1.0000	1/65 (64) 0.7143	0/65 (63) 1.0000	0/75 (74) 1.0000
Skin/Subcutis	Fibrosarcoma, malignant	1/75 (74) 0.8966	3/65 (65) 0.2625	1/65 (64) 0.7143	0/65 (63) 1.0000	2/75 (74) 0.5000
	Melanoma, amelanotic, malignant	2/75 (74) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
	Schwannoma, benign	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	1/75 (74) 0.5000
Small intestine, duodenum	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC

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Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
		Vehicle C	Low (L)	Mid (M)	High (H)	Water C (WC)
		P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. WC
Small intestine, ileum	Leiomyosarcoma, malignant	0/75 (74) 0.7218	1/65 (65) 0.4676	0/65 (64) NC	0/65 (63) NC	0/75 (74) NC
Testis	Leydig cell tumor, benign	3/75 (74) 0.7546	1/65 (65) 0.9227	1/65 (64) 0.9204	1/65 (63) 0.9181	5/75 (74) 0.3594
	Leydig cell tumor, malignant	0/75 (74) NC	0/65 (65) NC	0/65 (64) NC	0/65 (63) NC	2/75 (74) 0.2483
Thymus	Carcinoma, bronchioloalveolar, malignant	0/68 (67) 0.5877	0/58 (58) NC	3/59 (58) 0.0971	0/62 (60) NC	2/71 (70) 0.2592
Whole Body	Hemangioma, benign	2/75 (74) 0.1137	2/65 (65) 0.6398	1/65 (64) 0.8487	4/65 (63) 0.2673	2/75 (74) 0.6901
	Hemangiosarcoma, malignant	4/75 (74) 0.0000 *	9/65 (65) 0.0785	17/65 (64) 0.0005 **	31/65 (63) 0.0000 **	8/75 (74) 0.1836
	Comb_Hemangiosarcoma/ Hemangioma	6/75 (74) 0.0000 *	11/65 (65) 0.0928	18/65 (64) 0.0019 **	32/65 (63) 0.0000 **	10/75 (74) 0.2140
	Leukemia, malignant	1/75 (75) 1.0000	0/65 (65) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/75 (74) 1.0000
	Lymphoma, malignant	11/75 (74) 0.9563	9/65 (65) 0.6586	8/65 (64) 0.7408	4/65 (63) 0.9715	5/75 (74) 0.9694
	Sarcoma, histiocytic, malignant	2/75 (74) 0.5414	0/65 (65) 1.0000	1/65 (64) 0.8487	1/65 (63) 0.8454	3/75 (74) 0.5000

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.

Note: The p-values marked with an asterisk * indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Table 6B: Tumor Rates and P-Values for Trend and Pairwise Comparisons in Female Mice Compared with Vehicle Control

Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
		Vehicle C	Low (L)	Mid (M)	High (H)	Water C (WC)
		P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. WC
Bone marrow, sternum	Osteosarcoma, malignant	0/74 (74) NC	0/64 (63) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5034
		0/75 (75) 0.7191	1/65 (64) 0.4604	0/65 (63) NC	0/65 (65) NC	0/75 (75) NC
Gland, adrenal	Adenoma, adrenocortical, benign	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Adrenal tumor, subcapsular, benign	2/75 (75) 0.5685	0/65 (64) 1.0000	0/65 (63) 1.0000	1/65 (65) 0.8491	1/75 (75) 0.8775
	Adrenal tumor, subcapsular, malignant	0/75 (75) 0.7191	1/65 (64) 0.4604	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Pheochromocytoma, benign	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
Gland, clitoral	Adenocarcinoma, malignant	0/75 (75) 0.2434	0/65 (64) NC	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC
Gland, harderian	Adenocarcinoma, malignant	3/75 (75) 0.9462	0/65 (64) 1.0000	1/65 (63) 0.9160	0/65 (65) 1.0000	0/75 (75) 1.0000

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Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
	Adenoma, benign	3/75 (75) 0.9177	5/65 (64) 0.2749	1/65 (63) 0.9160	1/65 (65) 0.9207	1/75 (75) 0.9400
Gland, mammary	Adenocarcinoma, malignant	2/75 (75) 0.5419	3/65 (64) 0.4250	2/64 (62) 0.6153	2/65 (65) 0.6344	6/75 (75) 0.1378
	Adenoma, benign	0/75 (75) NC	0/65 (64) NC	0/64 (62) NC	0/65 (65) NC	1/75 (75) 0.5000
	Carcinoma, adeno squamous, malignant	4/75 (75) 0.9836	1/65 (64) 0.9571	1/64 (62) 0.9538	0/65 (65) 1.0000	2/75 (75) 0.8954
	Carcinosarcoma, malignant	2/75 (75) 1.0000	0/65 (64) 1.0000	0/64 (62) 1.0000	0/65 (65) 1.0000	1/75 (75) 0.8775
Gland, pituitary	Adenoma, pars distalis, benign	7/75 (75) 0.9988	6/64 (63) 0.5973	3/64 (62) 0.9116	0/65 (65) 1.0000	2/75 (75) 0.9832
	Adenoma, pars intermedia, benign	0/75 (75) 0.2453	0/64 (63) NC	0/64 (62) NC	1/65 (65) 0.4643	0/75 (75) NC
Hindlimb	Osteosarcoma, malignant	0/75 (75) 0.2434	0/65 (64) NC	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC
Kidney	Adenoma, renal cell, benign	0/75 (75) 0.2434	0/65 (64) NC	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC
Liver	Adenocarcinoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Adenoma, hepatocellular, benign	1/75 (75) 0.3698	0/65 (64) 1.0000	1/65 (63) 0.7064	1/65 (65) 0.7148	2/75 (75) 0.5000
	Carcinoma, hepatocellular, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	1/75 (75) 0.7517
	Osteosarcoma, malignant	0/75 (75) 0.7191	1/65 (64) 0.4604	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
Lung	Adenocarcinoma, malignant	3/75 (75) 0.9699	2/65 (64) 0.7636	1/65 (63) 0.9160	0/65 (65) 1.0000	2/75 (75) 0.8167
	Adenoma, bronchioloalveolar, benign	11/75 (75) 0.1130	10/65 (64) 0.5301	7/65 (63) 0.8076	14/65 (65) 0.2010	12/75 (75) 0.5000
	Carcinoma, adeno squamous, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
	Carcinoma, bronchioloalveolar, malignant	6/75 (75) 0.1888	4/65 (64) 0.7643	3/65 (63) 0.8678	7/65 (65) 0.3916	8/75 (75) 0.3900
	Carcinoma, hepatocellular, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
	Carcinosarcoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Choriocarcinoma, malignant	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Fibrosarcoma, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
	Mesothelioma, malignant	0/75 (75) 0.4794	0/65 (64) NC	1/65 (63) 0.4565	0/65 (65) NC	0/75 (75) NC
	Osteosarcoma, malignant	0/75 (75) 0.2910	1/65 (64) 0.4604	0/65 (63) NC	1/65 (65) 0.4643	2/75 (75) 0.2483
Lymph node, iliac	Adenocarcinoma, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/64 (64) 1.0000	0/75 (75) 1.0000

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Organ name	Tumor name	0 mg/kg/day	2 mg/kg/day	6 mg/kg/day	20 mg/kg/day	0 mg/kg/day
		Vehicle C	Low (L)	Mid (M)	High (H)	Water C (WC)
		P - Trend	P - VC vs. L	P - VC vs. M	P - VC vs. H	P - VC vs. WC
Lymph node, mandibular	Adenocarcinoma, malignant	1/75 (75)	0/65 (64)	0/65 (63)	0/64 (64)	0/74 (74)
		1.0000	1.0000	1.0000	1.0000	1.0000
Mediastinum/Pleura	Carcinosarcoma, malignant	0/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	1/75 (75)
		NC	NC	NC	NC	0.5000
	Mesothelioma, malignant	0/75 (75)	0/65 (64)	2/65 (63)	0/65 (65)	0/75 (75)
		0.4833	NC	0.2066	NC	NC
	Osteosarcoma, malignant	0/75 (75)	0/65 (64)	0/65 (63)	1/65 (65)	0/75 (75)
		0.2434	NC	NC	0.4643	NC
Mesentery /Peritoneum	Osteosarcoma, malignant	0/75 (75)	1/65 (64)	0/65 (63)	0/65 (65)	0/75 (75)
		0.7191	0.4604	NC	NC	NC
Nerve, sciatic	Fibrosarcoma, malignant	0/75 (75)	0/65 (64)	1/65 (63)	0/65 (65)	0/75 (75)
		0.4794	NC	0.4565	NC	NC
Nose	Adenocarcinoma, malignant	1/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	0/75 (75)
		1.0000	1.0000	1.0000	1.0000	1.0000
Ovary	Choriocarcinoma, malignant	0/73 (73)	0/65 (64)	0/63 (61)	0/64 (64)	1/75 (75)
		NC	NC	NC	NC	0.5068
	Cystadenoma, benign	4/73 (73)	1/65 (64)	0/63 (61)	1/64 (64)	0/75 (75)
		0.8445	0.9598	1.0000	0.9598	1.0000
	Ovarian sex cord-Stromal tumor, benign	1/73 (73)	4/65 (64)	2/63 (61)	1/64 (64)	2/75 (75)
		0.7321	0.1446	0.4325	0.7179	0.5102
	Stromal tumor, gonadal, malignant	1/73 (73)	0/65 (64)	0/63 (61)	0/64 (64)	0/75 (75)
		1.0000	1.0000	1.0000	1.0000	1.0000
Pancreas	Adenoma, islet cell, benign	0/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	1/75 (75)
		NC	NC	NC	NC	0.5000
	Carcinoma, islet cell, malignant	0/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	1/75 (75)
		NC	NC	NC	NC	0.5000
Skin	Carcinoma, basal cell, malignant	1/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	0/75 (75)
		1.0000	1.0000	1.0000	1.0000	1.0000
	Papilloma, squamous cell, benign	0/75 (75)	2/65 (64)	0/65 (63)	1/65 (65)	1/75 (75)
		0.4116	0.2102	NC	0.4643	0.5000
Skin/Subcutis	Fibrosarcoma, malignant	1/75 (75)	0/65 (64)	2/65 (63)	0/65 (65)	1/75 (75)
		0.6677	1.0000	0.4345	1.0000	0.7517
	Osteosarcoma, malignant	0/75 (75)	0/65 (64)	0/65 (63)	0/65 (65)	2/75 (75)
		NC	NC	NC	NC	0.2483
Small intestine, duodenum	Polyp, glandular, benign	0/75 (75)	0/65 (64)	0/65 (63)	1/65 (65)	0/75 (75)
		0.2434	NC	NC	0.4643	NC
Spleen	Adenocarcinoma, malignant	0/75 (75)	0/65 (64)	0/65 (63)	0/64 (64)	1/75 (75)
		NC	NC	NC	NC	0.5000
Stomach	Papilloma, squamous cell, benign	0/75 (75)	0/65 (64)	1/65 (63)	0/65 (65)	0/75 (75)
		0.4794	NC	0.4565	NC	NC
Thymus	Adenocarcinoma, malignant	0/75 (75)	0/63 (62)	0/60 (58)	0/61 (61)	1/73 (73)
		NC	NC	NC	NC	0.4932
	Carcinoma, bronchioloalveolar,	0/75 (75)	0/63 (62)	0/60 (58)	1/61 (61)	1/73 (73)

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Organ name	Tumor name	0 mg/kg/day Vehicle C P - Trend	2 mg/kg/day Low (L) P - VC vs. L	6 mg/kg/day Mid (M) P - VC vs. M	20 mg/kg/day High (H) P - VC vs. H	0 mg/kg/day Water C (WC) P - VC vs. WC
	malignant	0.2383	NC	NC	0.4485	0.4932
Urinary bladder	Adenocarcinoma, malignant	1/75 (75) 1.0000	0/64 (63) 1.0000	0/65 (63) 1.0000	0/64 (64) 1.0000	0/75 (75) 1.0000
Uterus/Cervix	Adenocarcinoma, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	2/75 (75) 0.5000
	Granular cell tumor, benign	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
	Leiomyoma, benign	3/75 (75) 0.9462	0/65 (64) 1.0000	1/65 (63) 0.9160	0/65 (65) 1.0000	1/75 (75) 0.9400
	Polyp, endometrial stromal, benign	1/75 (75) 0.8723	7/65 (64) 0.0181	3/65 (63) 0.2471	1/65 (65) 0.7148	3/75 (75) 0.3100
	Polyp, glandular, benign	0/75 (75) NC	0/65 (64) NC	0/65 (63) NC	0/65 (65) NC	1/75 (75) 0.5000
	Schwannoma, malignant	0/75 (75) 0.2910	1/65 (64) 0.4604	0/65 (63) NC	1/65 (65) 0.4643	0/75 (75) NC
	Stromal sarcoma, endometrial, malignant	3/75 (75) 0.3720	0/65 (64) 1.0000	0/65 (63) 1.0000	2/65 (65) 0.7691	2/75 (75) 0.8167
Vagina	Stromal sarcoma, endometrial, malignant	1/74 (74) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
Whole Body	Hemangioma, benign	1/75 (75) 0.0060	0/65 (64) 1.0000	1/65 (63) 0.7064	5/65 (65) 0.0748	1/75 (75) 0.7517
	Hemangiosarcoma, malignant	4/75 (75) 0.0001 *	5/65 (64) 0.4006	14/65 (63) 0.0034 **	18/65 (65) 0.0003 **	2/75 (75) 0.8954
	Comb_Hemangiosarcoma/ Hemangioma	5/75 (75) 0.0001 *	5/65 (64) 0.5241	14/65 (63) 0.0081 **	19/65 (65) 0.0004 **	3/75 (75) 0.8622
	Leukemia, malignant	1/75 (75) 1.0000	0/65 (64) 1.0000	0/65 (63) 1.0000	0/65 (65) 1.0000	0/75 (75) 1.0000
	Lymphoma, malignant	26/75 (75) 0.9239	25/65 (64) 0.3593	19/65 (63) 0.7715	17/65 (65) 0.8987	19/75 (75) 0.9232
	Sarcoma, histiocytic, malignant	0/75 (75) 0.4116	2/65 (64) 0.2102	0/65 (63) NC	1/65 (65) 0.4643	1/75 (75) 0.5000

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed; NC = Not calculable.

Note: The p-values marked with an asterisk * indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk ** indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Figure 1A: Kaplan-Meier Survival Functions for Male Rats

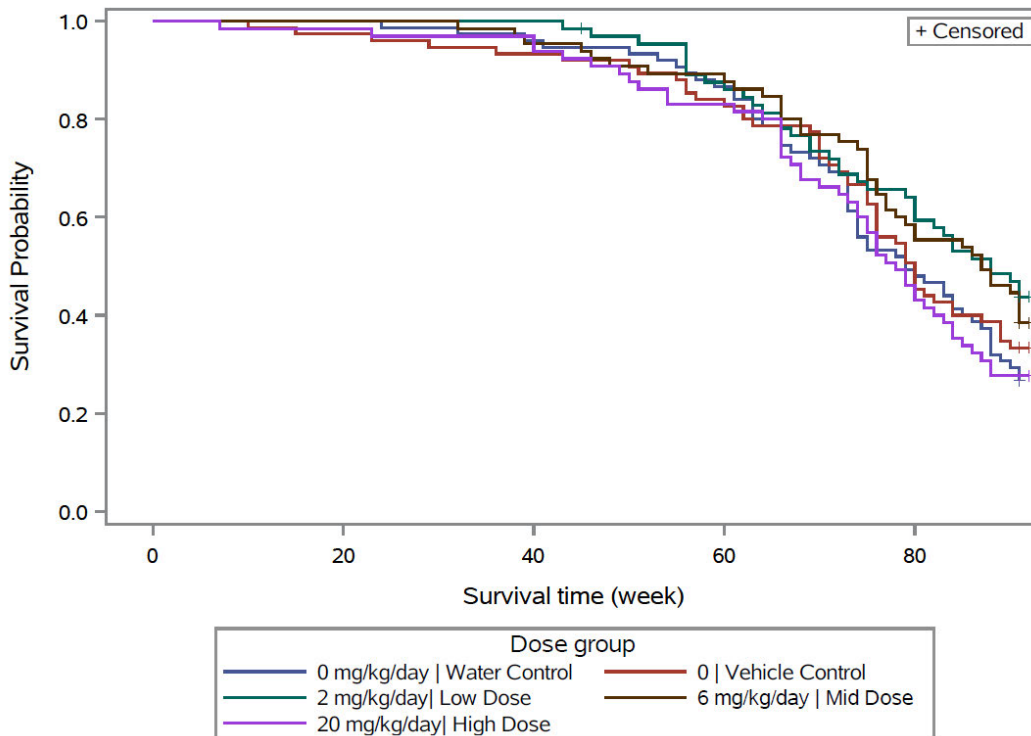


Figure 1B: Kaplan-Meier Survival Functions for Female Rats

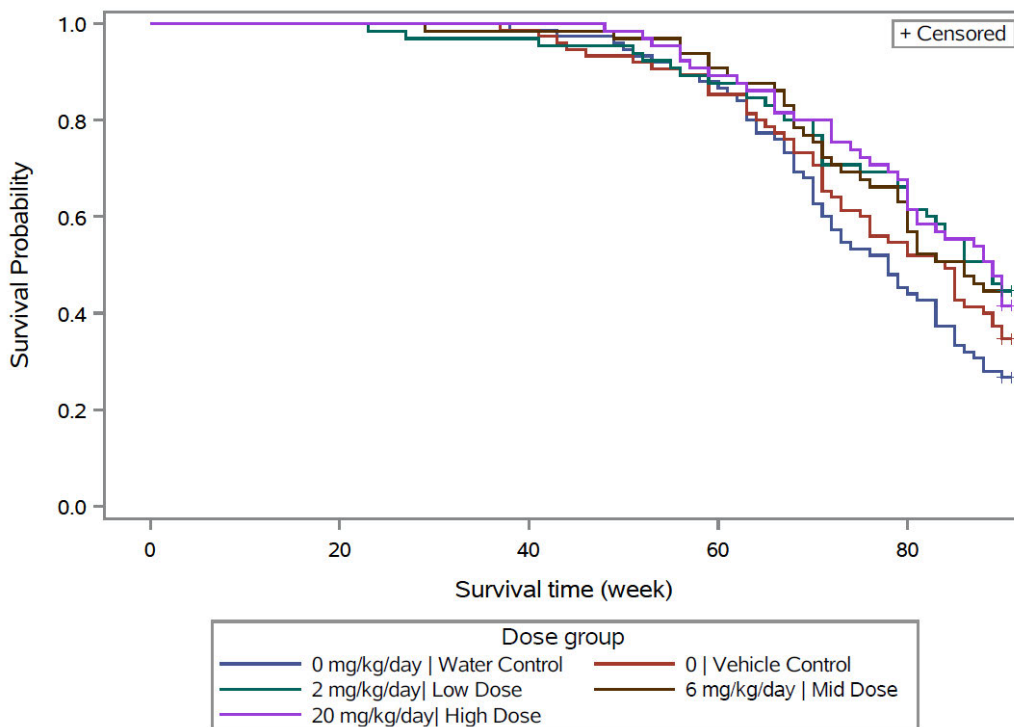


Figure 2A: Kaplan-Meier Survival Functions for Male Mice

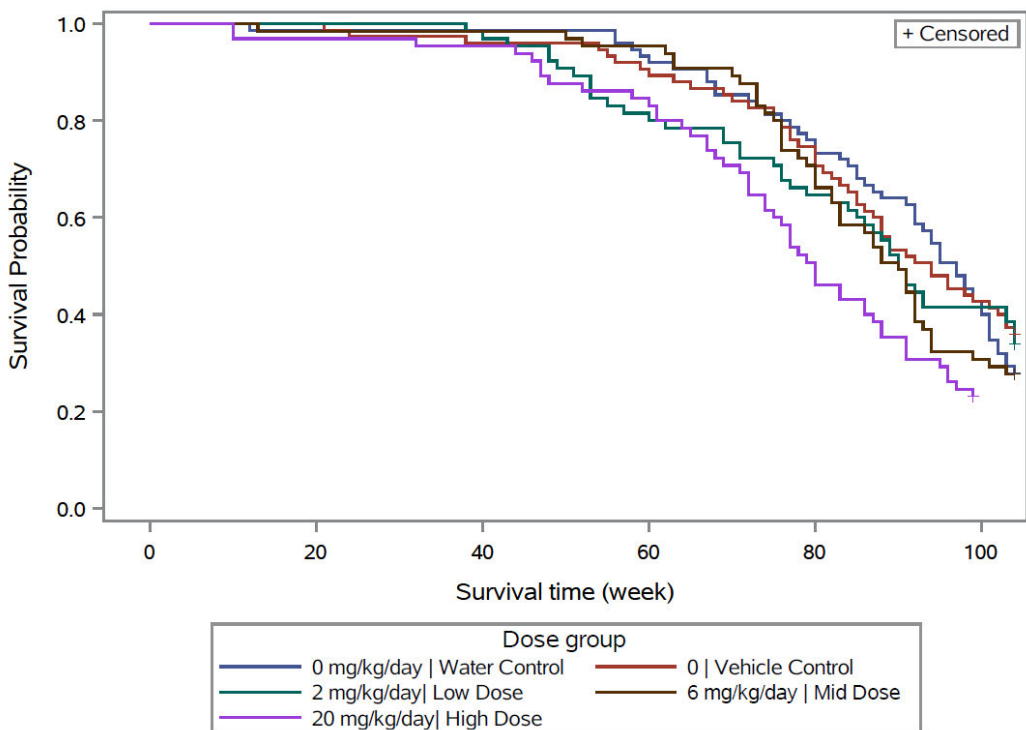
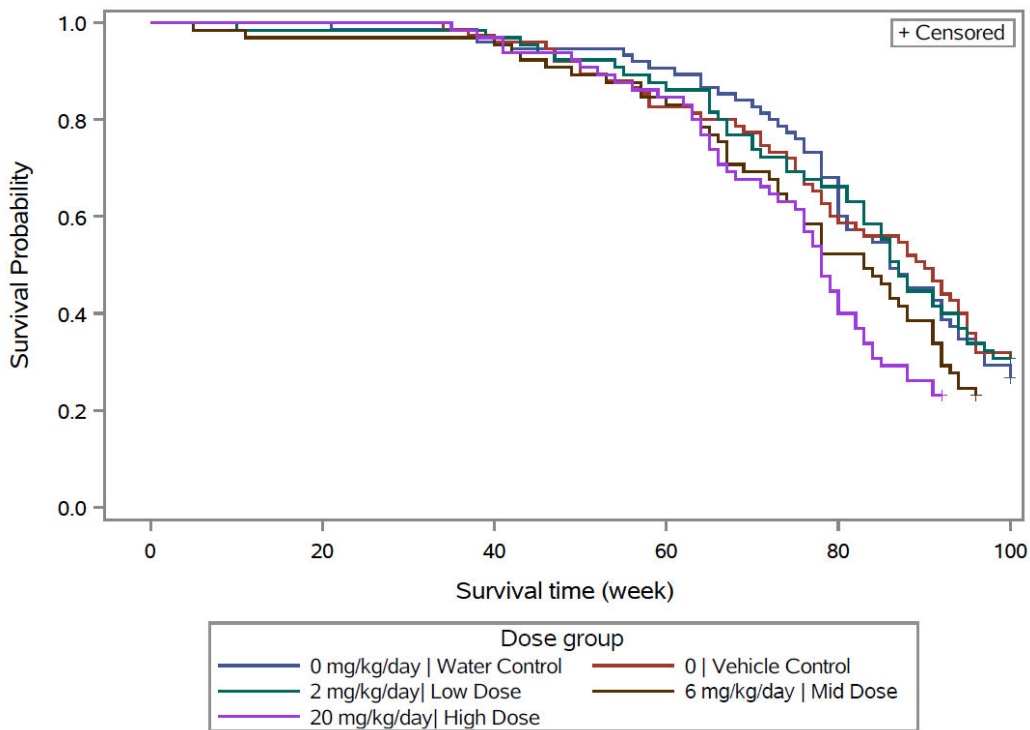


Figure 2B: Kaplan-Meier Survival Functions for Female Mice



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